Chapter 2
Targeting Chemotherapy Resistance in Glioblastoma Through Modulation of ABC Transporters

Amanda Tivnan

Abstract Glioblastoma (GBM) is a highly aggressive Grade IV solid central nervous system neoplasm with an incidence rate of 3–4 per 100,000 people worldwide and the average 5-year survival rate of GBM patients is less than 5 %, leading to the fact that GBM is the most lethal form of brain tumor. The presence of several adenosine triphosphate-binding cassette (ABC) transporters is thought to contribute to the sustained progression of GBM tumours, inhibiting and rapidly removing anticancer drugs from GBM tumour cells. ABC transporters are transmembrane pumps which use ATP hydrolysis to facilitate translocation of substrates across cellular membranes. Overexpression of ABC transporters including P-gp (ABCB1), ABCCs, or MRPs and breast cancer-resistance protein (BCRP, ABCG2) on the GBM cells themselves is thought to instill chemoresistance and active drug extrusion at the tumor site rendering the temporal effect of successfully administered drugs negligible, if at all. In this regard, the role of individual ABC transporters and their contribution to chemoresistance and potential as targeted therapies of GBM chemosensitization will be discussed in this chapter.

Keywords Adenosine triphosphate-binding cassette (ABC) transporters • Chemoresistance • Glioblastoma

Abbreviations

ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
B-BB	Blood-brain barrier

A. Tivnan, PhD (✉)
Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland
e-mail: amandativnan@rcsi.ie

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B-CFB	Blood-cerebrospinal fluid barrier
B-TB	Blood-tumor barrier
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
EGF	Epithelial growth factor
Gy	Gray (unit of ionizing radiation)
PD-1	Programmed cell death protein 1
VEGF	Vascular endothelial growth factor

2.1 Introduction

Glioblastoma (GBM) is a highly aggressive Grade IV solid central nervous system neoplasm with an incidence rate of 3–4 per 100,000 people worldwide [1], and an average 5-year survival rate of less than 5 %, leading to the fact that GBM is the most lethal form of brain tumor (http://www.braintumourresearch.org/our-reports). GBM represents approximately 15 % of all primary brain tumors diagnosed annually in the USA, increasing in frequency with age and showing more prevalence in men than women (http://globocan.iarc.fr/). Although the incidence has decreased in the context of primary and CNS tumors when determined histologically since 1995, it has remained the highest recorded type of glioma, accounting for 55.1 % diagnosed with respect to all other histological glioma subtypes between 2008 and 2012 [13]. Although the incidence rates for brain and nervous system tumors have been collated across Europe [14], the incidence rates for specific brain tumor types such as GBM have yet to be compiled.

Despite decades of ongoing clinical research, the median survival rate for GBM patients beyond 12 months has not changed significantly. Initially, standard clinical care involved extensive surgical resection followed by adjuvant radiation therapy (RT), which included 45–50 Gy of deep RT to the tumor site, given daily over a period of 4 to 5 weeks, resulting in a doubling in median survival time from 4–6 to 10–11 months [15]. Whole-brain RT became the standard of care, as non-specific targeting of uncontrollably growing cells proved to be efficacious in GBM treatment. Understandably, the brain contains many neural structures which are very sensitive to RT, limiting the amount of tolerated RT which could be used in patients; however, alternative RT regimen did not alter survival rates. Up until the mid-1990s, nitrosoureas, alkylating agents used in chemotherapy, showed benefit to patient survival by approximately 2 months [16]. Systemically administered bis-chloroethylnitrosourea (BCNU) forms interstrand cross-links in DNA, preventing replication and transcription. BCNU administration was the standard of care for adjuvant chemotherapy at that time and was administered at the time of surgery to the resection cavity. The BCNU studies span over four decades, until Stupp and colleagues established a new system of chemotherapy, the current standard of care. The Stupp protocol involves tumor resection followed by RT in

combination with adjunct and concomitant temozolomide (TMZ) chemotherapy, increasing the median survival rates from 12.1 months with RT alone to 14.6 months for TMZ/RT treatment; however, little improvement has been made since this time. Despite treatment, GBM recurrence at distal sites is typically 6.9 months [17], and in instances where repeat resection is not a viable option, adjunct chemotherapy is ineffective at stopping tumor progression and morbidity, with several studies attributing such treatment resistance to increased expression of multidrug resistance proteins including members of the ATP-binding cassette (ABC) family [6, 18–20].

2.2 Adenosine Triphosphate-Binding Cassette (ABC) Superfamily

Adenosine triphosphate-binding cassette (ABC) transporters are transmembrane pumps which consist of multiple subunits of transmembrane and membrane-associated ATPases, the latter of which uses ATP hydrolysis to facilitate translocation of substrates across cellular membranes. Substrates of these transporters can be both organic and inorganic, ranging from amino acids, lipids, and sterols to primary and secondary metabolites and drugs [21]. ABC genes, of which 48 have been identified in humans, are essential for human processes [22] with mutations being linked to a myriad of diseases such as cystic fibrosis [23, 24], Stargardt's disease [25–27], and Dubin-Johnson syndrome [28, 29] and contributing to multiple drug resistance through transporter protein overexpression in several cancers [30–33].

2.2.1 Structure

The primary sequence of the ABC transporter family is highly conserved throughout evolution with four subunits or domains, two nucleotide-binding domains (NBDs) which are ATP-binding domains and two transmembrane domains (TMD1 and TMD2). The NBD domains contain Walker A (or P-loop), Walker B motif, a Q-loop, and H motif, and an α-helical signature (C) domain "LSGGQ" [21]. The Walker A and the second Walker B domains are found in all ATP-binding proteins; however, the C motif is specific to ABC transporters. The TMDs which consist of between six and ten transmembrane α-helices, depending on the transporter class [21], form a transmembrane "pore" which can be classified as inward (open to the cytoplasm) or outward (open to the exterior) facing, with no known sequence homology resulting in the diversity of substrate binding. Genes encoding these transporters are organized as either full proteins (*2 NBF and 2 TMDs*) or half transporters which are later assembled as homo- or heterodimers.

2.2.2 *Mechanism of Transport*

ABC transporters typically have to pump substrates against a chemical gradient, requiring energy to fuel this process, provided as a result of ATP hydrolysis. In brief, ABC transporters undergo a catalytic cycle from a ground to activated state whereby direct binding of a specific substrate to the TMDs occurs in conjunction with two ATP molecules binding to the NBDs. TMDs change conformation from either inward to outward facing or vice versa; ATP is hydrolyzed with the result of ATP and phosphate; and reduced TMD substrate affinity leads to solute release. At this point, NBDs dissociate and return to the inactivated/ground state. This widely accepted theory of ABC transporter function is known as the "alternating access model" [34].

More specifically, the structure of NBDs in ABC transporters is an ongoing area of debate, with theories speculating that the resting state is composed of monomer NBDs requiring the binding of free ATP prior to dimerization or, alternatively, the maintenance of a ground inactivated dimer state with ATP preloaded requiring stimulation through substrate binding to the TMD before functional dimerization and transport activity. The mechanism through which TMDs change conformation after substrate binding is thought to involve coupling helices called intercellular loops (ICLs) which are found in close contact with the helical domains, in a groove between the two lobes of the NBD [35]. A Q-loop extends up from the TMD and overlaps a structurally diverse region (SDR) of 30 nucleotides with a downstream X-loop from the NBD [36]. Once bound, the substrate stimulates a change in the ICLs, causing the Q-loop to move. This movement can then, in theory, increase the affinity of the NBDs for unloaded ATP leading to dimerization of the NBD itself through ATP hydrolysis or promote dimerization of the inactive-dimer-ATP-bound ground state.

Although this process varies among ABC transporter classes, the basic steps of ATP-dependent NBD dimerization and TMD conformational switching are shared constitutively leading to translocation of a particular substrate across a membrane against a chemical gradient.

The ABC superfamily is the largest family of transmembrane proteins with seven subfamilies designated A–G based on sequence homology [37]. ABCA proteins are predominantly expressed in the central nervous system (CNS) and the hematopoietic system involved in lipid transport and homeostasis with 12 members being identified [37–40]. The *ABCB* gene is primarily expressed in the blood-brain barrier (B-BB) and liver involved in toxin extrusion; however, overexpression results in multiple drug resistance [22]. The most extensively studied is ABCB1, also known as P-glycoprotein (P-gp) or multidrug resistance protein 1 (MDR1), which is discussed in detail in Sect. 2.3 in the context of its contribution to GBM drug resistance. The ABCC subfamily contains 13 members, nine of which are also referred to as multidrug resistance proteins (MRPs) involved in transport, toxin excretion, and signal transduction [41], the role of which in chemotherapy-resistant GBM is outlined in Sect. 2.4 of this chapter. The ABCD subfamily is exclusively expressed in the peroxisome and the role of ABCD1 in fatty acid metabolism has been linked to adrenoleukodystrophy (ALD), a neurodegeneration and adrenal deficiency disease [42–44].

ABCE and *ABCF* genes contain ATP-binding domains; however, these genes do not encode transmembrane regions. The ABCG subfamily has an orientation opposing all other ABC genes with an ATP-binding domain in the N terminus and transmembrane at the C terminus. ABCG2 is also known as the breast cancer-resistance protein (BCRP), and although its native function is not known, chromosomal translocation resulting in ABCG2 amplification causes drug resistance to common anticancer drugs such as topotecan, mitoxantrone, and doxorubicin [45–47]. The role of BCRP in GBM drug resistance is further discussed in Sect. 2.5.

With respect to glioblastoma, tumor sustainability may be due to the inability of several anticancer drugs to cross the B-BB, blood-cerebrospinal fluid barrier (B-CFB), and blood-tumor barrier (B-TB) due to the presence of several ABC transporters [2–5]. In addition, overexpression of ABC transporters including P-gp (ABCB1), ABCCs or MRPs, and breast cancer-resistance protein (BCRP, ABCG2) on the GBM cells themselves is thought to instill chemoresistance and active drug extrusion at the tumor site rendering the temporal effect of successfully administered drugs negligible, if at all [6–12]. In this regard, the role of individual ABC transporters and their contribution to chemoresistance and potential as targeted therapies of GBM chemosensitization will be discussed in this chapter.

2.3 P-Glycoprotein (P-gp/Pgp/ABCB1/MDR1/CD243)

P-Glycoprotein is encoded by two multidrug resistance 1 genes (*MDR1 and MDR3*). The predominant protein isoform is the multidrug resistance protein 1 (MDR1), also known as ATP-binding cassette subfamily B member 1 (ABCB1) or cluster of differentiation 243 (CD243). The MDR3 product is not known to confer substrate resistance in humans [48]. P-gp was the first identified ABC transporter and is, therefore, the most extensively studied, showing a very broad range of substrate specificity (Table 2.1).

Although the presence of the B-BB acts as a major impediment to the therapeutic effect of several drugs on brain cancers, the active efflux of anticancer drugs by ABC transporters further reduces any effect which may be noted by such chemotherapeutics. In 2005, the FDA approved the concomitant use of temozolomide (TMZ) with radiotherapy for the treatment of newly diagnosed GBM, a standard of care which is maintained today, known as the Stupp protocol [49]. As TMZ is the current first-line treatment option for GBM, identification of mechanisms of TMZ resistance is an important avenue of research, holding the potential to improve clinically used chemotherapeutic agents. TMZ resistance, in many cancers, has been proven to be multifactorial, including changes in cell cycle in response to treatment, increased mismatch repair genes, and increased expression of O-6-methylguanine transferase (MGMT) expression [50, 51]. Although TMZ has been the only chemotherapy which has proven to increase survival rates, an achievement attributed to the drugs' ability to traverse the B-BB with ease, the presence of P-gp and several other ABC transporters whose substrate specificity extends to TMZ lends to one of the mechanisms which contribute to

Table 2.1 MRP transporter substrate and inhibitor lists

Transporter name	Distribution	Substrate	Inhibitors
P-Glycoprotein (P-gp/Pgp/ABCB1/CD243/MDR1)	• Intestinal epithelium • Liver cells • Proximal tubule of the kidney • Capillary endothelial cells (B-BB and blood-testis barrier) • Cancer cells	Aldosterone Amprenavir Bilirubin Cimetidine Colchicine Cortisol CPT-11 Cyclosporine Doxorubicin Dexamethasone Digoxin Diltiazem Domperidone Etoposide Estradiol-17B-D-glucuronide Erythromycin Fexofenadine Quinidine Tacrolimus Temozolomide Vinblastine Immunosuppressive agents HIV type I antiretroviral therapies Lipids Xenobiotics	Amiodarone Azithromycin Atorvastatin, bromocriptine, carvedilol, cyclosporine, captopril Clarithromycin Erythromycin, GF120918, itraconazole, ketoconazole, LY335979, meperidine Methadone Nelfinavir, pentazocine Progesterone Piperine Quercetin Quinidine Quinine Reserpine Reversan Ritonavir Tariquidar Verapamil

ABCC1 (MRP1)	• Ubiquitous Highest in • Intestine • Testes • Kidney • Malignant cancer cells of the brain • Capillary endothelial cells (B-BB and blood-testis barrier)	Adefovir Indinavir Saquinavir Ritonavir Methotrexate, edatrexate ZD1694 Doxorubicin Daunorubicin, epirubicin Idarubicin Etoposide, vincristine, vinblastine, paclitaxel Irinotecan Hydroxyflutamide, FR901228 NSC-630176 Difloxacin, grepafloxacin N-Ethylmaleimide, thiotepa, cyclophosphamide, melphalan, chlorambucil, ethacrynic acid, metolachlor Atrazine, sulforaphane, aflatoxin Arsenic NNAL SN-38 E3040S	Reversan MK571, indomethacin, benzbromarone, euchrestaflavanone, sophoraflavanone, cyclosporine A, quercetin

(continued)

Table 2.1 (continued)

Transporter name	Distribution	Substrate	Inhibitors
ABCC2 (MRP2)	• Intestine • Liver • Kidney • Brain	Indinavir Cisplatin Vinblastine Doxorubicin Methotrexate Etoposide, mitoxantrone, valsartan Olmesartan, glucuronidated SN-38	Cyclosporine Delavirdine, efavirenz, emtricitabine, benzbromarone
ABCC3 (MRP3)	• Kidney • Intestines • Adrenal gland • Liver • Pancreas • Capillary endothelial cells (B-BB and blood-testis barrier)	Etoposide Methotrexate Teniposide Fexofenadine, methotrexate, vincristine, Teniposide, acetaminophen, glucuronide	Delavirdine, efavirenz, nevirapine, emtricitabine, lamivudine, tenofovir, indomethacin, furosemide, probenecid, MK751
ABCC4 (MRP4)	• Bladder • Lung • Muscle • Pancreas • Prostate • Gonads • Capillary endothelial cells (B-BB and blood-testis barrier)	6-Mercaptopurine 6-Thioguanine and metabolites Methotrexate Acyclovir Ritonavir Tenofovir Topotecan PMEA Furosemide, ceftizoxime Cefazolin	MK571 Indomethacin, diclofenac, celecoxib, sulfinpyrazone, quercetin

ABCC5 (MRP5)	• Ubiquitous • Highest in • B-BB • Heart • Liver • Lung • Urethra • Placenta • Skeletal muscles	6-Mercaptopurine 6-Thioguanine and metabolites Methotrexate PMEA 5-Fluorouracil, rosuvastatin, atorvastatin	Sulfinpyrazone, benzbromarone Trequinsin Dipyridamole Zaprinast NPPB
ABCC6 (MRP6)	• Kidney • Liver • Low expression in the lung, intestines, retina, skin, and vessel walls	Cisplatin Daunorubicin Etoposide Anthracyclines Epipodophyllotoxins Leukotriene C4	Benzbromarone Indomethacin Probenecid
ABCC7 (MRP10)	• Ubiquitous expression at a low level • Highest expression in • Pancreas • Prostate cancer, hepatocellular cell lines • Breast cancer	Taxanes Epothilone B Vinca alkaloids Cytarabine Tamoxifen	Cepharanthine Lapatinib Erlotinib Nilotinib Imatinib Sildenafil Vardenafil

(continued)

Table 2.1 (continued)

Transporter name	Distribution	Substrate	Inhibitors
Breast cancer resistance protein (BCRP/ ABCG2)	• Intestine • Liver • Breast • Placenta • Brain	Daunorubicin Doxorubicin Topotecan Rosuvastatin Sulfasalazine Mitoxantrone Irinotecan Imatinib Methotrexate Anthracyclines SN-38 Nucleoside analogues, prazosin, pantoprazole Statins	Elacridar (GF120918) Abacavir Amprenavir Aripiprazole Atazanavir Atorvastatin Cerivastatin Daunomycin Dehydroepiandrosterone sulfate Delavirdine Efavirenz Erlotinib Fluvastatin Ko143 Lopinavir Nelfinavir Nilotinib Pitavastatin Prenylflavonoid Rosuvastatin Saquinavir Simvastatin SN-38 Sulfasalazine

the acquired resistance of TMZ transport into the brain. ABC transporters on the luminal surface of the B-BB, such as P-gp, transport drugs from the endothelial cells of the B-BB back into blood circulation, thereby reducing their bioavailability, reducing the drugs' capacity to elicit DNA damage, and having a profound effect on TMZ-induced tumor death. In addition, increased expression of P-gp on the tumor itself has a direct correlation to the therapeutic effect of the P-gp substrate anticancer drug [8, 52–54].

It has also been shown that treatment of GBM cells with TMZ led to increased expression of P-gp itself through epidermal growth factor (EGF) regulation of the *MDR1* gene [55]. This increased P-gp expression leads to increased drug extrusion and increased resistance. The diverse localization of P-gp has been found to contribute to multidrug resistance in many different forms of cancer [56–59]. The contribution of P-gp to GBM leads to rapid and prolonged resistance resulting in short progression-free survival for patients [55]. From a clinical perspective, P-gp-positive cells from surgical specimens increase with respect to malignancy grade, i.e., from low-grade glioma to high grade or GBM [60]. In addition to P-gp overexpression, genetic alterations such as MDR1 polymorphisms play an important role with respect to GBM patient's response to chemotherapeutic regimes.

In principle, P-gp-induced multidrug resistance may be overcome using treatment combinations of chemotherapy and P-gp inhibitors or, alternatively, non-P-gp substrate anticancer agents. Researchers have evaluated the potential of P-gp inhibition, which is used in several other forms of cancer [61–65], with respect to increasing therapeutic transport into the brain [12, 66–69]. The two most commonly used P-gp inhibitors, verapamil and cyclosporine A, are first-generation P-gp inhibitors, which have been assessed with respect to their ability to inhibit P-gp-mediated transport and drug resistance in glioblastoma models [66, 70–74]. Second- and third-generation P-gp reversal agents have also been developed and evaluated in cancers other than glioma: valspodar, dexverapamil and tariquidar, biricodar, elacridar, OC144-093, and R101933 [75–81]. Additional approaches include P-gp monoclonal antibodies, an immunotherapy-based approach [82–85]; however, this has yet to be applied to glioblastoma. There are a very limited number of glioma clinical trials which involve P-gp and are mainly focused on the assessment of P-gp expression or polymorphism contribution to novel drug resistance (Table 2.2). A considerable disadvantage to the use of such inhibition, specifically for brain cancer, is the fact that increased permeability of the B-BB to such a broad range of substrates may lead to drug neurotoxicity and restricted dosage limitations.

Although the role of the tumor microenvironment and hypoxia to apoptosis resistance has been readily studied [120–123], recent studies have linked hypoxia to chemodrug resistance through the *ABCB1* gene. The P-gp-encoded gene contains a hypoxia-responsive element in the promoter region and can, therefore, be regulated by hypoxic microenvironment conditions via HIF1α [124]. Increased hypoxia or tumor cycling hypoxia which occurs as the tumor establishes a reliable blood flow to facilitate tumor expansion can lead to increased P-gp expression and increased drug resistance. This pathway is particularly applicable to GBM drug resistance pathways as these highly heterogeneous tumors quite often have a necrotic and hypoxic core which, in addition to radiotherapy resistance [125], may also be multidrug resistant due to increased P-gp expression [124].

Table 2.2 Clinical trial involving ABC transporters

Link to ABC transporters	NCT number	Cancer type	Status	Research-relevant and trial result references
P-gp imaging	NCT01281982	Glioma	Terminated December 2015	[86–88]
P-gp expression correlation to prognosis	NCT02197637	Glioma	Open. Due to finish March 2017	[89, 90]
P-gp antagonist PSC 833	NCT00001302	Breast, kidney, neoplasm, lymphoma, metastasis, ovarian	Completed in 2002	[76, 78–81, 91–100]
P-gp as a stratification factor	NCT01459484	Osteosarcoma	Estimated completion January 2020	[101–103]
ABCC1 polymorphisms	NCT00898456	Myeloid leukemia	Estimated completion January 2020	[104, 105]
Multidrug resistance genes	NCT00898404	Acute lymphoblastic leukemia	Estimated completion January 2020	[106–108]
Genetic variations of ABC transporters ABCC1, P-gp	NCT01282658	Colorectal cancer	Completed May 2013	[109–111]
Genetic variations of ABC transporters ABCC1, P-gp	NCT01280448	Lung cancer	Completed September 2013	[112, 113]
Prediction response to chemotherapy. MRP1 P-gp	NCT00551798	High-grade lymphoma Hodgkin's disease	Completed January 2011	[114, 115]
Correlation to drug resistance (P-gp, MRP1, BCRP, and MDR-3)	NCT00753207	Breast	Completed January 2014	[116–119]

2.4 Multidrug Resistance Proteins

This family of ABC transporters contains six members, MRP1–6 (ABCC1–6) which have progressively been identified since 1992 [126–132] and are known to contribute to ATP-dependent decrease in anticancer drug efflux and, therefore, multidrug resistance. In this chapter, the author has focused on multidrug resistance proteins 1 (ABCC1, MRP1), 3 (ABCC3, MRP3), and 5 (ABCC5, MRP5) with regard to their contribution to glioblastoma drug resistance.

2.4.1 Multidrug Resistance Protein 1 (MRP1, ABCC1, MRP)

ABCC1 was first identified by Cole et al. in 1992 [126] as a glutathione-conjugated toxic compound unidirectional transporter. Further to its role in multidrug resistance, the ABCC1 transporter is thought to contribute to the avoidance of xenobiotic accumulation and toxicity [133, 134], aid in the transport of inflammatory mediators such as LTC4 [135], and protect against oxidative stress [136–138]. The expression in nonmalignant tissue is ubiquitous, with the highest mRNA noted in the basolateral cellular surface of tissues such as the lungs, kidneys, skeletal muscle, and testes (Table 2.2); however, there have been no definitive studies which attribute the expression of MRP1 in these tissues to drug elimination or absorption, rather than tissue distribution and toxicity avoidance. Notably, it is the contribution which MRP1 plays to multidrug resistance in cancer cells which is of most interest.

The role of MRP1 and polymorphic variants of ABCC1 with respect to patient response to several novel or clinical chemotherapeutic agents has been completed and is currently underway in many clinical trials for a myriad of cancers (Table 2.2). Many of these studies assess the expression of both P-gp and MRP1 in comparison to clinical prognosis due to the broad substrate overlap which is noted between these two transporters.

With regard to brain cancer, Abe et al. carried out several studies into the correlation of MRP1 and P-gp expression and glioma grading [6, 139] with confirmation in 1998 [140] that the increased expressions of ABCC1 and P-gp between pre- and post-chemotherapy suggest a role of these transporters in acquired and intrinsic drug resistance in glioma. MRP1 has been shown to only be highly expressed in high-grade gliomas (HGGs), especially GBM evident in over 55 % of samples in a study by Pinto de Faria et al. [141]. Since then, the role of MRP1 in glioblastoma drug resistance has been evaluated [8, 10, 128, 142] with recent findings of MRP1 expression on tumor-associated microvessel endothelial cells [11] providing support to the role of MRP1 in GBM intrinsic multidrug resistance. In 2015, the author identified the role of MRP1 in sensitization to two clinically relevant chemotherapeutic agents, vincristine and etoposide, in both primary and recurrent patient-derived GBM cell lines [12]. Additionally, the author assessed two nonspecific MRP1 small-molecule inhibitors, reversan and MK571, whose role in alternative cancers, such as neuroblastoma, has been assessed with promising results [37, 143, 144]. The findings suggest that specific MRP1 inhibition by targeted short interfering (si)RNA molecules in both primary and recurrent patient-derived GBM lines leads to chemosensitization to vincristine and etoposide treatment; however, temozolomide-induced cell death can only be achieved by additional inhibition of P-gp and/or breast cancer resistance protein (BCRP/ABCG2) supporting the fact that temozolomide is not a substrate for MRP1 [142].

2.4.2 Multidrug Resistance Protein 2 (MRP2, ABCC2, cMOAT, cMRP)

ABCC2 is also known as the canalicular multispecific organic anion transporter (cMOAT) due to its expression in the canalicular, or apical, section of the hepatocyte, being responsible for biliary transport in the liver [145–147]. MRP2 mutations have been proven to contribute to Dubin-Johnson syndrome, a typically asymptomatic autosomal recessive condition whereby the patient has an increase in conjugated bilirubin devoid of liver enzyme elevation [148–150]. Additional expression in the endothelial cells of the proximal renal tube highlights the role of MRP2 in small organic anion transport [151, 152], and clinically MRP2 transport inhibition may lead to iatrogenic Fanconi syndrome, an inhibition of mitochondrial DNA synthesis as a result of increased organic anion buildup in the kidneys [153].

Although the role of MRP2 in the native function of the human B-BB has been readily studied [154–157], the implication of MRP2 expression in glioma [10, 158] is the identification of topoisomerase II inhibitors as a substrate for this ABC transporter. Topoisomerase inhibitors are drugs which interfere with the action of the topoisomerase enzyme, responsible for controlling DNA structural formation during cell cycle events. These inhibitors include etoposide and teniposide, which are used clinically for recurrent GBM treatment, as well as several other epipodophyllotoxins, aminoacridine, and mitoxantrone. Increased MRP2 expression correlates to increased resistance to topoisomerase inhibitors in patient-derived GBM cells [159]. Although specific MRP2 inhibition in GBM has not been studied to date, successful pan inhibition of MRP1, 2, and 3 in HIVE-viral therapy studies in canine kidney cell lines has been developed [160], a technique which may be applicable for GBM cell chemosensitization.

2.4.3 Multidrug Resistance Protein 3 (MRP3, ABCC3, MOAT-D, cMOAT-2, MLP-2)

ABCC3 was first identified in the liver [161]; however, its noted expression in many tissues including the adrenal glands, kidney, small intestine, colon, pancreas, placenta, gallbladder, lungs, spleen, stomach, brain, and tonsils signifies its importance in xenobiotic and drug efflux. ABCC3 polymorphisms have been associated with negative clinical outcome in several cancer forms and arthritis [162–168]. Although it has been suggested that the contribution which MRP3 plays to drug resistance is minor compared to its nearest homologue MRP1 (ABCC1) [8], increased MRP3 expression in GBM biopsies correlated with a higher risk of mortality [169], and researchers have postulated the use of this multidrug resistance protein for targeted antibody therapy of malignant gliomas such as glioblastoma [169, 170]. Although the concept of MRP3 inhibitors may seem favorable with respect to improved drug response in high-grade

malignant glioma, the role of MRP3 in transporting bilirubin glucuronides into the blood under conditions of impaired biliary bilirubin excretion requires an air of caution for systemic administration of such inhibitor-based therapeutics.

2.4.4 *Multidrug Resistance Protein 4 (MRP4, ABCC4, MOAT-B)*

ABCC4 is the smallest ABC transporter and is a known mediator of secondary messenger signaling through cAMP translocation in several different cell and tissue types [171–176]. Dysregulation of MRP4 expression has been connected to multidrug resistance through modulated transport of anticancer drug substrates [37, 177–179] with various levels of MRP4 expression noted in glioblastoma, retinoblastoma, neuroblastoma, and prostate, lung, ovarian, and pancreatic tumor cell lines [180–182]. With respect to glioblastoma, very few studies have focused on MRP4 expression. Studies by Rama et al. showed that GBM-initiating cells express little or no ABC transporters [183]; however, drug-resistant cancer stem cells from differentiated malignant patient tumors were noted to express increased levels of MRP4, in addition to P-gp, MRP2, and BRCP [184] supporting a role for ABC transport inhibition in drug effect enhancement. The role of MRP4 in cancers such as neuroblastoma has been found to be a prognostic indicator of progression-free survival independent of drug efflux potential [37, 144, 179, 182], a role which has yet to be investigated in additional cancers including glioblastoma.

2.4.5 *Multidrug Resistance Protein 5 (MRP5, ABCC5, MOAT-C, Pabc-1)*

Multidrug resistance protein 5 (MRP5) is a 160 kDa protein with a broad range of substrate specificity, overlapping with several other members of the ABC subfamily of transporters (Table 2.1). Highest expression patterns in naïve tissues include the heart, urethra, astrocytes, and pyramidal neurons of the brain and the B-BB. Although very few studies have been carried out on the role of MRP5 in glioblastoma, as noted by Alexiou et al., MRP5 expression in GBM tumor specimen was noted in <45 % of patients, with increased expression correlating to reduced survival. This correlation was identified to be an independent prognostic indicator for GBM survival [185], making MRP5 protein or ABCC5 transcript inhibition a viable target in an attempt to reduce GBM chemoresistance.

2.4.6 Multidrug Resistance Protein 6 (MRP6, ABCC6, MLP-1) and Multidrug Resistance Protein 7 (MRP7, ABCC10, EST182763, SIMRP7)

The final ABCC family members discussed in this chapter are MRP6 (ABCC6) and a newly included member MRP7 (ABCC10) in 2001 [186]. Although the roles of MRP6 [187–190] and MRP7 [186, 191–197] have been investigated in many forms of cancer, their role in adult brain cancer has yet to be elucidated. Clinically, mutations of the ABCC6 gene lead to the accumulation of calcium and mineralization of the elastic fibers in the connective tissue of the body [198], while ABCC10 mutations have been noted in patients with kidney tubular dysfunction [199]; however, their expression profiles with respect to glioma have not yet been elucidated.

2.5 Breast Cancer Resistance Protein (BCRP, ABCG2, Cdw338)

All genes which are members of the ABC family encode for transporter proteins responsible for transporting solutes, drugs, and xenobiotics across cell membranes. The ABC gene family is divided into seven distinct subfamilies named ABC1, ABCB or MDR/TAP, ABCC or MRPs, ABCDs or ALD, ABCE (OABP), ABCF (GCN20), and ABCG or White genes, of which the breast cancer resistance protein (BRCP) is a member of the White subfamily [200]. BCRP was initially discovered in multidrug-resistant breast cancer cell lines conferring resistance to a variety of chemotherapeutic agents including most topoisomerase I and II inhibitors such as topotecan, irinotecan, and doxorubicin [201]. The BCRP transporter plays a significant role in barrier function, being highly expressed in the intestine, B-BB, placenta, and liver, preventing drug transport into tissues such as the brain, gut, and also tumors. Similar to MRP2 (ABCC2), BRCP is also involved in biliary and renal excretion of drugs [202–207].

In brain cancer cells, as discussed by Bleu et al. [208], increased ABCG2 expression can occur through several mechanisms including activation of the PI3K/Akt pathway, PTEN deletion, and hypoxia. In glioma, amplification of growth factor receptors can lead to activation of the PI3K/Akt pathway which facilitates translocation of the ABCG2 transporter from the cytoplasm to the cell membrane. Similarly, PTEN deletion, as is noted in several cancer forms [209–213], leads to the increased expression of ABCG2 through notch activation, translocation into the nucleus, and activation of ABCG2 transcription. Translocation of hypoxia-induced HIF1 and HIF-2α to the promoter region of the ABCG2 gene leads to increased transcription and expression. In this vein, BCRP was found to be localized to the nuclear membranes of both glioblastoma cells and patient biopsy samples [214], and microvessel endothelium of human brain and glioma cells [215, 216], highlighting its contribu-

tion to chemodrug resistance. In addition, BCRP expression has been associated with increasing glioma grading suggesting a role of BCRP as a prognostic marker for progressive astrocytoma [217].

2.6 Targeting Chemoresistance

As discussed in this chapter, the ability of many members of the ABC transporter family to confer drug resistance in various cancer forms has been well established in vitro, and although correlations between expression levels and clinical outcome in glioma patient samples have been verified, a clinically relevant role for ABC transporters in GBM treatment has yet to be definitively confirmed. In this section, we will discuss the use of ABC-targeted therapies, including RNA interference (RNAi)-based studies, microRNA (miRNA)-based therapeutics, small-molecule inhibitors, and immunotherapy-based approaches to inhibiting ABC transporters and increasing anticancer bioavailability in GBM cells in vivo and clinically.

In vivo studies by Parrish et al. [218] proved that although P-gp and BCRP inhibition using elacridar improved palbociclib (PD-0332991, a cyclin-dependent kinase 4/6 inhibitor) distribution in the brain, it was ineffective at improving orthotopic tumor burden in a patient-derived GBM model. This was also the case for the chemotherapy sunitinib where elacridar improved brain distribution but did not alter the efficacy of sunitinib to hinder tumor progression [219] and likewise for imatinib mesylate (Gleevec) [220]. Although the P-gp inhibitor PSC833 has been assessed in several forms of cancer including breast, kidney, and ovarian cancer and lymphoma (Table 2.2), its role in targeting chemoresistance in GBM has, to date, only been assessed in vitro or in vivo with respect to brain distribution studies and has yet to progress to tumor burden impact studies [73, 221–223].

Specific targeting of ABC transporters using RNA interference (RNAi)- or microRNA (miRNA)-based therapeutics has been extensively studied in vitro [12, 142, 224–228], and although progression to in vivo studies holds several challenges with regard to tumor delivery including biodistribution and limited delivery of effective intratumoral doses, many researchers have shown extremely promising results [229–237]. Researchers are attempting to overcome such delivery issues through direct RNAi administration to either the tumor itself or the cranial cavity post-tumor excision in surgical resection orthotopic models of glioblastoma [238]. Notably, nanoparticle-mediated delivery of short interfering (si) RNA molecules in combination with chemotherapeutic agents in models of glioblastoma [239–247] holds great promise, with encouraging preliminary results from nanoparticle-delivered ABCC-specific siRNA [248]. Of particular interest is the use of noninvasive intranasal delivery of RNAi molecules for glioblastoma in an orthotopic murine model of GBM [249]. Such techniques would facilitate researchers to circumvent the drug efflux effects of ABC transporter expression

at the B-BB and allow direct targeting of the tumor itself. Notably, however, nanoparticle encapsulation and surface modification may also assist in overcoming intrinsic ABC transporter expression which would still pose a challenge with regard to intratumoral uptake of native drugs.

Immunotherapy is, by definition, the use of a patient's immune system to treat and prevent malignant tumor growth and progression. This approach to inhibiting tumor growth in glioblastoma is readily underway in Phase II/III clinical trials for targets such as epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and immune checkpoint inhibitors such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), along with adoptive cell therapy and peptide vaccine assessment for this aggressive brain tumor [250–257]. To date, however, there have been no studies to evaluate ABC-based immunotherapies in GBM. P-gp monoclonal antibodies (mAbs) have been shown to induce chemosensitization in lymphoma [83, 84], and MRP1 mAbs for therapeutic use have been successfully developed and characterized [258, 259] providing potential for further research of these molecules in GBM.

2.7 Conclusion

This chapter is a concise review of the functional role of ABC transporters in the aggressive brain cancer, glioblastoma, and the chemoresistant implication of the expression of these proteins at both the blood-brain barrier and also within the tumor cells themselves. This expression provides intrinsic chemoresistance to several clinically suitable chemotherapeutic agents, and their increased modulation in response to drug exposure, hypoxia, tumor progression, and genetic mutation results in highly chemoresistant recurrent tumors which are devoid of treatment response. This chapter provides a detailed list of all known ABC transporter substrates and inhibitors (Table 2.1) in addition to currently active and completed clinical trials involving ABC transporters in various cancer types (Table 2.2).

Latest developments in drug delivery into the brain using nanoparticle technology have provided an opportunity for researchers to evaluate the effects of glioma-targeted delivery of several novel and clinically relevant drugs in vivo in higher dosages devoid of off-target neurotoxic effects. In this regard, it would be of great interest to evaluate the role of ABC inhibition through noninvasive intranasal ABC-targeted drug delivery in combination with systemic chemotherapy administration.

The aggressive nature of this form of cancer, and the dismal prognosis currently available for these patients, requires novel approaches to drug delivery methodologies with ABC transporter modulation holding an extremely promising avenue for treatment progression.

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