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Discussion

Harnessing drug resistance: Using ABC transporter proteins to target cancer cells

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ABSTRACT

The ATP-binding cassette (ABC) class of proteins is one of the most functionally diverse transporter families found in biological systems. Although the abundance of ABC proteins varies between species, they are highly conserved in sequence and often demonstrate similar functions across prokaryotic and eukaryotic organisms. Beginning with a brief summary of the events leading to our present day knowledge of ABC transporters, the purpose of this review is to discuss the potential for utilizing ABC transporters as a means for cellular glutathione (GSH) modulation. GSH is one of the most abundant thiol antioxidants in cells. It is involved in cellular division, protein and DNA synthesis, maintenance of cellular redox status and xenobiotic metabolism. Cellular GSH levels are often altered in many disease states including cancer. Over the past two decades there has been considerable emphasis on methods to sensitize cancer cells to chemotherapeutics and radiation therapy by GSH depletion. We contend that ABC transporters, particularly multi-drug resistant proteins (MRPs), may be used as therapeutic targets for applications aimed at modulation of GSH levels. This review will emphasize MRP-mediated modulation of intracellular GSH levels as a potential alternative and adjunctive approach for cancer therapy.

1. The discovery of ABC transporters

ABC protein history is rooted in bacterial substrate transport studies beginning in the 1960s. Early investigations in many laboratories suggested that mechanisms for substrate transport by bacteria could be divided into three broad classifications: (1) proton-motive-force-dependent (PMFD) systems or osmotic shock-insensitive systems; (2) group translocation systems or phosphotransferase systems; and (3) binding protein-dependent (BPD) systems or osmotic shock-sensitive systems [1]. In 1974, Berger and Heppel [2] demonstrated that, unlike PMFD systems, which coupled transport to the "energized membrane state" (i.e., electrochemical gradients), the energy for transport in BPD systems required ATP. This

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Abbreviations: ABC, ATP-binding cassette; BPD, binding protein-dependent; BSO, L-buthionine sulfoximine; 2′,5′-DHC, 2′,5′-dihydroxychalcone; γ-GCL, γ-glutamylcysteine ligase; GSH, glutathione; MRP, multi-drug resistant protein; NBD, nucleotide-binding domain; PMFD, proton-motive-force-dependent; TMD, trans-membrane domain

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report was the first to implicate an entire group of transport proteins with a functional requirement for ATP.

In 1982, the sequences of two members from the BPD class of transporters, one from the histidine transporter in *S. typhimurium*, and another from the maltose transporter in *E. coli*, were reported and found to be highly similar [3,4]. This recognition led to the inference that the BPD transporters may comprise a highly conserved system. In the same year, Walker et al. [5] described the nucleotide binding motifs for ATP synthase (Walker A and B domains). Two membrane bound protein regions in the histidine permease of *S. typhimurium* were later found capable of binding ATP, suggesting that they may be involved in coupling energy for the transport process [6]. Subsequently, Higgins et al. [7] identified nucleotide sequences of multiple BPD transporters that bind ATP and recognized that these regions included highly conserved consensus sequences similar to those previously described for ATP synthase.

These discoveries provided definitive evidence regarding ATP coupled transport in the BPD transporters as set forth by Berger and Heppel. In a 1986 Letters to Nature review, Higgins et al. [8] compiled the evidence concerning the BPD transporters and suggested to classify them as a superfamily of ATP-dependent transporters. The transporters were finally recognized as a superfamily when 3 years later ATP-hydrolysis was definitively demonstrated [9,10]. Readers are referred to the Academic Press publication ''ABC Proteins from Bacteria to Man'' for a more detailed history [11].

2. ABC transporters and drug resistance

The two highly conserved nucleotide-binding domains (NBDs) mentioned above define the membership to the ABC protein superfamily. Substrate recognition, on the other hand, is a function of the trans-membrane domains (TMDs), and sequence and protein homologies in this region define which subfamily the ABC proteins belong [12]. The first classification was based on the sequence mapping completed by Allikmets et al. [13]. A new nomenclature was established by the Human Genome Organization's Gene Nomenclature Committee (HGNC) in the 1990s (http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html) and adopted by the National Center for Biotechnology Information (NCBI) in 1999. ABC proteins are classified in seven subfamilies (common names are in parenthesis): ABCA (ABC1), ABCB (MDR), ABCC (CFTR/MRP), ABCD (ALD), ABCE (OABP), ABCF (GCN20) and ABCG (WHITE). Since the discovery of the first human ABC transporter ABCB1 (P-glycoprotein or MDR1) in 1976, 47 additional ABC proteins have been identified in the human genome [14,15]. The number of TMDs varies from two to three for most members of the ABC superfamily. Yet, some of the new members of the ABC superfamily do not contain TMDs, and therefore they may not be involved in transport. Examples of this latter category include members of the ABCE and ABCF subfamilies for which functions remain unclear.

Among other physiological functions, ABC transporters have an important role in responding to environmental and biological assaults [11,16]. This is, however, a problem for cancer chemotherapy, since cancer cells have the ability to develop resistance to therapeutics by over-expressing some of these transporters. The majority of transporters that confer a drug resistant phenotype were first called multi-drug resistant proteins (MRPs) and later classified as members of the ABCC subfamily. This subfamily includes ABCC1 (MRP1, discovered in 1992), ABCC2 (MRP2), ABCC3 (MRP3), ABCC4 (MRP4), ABCC5 (MRP5), ABCC6 (MRP6), ABCC10 (MRP7), ABC11 (MRP8), and ABC12 (MRP9) (Table 1). ABCB1 (MDR1) and ABCG2 (BCRP) are also known for imparting a multidrug resistant phenotype [17]. The substrates that these transporters recognize are very diverse in structure. MRP1 alone recognizes dozens of naturally occurring and synthetic compounds [18]. Although the study of MRPs is usually focused on their expression and function in cancer cells, many normal tissues also express these transporters to varying degrees (Table 2).

3. MRP distribution in normal tissue

In normal physiology, MRPs are often expressed to the greatest extent in tissues that require special protection from chemical assaults. Such tissues include the liver, intestines and kidney [19–29]. In general, the major routes of xenobiotic excretion are through urine and feces, although the lung can also expel xenobiotics via exhalation. The detoxification process often involves multiple enzymatic reactions and conjugations that result in more water-soluble and easier to transport xenobiotics.

Expression profiles of ABC transporters in multiple normal human tissues have been compiled using methods that include affymetrix, real-time reverse transcriptase polymerase chain reaction (RT-RTPCR), Western blotting and immunohistochemisty. These last two methods remain the most reliable, but can be time consuming and expensive. The affymetrix and RT-RTPCR methods allow for the greatest number of tissue types and target genes to be analyzed at one time, thus easily generating extensive databases. Table 2 is a compilation of a representative profile of MRP tissue expression as completed by Nishimura and Naito [25] using RT-RTPCR, and shows that MRP expression in normal tissues is highly variable. For example, the kidney exhibits high expression of MRP4, moderate expression of MDR1 and MRP2, low expression of MRP1, MRP3, MRP5, and MRP7 and very low expression of BCRP. In the 23 tissues profiled, MRP4 is highly expressed in 3, moderately expressed in 5, expressed low in 12, and shows very low to no expression in 3 tissues. In addition, this MRP tissue expression is variable among species [19].

The importance of MRP expression in normal tissues has proven significant. Many important endogenous compounds such as nucleotides, folate, steroids and eicosinoids rely on ABC transporters to be secreted. The importance of MRPs in xenobiotic detoxification is also well established. The heart represents an example where low expression of MRPs likely limits its ability to respond to exogenous chemical insult. Cardiotoxicity often dictates safety margins for the use of chemotherapeutics such as the anti-cancer agent doxorubicin.

Table 1 – ABC genes with associated drug resistance phenotypes

Gene name (common names)	Substrates [16,37]	Drug resistance and/or disease phenotypes [16]	Glutathione transport [34,37]
ABCB (MDR/TAP)			
ABCB1 (PGY1, PG-P, MDR1, GP170)	Colchicine, doxorubicin, vinblastine, digoxin, saquinivir, paclitaxel, verapamil, PSC8233, GG918, V-104, Pluronic L61	Multidrug resistance phenotype, ivermectin susceptibility, digoxin uptake, HIV protease inhibitor resistance	Unknown
ABCC (CFTR/MRP)			
ABCC1 (MRP1, MRP, ABCC, GS-X, ABC29)	Doxorubicin, daunorubicin, vincristine, colchicines, etoposide, rhodamine, cyclosporin A, V-104	Multidrug resistance phenotype	GSH and GSH S-conjugates are transported. Some substrates stimulate GSH transport without being transported themselves.
ABCC2 (MRP2, cMOAT)	Vinblastine, sulfinpyrazone, etoposide, cisplatin	Multidrug resistance phenotype, Dubin–Johnson syndrome	GSH and GSH S-conjugates are transported
ABCC3 (MRP3)	Methotrexate, etoposide	Multidrug resistance phenotype	GSH S-conjugates are transported
ABCC4 (MRP4)	Cyclic nucleotides, prostaglandins, antiretrovirals, purine analogs	Multidrug resistance phenotype, nucleoside/tide reverse transcriptase inhibitor resistance	GSH and GSH S-conjugates are transported
ABCC5 (MRP5)	Cyclic nucleotides, antiretrovirals, purine analogs	Multidrug resistance phenotype	GSH and GSH S-conjugates are transported
ABCC6 (MRP6)	Leukotriene C_4, N-ethylmaleimide-GSH	Mutated in *Pseudoxanthoma elasticum*	Conflicting data about transport of GSH S-conjugates
ABCC10 (MRP7)	Glucuronate conjugates, leukotriene C_4	Multidrug resistance phenotype	Unknown
ABCC11 (MRP8)	Cyclic nucleotides	Unknown physiological role	Unknown
ABCC12 (MRP9)	Unknown	Associated with paroxysmal kinesigenic choreoathetosis	Unknown
ABCG (WHITE)			
ABCG2 (BCRP, MXR1, ABCP)	Mitoxantrone, topotecan, doxorubicin, daunorubicin, CPT-11, rhodamine, glucoronate conjugates	Multidrug resistance phenotype	Unknown

4. MRP distribution in cancer cells

Szakacs et al. [30] profiled cancer cells available through the National Cancer Institute using microarray technology and Table 3 is a select compilation for MRP expression of a few cancer cell lines that they arrayed. Similar to the expression of MRPs in normal tissues, the expression profile of MRPs in tumors is highly variable. As shown in Table 3, this variability is true both between tumor families and within tumor families. For example, MRP3 is highly expressed in renal cancer cell lines but has low expression in leukemias. MRP4 expression in lung cancer cell lines varies from high levels in the A549-ATCC cell line to very low levels in the NCI-H460 cell line. Normal lung tissue has very little MRP2 expression whereas most of the lung cancer cell lines, MRP2 expression varies between moderate to high expression. Conversely, normal lung tissue has high expression of MRP4, but four of the six lung cancer cell lines reported in Table 3 exhibit very low levels of MRP4.

Overall, MRP expression in cancer cells remains largely unpredictable. However, it is important to make a distinction between general MRP expression of a cell and the ability of a cell to respond to a chemical insult by over-expressing specific MRPs. Indeed, clinical studies suggest that cancer cells adapt better to chemical insults than normal cells due, in part, to their ability to rapidly modulate their MRPs in response to multiple stimuli [31,32].

5. MRPs and glutathione

Cells use MRPs at the cost of ATP, but often at the cost of glutathione (GSH) as well. GSH is a tripeptide, L-γ-glutamyl-L-cysteinyl-glycine, and the major low molecular weight thiol compound in plants and animals [33]. GSH steady-state levels are determined by rates of synthesis, reactivation, utilization and transport. The cell utilizes GSH in a number of detoxification pathways. The thiol group is utilized for xenobiotic metabolism (glutathione-S-transferases), as a source of reducing power for detoxifying peroxides (glutathione peroxidases) and for protein repair (glutaredoxin). In addition, GSH is a major determinant of tissue redox status, which is determined by the ratio of reduced to oxidized glutathione (GSH/GSSG). GSH serves many important cellular roles as a redox regulator, co-factor, substrate and antioxidant.

Several members of ABC transporters that play a major role in the extrusion of xenobiotics depend on GSH, yet the involvement of GSH appears to be remarkably complex. GSH is often required either as S-conjugates or as a co-factor (or both). For example, GSH is required for the transport of the anti-cancer agent etoposide by MRP1 and MRP2. However, transport of the same molecule by MRP3 is independent of GSH [34]. Also, whereas some drugs stimulate GSH transport through MRP1 (i.e., doxorubicin,

Table 2 – Compilation of mRNA expression of multidrug resistant ABC transporters in normal tissue as reported by Nishimura and Naito [25]

Tissue	Transporter[a]							
	ABCB1 (MDR1)	ABCC1 (MRP1)	ABCC2 (MRP2)	ABCC3 (MRP3)	ABCC4 (MRP4)	ABCC5 (MRP5)	ABCC10 (MRP7)	ABCG2 (BCRP)
Adrenal gland	+++	+	±	+++	±	+	+	+
Bone marrow	±	+	±	±	++	+	+	+
Brain	±	±	±	±	+	+	+	+
Colon	+	+	±	+++	+	+	+	±
Heart	±	+	±	±	+	++	+	±
Kidney	++	+	++	+	+++	+	+	±
Liver	+	±	+++	+++	±	±	±	+
Lung	±	+	±	+	+++	+	+	+
Pancreas	±	±	±	++	+	±	+	±
Peripheral leukocytes	±	+	±	+	+	+	+	±
Placenta	+	+	±	+	±	±	++	++
Prostate	±	++	±	+	+++	+	+	+
Salivary gland	±	±	±	±	+	+	+	±
Skeletal muscle	±	+++	±	±	++	+++	+	±
Small intestine	+	+	+	++	+	±	+	+
Spinal cord	+	+	±	±	+	+	+	+
Spleen	±	+	±	+	+	+	+	±
Stomach	±	+	±	+++	+	+	+	±
Testis	±	+	±	±	++	++	+	+
Thymus	±	+	±	±	+	+	+	±
Thyroid gland	±	+	±	±	++	±	+	±
Trachea	±	++	±	+	++	+	+	±
Uterus	+	+	±	±	+	+	±	+

[a] High expression (+++); moderate expression (++); low expression (+); very low to no expression (±).

etoposide, vincristine, vinblastine), others do not (i.e., daunorubicin).

Observations in recent years suggest that the function of MRPs is not limited to the extrusion of xenobiotics, but that MRPs may also play an important role in modulation of GSH levels, both intracellularly and extracellularly. Elevated GSH levels have been observed in tissues of MRP1 knockout (−/−) mice [35], and CFTR-mediated GSH extrusion may play an important role in protecting the lungs during infection [36]. Whereas most MRPs are localized to baso-lateral membranes, MRP2 and CFTR are localized to apical membranes, thus exporting GSH into different extracellular compartments [37]. MDR1 has not been shown to transport GSH or GSH S-conjugates, but its expression appears to be modulated by intracellular levels of GSH [38].

In 2000, the Ca^{2+} channel blocker verapamil was reported to stimulate GSH transport on MRP1 without itself being transported [39]. It was later found that some flavonoids have the same effect on MRP1 [40]. Hydroxychalcones, the precursors of flavonoids, were also found to induce GSH depletion, although the MRP(s) involved remain to be identified [41].

Among the twelve identified members of the ABCC subfamily, five have been found to transport reduced GSH, i.e., MRP1, MRP2, MRP4, MRP5 and CFTR (Table 1) [37]. Of the remainder of the ABCC subfamily, only MRP3 has been found to transport GSH S-conjugates, and there is not yet evidence that MRP3, MRP6, MRP7, MRP8 and MRP9 transport GSH directly. It remains to be determined whether GSH transport in MRP2, MRP4, MRP5 and CFTR can be stimulated by compounds such as flavonoids. Finally, little is known at the molecular level about GSH transporters. According to site-directed mutagenesis studies on MRP1, lysine^{332} appears to be essential for both GSH binding and transport, whereas two other lysines (lysine^{319} and lysine^{347}) contribute to GSH transport [42].

6. Cancer and redox state modulation

Growing data suggests that cancer cells exist in a state of oxidative stress [43]. In fact, for a number of anti-cancer agents, including cisplatin and doxorubicin, their efficacy is partly based on their ability to induce the formation of reactive oxygen species (ROS) in the mitochondria and to affect the mitochondrial membrane potential, thus triggering programmed cell death (apoptosis) and necrosis [44]. How alkylating agents induce the production of ROS remains unclear. One hypothesis is that they interfere directly with the mitochondrial respiratory chain, similar to the proposed mechanism for rotenone. We recently suggested that, by forming mitochondrial DNA adducts, cisplatin might alter the assembly of the mitochondrial respiratory chain, thus leading to an increase in superoxide production [45]. It is also worth noting that one mechanism cancer cells use for adapting to oxidative stress is by elevating their intracellular concentrations of GSH [46].

A continuing avenue of research is to combine agents to synergize anti-tumor effects without increasing damage to normal tissue. A number of studies have shown that lowering cancer cell defenses against alkylating agents or ionizing radiation can synergize anti-tumor effects. Early attempts to target the antioxidant defenses of cancer cells involved the use

Table 3 – Expression of multidrug resistant ABC transporters in cancer cells as previously reported [30]

Cell type	Transporter[a]							
	ABCB1 (MDR1)	ABCC1 (MRP1)	ABCC2 (MRP2)	ABCC3 (MRP3)	ABCC4 (MRP4)	ABCC5 (MRP5)	ABCC10 (MRP7)	ABCG2 (BCRP)
Breast								
BT-549	+	+	++	+	+++	++	++	+
HS578T	++	+	+	+++	+	+++	+++	++
MCF7	±	+	±	±	±	+++	++	+++
NCI_ADR_RES	+++	++	±	±	±	++	+++	±
Colon								
COLO205	±	++	+++	+++	+	+	±	+++
HCC-2998	±	+	+++	+++	±	+++	±	+++
HCT-116	±	++	+++	++	++	++	+++	+
HCT-15	+++	+	±	±	+	++	+	±
HT29	±	++	±	+++	++	±	++	+++
Lung								
A549-ATCC	±	+	++	+	+++	++	+++	+
NCI-H226	±	++	++	+++	++	±	+	±
NCI-H23	±	++	+++	±	+	±	±	+++
NCI-H322M	±	+++	+++	+++	+	++	+++	+++
NCI-H460	+++	++	+++	+++	±	+	±	+++
NCI-H522	+	++	±	+	+	+++	++	±
Leukemia								
HL-60	++	++	±	±	+++	+++	+	±
MOLT-4	±	++	±	±	+	+++	+	±
RPMI-8226	+	++	±	±	+++	+	+	+++
SR	±	+	±	+	+	±	+	±
Ovarian								
IGROV1	+++	++	+	±	+++	+++	±	±
OVCAR-3	±	++	±	±	+	++	++	±
OVCAR-4	±	±	+	±	++	++	+++	+
OVCAR-5	±	++	±	+++	+++	++	+	±
OVCAR-8	+++	++	±	±	++	++	++	±
Prostate								
DU-145	+	++	+++	+++	++	+	+	++
PC-3	±	++	±	++	+	+++	++	±
Renal								
786-0	+++	++	±	+++	+	++	++	+++
A498	+++	+	+++	+++	++	±	±	±
ACHN	+++	+	±	+++	++	±	++	+
CAKI-1	+++	+	+	+	+	++	++	±
RXF-393	±	±	+	++	+++	+	+++	+
UO-31	+++	+	±	+++	++	++	+++	±

[a] High expression (+++); moderate expression (++); low expression (+); very low to no expression (±).

of inhibitors to block GSH synthesis or glutathione disulfide reductase that recycles the oxidized form of glutathione (GSSG) back to the active reduced form (GSH). BCNU (carmustine) is an anti-cancer drug that is an alkylating agent and an inhibitor of glutathione reductase [47]. Ethacrynic acid is an approved drug that inhibits glutathione-S-transferase and was shown to potentiate the cytotoxic effects of alkylating agents including BCNU, chlorambucil and melphalan [48]. L-Buthionine sulfoximine (BSO) is an example of an inhibitor of cellular GSH synthesis that has been studied in combination with ionizing radiation [49], and is currently in phase II clinical trials in combination with melphalan. Another potential strategy for disrupting the antioxidant response of the cell is by targeting thioredoxin reductase, a component of several redox-regulated pathways [50].

A major problem associated with these approaches remains the lack of selectivity. Although MRP expression can vary dramatically from one tissue to another and from one cancer cell to another, MRPs tend to be over-expressed in late stage tumors and often confer resistance to anti-cancer drugs [31,32]. However, this potential obstacle to cancer therapy may also provide a targeting opportunity for cancer cell sensitization by stimulating GSH efflux and cellular GSH depletion.

7. Targeting GSH synthesis: key discoveries

Radiation therapy is an integral part of many cancer treatment regimes. One of the best-recognized concepts in radiation biology is that the presence of oxygen increases the efficacy of

radiation whereas GSH is a radioprotector. In 1949, it was reported that L-cysteine, a rate limiting substrate in GSH synthesis, protected rats from lethal radiation doses [51]. This study indicated an important role for GSH in radiation biology and seeded the idea of using GSH depletion as a method to sensitize cancer cells.

During the 1950s, researchers began to address the problem of drug resistance in chemotherapy, in particular the resistance to alkylating agents [52,53]. In 1966, Ball et al. [54] described the differential sensitivity of two Yoshida sarcoma cell lines to melphalan, and found that the melphalan resistant cells had higher protein-free thiol content compared to the sensitive cell line. In 1982, melphalan resistant leukemia cells were depleted of GSH by depriving them of L-cysteine and then treated with melphalan [55]. According to the authors, melphalan resistant cells were ''completely sensitized'' due to the depletion of GSH.

In the meantime, Griffith and Meister [56] explored compounds that would inhibit the enzymes involved in the synthesis of GSH. They started with methionine sulfoximine, which was quite toxic due to its ability to inhibit both glutamine synthetase and γ-glutamylcysteine ligase (γ-GCL). Its derivative S-*n*-propyl homocysteine sulfoximine, however, appeared to selectively inhibit γ-GCL [57], and the analog S-*n*-butyl homocysteine sulfoximine (L-buthionine sulfoximine or BSO) proved to be the most potent inhibitor [58].

These early studies set the stage for the growing interest in targeting GSH as a potentially useful cancer therapeutic approach. In the past two decades, dozens of publications have reported the ability of BSO to sensitize cancer cells to anti-cancer agents (including cisplatin and 2-deoxy-D-glucose) and ionizing radiation [49,59–61]. Furthermore, cytosolic GSH depletion has been reported to cause mitochondrial dysfunction, including the increased production of ROS by mitochondrial respiratory complex III [62].

8. BSO: animal studies and clinical trials

Animal studies combining BSO with melphalan were carried out and produced a 72% increase in median survival of tumor-bearing nude mice as compared to melphalan alone treated mice [63]. However, preclinical data in mice indicated that normal tissue also experienced GSH depletion when BSO was administered [64]. After a single bolus dose, mice bearing tumors were reported to show GSH depletion by 5 h in the liver (74% depletion) and kidney (80%), by 8 h in the lung (40%) and bone marrow (83%), by 12 h for red blood cells (13%) and by 24 h for the heart (54%). It was also reported that the greatest GSH depletion in tumors (55–65%) occurred between 10 and 12 h. Interestingly, GSH recovery occurred in the tumors 48 h after BSO treatment. In contrast, the liver, kidney and lung required less than 48 h to recover their GSH levels, but the bone marrow, red blood cells and heart required longer than 48 h for GSH recovery.

The first clinical trial using BSO to enhance cancer therapy efficacy was published in 1994 by Bailey et al. [65]. Since then, three Phase I clinical trials using BSO in combination with melphalan have been reported [66–68]. One Phase I trial showed significant responses to the BSO/melphalan combination [67], and two clinical trials using the same combination are currently being conducted, one at the Phase I level (New Approaches to Neuroblastoma Therapy Consortium, USA. Phase I Study of Melphalan and Buthionine Sulfoximine followed by Autologous Bone Marrow or Peripheral Blood Stem Cell Transplantation in Treating Children with Resistant or Recurrent Neuroblastoma) and one at the Phase II level (McGill Center for Translational Research in Cancer: Quebec, Canada. A Phase II Trial of IV-L-PAM and BSO in Patients with Relapsed or Refractory Ovarian Cancer). Reported side effects often included myelosupression and nausea/vomiting along with a single case of hepatotoxicity [67]. Melphalan alone has been associated with nausea/vomiting, but given the previously reported animal data, it can be speculated that some of the side effects were exacerbated by BSO.

In theory, sensitizing cancer cells to treatments that have pro-oxidant effects such as alkylating agents or radiotherapy will allow one to decrease the effective concentrations of treatment doses, thereby limiting potential side effects. BSO is a promising adjunctive agent for cancer treatment, but may be limited by its lack of specificity toward cancer cells. Using compounds that act like BSO or can synergistically increase its efficacy specifically in cancer cells could translate into further lower efficacious doses.

9. MRP-mediated GSH depletion and cancer cell sensitisation

In the past decade, several studies have linked the ability of verapamil to sensitize cancer cells by MRP-mediated GSH depletion rather than inhibition of drug efflux [69–72]. To our knowledge, the first report of this phenomenon came in 1998 from a study by Grech et al. [69] by combining etoposide and verapamil in human leukemia cells over-expressing MRP1. Whereas verapamil did not induce a significant accumulation of etoposide in the cells, its presence greatly enhanced the cytotoxicity of etoposide, which, the authors thought, was due to the ability of verapamil to stimulate GSH extrusion. Since then, several studies have associated verapamil-induced GSH depletion with apoptosis [70–72].

We recently showed that, in three separate cancer cell lines, simple naturally occurring compounds such as chrysin and 2′,5′-dihydroxychalcone (2′,5′-DHC) were more effective than verapamil in depleting cytosolic GSH [73]. In human lung adenocarcinoma (A549) cells, non-toxic concentrations of chrysin and 2′,5′-DHC (10–25 μM) were remarkably effective in both depleting GSH and potentiating the toxicity of rotenone, an agent that interferes directly with mitochondrial respiratory complex I. Chrysin and 2′,5′-DHC also potentiated the toxicity of cisplatin in A549 cells, and more efficiently than BSO [45]. The potentiation effects were blocked by adding exogenous GSH to the culture media, and likely mediated by the mitochondria. Whereas flavonoids stimulate GSH transport through MRP1, preliminary results in our laboratory suggest that distinct and possibly different MRPs are involved in the transport of GSH induced by chalcones.

A recent study compared verapamil and the flavonoid apigenin with BSO in their ability to induce GSH depletion and apoptosis in five cancer cell lines, including two MRP1-over-expressing cell lines [72]. These two were more sensitive to

verapamil and apigenin-induced toxicities, but also to BSO, thus supporting that MRP levels play an important role in modulating GSH levels. The authors concluded that over-expression of MRP1 contributes to cell death by oxidative stress through drug-enhanced GSH efflux.

It is finally worth noting that cancer cells have a variety of ways to adapt to oxidative stress, including increased synthesis of GSH and the over-expression of catalytic antioxidants that do not require GSH such as catalase. Interestingly, both the expression of MRPs and the synthesis of GSH appear to be controlled by the transcription factor Nrf2 [74]. p53-mediated down-expression of MRPs cannot be discarded as another mechanism to adapt to higher levels of ROS [75], but that would be at the cost of drug extrusion.

10. Pharmacogenetics and personalized medicine

A major drawback to chemotherapy is the onset of adverse side effects from the drug combinations prescribed, largely due to their broad specificity for all rapidly dividing cells. In addition, it is difficult to predict which combinations of drugs will produce the greatest outcome between individuals since each individual's cancer is genetically unique. The vast genetic diversity that exists among cancer cell lines in their expression of ABC transporters is a case in point (Table 3). However, because MRPs are often over-expressed in advanced cancer [26,31,32], we contend that these transporters can be used to target tumor specific GSH depletion, thereby providing a more specific approach to sensitize cancer cells.

MRP-mediated GSH extrusion from cancer cells may potentiate cancer therapies that have pro-oxidants effects, including alkylating agents and radiotherapy, by at least three separate mechanisms (Fig. 1). The first mechanism involves the active transport of intracellular GSH, which lowers the cell's antioxidant capacity to defend against alkylating agents and radiotherapy. The second mechanism involves sensitizing the mitochondria to treatments that induce the over-production of ROS in this organelle, making it easier for the cell to undergo apoptosis or necrosis. The third mechanism is by limiting the availability of cellular GSH needed for metabolizing and transporting some alkylating agents out of the cell.

Five MRPs have so far been identified as transporters of GSH. Among them, MRP1 mediates the extrusion of GSH when activated by certain flavonoids. These natural compounds have already been shown to be nontoxic and chemopreventive, making them ideal for continued development as potential GSH depleting agents. The development of new and more efficient inducers of GSH depletion may significantly impact cancer treatment by allowing the use of smaller treatment doses, therefore resulting in better patient tolerance and compliance. Because tumors exhibit such great variation in MRP expression, targeting MRPs for chemotherapy would require individualized medicine approaches. One way to design a tailored approach directed toward MRPs would be by choosing substrates for the specific transporters that are over-expressed in the cancer cells to be targeted. One could envision the use of super arrays to genotype the patient's tumor and select a customized therapy based in part on MRP expression.

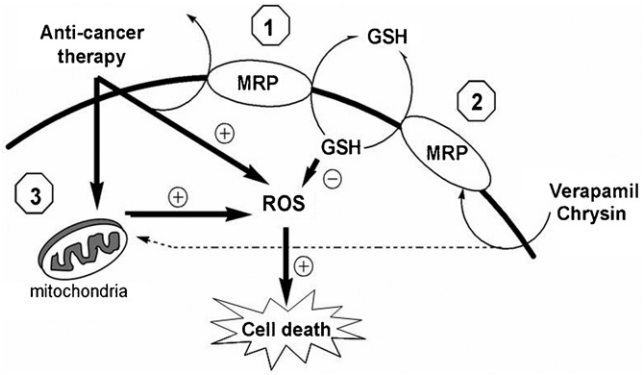

Fig. 1 – MRP-mediated GSH extrusion from cancer cells may potentiate cancer therapies that have pro-oxidant effects, including alkylating agents and radiation therapy, by at least three separate mechanisms: (1) limiting the availability of cellular GSH needed for metabolizing and transporting some alkylating agents out of the cell. Direct effects of some flavonoids on mitochondria function cannot be discarded (discontinued arrow); (2) active transport of intracellular GSH, which lowers the cell's antioxidant capacity to defend against alkylating agents and radiotherapy; and (3) sensitizing the mitochondria to treatments that induce the over-production of ROS in this organelle, making it easier for the cell to undergo apoptosis or necrosis.

In summary, the growing evidence that MRP-mediated GSH extrusion can potentiate the efficacy of anti-cancer treatments raises the hope for the development of a new adjunctive and more specific approach of cancer cell sensitization. Furthermore, the use of agents such as flavonoids that can deplete GSH through activation of specific MRPs may open the perspective to tailor-making chemotherapeutic combinations based on affymetrix analysis of individual tumors and identification of MRP expression. Analysis and treatment of this type would allow physicians to build more effective cancer regimes for individual patients and advancing cancer therapy into the realm of real-time pharmacogenetics.

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