The ultimate cause of treatment failure for many forms of malignant and infectious diseases is the development of resistance to a broad range of cytotoxic drugs. Hence, despite significant advances in cancer treatment, multidrug resistance (MDR) remains a major clinical problem and leads to limited therapeutic options and poor patient outcome in a number of cancers, including high-risk neuroblastoma, leukaemia and carcinomas of the prostate, breast, lung and ovary. Therefore, gaining a better understanding of the mechanisms that lead to drug resistance is of utmost importance in order to improve the current chemotherapy regimes in cancer treatment.

While MDR may develop in response to a specific drug or drug combination, the resultant phenotype typically confers resistance to a variety of agents, often with diverse mechanisms of action. A large body of research devoted to exploring cellular MDR has revealed that resistance most commonly arises through (a) insufficient uptake of water-soluble drugs by specific transporters, (b) alterations in cellular pathways that result in a reduced capacity of cytotoxic drugs to kill cells, or (c) increased drug efflux from cancer cells. This review deals with the latter of these mechanisms, which is most commonly associated with overexpression of one or more membrane-bound transporter proteins that act as extrusion pumps for a diverse array of substrates (1,2).

The ABCC Transporter, MRP1

Despite the multifactorial nature of MDR, a major breakthrough in the field came with the first demonstration that resistance to a significant number of both structurally and mechanistically diverse compounds could be attributed to overexpression of a single protein, P-glycoprotein (P-gp/MDR1) (3). P-gp belongs to the ATP-binding cassette (ABC) superfamily of proteins, which function as energy-dependent efflux pumps. In humans, the ABC superfamily includes 49 genes distributed in a seven-branch family tree (from A to G) with P-gp/MDR1 belonging to the ABCB family (4). The ABCC/multi-drug resistance-associated protein (MRP) subfamily is composed of at least 12 members (MRP1-12), and contains the great majority of transporters so far known to confer cellular resistance to clinically important chemotherapeutic agents. In particular, MRP1/ABCC1 was originally identified as being responsible for MDR in a doxorubicin-selected human small cell lung carcinoma cell line. Even though these cells displayed an MDR phenotype similar to that previously associated with P-gp overexpression, resistance was found to be P-gp-independent (reviewed in (4)). MRP1 has since been closely linked to the development of clinical MDR in several types of cancer (1,5) and an extraordinarily wide variety of transport substrates have been identified (Fig. 1). While there is significant overlap with the substrate specificity of P-gp, MRP1 transports a broader range of xenobiotics used as antineoplastic or therapeutic agents, including folate-based antimetabolites, anthracyclines, vinca alkaloids, antiandrogens, and numerous glutathione (GSH)- and glucuronide-conjugates of these compounds as well as organic anions and heavy metals. MRP1 also transports diverse physiological substrates such as folates, GSH and GSH disulfide (GSSG), as well as sulphate-, GSH- and glucuronide-conjugates of steroids, leukotrienes and prostaglandins (5,6). These activities of MRP1 are important in normal cellular processes such as export of endogenous intermediates and protection from toxic insult. MRP1 overexpression in cancer cells, however, allows the elimination of a wide range of therapeutic agents. Furthermore, through its ability to export GSH and its derivatives, MRP1 has the potential to influence cellular GSH-GSSG balance and could therefore play a role in the cellular response to oxidative stress induced by drugs or associated with various disease states.

MRP1 and Multi-Drug Resistance in Adult Cancers

Although MRP1 is widely expressed in normal tissue, numerous studies have shown upregulation of MRP1 in a variety of solid tumours such as those of the lung, breast and prostate and this transporter has been clearly implicated as having a role in the clinical drug resistance behaviour of several cancers. Non-small cell lung carcinoma (NSCLC) accounts for over 75% of lung cancer cases and is typically chemoresistant. MRP1 was found to be frequently overexpressed in a large proportion of tumours prior to treatment exposure. Moreover, MRP1 expression was found to be a highly significant indicator of poor response to chemotherapy and poor overall survival (reviewed in (4)). MRP1 expression was also predictive of poor response to chemotherapy in small cell lung carcinoma (SCLC) (4). Expression levels of both MRP1 and P-gp were increased in SCLC metastases detected at relapse, suggesting a role for these transporters in cell survival during metastasis and chemotherapy (7). MRP1 expression also constitutes a negative prognostic marker for early-stage breast cancer, with several studies revealing a strong association between expression level and reductions in both time to relapse and overall survival (reviewed in (4)). Reports of increased MRP1 expression have also been noted in other cancers, such as prostate, colorectal, glioblastoma, and ovarian cancer, highlighting its potential as a biomarker for drug resistance and therapeutic target.

The MRP1/ABCC1 Multi-drug Transporter Protein and Cancer
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SHOWCASE ON RESEARCH

The ABCC Transporter, MRP1

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expression in breast cancer lymph node metastases suggest a potential role for MRP1 in metastatic disease (8). Importantly, MRP1 is known to mediate transport of certain drugs used to treat metastatic breast cancer, such as methotrexate and cyclophosphamide.

In prostate cancer, the levels of MRP1 tend to increase with disease stage and invasiveness. Furthermore, MRP1 overexpression conferred chemoresistance in prostate cancer cell lines exposed to doxorubicin (4). Other in vitro studies demonstrated the ability of MRP1-overexpressing cells to expel therapeutic antiandrogen compounds, suggesting that MRP1 may be an important factor in failure of antiandrogen therapy (9).

**MRPs and Drug Resistance in Neuroblastoma**

Neuroblastoma is the most common extracranial solid tumour in children. It arises in cells of neural crest origin and the prognosis for patients with widely disseminated disease at diagnosis is often dismal despite intensive treatment (10). Amplification of the MYCN oncogene is currently one of the most powerful adverse prognostic indicators for neuroblastoma (11). MYCN amplification occurs in 20–25% of primary untreated neuroblastomas and is associated with rapid tumour progression, advanced clinical stage and poor outcome (10,11). In vitro studies have shown that N-Myc is involved in growth and differentiation of neuroblastoma cells. Furthermore, transgenic mice constitutively expressing N-Myc in neural crest cells develop aggressive neuroblastoma that closely mirrors the human disease (5).

There is increasing evidence that MRP1 is a MYCN target gene involved in the MDR phenotype of neuroblastoma tumours (12). We and others have demonstrated that high MRP1 expression strongly correlates with amplification and expression of the MYCN gene in primary human neuroblastoma tumours, in tumours from MYCN transgenic mice and in neuroblastoma cell lines (5). Several studies of primary untreated neuroblastoma specimens point to a clear role for MRP1 in drug-refractory neuroblastoma, demonstrating that, as for MYCN, high MRP1 expression is strongly associated with reduced overall survival and event-free survival (13). Importantly, however, following adjustment for MYCN amplification and other prognostic factors using multivariate analysis, MRP1 expression remains a significant prognostic indicator in neuroblastoma. MRP1 expression also predicts outcome in patients bearing MYCN non-amplified tumours (14).

**XENOBIOTICS**

- **Folate-based antimetabolites**: Methotrexate
- **Anthracyclines**: Doxorubicin
- **Plant alkaloids**: Etoposide, vincristine, vinblastine, paclitaxel, irinotecan
- **Antiandrogens**: Flutamide, hydroxyflutamide
- **Antivirals**: Saquinavir, ritonavir
- **Antibiotics**: Difloxacin, grepafloxacin
- **Metalloids**: Sodium arsenite, potassium antimonite
- **Toxicants**: Aflatoxin B1, methoxychlor

**ENDOBIOTICS**

- **Folates**: Folic acid, L-leucovorin
- **Other metabolites**: GSSG, GSH, bilirubin

**GSH- Glucuronide- and sulphate-conjugates**

- Etoposide-gluc, SN-38-gluc
- 2,4-Dinitrophenyl-SG, doxorubicin-SG, cyclophosphamide-SG, atrazine-SG, aflatoxin B1-epoxide-SG, 4-nitroquinoline 1-oxide-SG
- 17β-Estradiol-17β-D-gluc, glucuronosylbilirubin
- Leukotriene-C₄, prostaglandin A₂-SG, 15-Deoxy-A₁₂-1₄ prostaglandin J₅-SG
- Estrone 3-sulphate, sulphatolithocholate

Fig. 1. Diversity of compounds transported by MRP1. Some of the xenobiotic substances transported by MRP1 are drugs currently used in cancer treatment. Among the endobiotics, or naturally occurring metabolites, are some of the most relevant physiological substrates of MRP1.
Studies of the MRP1 promoter demonstrate that N-myc regulates MRP1 gene expression through interaction with a putative E-box element and other cis-acting factors in the MRP1 promoter region (Fig. 2). Neuroblastoma cells overexpressing MYCN were found to have enhanced levels of MRP1 and were resistant to the well-characterised MRP1 substrate vincristine, but not to the non-MRP1 substrate cisplatin. Importantly, vincristine resistance in these cells was reversed by MRP1 modulators (12). Further in vivo evidence for the importance of the interaction between N-myc and MRP1 in neuroblastoma therapy comes from the observation that tumours from NMYC transgenic mice with impaired MRP1 expression were significantly more responsive to therapy with MRP1 substrate cytotoxic drugs when compared to tumours wild-type for the MRP1 gene (5). Interestingly, MRP1 downregulation with antisense approaches in MRP1-overexpressing neuroblastoma cells also promoted cell differentiation and apoptosis, suggesting that apart from influencing cytotoxic drug response, MRP1 may have a role in preventing neuritic differentiation and cell death (5).

While there is strong evidence to support an important role for MRP1 in neuroblastoma, it is conceivable that other MRP family members might contribute to drug resistance in this disease. Indeed, overexpression of MRP4 was found to be significantly associated with poor clinical outcome in aggressive primary neuroblastomas, and to correlate significantly with MRP1 expression and MYCN amplification (15). Of nine MRP family members examined, only MRP1 and MRP4 expression levels were associated with disease outcome. Studies are currently underway to investigate whether N-Myc similarly regulates the expression of MRP4, since there are a number of potential N-Myc binding sites (E-boxes) in the MRP4 promoter (15).

High MRP4 expression has also been associated with drug resistance in vitro by means of efflux of a number of compounds, including the topoisomerase I poison camptothecin and its semi-synthetic derivatives irinotecan and topotecan, cyclophosphamide and rubitecan (15,16). In addition, in vivo studies suggest that MRP4 is involved in the transport of nucleoside-based analogues and may be responsible for acquired resistance to compounds belonging to this class of anticancer agents (17), and the antiviral acyclic nucleotide phosphonates, adenofovir and tenofovir (antiviral drugs) (18). MRP4 has also been associated with low-affinity transport of cyclic nucleotides (cGMP and cAMP) (19), and both in vivo and in vitro models have shown that MRP4 mediates transport of prostaglandins and conjugated steroids (DHEAS) (20-22), suggesting that in addition to its involvement in MDR, MRP4 may have a physiological role in signal transduction and prostaglandin homeostasis.

Conclusions and Future Prospects

MRP1 is an important component of drug resistance in a number of cancer types. In particular, the study of the role of MRP1 in neuroblastoma has provided important insights into the drug refractory behaviour of this disease, and available evidence indicates that elucidating the pathways involved in MRP1 signalling will create new approaches to improving patient outcome in this and other diseases. Given the clear association between high MRP1 expression and the malignant and drug-resistant phenotype of neuroblastoma both in vitro and in clinical neuroblastoma, MRP1 is potentially an important target for reversing chemotherapy resistance in neuroblastoma patients. Both in vitro and in vivo studies have demonstrated that downregulation of MRP1 in neuroblastoma cell lines or tumour xenografts can achieve significant chemosensitisation to clinically relevant MRP1 substrates (5,23,24). Such findings indicate that MRP1 modulation may be a viable approach to improving patient response in this disease.

For these reasons, and since it is widely believed that safe and potent inhibitors of the ABC pumps would be clinically efficacious in increasing the sensitivity of drug-resistant cancers to current chemotherapy approaches, MRP1 and other transporters such as P-gp have been actively pursued as targets for therapeutic suppression. Several MRP1-specific reversing agents have been identified in vitro and these include the leukotriene C4 antagonist MK571, the benzothipene LY329146, the dihydropiridine analogue NIK250, the quinolone antibiotic difloxacin, and the organic acids,
sulfinpyrazone, benz bromarone and probenecid (25). Another strategy has been to target the GSH-dependent nature of MRP1 transport of a number of substrates, either by blocking GSH synthesis or by developing GSH-conjugates as competitive inhibitors of MRP1 transport (4). Most recently, the PI3K inhibitor LY294002 was found to enhance doxorubicin-mediated killing of colon cancer conjugates as competitive inhibitors of MRP1 transport, with a concomitant increase in intracellular doxorubicin levels (26). The cyclooxygenase inhibitor indomethacin was shown to reduce MRP1 advances, the search for specific clinically relevant MRP1 modulators remains an important and ongoing area of research. Despite these encouraging advances, the search for specific clinically relevant MRP1 inhibitors remains an important and ongoing area of research.

To date, the most specific MRP1 inhibitors (tricyclic isoxazole derivatives) have been discovered via high-throughput screening approaches incorporating relevant cell-based read-outs (4). Screening of small molecule chemical libraries to identify specific inhibitors of the MRP1 transporter is currently underway in our laboratory and we have identified compounds that appear to be highly effective at enhancing sensitivity to MRP1-substrate drugs both in vitro and in vivo. Such compounds may potentially provide an effective new treatment approach for neuroblastoma and other malignancies in which MRP1 plays a clinically relevant role. Finally, the evidence so far obtained supporting the importance of other ABCC family members, such as MRP4, in neuroblastoma and other cancers warrants more thorough investigation of these molecules as potential therapeutic targets.

References