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Drug Transporters and Multiple Drug Resistance in the Most Common Pediatric Solid Tumors

Doriana Fruci[a*], William C.S. Cho[b*], Paolo Romania[a], Valerio Nobili[c], Franco Locatelli[a] and Anna Alisi[c*]

[a]*Paediatric Haematology/Oncology Department "Bambino Gesù" Children's Hospital and IRCCS, Rome, Italy;* [b]*Department of Clinical Oncology, Queen Elizabeth Hospital, Kowloon,* Hong Kong; [c]*Hepato-Metabolic Disease Unit, "Bambino Gesù" Children's Hospital and IRCCS, Rome, Italy*

William C.S. Cho

Abstract: Solid tumors account for approximately 30% of all pediatric cancers. Although chemotherapy has largely contributed to strongly improve patient outcome, multidrug resistance (MDR) remains one of the major mechanisms limiting the overall survival. The enhanced efflux rate of chemotherapeutic drugs from tumor cells through drug transporters is one of the most important mechanisms of MDR. Drug transporters play a pivotal role in preserving the balance between sensitivity and resistance of tumor cells to anti-neoplastic drugs. Their functional activities have been barely investigated in pediatric solid malignancies. Here, we provide evidence from the current literatures on drug transporters and MDR in the most common types of pediatric solid tumors, including neuroblastoma, Wilms' tumor, rhabdomyosarcoma, retinoblastoma, medulloblastoma and hepatoblastoma.

Keywords: ABC transporters, MDR1, MRP1, Multidrug resistance, neoadjuvant chemotherapy, pediatric solid tumors.

1. INTRODUCTION

Cancer is the leading cause of disease-related death among children in developed countries. In the United States, over 15,000 new cases of childhood cancers have been diagnosed in 2014 [1], with solid tumors accounting for 30-40% of all cancers. During the past 60 years, the outcome of cancer in children has improved considerably, with an overall survival (OS) at 5 years increasing from 30% to more than 80% [2]. This is due to the improvement of the therapeutic strategies from aggressive surgical approaches to multimodal approaches with neoadjuvant chemotherapeutic treatments before resection [3]. However, the use of these new drugs has led cancer cells to develop resistance, a phenomenon known as multidrug resistance (MDR), which is currently the leading cause of treatment failure in pediatric tumors. Resistance to chemotherapy drugs falls into two categories with respect to the tumor cells: extrinsic and intrinsic. Extrinsic resistance consists in the failure of drugs to reach their site of action in an active form. This can be due to several reasons such as short half-life or rapid clearance in kidney or liver. Intrinsic resistance includes processes such as removal of the drug from its site of action by increased efflux or decreased uptake, enzymatic modification and/or inactivation of drug targets within the cell. Drug transporters and drug-metabolizing enzymes play crucial roles in determining the toxicity produced by chemotherapeutic agents in normal tissues and in preserving the balance between sensitivity and resistance to anti-neoplastic drugs in tumor cells. In this review, we examine the MDR phenomenon and the role of drug transporters in the most common types of pediatric solid tumors, including neuroblastoma (NB), Wilms' tumor (WT), rhabdomyosarcoma (RMS), retinoblastoma (RB), medulloblastoma (MB) and hepatoblastoma (HB).

*Address correspondence to William Cho at the Department of Clinical Oncology, Queen Elizabeth Hospital, Kowloon, Hong Kong; Tel: 852-3506-5441; Fax: 852-3506-5455; E-mail: williamcscho@gmail.com

*Doriana Fruci at the Paediatric Haematology/Oncology Department "Bambino Gesù" Children's Hospital and IRCCS, Rome, Italy; Tel: 39-06-6859-2657; Fax: 39-06-6859-2904; E-mail: doriana.fruci@opbg.net

*Anna Alisi at the Hepato-Metabolic Disease Unit, "Bambino Gesù" Children's Hospital and IRCCS, Rome, Italy; Tel: +39-06-6859-2186; Fax: 39-06-6859-2904; E-mail: anna.alisi@opbg.net

2. DRUG TRANSPORTERS AND MDR

Drug transporters are membrane proteins known to regulate efflux and uptake of drugs by playing a key role in pharmacokinetics and pharmacodynamics. Currently, more than 420 drug transporters belonging to the solute carrier (SLC) and Adenosine 5-triphosphate (ATP)-binding cassette (ABC) transporter families have been identified in human. Most of them are known to confer MDR to cancer cells [4]. SLC and ABC transporters are expressed at high levels in epithelial cells to regulate the flux of endogenous metabolites and small-molecules into and out of different tissues. Studies in knockout mice and loss of function of genetic variants in humans have demonstrated the key role of transporters in drug disposition, treatment efficacy and adverse events [5, 6].

2.1. SLC Transporters

The SLC transporters are involved in influx and efflux of small molecules, such as ions, metabolites, toxins and drugs across biological membranes, without relying on the ATP hydrolysis [7]. To date, nearly 380 members have been identified, organized into 52 families based on their sequences, number of transmembrane α-helices and biological functions. SLC transporters are involved in a variety of cellular functions, often in association with other proteins, including receptors, enzymes and other transporters. Members of several SLC families are highly abundant in liver, kidney and blood-brain barrier, where they regulate drug absorption, distribution, metabolism and excretion. Mutations in SLC transporters have been associated with differences in drug response among individuals [8, 9]. However, the impact of SLC transporters on cancer therapy has not been extensively investigated in pediatric solid tumors. Therefore, in this review we will discuss data on the other drug transporters.

2.2. ABC Transporters

ABC transporters utilize energy resulting from ATP hydrolysis to mediate substrate efflux across the membrane out of cells [10, 11]. This group of drug transporters includes 48 functional members divided into 7 subfamilies, ABC-A to ABC-G, and additional subfamilies, depending on their structural differences and similari-

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ties [12]. The structure of ABC transporters consists of two transmembrane binding domains (TMD) that serve for substrate moving, and two nucleotide-binding domains (NBD) required for ATP hydrolysis and substrate translocation across cell membrane. ABC transporters are distinguished by the presence of a conserved consensus sequence of about 100 amino acids containing the Walkers A and Walkers B ATP-binding motifs, as well as the ABC signature (C motif). The substrates handled by ABC transporters include a variety of endogenous and exogenous compounds and different types of molecules, ranging from organic cations and anions to polypeptides or therapeutic agents. The key role of these drug transporters in cancer chemotherapy is well recognized. The MDR protein 1 (MDR1, P-gp, ABCB1), the MDR associated protein 1 (MRP1, ABCC1) and the breast cancer resistant protein (BCRP, MXR, ABCG2) are the best-characterized ABC transporters associated with a MDR phenotype in a variety of human tumors [13].

2.2.1. MDR1

MDR1 is involved in the transport and excretion of hydrophobic drugs out of cells. High levels of MDR1 expression have been detected in several tumors, including advanced gastrointestinal stromal tumor (GIST), non-small cell lung cancer (NSCLC), fallopian tube, ovarian and thyroid cancer. In all cases, MDR1 overexpression is known to confer a significant resistance to a number of chemotherapeutic drugs, including anthracyclines, taxanes, epipodophyllotoxins, vinca alkaloids and imatinib mesylate [4]. Interestingly, the absence of MDR1 expression in drug-resistant cancer cells has encouraged studies on further transporters, such as MRP subfamily and BCRP that may exert relevant efflux functions.

2.2.2. MRP Family

MRP subfamily can be further classified into two groups on the basis of their structural topology: one group including ABCC1/MRP1, ABCC2/MRP2, ABCC3/MRP3, ABCC6/MRP6, and ABCC10/MRP7, consists of three TMDs and two NBDs, while the other group, including ABCC4/MRP4, ABCC5/MRP5, ABCC11/MRP8 and ABCC12/MRP9, is composed by two TMDs and two NBDs [14]. Members of this family have been associated with MDR in several cancers treated with chemotherapeutic compounds, such as vincristine, doxorubicin, etoposide and irinotecan [15]. MRP members have high affinity for negatively charged hydrophobic drugs, including the transport of compounds formed after phase II metabolism, such as methotrexate, leukotrienes, prostaglandins and glutathione-conjugated molecules. Typical substrates of MRP2 are cisplatin and indinavir. MRP4 and MRP5 have been shown to transport nucleoside derivative drugs. Overexpression of MRP7 in HEK293 was found to confer resistance to several anticancer agents, including paclitaxel, docetaxel, vincristine, vinblastine, cytarabine, gemcitabine and epothilone B [16]. In NSCLC, MRP7 expression has been associated with vinorelbine and paclitaxel resistance [17].

2.2.3. BCRP Transporter

The BCRP transporter is expressed in various tissues including brain, testis and blood brain barrier and it is thought to have a physiological function in protecting against various dietary xenobiotics. Overexpression of BCRP confer MDR in different cell lines and solid tumors, including melanoma, breast, colon, small cell lung, ovarian, stomach and intestinal cancer, gastric, hepatocellular and endometrial carcinoma [18, 19]. Unlike MDR1 and MRP1, BCRP contains only one TMD and one NBD, and is thought to dimerize with itself (homodimerize) to perform its efflux function. BCRP substrates, in part overlapping with MDR1 and MRP1, include organic anion conjugates, nucleoside analogues, organic dyes, tyrosine kinase inhibitors, anthracyclines (such as doxorubicin), camptothecin-derived indolocarbazole topoisomerase I inhibitors, MTX, and flavopiridols.

2.3. LRP Transporter

Another important transporter that plays a role in MDR is the lung resistance-related protein (LRP), a non-ABC transporter that was identified as the major component of the vault complex. Vaults are highly conserved ribonucleoprotein particles with a hollow barrel-like structure involved in several cellular processes and linked to MDR in several tumors, such as glioblastoma and hepatocellular carcinoma [20]. LRP is largely distributed in different tissue districts, including bronchial epithelium, digestive tract, keratinocytes, adrenal cortex, macrophages, kidney, pancreas and germ cells. Unlike MDR1, MRP1 and BCRP, LRP is localized in vesicles and lysosomes to exclude cytotoxic agents from the cells by exocytosis [20]. Kitazono *et al*. reported that LRP mediates resistance to different drugs and plays a pivotal role in the transport of doxorubicin between the nucleus and the cytoplasm [21].

3. DRUG TRANSPORTERS IN THE MOST COMMON PEDIATRIC SOLID MALIGNANCIES

Pediatric solid tumors differ from adult solid tumors in several ways. First, they derive from DNA changes occurring in cells that take place in early life, they are not linked to lifestyle or environmental factors, and usually they respond better to chemotherapy due to the faster growth rate, less unfavorable mutations accumulated on MDR genes, and a better recovery after high doses of chemotherapy. In the next sections, we will discuss the association of most frequent pediatric solid tumors, including NB, WT, RMS, RB, MB and HB, to ABC transporters and MDR. A schematic representation of MDR proteins involved in these tumors is presented in Fig. (**1**).

3.1. Neuroblastoma and Drug Transporters

NB is the most common extracranial solid tumor of childhood and accounts for 15% of all pediatric cancer deaths [22]. Although in some cases, aggressive conventional multimodal therapies (e.g., chemo- and radio-therapy, surgery, autologous stem cell transplantation, differentiating therapy and immunotherapy) have been effective in eradicating metastatic NB, the clinical outcome for these patients remains very poor, with less than 40% long-term remissions. This is because conventional chemotherapy is hampered by the rapid emergence of MDR. Several biological and genetic factors play a pivotal role in NB development and prognosis, including age at diagnosis, tumor stage, unfavorable histology and the *MYCN* amplification status [22]. Amplification of the *MYCN* oncogene, found in approximately 20% of primary NB, predicts very poor outcome. To date, eleven ABC transporters, including MDR1, MRP1, MRP3, MRP4 and BCRP are known to be directly transcriptionally regulated by MYCN [23-25], supporting a role of these drug transporters in promoting MDR in NB.

3.1.1. MDR1

Several drugs used in NB therapy, including etoposide, vincristine, doxorubicin and irinotecan, are exported out of the cells by MDR1. MDR1 expression was increased in NB following chemotherapy and in nonresponsive tumors [26, 27]. Nevertheless, despite the direct involvement of MYCN in regulating MDR1 expression, a clear association between MDR1 expression and patient outcome as well as age at diagnosis, tumor stage or *MYCN* status was not detected [28-31]. However, a report provided evidence of a role of MDR1 in earlier stages of NB development showing that $MDR1^{high}$ NB tumor-initiating cells were associated with greater tumor growth *in vivo* compared to $MDR1^{low}$ cells [32].

3.1.2. MRP1

In retrospective and prospective studies, an increased MRP1 expression was strongly correlated with poor event-free survival and OS in NB patients [31, 33]. The link between MYCN and transcriptional regulation of MRP1 expression has been well established [34-36, 24]. MYCN directly regulates MRP1 activity by binding to

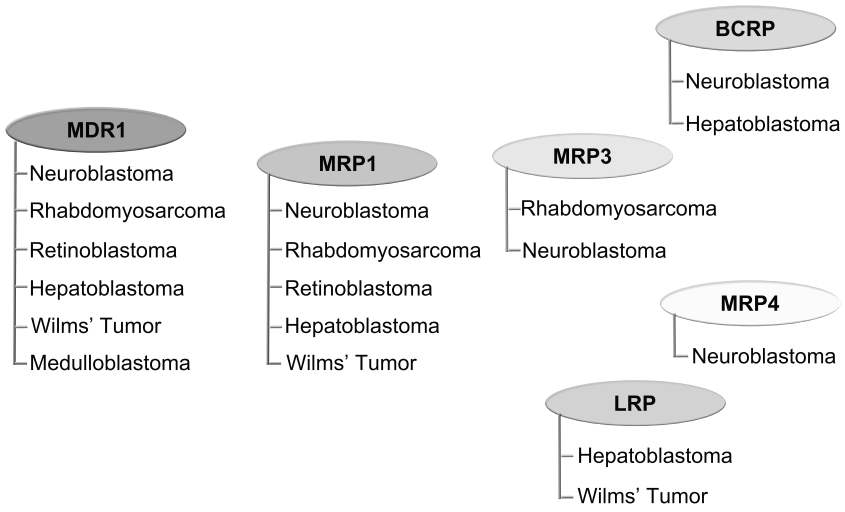

Fig. (1). Schematic representation of the MDR proteins expressed in neuroblastoma, Wilms' tumor, rhabdomyosarcoma, retinoblastoma, medulloblastoma and hepatoblastoma.

E-box elements within its promoter, immediately downstream of the transcriptional start site [24]. In high-risk NB, amplification of MYCN leads to increased MRP1 expression, which in turn is able to confer a MDR phenotype. MRP1 extrudes a large number of chemotherapeutic compounds relevant to NB treatment, including topotecan, etoposide, doxorubicin, and vincristine [35]. Genetic and pharmacological inhibition of MRP1 sensitizes NB to MRP1 substrate drugs both *in vitro* and *in vivo* [37, 38]. Studies in animal models confirm the role of MRP1 in the MDR phenotype of human NB and support the notion that this transporter may represent a potential therapeutic target to increase the efficacy of MRP1 substrates in NB [37, 38].

3.1.3. MRP4

MRP4 expression was significantly correlated with MRP1, and associated with MYCN amplification in NB patients. In a multivariate analysis of a cohort of 52 primary NB samples, Norris *et al*. found that MRP4 expression is a significant indicator of poor survival [39]. Moreover, the same authors demonstrated that high levels of MRP1 and MRP4 expression combined with low levels of MRP3 were associated with a poor prognosis in a large cohort of primary NB samples [40].

3.1.4. BCRP

The expression BCRP, although directly regulated by *MYCN* oncogene, is inversely correlated with poor outcome in NB patients [25], suggesting its minor contribution to a MDR phenotype in high-risk NB. However, in one study BCRP was expressed at high levels in primary NB cells resistant to cytotoxic drugs, such as mitoxantrone [41]. This discrepancy can be attributed to the system used or to the methylation status of BCRP promoter, which influences MYCN activity.

3.2. Wilms' Tumor

WT, also known as nephroblastoma, is the most common type of kidney cancer in children accounting for 1 child per 10,000 worldwide before the age of 15 years [42]. It arises from metanephric blastemal cells and recapitulates renal embryogenesis. Aberrations in 10 genes involved in the control of nephron progenitors have been found to characterize different subsets of WT. WT is a curable disease in most patients with 90% OS. However, a subset of patients fails to respond to chemotherapy as consequence of MDR acquisition [43]. The role of ABC transporters as prognostic factors in WT is still doubtful. Very few studies have investigated the role of these transporters in drug resistant WT.

3.2.1. MDR1, MRP1 and LRP

In one study expression of MDR1 and MRP1 was investigated together with that of p53, in 25 primary WT specimens by immunohistochemistry. Forty-eight percent of tissue samples were positive for MRP1 expression, 24% for MDR1 and 8% for p53. No correlation was detected between the expression of these MDR proteins and the wild-type p53 [44]. In a second study, 45% of WT patients were positive for MDR1 protein, of which only half relapsed suggesting that other factors than MDR1 are involved [45]. Using a tissue microarray technique, Fridman *et al*. found a prominent reduction of MRP1 expression in more than 50% of the WT samples studied as compared to normal kidneys [46]. Unfortunately, no indication on chemosensitivity of the tumor analyzed was provided. In another study, MDR1 was detected in tumor samples from 93 patients, 72 of which were collected after chemotherapy. No association between MDR1 expression and disease-free survival, stage, or grade was detected [47]. However, the authors suggested that MDR1 expression in endothelial cells of tumor vessels is one of the factors responsible for the MDR phenotype in WT. LRP was either weakly or not expressed at all in both WT and normal kidneys.

3.3. Rhabdomyosarcoma

RMS is the most common soft-tissue sarcoma in children accounting for 4-8% of all pediatric malignancies with an incidence of approximately 4.6 cases per million/per year [48]. RMS commonly originates from primitive mesenchymal cells that fail to completely differentiate into skeletal muscle, even though the same tumor may have non-myogenic origin [49]. RMS can be divided in two main forms: embryonal subtype (the most common) and alveolar subtype (the most aggressive). These two subtypes are heterogeneous and characterized by different genetic aberrations. Although the use of multimodal therapy (surgery, chemotherapy and radiation therapy) has improved the success rate to nearly 70%, the 5-year survival rate of children with metastatic disease is less than 30% [50].

3.3.1. MDR1, MRP1, MRP3 and LRP

The role of drug transporters in RMS outcome is controversial. In a comparative study between pediatric and adult RMS, Komdeur *et al*. found that the expression of MDR1 and MRP1 in embryonal and alveolar RMS did not differ significantly between children and adults [51]. On the contrary, LRP appears to have a higher expression in adults than in children, possibly explaining different response to chemotherapy [51]. Chan *et al*. demonstrated that MDR1 expression was an adverse prognostic factor in pediatric RMS, with

its reduced expression associated with a more favorable prognosis [52]. On the contrary, Kuttesch *et al*. [53] reported no significant association between the expression of MDR1 at diagnosis and clinical features or disease outcome in childhood RMS. These findings highlight the concept that MDR in RMS strongly depends on the differentiation stage. Resistance to actinomycin D resulted in increased MDR1 expression in a RMS cell line, as reported by two studies from the same group [54, 55]. By using *in vitro* cell lines and xenografts models of human alveolar RMS, Seiz *et al*. demonstrated that the up-regulation of MDR1 expression was associated with drug resistance [56]. More recently, we investigated the expression of MDR1, MRP1 and MDR3 in primary RMS tumors and in residual tumor after chemotherapy [57]. MRP1 was detected in 70% of the cases, followed by MDR3 and MDR1 (58% and 44%, respectively). Many samples exhibited co-expression of at least two of them. Furthermore, MDR3 was significantly associated with PAX3/PAX7-FKHR fusion transcripts typical of alveolar RMS. After chemotherapy expression of MRP1, MDR3 and MDR1 was enhanced in most of RMS tested [57].

3.4. Retinoblastoma

RB is the most common intraocular pediatric tumor with an incidence of approximately 9,000 newly affected every year worldwide. About 50% of RB patients carry germline mutations in the RB1 gene, which encodes the first recognized tumor suppressor protein [58]. Hundreds of mutations in the RB1 gene have been detected in monocular and binocular RB patients. The clinical management of RB is complex due to differential presentation of the disease [59]. Based on tumor size, therapeutic strategies may include conservative (non-enucleation) and non-conservative (enucleation) approaches combined with radiotherapy and chemotherapy.

3.4.1. MDR1, MRP1 and LRP

MDR is one of the major problems occurring after chemotherapy in RB. Chan *et al*. found an increased expression of MDR1 in four treated tumors that correlates with clinically relevant MDR [60]. More recently, the same authors reported an association between increased MRP1 expression and failure of chemotherapy [61]. In contrast, Krishnakumar *et al*. found an increased expression of MDR1 and LRP that did not correlate with invasion, differentiation, laterality of the tumors, or response to chemotherapy [62]. Expression and function of MDR proteins was investigated also in human RB cell lines. Ishikawa *et al*. demonstrated that the Y79 RB cell line expressed MDR proteins, but that only MRP1 and MRP2 are functional and that the efflux of doxorubicin from these cells may depend on a still unknown ATP-dependent transporter [63]. However, Y79 RB cell line is just a prototype for RB cells that differ from other RB cell lines in terms of sensitivity to anticancer drugs and response to cytokine exposure. An interesting *in vitro* study demonstrated that curcumin, a polyphenolic compound that inhibits ABC transporters [64], blocks MDR1 activity in Y79 RB cell line by a direct physical interaction [65]. Recently, moxifloxacin, a dual substrate of MDR1 and MRP2 efflux transporters, was found to regulate the cellular accumulation of cytotoxic drugs and their permeability by reducing MDR in RB cells [66].

3.5. Medulloblastoma

MB is the most common pediatric malignant brain tumor with an incidence of around 650 new cases per year in the European Union. MB histology and clinical presentation are similar to those observed in other tumors of the central nervous system [67]. Treatment begins with maximal surgical removal of the tumor followed by craniospinal and local boost radiotherapy and chemotherapy. Although cure efficacy for MB have improved significantly in the past 3 decades, recurrence of disease occurs in 30-40% of high-risk patients, suggesting that MDR could be the major cause of treatment failure.

3.5.1. MDR1

Chou *et al*. provided the first evidence that high expression of MDR proteins may be associated with chemoresistance and poor outcome in MB. The authors found an increased MDR1 expression (mRNA and protein) that significantly correlated with an adverse outcome in archival specimens from 29 children with MB receiving adjuvant chemotherapy [68]. Conversely, a study performed on 17 samples of MB and primitive neuroectodemic tumors demonstrated that the expression of resistance genes for MDR1, MRP1, MDR3, BCRP, or LRP had no impact on the OS of patients [69]. Studies in MB cell lines demonstrated an increased expression of MDR1 after treatment with different chemotherapeutic agents, including vinblastine and cyclophosphamide [70, 71]. Interestingly, Hussein *et al*. found in two MB cell lines the co-expression of MDR1 and the cancer stem cell marker CD133 that further increased upon etoposide treatment [72]. These cells are able to develop tumors similar to human MB in orthotopic xenograft models. More recently, the same authors tested several compounds to sensitize MB cells to chemotherapeutic drugs. They found that vardenafil may represent a good candidate to increase sensitivity of MDR1-expressing tumors to etoposide [73].

3.6. Hepatoblastoma

HB accounts for 79% and 65% of all primary malignant hepatic tumors in children under 3 years and 15 years of age, respectively. Although, its worldwide incidence rate has not been estimated due to ethnic differences, recent studies suggest an increasing trend during the last decades [74]. HB originates from hepatocellular progenitors and is characterized by different cellular and tissue morphologies resembling liver development phenotypes [75]. Surgical resection of the tumor mass is the mainstay of therapy for HB. The use of neoadjuvant cisplatin-based chemotherapy has strongly improved survival rate by increasing the number of tumors that can be resected. However, in some cases tumors cannot be removed by surgery even after chemotherapy, leaving liver transplantation as the only solution [76]. Given the limited availability of liver for transplant, an improvement of pre-surgical chemotherapy response is necessary.

3.6.1. MDR1, MRP1, BCRP and LRP

Although the majority of HB tumors are chemosensitive, some of them can develop MDR [77, 78]. Several *in vivo* and *in vitro* models of HB exhibited an up-regulation of MDR1 expression after doxorubicin and cisplatin treatments [79, 80]. Warmann *et al*. found an increased MDR1 expression in a child with multifocal HB, during both pre- and post-chemotherapy [81]. The same authors showed that *in vitro* and *in vivo* treatment of HB with MDR modulators, such as cyclosporine derivatives and verapamil increased response to chemotherapy by modulating MDR1 expression [82, 83]. Another study reported up-regulation of MDR1 and MRP1 expression and *ex novo* expression of LRP following chemotherapy in HB tumors [84]. Finally, up-regulation of BCRP was also detected in HB cells and tumors, even if, in these cases, no changes of the MDR1 or other ABC transporters were found [85]. Eicher *et al*. developed a three-dimensional culture system to study MDR in HB [86]. Expression of MDR1, MRP1 and BCRP in this model were similar to that observed in previous *in vitro* studies. The multiple phenotypes of HB cells, which reflect tumor heterogeneity, may explain the differences observed in the MDR protein expression in this tumor type. However, a further explanation for this heterogeneity in HB could be provided by the origin of the tumor and the presence of diverse cell subpopulation with characteristics of cancer stem cells [87, 88].

4. FUTURE PERSPECTIVES AND CONCLUSIONS

Pediatric solid malignancies are a group of heterogeneous tumors, which differ in term of cellular origins, developmental stages and clinical features. Although these tumors respond even better

than adult tumors to chemotherapeutic treatments, relapse events suggest the involvement of chemoresistance mechanism. An enhanced understanding of drug transporter expression profiles could significantly improve strategies to overcome MDR and increase patient survival. However, to date, the expression of MDR proteins and in particular that of ABC transporters, has been only barely investigated in these tumors. Several studies, carried out with old techniques based on RNA expression and in a small number of samples, provided controversial results (Table **1**). Recent technological progresses from microarrays to transcriptome deep-sequencing analyses, as well as from gene knockouts to RNA interference strategies could provide novel tools for the identification and quantification of MDR-associated genes [89]. For each type of pediatric tumors, expression profiles of MDR proteins should be correlated with time of diagnosis, MDR phenotype onset and type of relapse. At diagnostic level, molecular subtyping could allow the characterization of MDR protein expression in pediatric solid tumors revealing cancer cell sensitivity to a specific drug, and thus contributing to the choice of the best protocol of treatment.

The prediction of which tumors will become resistant at what stage and after which type of treatment is another important clinical challenge that could be faced with the identification of biomarkers associated to MDR. Moreover, the increasing popularity of circulating miRNAs as prognostic or predictive tool in cancer makes these molecules useful non-invasive biomarkers to predict the patient response to chemotherapy providing insights about mechanism of

Table 1. Clinical evidence of MDR transporter in neuroblastoma, Wilms' tumor, rhabdomyosarcoma, retinoblastoma, medulloblastoma and hepatoblastoma.

TUMOR TYPE	NUMBER OF PATIENTS	TRANSPORTER(S)	CLINICAL ASSOCIATION	EVIDENCE	REF
Neuroblastoma	41	MDR1	Chemoterapy resistance	RNA	[26]
	49	MDR1	Chemoterapy resistance	RNA	[27]
	84	MDR1	No significant correlation	RNA	[28]
	34	MDR1	No significant correlation	RNA	[29]
	23	MDR1	No significant correlation	ICC	[30]
	209	MDR1 MRP1	No significant correlation Event free survival, overall survival	RNA RNA	[31]
	60	MDR1 MRP1	No significant correlation Event free survival, overall survival	RNA RNA	[33]
	54	MRP4	Overall survival	RNA	[39]
	208	MRP1, MRP4, MRP3	Event free survival	RNA	[40]
Wilms' tumor	40	MDR1	No significant correlation	IHC	[45]
	14	MDR1, LRP	No significant correlation	IHC	[46]
	93	MDR1	Disease free survival for intratumoral endothelial cells	IHC	[47]
Rhabdomyosarcoma	45	MDR1, MRP1, LRP	No significant correlation	IHC	[51]
	30	MDR1	Event free survival, overall survival	IHC	[52]
	76	MDR1	No significant correlation	IHC	[53]
	43	MDR1, MRP1, MDR3	No significant correlation	IHC	[57]
Retinoblastoma	4	MDR1	Chemoterapy resistance	IHC	[60]
	18	MDR1, MRP1	Chemoterapy resistance	IHC	[61]
	60	MDR1, LRP	No significant correlation	IHC	[62]
Medulloblastoma	29	MDR1	Overall survival	IHC/RNA	[68]
	17	MDR1, MRP1, MDR3, BCRP, or LRP	No significant correlation	RNA	[69]
Hepatoblastoma	9	MDR1	Chemoterapy resistance	RNA	[80]
	24	MDR1, MRP1, LRP	Chemoterapy resistance	IHC	[84]
	7	MDR1, BCRP	No significant correlation	IHC	[85]

MDR [90]. Furthermore, profiling of ABC gene variants may also contribute to identify individual predispositions to develop MDR, thus providing crucial information for the choice of the treatment [91].

In addition to the establishment of a complete MDR phenotyping, a further goal will be to overcome this phenomenon. It is hope that combining chemotherapy with targeted therapy using advanced technologies could help to reduce the likelihood of MDR in pediatric solid tumors. An effective treatment of MDR should require the use of specific therapies that i) inhibit the ABC transporters on the cell membrane, ii) block pathways involved in the regulation of these transporters, and iii) directly target transcription factors regulating their expression.

Some efforts have been made to develop potent and selective compounds to inhibit MDR proteins [92]. Unfortunately, these studies have encountered a number of issues related to toxicity, dose reduction of anticancer drugs, and perturbation of key barrier tissues. Furthermore, response to MDR modulators may be strongly affected by genetic variants in ABC transporters and may depend on genetic background of patient, an issue that was observed also for other drugs.

Therapeutic approaches based on nanoparticles represent an effective way to overcome efflux-mediated resistance. A recent study has shown that the encapsulation into rHDL nanoparticles of fenretinide, which has been shown to sensitize MDR human NB cells to natural killer cell cytotoxicity [93], can achieve a 100-fold overall improvement of therapeutic efficiency [94]. As a proof-of-concept, a recent phase-I clinical trial has demonstrated that fenretinide delivered in an oral powderized lipid complex exhibited an evident anti-tumor activity with minimal toxicity in NB patients [95]. The targeted therapy with NVP-BEZ235, an inhibitor of PI3K/mTOR signaling pathway whose activation may induce proliferation in malignant cells and confer MDR properties, can significantly enhance doxorubicin-induced apoptosis in NB. In addition, it has been found that the use of multiple inhibitors to target the PI3K/Akt/mTOR network in an optimal sequential dosing can further enhance chemo-sensitization [96]. Michaelis *et al*. show that nutlin-3 acts as a MDR1 and MRP1 substrate in NB and RMS enhancing the sensitivity of these cells to different chemotherapeutic drugs [97]. In MB cells, selective inhibitors of the tumor-promoting sonic hedgehog signaling pathway were found to reduce the expression of ABC transporters [98, 99].

A promising strategy to reduce adverse events associated to pharmacological inhibition of ABC transporters could be the use of natural products. Many natural products (i.e., flavonoids) are excellent modulators of major functions of ABC drug transporters [100]. However, we still need a systematic high-throughput approach to screen natural products that may act as nontoxic, potent and selective inhibitors of MDR proteins.

An alternative strategy to overcome and exploit clinical MDR in solid pediatric tumors could be to take advantage of collateral sensitivity that consists in the identification of molecules/drugs that selectively kill MDR cells by preserving the non-resistant parental cells from which they are derived [101]. Even limited studies are available, an additional interesting strategy to treat pediatric solid malignancies with MDR is the use of immunotherapy as extensively reviewed by Curiel [102].

Taken together, this review highlights the concept that targeted therapy in combination with conventional anti-cancer drugs will lead to an improved treatment for MDR cancers once a better understanding of the phenomenon will be gained. Because MDR pathway seems to be crucial for several pediatric solid cancers, efforts are required to advance drugs that are less prone to resistance induction.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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