Determination of P-Glycoprotein, MDR-Related Protein 1, Breast Cancer Resistance Protein, and Lung-Resistance Protein Expression in Leukemic Stem Cells of Acute Myeloid Leukemia

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Background: The most primitive leukemic precursor in acute myeloid leukemia (AML) is thought to be the leukemic stem cell (LSC), which retains the properties of self-renewal and high proliferative capacity and quiescence of the hematopoietic stem cell. LSC seems to be immunophenotypically distinct and more resistant to chemotherapy than the more committed blasts. Considering that the multidrug resistance (MDR) constitutive expression may be a barrier to therapy in AML, we have investigated whether various MDR transporters were differentially expressed at the protein level by different leukemic subsets.

Methods: The relative expression of the drug-efflux pumps P-gp, MRP, LRP, and BCRP was evaluated by mean fluorescence index (MFI) and the Kolmogorov-Smirnov analysis (D values) in five leukemic subpopulations: $CD34^+CD38^-CD123^+$ (LSCs), $CD34^+CD38^+CD123^-$, $CD34^+CD38^+CD123^+$, $CD34^+CD38^+CD123^-$, and $CD34^-$ mature cells in 26 bone marrow samples of $CD34^+$ AML cases.

Results: The comparison between the two more immature subsets (LSC versus $CD34^+CD38^-CD123^-$ cells) revealed a higher P-gp, MRP, and LRP expression in LSCs. The comparative analysis between LSCs and subsets of intermediate maturation ($CD34^+CD38^+$) demonstrated the higher BCRP expression in the LSCs. In addition, P-gp expression was also significantly higher in the LSC compared to $CD34^+CD38^+CD123^-$ subpopulation. Finally, the comparative analysis between LSC and the most mature subset ($CD34^-$) revealed higher MRP and LRP and lower P-gp expression in the LSCs.

Conclusions: Considering the cellular heterogeneity of AML, the higher MDR transporters expression at the most immature, self-renewable, and quiescent LSC population reinforces that MDR is one of the mechanisms responsible for treatment failure. © 2008 Clinical Cytometry Society

Key terms: P-glycoprotein; multidrug resistance; leukemic stem cells; acute myeloid leukemia

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The population of blasts of acute myeloid leukemia (AML) infiltrating the bone marrow (BM) at diagnosis is frequently heterogeneous regarding their immunophenotypic features and engraftment capacity in xenotransplant models, reflecting the fact that the leukemic cells are blocked at different maturational stages (1,2). The more primitive cells have recently been called leukemic stem cells (LSCs) by analogy with the normal hematopoietic stem cells (HSCs), since they retain similar properties of self-renewal, high proliferative capacity, and predominant quiescent cell cycle status (2). The immunophenotype and isolation of LSCs from primary human AML samples were first described by Lapidot et al. (3), and later studies have shown that LSCs can be defined as $CD34^+$, $CD38^-$, $HLA-DR^-$, $CD90^-$, $CD117^-$, and $CD123^+$ (4). Some of these markers are also detected in HSCs, but the expression of CD123 seems to be leukemic-specific (5,6). In vivo studies using the nonobese diabetic/severe combined immunodeficient mouse (NOD/SCID) model have shown that long-term culture-initiating cells in AML are found in the $CD34^+CD38^-$ and not in the more differentiated $CD34^+CD38^+$ subpopulation, and that cytogenetic markers are detected in this primitive subset (7). In addition to their relevance in AML pathogenesis, LSCs have a pivotal role in disease relapse and resistance to treatment, since they are more resistant to proapoptotic stimuli than HSCs and differentiated leukemic blasts and, experimental data suggest that current therapies are unable to eradicate LSCs thus leading to disease relapse (8–10).

Although the importance of drug efflux pumps in resistance to chemotherapy is well established in literature (11,12), the contribution of these mechanisms to LSCs survival advantage is not well characterized. de Grouw et al. (13) have described higher mRNA levels of 27 out of 40 ATP binding cassette (ABC) transporters in the $CD34^+CD38^-$ cells compared to the more committed $CD34^+CD38^+$ cells from 12 untreated AML patients. Moreover, higher gene expression of ABC transporters was also detected in the HSCs $CD34^+CD38^-$ compared to the more committed progenitors (13,14). The *ABCB1* gene encodes for the P-glycoprotein (P-gp) which is the best characterized mechanism of multidrug resistance (MDR) and is highly expressed by stem cells during normal hematopoiesis (15). Contradictory results have been obtained by gene expression and functional studies concerning *ABCB1*/P-gp in leukemic and normal progenitors. Despite higher levels of gene expression, leukemic cells were shown to present lower P-gp mediated mitoxantrone efflux compared to normal progenitors (16). These discrepancies may reflect the immunophenotypic and biological diversity of malignant cells found in AML, which consist of blasts at different stages of myeloid maturation and rare immature LSCs. Moreover, additional transport mechanisms may lead to incomplete chemotherapy eradication of these primitive cells. These could include the MDR-related protein 1 (MRP1/*ABCC1*), the breast cancer resistance protein (BCRP/*ABCG2*) and the lung-resistance protein (LRP). Our aim was to determine whether the various MDR transporters were differentially expressed at the protein level by the LSC compared to more mature leukemic cell subsets in AML.

MATERIALS AND METHODS
Patients

BM samples were obtained from 26 patients with de novo AML and eight "normal" controls (four patients with immune thrombocytopenia and four individuals submitted to BM collection during orthopedic surgery) after written informed consent. Only AML cases with $CD34^+$ blasts were selected because high P-gp expression has been associated with this phenotype (17). Diagnosis of AML was based on standard morphological and immunophenotypical criteria according to the World Health Organization (WHO) classification (18). Total RNA was extracted from BM samples and reverse transcription-polymerase chain reaction (RT-PCR) was performed for assessment of *PML-RARα*, *AML1-ETO*, and *CBFβ-MYH11* fusion genes expression in 25 AML cases using reagents and primers from Invitrogen and the BIOMED-1 protocol.

Flow Cytometry

BM samples preparation. Mononuclear cells were isolated by Ficoll Hypaque density gradient centrifugation (Sigma-Aldrich, St. Louis, MO) and cryopreserved in RPMI 1640 with 10% of fetal bovine serum (both from Invitrogen, Carlsbad, CA) and 10% dimethyl sulfoxide. After thawing, cells concentration was adjusted to 1×10^6 cells per tube. Cell suspensions were then stained with saturating amounts (previously titrated) of monoclonal antibodies (MAb) anti-P-gp (clone MRK16), MRP (clone 33A6), BCRP (clone BXP-21), or LRP (clone MVP-37) (Kamiya Biomedical, Seattle, CA) for 30 min at $4°C$. Staining with anti-MRP, BCRP, and LRP, which recognize internal protein epitopes, required previous permeabilization with "Fix & Perm" reagent (Caltag Lab, Burlingame, CA). Cells were not permeabilized before MRK16 staining since it is P-gp specific and recognizes an external epitope of the protein. Between all specific and isotypic MAb incubation steps, cells were washed with phosphate buffered saline with 0.1% sodium azide. A goat anti-mouse antibody conjugated with fluorescein isothiocyanate (GAM-FITC, Becton and Dickinson, San Jose, CA) was used as secondary antibody. GAM-FITC free binding sites were blocked with mouse immunoglobulin for 20 min at $4°C$ and then cells were simultaneously incubated for 20 min at $4°C$ with anti-CD34-allophycocyanin (APC), CD38-phycoerythrin (PE) and CD123-phycoerythrin-Cy5 (PE-Cy5) (all purchased from Becton and Dickinson). After staining procedures, at least one hundred thousand events were acquired in a FACScalibur flow cytometer (Becton and Dickinson) and analysis was performed using the Cell Quest software.

Assessment of MDR transporters expression. To select the different cell subpopulations for analysis, viable mononuclear cells were gated by FSC and SSC
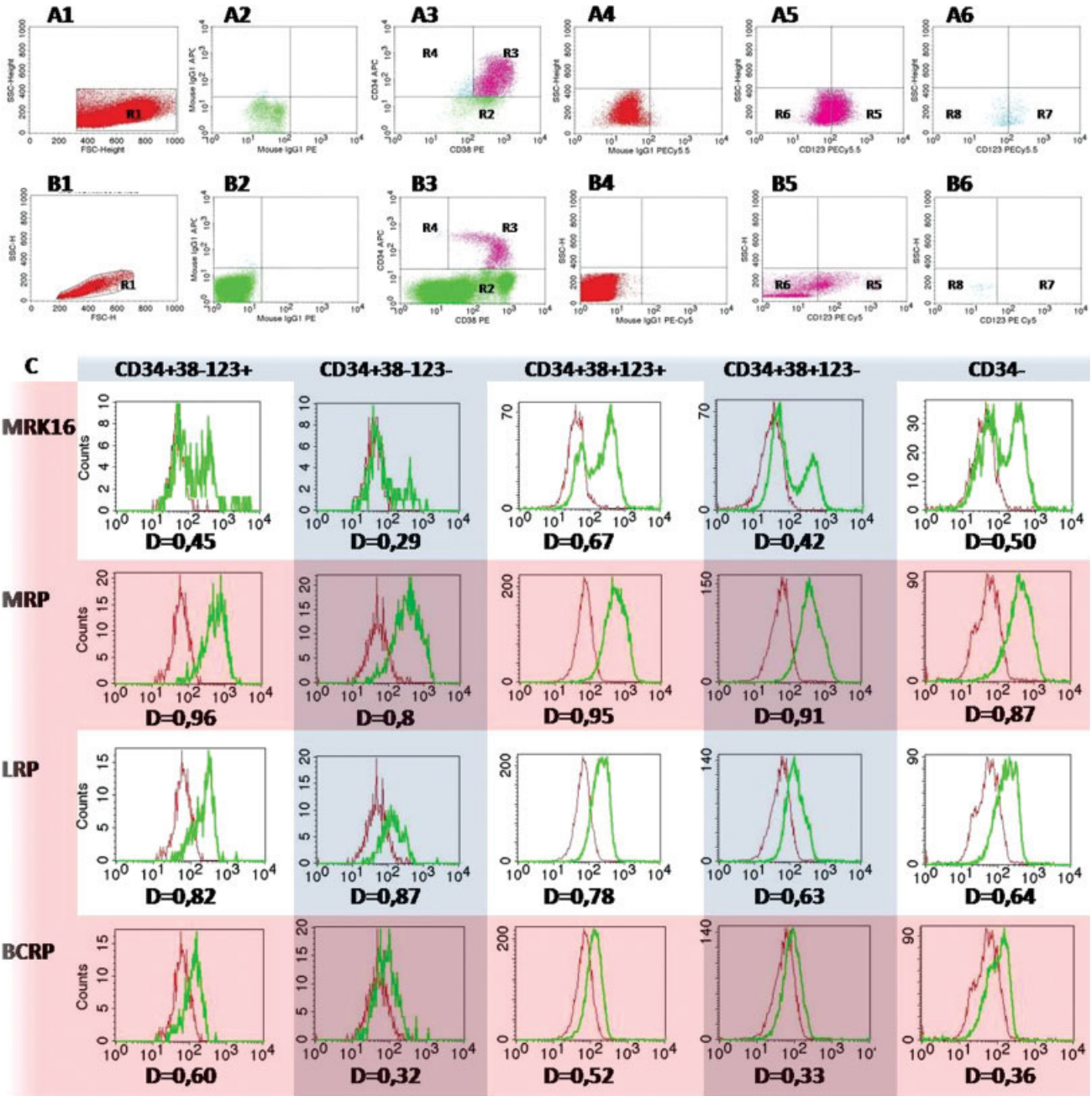
FIG. 1. Illustrative example of the method of analysis of the different cell subsets explored in normal and leukemic bone marrow and of the evaluation of MDR transporters P-gp, MRP, LRP, and BCRP expression. Viable mononuclear cells were gated by FSC and SSC parameters, selected according to CD34 and CD38 expression, followed by the identification of CD123 positive cells within the subsets $CD34^{+}CD38^{-}$ and $CD34^{+}CD38^{+}$. The gating strategy was identical for both leukemic (graphs A1–A6) and non-neoplastic (graphs B1–B6) samples. Viable mononuclear cells, more mature $CD34^{-}$ cells, $CD34^{+}CD38^{+}$, and $CD34^{+}CD38^{-}$ immature progenitors were selected as gates R1, R2, R3, and R4, respectively. The expression of CD123 was verified in $CD34^{+}CD38^{+}$ subpopulation, generating the gates R5 ($CD34^{+}CD38^{+}CD123^{+}$) and R6 ($CD34^{+}CD38^{+}CD123^{-}$). Similarly, gates R7 and R8 represent $CD34^{+}CD38^{-}CD123^{+}$ and $CD34^{+}CD38^{-}CD123^{-}$ cells, respectively, inside the immature $CD34^{+}CD38^{-}$ blasts. As shown in the upper panels (A and B), LSCs ($CD34^{+}CD38^{-}CD123^{+}$) were rarely identified in normal bone marrow samples (graph B6) in contrast to AML (graph A6). The fluorescence intensity of ABC transporters, measured by the Kolmogorov-Smirnov method as D values, is shown in panel C and was determined in the leukemic subsets: $CD34^{+}CD38^{-}CD123^{+}$ (LSCs), $CD34^{+}CD38^{-}CD123^{-}$, $CD34^{+}CD38^{+}CD123^{+}$, $CD34^{+}CD38^{+}CD123^{-}$ and $CD34^{-}$. Red lines represent fluorescence intensity detected in isotypic controls and green lines in leukemic samples labeled with MRK16, MRP, LRP, and BCRP monoclonal antibodies.

parameters, selected according to CD34 and CD38 expression, followed by the identification of CD123 positive cells within the subsets $CD34^{+}CD38^{-}$ and $CD34^{+}CD38^{+}$. Figure 1 illustrates how gates were defined in normal and leukemic samples. Viable mononuclear cells, more mature $CD34^{-}$ cells, $CD34^{+}CD38^{+}$, and $CD34^{+}CD38^{-}$ immature progenitors were selected as gates R1, R2, R3, and R4, respectively. The expression of

CD123 was verified in CD34$^+$CD38$^+$ subpopulation, generating the gates R5 (CD34$^+$CD38$^+$CD123$^+$) and R6 (CD34$^+$CD38$^+$CD123$^-$). Similarly, gates R7 and R8 represented CD34$^+$CD38$^-$CD123$^+$ and CD34$^+$CD38$^-$CD123$^-$ cells, respectively, inside the immature CD34$^+$CD38$^-$ blasts. The expression of these surface antigens was established based on the staining of each sample with an isotype control, and cursors were placed based on the fluorescence intensity excluding 98% of the negative cells. The gating strategy was identical for both leukemic and non-neoplastic samples. These latter were included in the study to define and quantify the LSC subpopulation in normal BM samples, but were not evaluated in respect to MDR proteins expression. The fluorescence intensities of ABC transporters (P-gp, MRP, and BCRP) and LRP were determined in five subpopulations: the more immature progenitors (a) CD34$^+$CD38$^-$CD123$^+$ (LSCs) and (b) CD34$^+$CD38$^-$CD123$^-$, the more committed (c) CD34$^+$CD38$^+$CD123$^+$ and (d) CD34$^+$CD38$^+$CD123$^-$, and more mature (e) CD34$^-$ cells. Since all selected AML cases presented more than 70% of CD34$^+$ blasts, it should be pointed out that the CD34$^-$ subset analyzed was part of the blast population from the same AML samples. The relative values for expression levels of the transporters were based on the median intensity of fluorescence and were analyzed using: (1) the Kolmogorov-Smirnov statistic, categorizing D value for descriptive purposes as high if $D \geq 0.30$, low if $0.2 \leq D < 0.3$ and negative if $D < 0.20$; and (2) the mean channel fluorescence index (MFI), defined as the difference between the mean channel of fluorescence (MCF) for each antigen and the MCF of the isotypic control.

Statistical Analysis

MDR protein expression in the leukemic subsets was measured using a modification of the Kolmogorov-Smirnov statistic, denoted D, which measures the difference between two distribution functions and generates a value ranging from 0 to 1.0 (19). Differences in fluorescence intensity and D values of MDR transporters (MRK16, MRP, LRP, or BCRP/isotype control) between the LSC and the other leukemic populations were estimated by the Wilcoxon's nonparametric test for paired samples. The frequency of AML cases presenting D values ≥ 0.2 were compared using Chi-square test. In all analysis, a significance level of $P < 0.05$ was adopted. Statistical analysis was performed using SPSS 13.0 software.

RESULTS

According to the WHO Classification for Myeloid Neoplasms six cases were classified as AML associated with recurrent cytogenetic abnormalities (three harbored the t(8;21)/*AML1/ETO*, three the inv(16)/*CBFβ/MYH11*) and 20 cases as not otherwise categorized of which six were minimally differentiated AML, eight AML without maturation, and six AML with maturation. The *PML/RARα* gene rearrangement was not detected. No difference in the expression of P-gp, MRP, LRP, or BCRP was detected among WHO subgroups or among cases harboring or not gene rearrangements.

Five subpopulations of leukemic blasts were defined and analyzed: (a) CD34$^+$CD38$^-$CD123$^+$ (LSCs), (b) CD34$^+$CD38$^-$CD123$^-$, (c) CD34$^+$CD38$^+$CD123$^+$, (d) CD34$^+$CD38$^+$CD123$^-$, and (e) CD34$^-$ cells. We have selected only de novo AML cases with more than 70% of CD34$^+$ blasts infiltrating the BM. The mean percentage (range) of LSCs (CD34$^+$CD38$^-$CD123$^+$) was 8.83% (0.10–64.52%) of the total leukemic population, and these cells corresponded to 42% of the CD34$^+$CD38$^-$ subpopulation (Table 1). In normal samples, the most primitive CD34$^+$CD38$^-$CD123$^+$ subset corresponded to 0.3% (0.08–0.70%) of the gated mononuclear cells, with a sensitivity level for detection of cells with this specific immunophenotypic profile of 0.01%.

The comparison of the number of positive cases (D values ≥ 0.2) for MRP, LRP, or BCRP expression did not show significant differences among the blasts subpopulations. When the analysis was based on any of the four CD34$^+$ subsets, the frequency of P-gp positive cases ranged from 42 to 58%, and the difference was not significant (Table 1). In contrast, when the analysis was performed based on the CD34$^-$ subpopulation, a significantly higher number of samples (84%, $P = 0.017$) was classified as P-gp positive (Table 1).

The expression of MDR transporters in LSCs was compared to the different blasts subsets as defined earlier (Table 1) and analyzed either by D or MFI values. The comparison between MFI values of the two more immature subsets (LSC versus CD34$^+$CD38$^-$CD123$^-$ cells) revealed a higher P-gp ($P = 0.044$), MRP ($P = 0.008$), and LRP ($P = 0.001$) expression in LSCs. These findings were confirmed by the comparison between D values for P-gp and LRP ($P = 0.046$ and 0.028, respectively), but not for MRP. The comparative analysis between D values of LSCs and subsets of intermediate maturation (CD34$^+$CD38$^+$) demonstrated the higher BCRP expression in the LSCs ($P = 0.011$ and 0.006 for the comparison between LSC versus CD34$^+$CD38$^+$CD123$^-$ and CD34$^+$CD38$^+$CD123$^+$ cells, respectively). In addition, P-gp expression was also significantly higher ($P = 0.047$) in the LSC compared to CD34$^+$CD38$^+$CD123$^-$ subpopulation. Finally, the comparative analysis between LSC and the most mature subset (CD34$^-$) revealed higher MRP ($P = 0.005$) and LRP ($P = 0.007$) and lower P-gp ($P = 0.001$) expression in the LSCs.

DISCUSSION

To our knowledge, this is the first study to compare simultaneously the expression of MDR transporters at protein level among different leukemic subsets in AML. Since the MDR phenotype may hamper cure, the precise characterization of P-gp, MRP, LRP, and BCRP expression in the various AML leukemic subpopulations may contribute to the identification and eradication of resistant clones. It should be pointed out that we have included the expression of the Interleukin-3 receptor alpha chain (CD123) as a criterion for the definition of LSC. Jordan

Table 1
Analysis of Multidrug Resistance Transporters Expression in Different Subsets of Blasts in 26 Cases of de novo AML

		CD34$^+$CD38$^-$CD123$^+$ (LSC)	CD34$^+$CD38$^-$CD123$^-$	CD34$^+$CD38$^+$ CD123$^+$	CD34$^+$CD38$^+$ CD123$^-$	CD34$^-$
P-GP	% of blasts	8.83 ± 15.56	7.63 ± 18.19	27.76 ± 23.78	28.76 ± 27.72	28.08 ± 23.43
	% of cases with $D \geq 0.2$	13 (50%)	11 (42%)	15 (58%)	12 (46%)	22 (84%)[a]
	D	0.33 ± 0.27	0.27 ± 0.27[b]	0.33 ± 0.24	0.25 ± 0.22[c]	0.38 ± 0.22
	MFI	23.24 ± 97.7	4.17 ± 9.25[d]	10.94 ± 33.45	5.35 ± 10.21	32.9 ± 53.95[e]
MRP	% of cases with $D \geq 0.2$	21 (81%)	21 (81%)	20 (77%)	21 (81%)	21 (81%)
	D	0.64 ± 0.35	0.61 ± 0.36	0.63 ± 0.35	0,62 ± 0.35	0.56 ± 0.31[f]
	MFI	149.17 ± 172.85	112.97 ± 125.40[g]	158.33 ± 182.35	140.28 ± 156.34	131.68 ± 143.05
LRP	% of cases with $D \geq 0.2$	21 (81%)	20 (77%)	20 (77%)	21 (81%)	21 (81%)
	D	0.58 ± 0.32	0.52 ± 0.31[h]	0.53 ± 0.31	0.53 ± 0.31	0.49 ± 0.26[i]
	MFI	49.99 ± 54.51	33.00 ± 37.69[j]	56.15 ± 61.78	52.30 ± 63.93	43.60 ± 42.24
BCRP	% of cases with $D \geq 0.2$	21 (81%)	20 (77%)	20 (77%)	19 (73%)	18 (69%)
	D	0.45 ± 0.26	0.40 ± 0.25	0.37 ± 0.22[k]	0.36 ± 0.22[l]	0.34 ± 0.21[m]
	MFI	14.43 ± 15.31	11.35 ± 11.06	16.33 ± 17.97	15.10 ± 14.27	21.42 ± 18.47

The expression of MDR transporters was analyzed in leukemic stem cells (LSCs) in comparison to different blasts subsets. Statistical analysis of the comparisons was performed by the Wilcoxon's nonparametric test for paired samples. The frequency of AML cases presenting D values ≥0.2 was compared by Chi-square test. The P-values refer to the comparison between LSCs and the indicated subset.

Lower frequency of P-gp positive cases compared to the CD34$^-$ subpopulation ([a]$P = 0.017$)
Higher P-gp D values compared to the CD34$^+$CD38$^-$CD123$^-$ ([b]$P = 0.046$) and CD34$^+$CD38$^-$CD123$^-$ ([c]$P = 0.047$) subpopulations.
Higher P-gp MFI values compared to the CD34$^+$CD38$^-$CD123$^-$ ([d]$P = 0.044$) subpopulation
Lower P-gp MFI values compared to the CD34$^-$ ([e]$P = 0.001$) subpopulation
Higher MRP D values compared to the CD34$^-$ ([f]$P = 0.005$) subpopulation
Higher MRP MFI values compared to the CD34$^+$CD38$^-$CD123$^-$ ([g]$P = 0.008$) subpopulation
Higher LRP D values compared to the CD34$^+$CD38$^-$CD123$^-$ ([h]$P = 0.028$) and CD34$^-$ ([i]$P = 0.007$) subpopulations
Higher LRP MFI values compared to the CD34$^+$CD38$^-$CD123$^-$ ([j]$P = 0.001$) subpopulation
Higher BCRP D values compared to the CD34$^+$CD38$^+$CD123$^+$ ([k]$P = 0.006$), CD34$^+$CD38$^+$CD123$^-$ ([l]$P = 0.011$) and CD34$^-$ ([m]$P < 0.001$) subpopulations
Kolgomorov-Smirnov (D) and Mean Fluorescence Index (MFI) values are represented as mean ± Standard Deviation

(6) reported that CD123 was strongly expressed in CD34$^+$CD38$^-$ cells (98% ± 2% positive) from 16 of 18 primary AML specimens, and virtually absent in CD34$^+$CD38$^-$ cells from normal bone marrow. In addition, CD34$^+$CD123$^+$ leukemia cells transplanted into NOD/SCID mice were able to establish and maintain leukemic populations in vivo (5). Neoplastic stem cells defined as CD34$^+$CD38$^-$CD123$^+$ in various myeloid neoplasms appear to express target antigens such as CD33 (20), CD13 and CD44 (21), which may be used in future immunotherapeutic approaches. In our study, the CD34$^+$CD38$^-$CD123$^+$ subpopulation was detected in a very low frequency in normal BM specimens, thus reinforcing the relevance of CD123 for the distinction between normal and leukemic progenitors. This is in accordance with Jordan et al. (5) who reported that CD123 expression corresponds to less than 1% of CD34$^+$CD38$^-$ normal BM samples. We have not observed significant higher expression of MRP or LRP within the immature LSC subset compared to the more mature CD34$^+$CD38$^+$ subpopulations irrespective of CD123 positivity. On the other hand, P-gp and BCRP expression was higher in the LSC compared to CD34$^+$CD38$^+$123$^-$ subset, the latter transporter being also significantly less expressed by the CD34$^+$CD38$^+$123$^+$ cells. de Grouw et al. (13) compared the gene expression profile of ABC transporters in CD34$^+$CD38$^+$ and CD34$^+$CD38$^-$ AML cells and reported

a preferential expression of *ABCB1*/P-gp, *ABCC1*/MRP1, and *ABCG2*/BCRP in the latter subset. Our results concerning P-gp and BCRP at the protein level corroborate these gene expression findings. Nevertheless, we describe here relevant differences in ABC transporters expression between CD34$^+$CD38$^-$CD123$^+$ and CD34$^+$CD38$^-$CD123$^-$ immature progenitors not previously reported. Since CD34$^+$CD38$^-$CD123$^-$ cells are thought to be the normal counterparts of LSCs, the higher expression of P-gp and BCRP may be directly associated with malignant transformation.

The higher BCRP expression in the LSCs corroborates previous evidences showing that this protein was preferentially expressed and contributed to mitoxantrone efflux in leukemic CD34$^+$CD38$^-$ stem cells (22). Although BCRP seems to contribute to the resistant phenotype, selective blockage of BCRP-mediated drug extrusion by the novel fumitremorgin C analog KO143, the most potent BCRP inhibitor currently available, resulted in increased intracellular mitoxantrone accumulation in leukemic CD34$^+$CD38$^-$ cells in most AML patients but did not increase chemosensitivity to this agent. Therefore, substantial drug efflux in these leukemic cells in the presence of BCRP inhibition suggests that other drug transporters may contribute to the resistant phenotype (22).

In most of the studies investigating de novo or secondary adult AML, *ABCB1*/P-gp is an independent prognostic

factor associated with reduced remission rates with anthracycline containing regimens, and in some reports, inferior leukemia-free and overall survival (23–26). In contrast, functional MDR assays have failed to show greater prognostic value in larger studies (12). P-gp is the major molecular determinant of mitoxantrone and rhodamine-123 efflux in $CD34^+CD38^-$ cells in normal BM. In our study, P-gp expression in LSCs was higher than that in $CD34^+CD38^-CD123^-$ and $CD34^+CD38^+CD123^-$ cells. On the other hand, P-gp MFI values were significantly higher in the more mature $CD34^-$ cells. Accordingly, a significantly higher number of AML cases was classified as P-gp positive when the analysis was based on $CD34^-$ cells compared to when it was based on $CD34^+$ subsets. In the literature (10,17), P-gp expression and function has been shown to be higher in $CD34^+$ compared to $CD34^-$ cases. However, in the present study the $CD34^-$ subset is a small part of the blast population (<30% of all blasts) from the same AML samples. Alternatively, these findings may imply that P-gp expression varies along maturation, being higher in the most immature, decreasing in intermediate and increasing again in the more mature AML leukemic cells. If that is the case, the $CD34^-$ subset may also contribute to drug resistance.

In conclusion, we described the differential expression of P-gp, MRP, LRP, and BCRP in LSCs compared to the more committed $CD34^+$ blasts subsets. Considering the cellular heterogeneity of this disease, the higher MDR transporters expression at the most immature, self-renewable, and quiescent cell population in AML provides convincing evidence that MDR is one of the mechanisms responsible for treatment failure. Moreover, the differential expression amongst the various leukemic subsets may be associated with the conflicting results yielded by clinical trials investigating MDR antagonists. Our findings suggest that simultaneous modulation of several transporters is probably required to eradicate the leukemic clone.

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