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Importance of Detecting Multidrug Resistance Proteins in Acute Leukemia Prognosis and Therapy

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Multidrug resistance (MDR) is a multifactorial phenomenon and the role of these proteins in generating the MDR phenotype is controversial. With this in mind, this review compiled the current data on the role of ABCB1, ABCC1, and LRP proteins in the prognosis of hematologic neoplasms and their influence on the choice of therapy. Literature showed that the detection of these proteins, mainly ABCB1, is important in the AL prognosis. However, there is controversy regarding the methodology used for their detection. In summary, the expression and activity profiles of ABCB1, ABCC1, and LRP, proteins capable of promoting the efflux of a variety of chemotherapeutic agents from the cell cytoplasm represent one of the greatest causes of failure in AL treatment. J. Clin. Lab. Anal. 27:62–71, 2013.

Key words: multidrug resistance; ABCB1; ABCC1; LRP; acute leukemia

INTRODUCTION

Acute leukemias (ALs) constitute a heterogeneous group of malignant hematologic diseases characterized by the clonal expansion of bone marrow hematopoietic cells with increased blasts and aggressive clinical course. ALs may be of lymphoid or myeloid lineage, and vary in differentiation (1).

The goal of leukemia treatments is to eradicate the leukemic clone and, thus, reestablish normal hematopoiesis. Several therapy methods have been employed in the treatment of leukemia, such as radiotherapy, chemotherapy, immunotherapy, and bone marrow transplant. Although bone marrow transplant is considered an important therapeutic weapon to achieve complete leukemia remission, this procedure has clinical and socioeconomic restrictions. For this reason, chemotherapy is the most common antileukemic therapy employed today, even with the high morbidity associated to it (2).

In spite of progress in chemotherapy, antitumor drug efficacy is still limited by three main factors: (i) by the drug's pharmacokinetic characteristics in terms of reaching the target cell in adequate amounts; (ii) by the adverse events they cause in normal tissues and cells; and (iii) by the resistance of tumors to the cytotoxic agents administered. The latter remains a primary problem in treating AL.

Multidrug resistance (MDR) might be an inherent phenomenon seen prior to medication therapy or acquired after an initially successful therapy begins (3). The MDR phenotype is characterized by the simultaneous resistance against different drugs that have no structural similarity and act on different molecular targets. It is a multifactorial phenomenon with biochemical resistance mechanisms in common, which can include the reduction of intracellular drug concentrations by changes in its influx/efflux (4).

The extracellular efflux of chemotherapy agents fundamentally involves mechanisms mediated by a superfamily of transport proteins called ATP-binding Cassette, including ABCB1 (P-Glycoprotein), ABCC1 (MDR-associated

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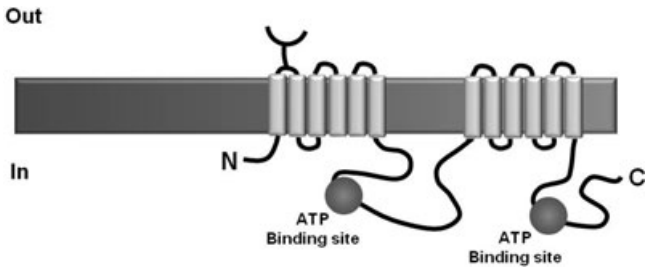

Fig. 1. Schematics of protein ABCB1 (Adapted from 14).

protein [MRP] 1), and a protein involved in the nucleus-cytoplasm molecule transport called LRP (lung resistance protein; 4, 5).

Protein ABCB1 (P-Glycoprotein/PgP/MDR1)

ABCB1 (P-Glycoprotein or MDR1) is a glycosylated protein encoded by the gene *abcb1*, which is located in chromosome 7q21.12 (6, 7). This protein acts as an ATP-dependant drug efflux pump and belongs to the ABC-transporter superfamily, which includes proteins responsible for the transport of a broad range of substrates, such as sugars, amino acids, peptides, organic ions, and several hydrophobic and metabolic compounds (8).

ABCB1 was first described by Juliano and Ling (1976) who, by investigating MDR profiles in Chinese hamster ovary cell strains, identified a 170 kDa glycoprotein involved in the change in permeability to drugs; hence it was named P-Glycoprotein (9). Later, Fojo and colleagues (1985) characterized the presence of a DNA sequence commonly amplified in cells with MDR phenotype, called *mdr1*, which was responsible for coding a 170 kDa peptide (10). Chen and colleagues (1986) sequenced this *mdr1* gene and confirmed that it coded a 1,280-amino acid protein consistent with the estimated size for P-Glycoprotein or ABCB1, proving the *mdr1* gene was responsible for coding protein ABCB1 (11).

ABCB1 is structured in two homologous and symmetric halves, each composed of a transmembrane region made up by an N-terminal domain, a cytosolic C-terminal domain and two ATP-binding sites (Fig. 1; (12). ATP hydrolysis by ABCB1 provides the energy needed for translocating substrates through the cell membrane (13).

Protein ABCB1 can be detected in cells from normal tissues involved in physiological absorption and excretion of compounds (14), such as the liver, the kidneys (15), the placenta, the blood–brain barriers, blood–cerebrospinal fluid barriers, and blood–testis barriers (16). ABCB1 is also related to processes involving the regulation of cell differentiation, proliferation (17), and apoptosis, acting in blocking the activation of caspase 8 and caspase 3 (18). Moreover, some studies show that protein ABCB1 plays a role in immune responses, being involved in inflammatory processes. This last hypothesis is corroborated by studies showing that some immune system cells express ABCB1 constitutively (19) and that this protein is related to the transport of cytokines, such as interleukin-2, interleukin-4, and interferon-γ (20).

Protein ABCB1 is well characterized as an efflux pump capable of extruding several drugs out of the cells, as doxorubicin, daunorubicin, vinca alkaloids, actinomycin D, paclitaxel, teniposide, and etoposide (6, 21). Several models have been proposed to explain the efflux mechanism promoted by protein ABCB1. One of the most well-known models is called "hydrophobic vacuum cleaner" (22), where the two subunits of protein ABCB1 form a single transport channel through which the drug is expelled, being it in its neutral or charged forms. An adaptation of this model considers the occurrence of conformational changes in the protein through ATP hydrolysis. These conformational changes lead to the opening of a channel through which the drug is expelled. Higgins (1994) proposed that the action of protein ABCB1 is similar to that of a flippase enzyme, which is present in the cell membrane and aids the transport of phospholipid molecules (23). Currently, the most accepted model is the one that describes the partitioning of the substrate from the lipid bilayer to an inner part of the protein, called "inner leaflet," where the substrate-binding site is localized. When ATP binds to the nucleotide-binding site (NBS), a great conformational change occurs, exposing the substrate-binding site to the extracellular space, thus allowing for the drug extrusion process to take place (24).

Due to the drug extrusion mechanism, a marked expression of protein ABCB1 has been related to resistance to chemotherapy. Gottesman (2002) highlighted that protein ABCB1 plays an important role in chemotherapy tumor treatment, since (i) the levels of ABCB1 expression in several tumors are high enough to provide significant resistance to drugs; (ii) the acquisition of resistance after chemotherapy is associated with an increase in the levels of this protein; (iii) the expression levels of protein ABCB1 in some tumors can be used to predict the poor response to chemotherapy, when the drugs employed are ABCB1 substrates (25).

Several pieces of evidence indicate that the expression of gene *abcb1* contributes to the resistance of leukemia cells against antineoplastic agents (26). In this context, studies have shown that patients with acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) who do not overexpress protein ABCB1 in their cells had a full remission rate after the induction therapy of 89% and 93%, respectively. On the other hand, this rate dropped to 53% and 56% in patients with AML and ALL, respectively, who overexpressed protein ABCB1 (27, 28).

Although many studies have shown the relationship between ABCB1 expression and the prognosis of several neoplasms (29), other studies have shown exactly the opposite (30), making the role of protein ABCB1 in MDR controversial. Nevertheless, the expression of protein ABCB1, as well as gene *abcb1*, is becoming increasingly relevant and recognized in deciding the most appropriate treatment for patients with neoplasms (6). Therefore, many researchers focus their investigations on improving the response to antineoplastic agents in patients who overexpress protein ABCB1, and/or gene *abcb1*.

Protein ABCC1 (MDR-Associated Protein 1/MRP1)

ABCC1 (MRP1) is an ATP-dependant transmembrane protein encoded by gene *abcc1*, located in chromosome 16p13.12 (7). Protein ABCC1 also belongs to the ABC-transporter superfamily (31) and is involved in the transport of hydrophobic and anionic substances, and also organic anions conjugated with reduced glutathione (GSH), glucuronide, and sulfate (32).

The discovery, characterization, and identification of protein ABCC1 and the gene responsible for its coding follow the same paths of protein ABCB1. Several studies have shown that some tumor cell strains have a MDR phenotype without expressing protein ABCB1, which reinforces the hypotheses of another protein being involved in the efflux of xenobiotics (33–35). In 1992, Cole and colleagues identified the amplification of a gene sequence as being responsible for the MDR phenotype in ABCB1-negative tumor cell strains (36). Later, the sequencing of this region identified the coding gene of the MRP, called *abcc1*. After the discovery of MRP, other five homologous proteins (MRP2–MRP6) were characterized and considered members of the same family. This family was then called ABCC/MRP transporter subfamily, while protein MRP was then called protein ABCC1/MRP1 (37). In 1994, Grant and colleagues characterized ABCC1 as a whole-membrane, glycosylated protein with molecular weight around 190 kDa (38).

Structurally, protein ABCC1 has two membrane spanning domains (MSD1 and MSD2), each of them with six transmembrane helixes, and a third membrane spanning domain, with approximately 200 amino acids, which is formed by five transmembrane helixes and an amino-terminal region (Fig. 2; 39). It is believed that the protein binding to its substrates happens by the interaction of these transmembrane helixes (40). ABCC1 is made up of two NBSs, located in the cytoplasmic portion of the protein (41). It must be highlighted that NBS1 has a greater ATP affinity and NBS2 has a greater ATP-hydrolysis capacity (42).

The constitutive expression of protein ABCC1 happens in hematopoietic cells of peripheral blood, lung, testicle, placenta, brain, kidneys, adrenal gland, duodenum, heart, colon, and skeletal muscle (7, 37, 43, 44). Protein ABCC1 acts in the transport of physiologic substrates such as leukotriene C4 and oxidized glutathione (GSSG) (45). Moreover, protein ABCC1 protects cells, playing a role in the extrusion of xenobiotic toxic metabolites with the goal of preventing their accumulation (15). In this way, ABCC1 plays an important physiological role in detoxifying cells both from metabolites produced by normal cellular processes and from exogenous toxic agents, such as chemotherapy drugs, which favors the resistance mechanism. Vinca alkaloids, anthracyclines, epipodophyllotoxins, methotrexate, daunorubicin, and doxorubicin are some of the most well-known antineoplastic agents that are substrates for ABCC1 (37).

The likely MDR mechanism induced by ABCC1 is associated with the cotransport of GSH-conjugated antineoplastic agents, for example, protein ABCC1 acts as a GSH-dependant drug carrier (46). The importance of this co-transport mechanism was verified by several studies that show a reduction in the transport of many substrates by protein ABCC1 when GSH was absent or GSH production was inhibited (47). Some studies enabled the confirmation of a direct relationship between the increase in ABCC1 expression and GSH in tumor cells (48, 49). The interaction mechanism between GSH and ABCC1 is complex and not completely understood, but it is believed that these two molecules can interact by means of four different mechanisms. The first mechanism suggests GSH works as a substrate for ABCC1; in the second, GSH plays the role of a co-transporter for some ABCC1 substrates. Other possible mechanisms show that the enhancement of the ABCC1 transport activity can be mediated by GSH or by other compounds that are not ABCC1 substrates (47).

Several studies in the literature show that the expression of protein ABCC1 is associated with resistance to treatment of different types of cancer (50–52). Regarding ALs, there is controversy about the role of protein ABCC1 when it is expressed. While some studies claim to find an association of ABCC1 with MDR (53), others show that protein expression seems to have no influence on resistance to treatments (54). One possible explanation for the

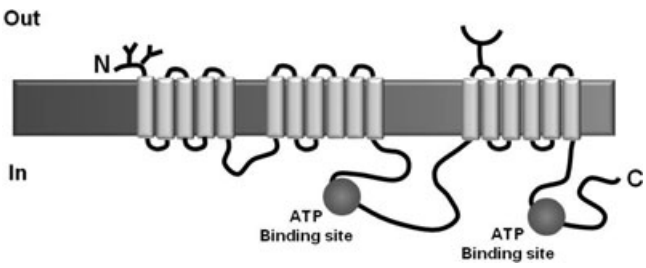

Fig. 2. Schematics of protein ABCC1 (Adapted from 99).

difficulty in determining the true clinical meaning of these proteins in ALs is based on the fact that the expression of gene *abcc1* takes place in all normal hematopoietic cell strains (55). It must be highlighted that many tumor cells co-express proteins ABCB1 and ABCC1. Considering this, it might be possible that leukemia cells express multiple membrane transporters and that multiple proteins participate in generating the MDR phenotype (56).

Protein LRP (Lung Resistance Protein)

Protein LRP was isolated in 1996 as a vesicular protein of molecular weight of 110 kDa, overexpressed in lung cancer cell strains that had the MDR phenotype. Its identification allowed for the classification of LRP as the main protein of a group of cell organelles discovered in the end of the 1980s called vaults. These proteins include a family of ribonuclear particles present in all eukaryotic cells, explaining why LRP is also called human major vault protein (MVP; 57).

Morphologically, vaults are organelles made up by four structures: the MVP itself, adenosine diphosphate ribose, telomerase-associated protein, and the small untranslated RNA. Despite their complex structure and composition, they are highly conserved among different phylogenetic species and are present in different cell types. It has been suggested that vaults perform basic functions in all cells, especially in mechanisms that involve the protection of the cell nucleus against toxic compounds and in the intracellular transport of ribosomes and steroid receptors, including those related to progesterone, estrogen, and glucocorticoids (4). There are reports of the expression of protein LRP in the bronchial epithelium and in the digestive tract, in keratinocytes, in the adrenal cortex, in macrophages, in the kidney proximal tubules, in the urothelium, in the pancreatic duct, in germinative cells, in fibroblasts, in Purkinje cells, and in the endothelium of different tissues (58).

Immunihostochemical evaluations reinforce the correlation between LRP levels and drug resistance in cell cultures, the aggressiveness of tumors, and the bad prognosis in cancer treatment. The molecular mechanism involved in MDR mediated by LRP has not been fully determined, but the early idea suggests that the protein acts in the transport of molecules from the nucleus to the cytoplasm, hypothesis generated due to the structural similarity and close fitting to the nuclear pores. Besides transporting toxic substances to the cytoplasm, it is believed that the proteic complex of vaults can arrest molecules in vesicles for later taking them out of the cells by exocytosis (Fig. 3; 58–60).

The hypothesis of drug arrest followed by its efflux by using proteic pumps has been explained by investigations involving cell signaling mechanisms and nuclear magnetic resonance spectroscopy. It has been proposed that the

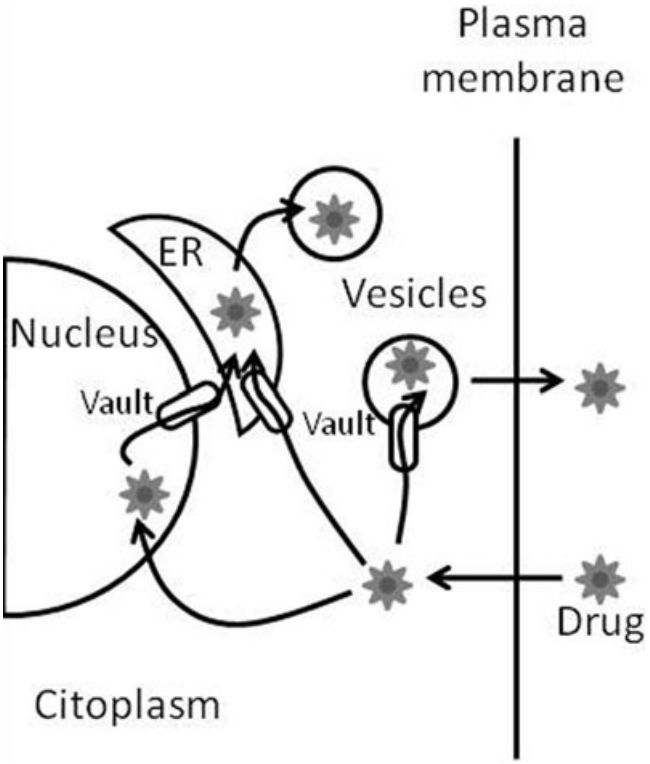

Fig. 3. Hypothetical role of LRP in mediating resistance against chemotherapy drugs. LRP/vaults act in the nucleocytoplasmic and vesicular transport of drugs. ER, endoplasmic reticulum (Adapted from 59).

vaults act as structured proteins in the signaling pathways of the epidermal growth factor. These proteins can take part in multiple protein–protein interactions, which result in a greater efficiency of the intracellular signaling mechanisms. This model suggests that the role played by LRP involves stimulation of the activation pathways of molecules associated with cell proliferation mechanisms and not necessarily the levels of LRP expressed in the cell (59, 60).

Studies employing ovarian carcinoma cells show that a high LRP expression is required, but not enough, for the development of the MDR phenotype in these cells. Moreover, no relation was seen between the resistance to drugs such as etoposide, daunorubicin, and vincristine and the overexpression of the vaults (61). Van Zon and colleagues (2004) assessed the efflux and the arrest of anthracyclines in vesicles and found these mechanisms to be independent of the increased LRP expression (62). The absence and the reduction of vaults expression were also assessed and were shown not to induce hypersensitivity to cytostatic drugs and not to affect the cells resistance ability (63).

However, other studies suggest the overexpression of LRP can be seen as a phenomenon related to resistance against several chemotherapy agents, such as doxorubicin,

vincristine, etoposide, and taxol in patients with solid tumors such as sarcomas, ovary, kidney, bladder, colon, and lung cancer (64). In the case of neoplasms of hematologic origin, LRP expression did not show any significant correlation with risk groups identified by initial white blood cell (WBC) count, gender, and age, while the presence of the MDR phenotype seems to be significantly influenced by them (65). The expression of LRP in AML, multiple myeloma, and diffuse large B-cell lymphomas was associated with reduced response to chemotherapy drugs and with a shorter life expectancy of patients (66). In ALL cases, LRP was shown to be related to the in vitro resistance against daunorubicin (67). Studies have also evaluated the contribution of LRP in MDR in children with ALL. These studies showed a positive correlation in pre-B and pro-B ALL and a negative correlation in the T-cell ALL cases (63, 68). The results are still contradictory, since some analyses revealed no difference between LRP levels in the initial diagnosis and after relapses, while others found significantly larger expressions in samples with multiple relapses (65, 69).

Several studies related the detection of LRP, and of other resistance proteins that belong to the same cellular group, to the MDR phenotype. While LRP and ABCB1 are rarely overexpressed simultaneously, the frequent concomitant expression of LRP and ABCC1 is seen in ABCB1-negative malignant cell strains. Due to the proximity in the location of genes *lrp* (16p11.2) and *abcc1* (16p13.1), it was initially believed that the gene *lrp* was co-amplified with gene *abcc1*. However, later studies reported the expression of LRP by itself, as well as determined that two proteins are rarely located in the same amplicon (58, 70).

Therefore, the contradictions seen in the studies published so far suggest the need for further investigations regarding LRP/MDR (63). The latest studies highlight the possibility of the involvement of LRP in MDR through the signaling regulation by kinase proteins traditionally regulated by extracellular growth factors (Ras/Raf/ERK) or by the phosphatidylinositol 3-kinase/serine-threonine kinase (PI3K/AKT) pathway. Among the phosphatases involved, Phosphatase and Tensin Homolog (PTEN) stands out, as it interacts with the pathways described above by promoting the regulation of gene expression and cellular growth (60). In this sense, mutations of the gene PTEN or the inhibition of this enzyme can silence it and promote the development of malignant neoplasms by the constitutive activation of AKT. In the hematopoietic strains, active Raf and AKT cause an MDR phenotype in cells by stimulating proliferation, apoptosis prevention, and increase of clonogenicity. In mammary cancer cells, Raf causes resistance against chemotherapy drugs by inducing the expression of ABCB1 and the antiapoptotic protein Bcl-2 (71, 72).

Uncertainties about the mechanism through which protein LRP is involved in the resistance against chemotherapy drugs reinforce the multifactorial nature of MDR. Several biochemical pathways can be simultaneously involved, as well as specific cell types are shown to be more susceptible to resistance against cancer treatment mediated by LRP (66).

FINAL CONSIDERATIONS

The development of new chemotherapy drugs, as well as the use of more aggressive therapeutic protocols, has improved the rates of AL cure. However, some patients do not respond to treatment and have relapses (73). It is believed that one of the causes for treatment failure takes place through the MDR mechanism. Extrusion of chemotherapeutic drugs out of the cell, mediated by overexpression or activation of transport proteins, is the most commonly involved mechanism of chemoresistance in hematologic neoplasms. Literature reports the increased expression of proteins ABCB1, ABCC1, and LRP would be related to the MDR phenotype, and several studies have been published aiming to elucidate the relation between the expression of proteins ABCB1, ABCC1, and LRP and the prognostic factors of ALs, such as age, WBC count at diagnosis, aberrant immunophenotypic markers in blasts cells, and CD34 expression (Table 1).

However, many of these studies are controversial when regarding the relationship between the overexpression of these proteins with worse patient prognosis, emphasizing the need for elucidation of resistance mechanisms operated by them (78).

Nowadays, the resistance mechanism mediated by protein ABCB1 is the one that has been best established. Some studies, by evaluating ABCB1 expression in AL patients, showed that the correlation of this protein with patient prognosis has no clinical relevance (65, 77, 80, 81, 86, 87). On the other hand, Fujimaki and colleagues (2002) reported that the expression of proteins ABCB1 and ABCC1 is more relevantly related in the studies involving AML (73). According to Figueiredo-Pontes and colleagues (2008), the heterogenicity of cells in AMLs and the increased expression of MDR transporters in most of the immature population of myeloid leukemia cells reinforces that MDR might be responsible for the failure in treating these neoplasms (85). However, these results must be interpreted with care, since in some studies the number of samples was small.

Other studies show that the isolated expression of protein LRP is determinant in the phenomenon of resistance against chemotherapy drugs in cell strains of ALL patients (64, 78). More specifically, Den Boer and colleagues (1998) suggest that LRP might contribute to MDR especially in pre-B ALL in pediatric patients (67). Olson and

TABLE 1. Studies on the clinical importance of the expression of genes *abcb1*, *abcc1*, and *lrp* and/or proteins ABCB1, ABCC1, and LRP in ALs

Author	Method	Sample	Results/conclusions
Dhooge et al. (1999) (74)	Immunohistochemistry	de novo ALL ($n = 102$) and relapse ALL ($n = 35$)	The expression of protein ABCB1 negatively influenced the prognosis, especially in de novo ALL cases
Wutcher et al. (2000) (75)	Flow cytometry	ALL ($n = 102$) and AML ($n = 121$)	The expression of protein ABCB1 did not negatively influence the prognosis
Fujimaki et al. (2002) (73)	Flow cytometry and RT-PCR	ALL ($n = 18$) and AML ($n = 26$)	The expression of gene and protein ABCB1 was more significant in AML patients, mainly in relapse cases. The expression of protein ABCC1 did not show clinical correlation
Schaich et al. (2004) (76)	RT-PCR	De novo or secondary AML ($n = 331$)	The expression of ABCB1 and ABCC1 negatively influenced full disease remission after treatment, while LRP did not negatively influence the prognosis
Suarez et al. (2004) (77)	Flow cytometry	De novo AML ($n = 90$)	The expression of ABCB1, ABCC1, and LRP did not negatively influence the prognosis
Valera et al. (2004) (78)	RT-PCR	ALL ($n = 30$)	Among the evaluation of proteins ABCB1, ABCC1, and LRP, only LRP negatively influence the prognosis
Benderra et al. (2005) (79)	Flow cytometry	De novo AML ($n = 85$)	The expression of ABCB1 was shown to influence treatment failure
Olson et al. (2005) (80)	Flow cytometry	Initial ALL ($n = 295$)	The overexpression of ABCB1, ABCC1, and LRP to diagnostics did not influence treatment failure
Anuchapreeda et al. (2006) (81)	RT-PCR	ALL ($n = 61$) and AML ($n = 14$)	The expression of gene *abcb1* was statistically similar in patients with relapse and patients who responded to treatment
Huh et al. (2006) (82)	Nested RT-PCR	ALL ($n = 32$) and AML ($n = 39$)	The expression of ABCB1, ABCC1, and LRP influenced full remission and the survival rate in AL patients, especially ABCB1 and LRP
Styczynski et al. (2007) (83)	Flow cytometry	Initial ALL ($n = 527$), relapse ALL ($n = 104$), initial AML ($n = 133$), and relapse AML ($n = 23$)	The expression of ABCB1, ABCC1, and LRP represented an adverse prognostic factor with relevance in de novo ALL cases
Yasunami et al. (2007) (64)	Flow cytometry and real-time RT-PCR	ALL-T ($n = 11$)	Among the evaluation of proteins ABCB1, ABCC1, and LRP, only LRP showed increased expression and function
Fedasenka et al. (2008) (84)	Real-time RT-PCR	Pre-B ALL with differentiated responses to CT ($n = 19$)	The expression of ABCC1 and LRP did not have a direct relation with minimum residual disease
Figueiredo-pontes et al. (2008) (85)	Flow cytometry	De novo AML $CD34^+$ ($n = 26$)	The overexpression of ABCB1, ABCC1, and LRP in more immature leukemia cell strains influenced treatment failure
Grotel et al. (2008) (86)	Flow cytometry and real-time RT-PCR	ALL-T ($n = 72$)	The expression of ABCB1, ABCC1, and LRP to diagnosis, in all cut-offs adopted, did not negatively influence prognosis
Svirnovski et al. (2009) (87)	Flow cytometry and RT-PCR	ALL ($n = 65$), relapse ALL ($n = 42$), AML ($n = 53$), and relapse AML ($n = 16$)	There was no significant difference between the expression of gene *abcb1* and protein ABCB1 in patients with de novo and recently diagnosed AL
El-Sharnouby et al. (2010) (88)	RT-PCR	All ($n = 34$)	The expression of ABCC1 and LRP were associated with poorer outcomes and worse two-year survival
Chauhan et al. (2012) (89)	Real-time RT-PCR	ALL ($n = 40$) and AML ($n = 45$)	High expression of ABCB1 in AML and ABCC1 in ALL was associated with poor response to induction chemotherapy
Scheiner et al. (2012) (90)	Flow cytometry	AML ($n = 109$)	ABCB1 expression did not show an impact on the response to remission induction therapy

ALL, acute lymphoblastic leukemia; ALL-T, acute lymphoblastic leukemia of lymphocytes T; Pre-B ALL, acute lymphoblastic leukemia of pre-B lymphocytes; AML, acute myeloid leukemia; CT, chemotherapy; RT-PCR, reverse transcriptase–polymerase chain reaction.

colleagues (2005) did not find a correlation between LRP and the worst prognostic of ALL patients (80).

Another parameter often evaluated in the literature is the relationship between MDR proteins and the presence of CD34 antigen, an immature cell marker and an important factor in AL prognosis. Studies investigating AML patients showed the activity and expression of proteins ABCB1, ABCC1, and LRP were more relevant in $CD34^+$ cells than in those with a negative phenotype for this marker (79, 85, 91). Grotel and colleagues (2008) showed that pediatric patients with LLA-T $CD34^+$ had shorter life expectancy (86). However, authors associated the short life expectancy of these patients with an increase in resistance protein activity, but not with an increased expression of genes *abcb1*, *abcc1*, and *lrp*. A possible explanation for this fact would be the existence of additional posttranscriptional regulatory mechanisms or the occurrence of posttranslational modifications necessary for protein activity.

The results in literature show that the methodology employed for the analysis of resistance proteins directly interferes on the interpretation of results. For example, while the study of Dhooge and colleagues (1999) showed by immunohistochemistry the existence of a strong correlation between the expression of protein ABCB1 and the adverse clinical course for ALL, Wutcher and colleagues (2000) used flow cytometry and obtained contrary results (74, 75). Fujimaki and colleagues (2002), in a study involving patients who were nonresponsive to the chemotherapy treatment, found higher levels of expression for gene *abcb1*, determined by reverse transcriptase–polymerase chain reaction (RT-PCR), than of expression for protein ABCB1, determined by flow cytometry (73). This study shows that the results obtained by flow cytometry and RT-PCR are not correlated in all cases. This is due both to the complexity of the mechanisms involved in translation of the functional protein and to the sensibility of the method and its standardization, since, according to some authors, the sensibility of RT-PCR is higher for determining the MDR phenotype (64, 73).

As it can be seen, the influence of the MDR phenotype is controversial in AL prognosis. The contradictory results can be justified by use of different cut-off values, existence of demographic variations among samples, involvement of other resistance mechanisms, and use of different analysis methodologies (78).

The use of methodologies that directly quantify the expression of the gene seems to be the most adequate for defining its participation in the resistance phenotype against chemotherapy drugs in oncohematologic diseases. However, RT-PCR only evaluates the gene expression, while flow cytometry can bring up more information regarding AL prognosis as it allows for trials that evaluate the activity of MDR proteins and the phenotypic detection of additional markers typical of hematologic neoplasms.

Since the discovery of MDR proteins, investigations have been carried out to establish their role in the prognosis of hematological malignancies and discover drugs capable of antagonizing their role in chemoresistance. Although relative success was achieved in determining the biological role of MDR proteins (89, 90, 92, 93), little success has been obtained in demonstrating the benefits of their pharmacological modulation (93–95).

Among the possible target proteins related to the MDR phenotype, inhibitors of ABCB1 protein are the ones to have been most thoroughly investigated. Based on sequential refinements in the pharmacodynamic properties of ABCB1 competitive inhibitors, they are categorized into three generations of drugs (95). Studies that used cyclosporine A (CSA), a first-generation ABCB1 inhibitor, showed a response and survival advantage for its use in relapsed and refractory AML (96). However, subsequent randomized trials of CSA or valdospar, a second-generation ABCB1 inhibitor, failed to demonstrate an improvement in outcomes (94, 97). Zosuquidar, a third-generation inhibitor, also demonstrated promising results at first (98), but Cripe and colleagues (2010) failed to demonstrate the same benefits from the addition of zosuquidar to standard induction chemotherapy (93). Despite the failure to find an ABCB1 inhibitor of proven clinical efficacy, studies that seek inhibitors of MDR proteins have not been completely abandoned, since it is impossible to ignore the connection between MDR proteins and therapy outcome, especially in AML patients (95).

In summary, MDR is a multifactorial phenomenon. The expression and activity profiles of ABCB1, ABCC1, and LRP, proteins capable of promoting the efflux of a variety of chemotherapeutic agents from the cell cytoplasm represent one of the greatest causes of failure in AL treatment. Although there is a consensus in reporting that the detection of these proteins, mainly ABCB1, is important in the AL prognosis, there is controversy in the literature regarding the methodology used for their detection.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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