Introduction

Chronic myeloid leukemia (CML) is probably the most extensively studied human hematopoietic malignancy and accounts for approximately 20% of all leukemias diagnosed in adults (1). In CML, the number of CD34\(^+\) progenitor cells represents a useful diagnostic and prognostic indicator regarding the evolution of this disease (2). Furthermore, in CML, CD34\(^+\) hematopoietic progenitors carry the t(9;22) (q34;q11) reciprocal chromosomal translocation (chromosome Ph), which gives rise to the BCR-ABL proto-oncogene and its constitutively active protein tyrosine kinase product p210/BCR-ABL (3). Currently, treatment of CML is based on the administration of the tyrosine kinase inhibitors, which selectively inhibit BCR-ABL tyrosine kinase activity responsible for the pathogenesis of this disease (4). However, patients may experience resistance to tyrosine kinase inhibitors, thus limiting the long-term benefits of the drug. The most extensively studied mechanism of drug resistance is represented by point mutations in the BCR-ABL kinase domain that impair tyrosine kinase inhibitors binding (reviewed in ref. 5). Nonetheless, mutations have been found to mediate resistance only in a proportion of resistant patients. Other mechanisms have been invoked but very little is known of their actual role. In this regard, the intracellular concentration of tyrosine kinase inhibitors is a critical feature influencing drug efficacy. Successful therapy of CML is impeded by the development of resistance to a wide spectrum of chemotherapeutic drugs. In a number of cancers, a drug-resistant phenotype has been linked to
overexpression of some members of the highly conserved family of transmembrane proteins characterized by an ATP-binding cassette (ABC) domain, the so called ABC superfamily of transporters (6, 7). Some studies have shown that the ABCB1 (Pgp/MDR1) and ABCG2 members of this family are deregulated in imatinib-resistant cell lines and/or patients (8, 9). Less is known on the role of the ABC transporters in mediating resistance to the second-generation tyrosine kinase inhibitors (dasatinib and nilotinib) approved for imatinib-resistant or intolerant CML patients (10–13). Moreover, besides their role in conferring drug resistance, a wide range of observations and correlative studies indicate that high levels of ABC transporters in tumors may determine a malignant progression and a more aggressive phenotype independently of their role in drug efflux (reviewed in ref. 14).

Misregulation of the ABC transporter genes in CML may occur through activity of specific transcription factors whose function is also altered in this disease. MYC dysregulation is considered an oncogenic event required for human tumorigenesis (15–17) and has also been related to the progression of myeloid leukemias (18). The proto-oncogene c-MYC encodes a basic helix-loop-helix leucine zipper transcription factor that, dimerizing with its partner MAX, controls multiple cell functions. Patients with myeloid leukemias are often characterized by the presence of double minute chromosomes that contain MYC amplification (19, 20), and a recent study suggests that several oncogenes, involved in myeloid tumor progression, induce leukemogenesis by activating c-MYC oncoprotein (21). Moreover, in CML, some studies have shown that BCR-ABL can indirectly activate c-MYC function via either the Janus-activated kinase (JAK2) pathway (22) or the mitogen-activated protein kinase/heterogeneous nuclear ribonucleoprotein K (MAPK/HNRPK) pathway (23) causing increased c-MYC mRNA translation. Furthermore, c-MYC is located on chromosome 8 (8q24), and trisomy of chromosome 8 is one of the most frequent additional abnormalities detected in CML patients (24). Overall, these findings point to c-MYC as a main downstream actor in leukemias, and in particular in CML pathogenesis, possibly by modulating transcription of a large set of genes.

Recently, we have established that MYCN is responsible for directing and coordinating the transcription of ABC genes in neuroblastoma tumors (25). Here, we have investigated whether c-MYC can also orchestrate ABC transcription in myeloid leukemias. Importantly, we found that c-MYC is highly expressed in CD34+ progenitor cells from newly diagnosed chronic phase (CP)-CML patients and that its transcriptional level correlates with that of specific ABC

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**Figure 1.** c-MYC regulates transcriptional activity of a large group of ABC drug transporter genes in HL-60 cells. A, top, modulation of c-MYC expression by DMSO in HL-60 cells was monitored at the protein level using Western analysis. Bottom, cluster 3 (28) was used to generate an image map showing expression levels of ABC genes as a function of c-MYC downregulation mediated by DMSO treatment. Green indicates genes positively regulated by c-MYC, whereas red indicates those negatively regulated. Genes, the expression of which is totally absent in HL-60 cell line, are listed below the cluster. B, top, modulation of c-MYC expression following infection of HL-60 with viruses expressing shRNA ctrl and shRNA c-MYC after 10 and 12 days of selection with puromycin. Bottom, cluster 3 (28) was used to generate an image map showing expression levels of some ABC genes, selected from the previous analysis, as a function of c-MYC downregulation mediated by specific shRNA. Green indicates genes positively regulated by c-MYC, whereas red indicates those negatively regulated.
transporter genes. Our results show that c-MYC directly controls the transcription of a large subset of ABC transporter genes in myeloid leukemia cells. Finally, we have investigated how, besides c-MYC–mediated transcriptional events, epigenetic mechanisms can lead to dysregulation of ABCG2, a well-studied protein associated with multidrug resistance in CML.

Overall, c-MYC–driven transcription of ABC genes may provide insights into the molecular mechanisms of cancer drug resistance in CML and it may represent an unexpected target for the development of novel therapeutic strategies with important implications for the treatment of different types of leukemia.

**Materials and Methods**

**Cell lines and patient samples**

KG-1a cells were cultured in RPMI containing 20% FBS heat inactivated and 50 mg/mL gentamycin. HL-60 and K562 cells were cultured in RPMI medium 1640 containing 10% FBS heat inactivated and 50 mg/mL gentamycin.
Peripheral blood samples from 21 newly diagnosed CP CML patients were collected after written informed consent. Mononuclear cells were obtained by Ficoll–Hypaque density gradient centrifugation. CD34⁺ cells were selected by using the human CD34 microbead kit on an automatic immunomagnetic separator (AutoMacs, Miltenyi Biotec) according to manufacturer’s recommendations.

Retroviral production and infection of plasmids expressing short hairpin RNAs

To generate short hairpin RNA (shRNA) vectors for c-MYC, hairpin-encoding oligonucleotides were annealed and ligated into pCL (high titer) expression vector. For c-MYC downregulation, we used the following targeting sequence: 5′-GATGAGGAAGAAATCGATG-3′ previously established in the laboratory of Professor Martin Eilers (26). Retroviruses expressing c-MYC shRNA and nontargeting shRNA were produced in HEK293T cells after cotransfection of pCL expression vector and pC-ϕ amphi packaging vector. After 24 hours, supernatants containing the retroviruses are collected and filtered through a 0.45-μm pore size filter. Each supernatant was diluted in 2.5 volume of complete medium. Polybrene (8 μg) per milliliter of medium was added. One day before the infection, the

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**Figure 2. (Continued).**

**ABCC1**

**ABCA10**

**ABCC4**

**ABCB1**

**ABCE1**

**APEX**

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**Canonical E-boxes**  **Noncanonical E-boxes**  **Start Site**
HL-60 cells were seeded such to be at 50% of confluency on the day of infection. On the day of the infection, the medium was replaced with medium containing retrovirus and polybrene. Thirty-six hours after infection, the HL-60 cells were grown in the presence of 8 μg/mL puromycin to select for successful transfectants of the shRNAs. After 10 and 12 days of selection, cells were harvested and analyzed for proteins and RNA expression levels.

Luciferase assay
The pGL3-basic and Renilla-TK vectors were obtained from Promega. Promoters of selected ABC transporter genes were obtained using PCR and cloned into the pGL3-basic vector as described previously (25). The vectors were transfected in HL-60 previously infected with retroviruses expressing c-MYC shRNA and nontargeting shRNA. The transfection was carried out after 10 days of selection with puromycin by using Lipofectamine LTX Reagent according to manufacturer’s recommendations. The activity of firefly or Renilla luciferase was measured after 48 hours with the Dual Luciferase Assay Kit (Promega) according to instructions.

Gene expression analysis
RNA samples were prepared by using Tri-Reagent (Sigma) and treated with DNase (DNA-free, Ambion). Reverse transcription and PCR were carried out by using Thermoscript reverse transcriptase PCR (RT-PCR) kit (Invitrogen). Expression data were obtained both from real-time quantitative PCR (qRT-PCR). qRT-PCR was carried out by using iQ SYBR Green Supermix and the real-time quantitative PCR (qRT-PCR) kit (Sigma) and treated with DNase (DNA-free, Ambion).

Results

c-MYC is critical for expression of several ABC transporter genes in promyeloid leukemia cells
To investigate the potential role played by c-MYC in transcriptional regulation of ABC transporter genes in myeloid leukemias, we used the promyelocytic leukemia cell line HL-60. Although highly expressing Bcl-2, HL-60 cells were not selected for enhanced resistance to cytotoxic drugs (29). HL-60 cells lack expression of the well-defined human stem cell marker CD34 and they are characterized by c-MYC proto-oncogene amplification. Moreover, c-MYC overexpression can be turned off by treating cells with dimethyl sulfoxide (DMSO; ref. 30). By using this cell model system, we examined the expression level of all 48 human ABC drug transporters as a function of c-MYC silencing at 24- and 48 hours of DMSO treatment. Transcription profiles were determined by qRT-PCR and clustered by using the Cluster 3 program (31). Results show that DMSO-mediated differentiation can affect transcription of a large subset of ABC transporter genes (Fig. 1A). The analysis reveals 4 groups of ABC transporters depending on the correlation of their expression levels with that of c-MYC. For example, a high-c-MYC level correlates with increased expression of a specific group of ABC genes including ABCA2, ABCB9, ABCB10, ABCC1,
ABCC4, ABCE1, ABCF1, and ABCF2, a majority of which has been implicated in drug resistance. On the other hand, the expression of another group of ABC transporters such as ABCA7, ABCB2, ABCD1, ABCD3, and ABCG1 genes inversely correlated with expression of c-MYC. Two additional groups were also identified: one in which genes did not change their expression as a function of differentiation; and a second one in which genes were not at all expressed in HL-60 cells. To further determine whether c-MYC is required to activate or repress transcription of ABC genes and to rule out pleiotropic effects because of the use of DMSO, we have manipulated c-MYC expression directly and specifically by using a RNA interference approach. HL-60 cells were infected successfully with nontargeting shRNA (shctrl) or c-MYC targeting shRNA (shMYC) retroviruses. The cells were selected in presence of puromycin, and the expression of the subset of ABC genes previously showed to be responsive to treatment with DMSO was monitored after 10 days and 12 days postselection. Results confirm that c-MYC plays a key role in regulating transcription of a large subset of ABC transporter genes (Fig. 1B).

**c-MYC is a direct transactivator of a large group of ABC transporter genes**

Next, we investigated whether c-MYC directly contacts promoters of specific ABC transporter genes thus affecting their transcription. We mainly focused on those genes that were positively regulated by c-MYC and likely responsible for increased drug efflux. To address this issue, we carried out bioinformatic analysis of the ABC promoter regions and found out that many of the MYC responsive ABC gene promoters contain several canonical and noncanonical MYC binding sites (E-box sites), the majority of which are located in regions from -2,000 to +2,000 base pairs around the predicted transcription start site (Fig. 2). To confirm that c-MYC indeed binds these promoters in vivo, we carried out ChIP on HL-60 cells. Results show that promoters of ABCA2, ABCB9, ABCB10, ABCC1, ABCC4, ABCE1, ABCF1, and ABCF2, were occupied by the c-MYC/MAX complex (Fig. 2). As a control, ChIP analysis was also employed on HL-60 cells pretreated with DMSO for 3 days, thereby, depleted of the c-MYC protein and found that MAX but not c-MYC was associated with gene promoters (Supplementary Fig. S1). As additional controls, ChIP was carried out for ABCA10 that does respond to c-MYC, for ABCB1 and ABCG2 that are not expressed in HL-60 cells and for APEX-1, a well-known MYC positive target gene (Fig. 1).

Because direct binding of c-MYC to gene promoters is not per se sufficient to prove its effect on the transcription of target genes (32), we carried out experiments by using luciferase reporter constructs carrying the promoter regions of those ABC transporters bound by c-MYC in ChIP experiments. Recombinant reporters were transiently cotransfected into HL-60 cells, and luciferase activity was quantified as a function of c-MYC downregulation, obtained through an shRNA strategy and DMSO treatment, respectively (Fig. 3 and Supplementary Fig. S2). As expected, results show that for each of these gene promoters (ABCA2, ABCB9, ABCB10, ABCC1, ABCC4, ABCE1, ABCF1, and ABCF2), luciferase activity was indeed dependent on c-MYC expression. The promoter of ABCA10 transporter gene was cloned and used as negative control (Fig. 3). Overall, these data support the view that c-MYC may function as a direct transactivator of a large subset of ABC transporter genes.

**c-MYC controls expression of ABC transporters in CD34+ hematopoietic progenitors of CML patients**

The expression profiles of c-MYC responsive ABC transporter genes was analyzed by qRT-PCR in CD34+ progenitors cells versus mononuclear cells of 21 newly diagnosed CP CML patients. Results show that the ABC genes, whose promoters are bound by c-MYC, were highly expressed in the CD34+ cell population when compared...
with the population of mononuclear cells from which they have been purified, and that their expression strongly correlated with that of c-MYC (Fig. 4A). Furthermore, the GEPs of c-MYC and the ABC transporter genes were extrapolated from the whole transcriptome and compared with that of CD34⁺ cells from healthy donors. The 2 sets of data were compared by using the ΔΔCt method, with the GUSB (housekeeping gene) as internal reference and the medians of each gene expression values as calibrators. Both in (A) and (B) significant differences between the 2 cell populations were determined by using Mann–Whitney statistical tests.

Figure 4. c-MYC controls expression of ABC drug transporters in CD34⁺ hematopoietic progenitors. A, relative expression of c-MYC and ABC genes in the CD34⁺ cell population was compared with that of the entire population of mononuclear cells (MNC). Each sample was analyzed in duplicate and threshold cycle (Ct) values were averaged. Results are reported as delta Ct values which were calculated as the difference between Ct of the ABC mRNAs and Ct of the GUSB mRNA used for normalization. B, c-MYC and ABC gene expression of CML CD34⁺ cells was compared with that of CD34⁺ cells from healthy donors. The 2 sets of data were compared by using the ΔΔCt method, with the GUSB (housekeeping gene) as internal reference and the medians of each gene expression values as calibrators. Both in (A) and (B) significant differences between the 2 cell populations were determined by using Mann–Whitney statistical tests.

significantly higher levels of c-MYC and ABC transporter genes by comparison with healthy donor CD34⁺ cells (Fig. 4B). With regard to ABCG2, we recapitulated the same finding observed for those ABC genes selected as positively regulated by c-MYC. Indeed, we observed higher ABCG2 expression levels in the CML-CD34⁺ cells than in the mononuclear population (Fig. 5A). Interestingly, because ABCG2 promoter is regulated by epigenetic mechanisms, such as DNA methylation (33, 34), by using methylation-sensitive PCR, we found that the ABCG2
promoter was methylated on the MYC binding site, located in close proximity to its transcriptional start site, in CD34^+ cells of 5 patients out of the 21 ones that were analyzed (Fig. 5B). Based on this observation, we examined whether there may be a correspondence between the methylation data and the expression level of ABCG2 in our patient samples, and we found that indeed in those patients showing a methylated MYC binding site, the expression of ABCG2 is the lowest (data not shown). Considering the above, we have re-analyzed microarray data expression of ABCG2 only in those patients that carried an unmethylated ABCG2 gene and compared those data with those of healthy donors (data from GEO database). By doing so, the P value was definitely improved although it did not reach statistical significance (Fig. 5C, left). Indeed, this trend was then confirmed as statistically significant when ABCG2 expression was measured by qRT-PCR, a more sensitive technique than microchip arrays. In that case, expression of ABCG2 in CML-CD34^+ cells was compared with that of CD34^+ cells from healthy donors extrapolated from GEO database (left) or collected from 10 new healthy donors (right). Significant differences between cell populations were determined by using Mann–Whitney statistical tests.

**Figure 5.** c-MYC controls expression of ABCG2 in CD34^+ hematopoietic progenitors. A, relative expression of ABCG2 genes in the CD34^+ cell population was compared with that of the entire population of mononuclear cells (MNC). Each sample was analyzed in duplicate, and threshold cycle (Ct) values were averaged. Results are reported as delta Ct values which were calculated as the difference between Ct of the ABC mRNA and Ct of the GUSB mRNA used for normalization. B, ABCG2 E-Box methylation status of CD34^+ cells purified from a representative group of 10 patients affected by CML. C, ABCG2 gene expression of CML CD34^+ cells was compared with that of CD34^+ cells from healthy donors extrapolated from GEO database (left) or collected from 10 new healthy donors (right). Significant differences between cell populations were determined by using Mann–Whitney statistical tests.

Next, we determined the binding of c-MYC to the ABC transporter gene promoters in CD34^+ cells by carrying out ChIP assay. Ideally, ChIP should have been carried out on CD34^+ hematopoietic stem cells (HSC) of patients; however, this was not possible because of the limited amount of CD34^+ HSCs that can be purified from peripheral blood or bone marrow of donor patients. To overcome this problem, ChIP was carried out on K562 and KG-1a cell lines, which share several biological and biochemical features with myeloid leukemia cells. K562 derives from a CD34^-Ph^+ CML, whereas KG-1a derives from a CD34^+ AML. Results show that, in these cells, c-MYC can bind the promoter of all of the ABC transporter genes found to be positively regulated by c-MYC, fostering the view that c-MYC plays a crucial role in ABC transporter expression in myeloid leukemias (Fig. 6). In contrast, no binding was observed for ABCB1 or ABCA10, neither of which had been shown to be regulated by c-MYC.

c-MYC–mediated transcription of ABCG2 gene is controlled by its CpG island–promoter methylation status

ABCG2 is usually aberrantly overexpressed on primitive CML CD34^+ HSCs. Indeed, it should be noted that in contrast to what has been found in HL-60 and K562 cells, ABCG2 was expressed both in CD34^+ progenitor cells as well as in KG-1a cells, and its expression correlated with that of c-MYC in CD34^+ progenitors of CML patients (Fig. 5A). Nonetheless, c-MYC was found associated with the ABCG2 promoter in KG-1a CD34^+ cells but not in HL-60 and K562 cell lines (Fig. 6), thus revealing a complex pattern of mechanisms involved in controlling ABCG2 transcription. One possible explanation of these findings may rely on the evidence that the promoter of ABCG2 is often hypermethylated in cancer (33, 34). Moreover, we have previously shown that MYC cannot bind its cognate sites when methylated (35). Therefore, in
In the context of promoter methylation, c-MYC does not have access to the \textit{ABCG2} promoter and thus cannot drive \textit{ABCG2} transcription. In support of this hypothesis, it is worth noting that in the HL-60 cell line, c-MYC affects the luciferase activity of a reporter construct carrying the unmethylated promoter of \textit{ABCG2}, but cannot modulate its endogenous expression (Fig. 7A). To better investigate whether a distinct promoter DNA methylation pattern may explain the different \textit{ABCG2} transcriptional levels, KG-1a, HL-60, and K562 cells were treated with AZA, a DNA demethylating agent, and mRNA expression of \textit{ABCG2} was monitored by qRT-PCR. Despite the fact that 3 cell lines show same sensitivity to the treatment with AZA (Supplementary Fig. S3) only in HL-60 and K562 cells AZA treatment downregulates MYC expression (data not shown), thus technically preventing the execution of the ChIP assay. However, we still felt that determining the global methylation status of the \textit{ABCG2} promoter region is crucial for its silencing in some leukemic cell lines. To do that, the methylation status of the \textit{ABCG2} gene in KG-1a, HL-60, and K562 was determined by mCIP, by using a monoclonal antibody that specifically recognizes the methylated cytosines. As shown in Fig. 7C, the putative CpG island surrounding the transcription start site of \textit{ABCG2} promoter was found to be enriched in methylated cytosines in HL-60 and K562 cell lines compared with a distal region. As expected, the same region was found to be completely unmethylated in KG-1a cells (Fig. 7C). To confirm these results, the DNA methylation state of the

Figure 6. c-MYC is physically associated with promoters of ABC transporter genes in K562 and KG-1a cell lines. Quantitative crosslinking ChIP (qChIP) was carried out in K562 and KG-1a cell lines. The relative fold enrichment was determined by comparing the enrichment of sequences immunoprecipitated with anti-c-MYC or anti-MAX antibodies with that obtained with a preimmune serum (IgG). Results represent the mean ± SE of 5 independent qChIP experiments in which each region was amplified by qRT-PCR in triplicate.
ABC2G2 promoter was evaluated in HL-60 and K562 cells by Southern blot (Supplementary Fig. S4). Genomic DNA extracted from these 2 cell lines was digested with either the Msp or HpaII restriction enzyme and then hybridized with a DNA probe corresponding to the ABC2G2 promoter. Although HpaII and MspI recognize the same CCGG sequence, HpaII can cleave that sequence only when unmethylated. As shown in Supplementary Figure S4, the hybridization profile reveals that the ABC2G2 promoter region is resistant to cleavage with HpaII but not to the cleavage with MspI, indicating that the ABC2G2 promoter region is highly methylated in HL-60 and K562 cell lines.

Overall, the combined ChIP data along with the methylation data allow us to conclude that c-MYC can target ABC2G2 expression only when its cognate sites located within ABC2G2 promoter are unmethylated and importantly, in the context of different types of leukemia, this event seems to occur prevalently, if not exclusively, only in CD34+ progenitor hematopoietic cells as shown in Fig. 5B.

Discussion

Resistance to chemotherapeutic agents is a major obstacle for the successful treatment of cancer. The failure of the curative treatment of cancer patients often occurs as a result of intrinsic or acquired drug resistance of tumors to chemotherapeutic agents. Therefore, understanding how chemoresistance develops and eventually how it can be prevented, becomes crucial to fighting cancer effectively. Functional redundancy in the ABC transporters family complicates their utility as therapeutical targets. In this study, we have provided lines of evidence that c-MYC can target a wide spectrum of ABC transporter genes with different structural and functional features raising questions as to how their altered expression may be relevant for drug efflux and more in general for tumor biology. In particular, our data suggest that c-MYC may contribute to the multidrug resistance profile and malignant progression of myeloid tumors by dysregulating the transcription of specific ABC transporter genes prevalently in CD34+ hematopoietic...
progenitors of CML patients. The fact that BCR-ABL has been previously shown to increase c-MYC expression (22, 23) and that c-MYC in turn coordinates transcription of a large set of ABC transporter genes, reveals a novel role of BCR-ABL in potentially mediating drug efflux in CML. Numerous studies have shown that members of the ABC family shown here to be positively regulated by c-MYC may be involved in the development of a chemoresistance phenotype. ABCC1, ABCC4, and ABCG2 can confer resistance to several antineoplastic drugs such as antifolates, doxorubicin, and nucleotide analogs (36). In addition, the ABCA2 transporter has been associated with drug resistance in childhood T-cell acute lymphoblastic leukemia (37), whereas the ABCE1 gene, an inhibitor of Ribonuclease L, was amplified in several tumorigenic cell lines and its high expression correlated with resistance to macrolide inhibitors of protein synthesis (38). Furthermore, ABC transporters play specific roles in cancer biology beyond the efflux of cytotoxic drugs, contributing to some of cancer hallmarks, such as evasion of apoptosis, limitless replicative potential, tissue invasion, and metastasis (reviewed in ref. 14). Indeed, ABCC4 is involved in human dendritic cell migration (39) and its expression is affected during hematopoietic stem cell differentiation (40), whereas ABCF2 has been found amplified in ovary and breast cancers suggesting a possible role in malignant progression (41). Likewise, ABCG2 is highly expressed in a wide range of stem cells (reviewed in ref. 42) and in primitive hematopoietic CML CD34+ progenitors (9). ABCG2 is responsible to establish the side population phenotype of HSCs (43), blocking hematopoietic development, and maintaining the stem cell self-renewal potential. It has been shown that BCR-ABL TK inhibitors exhibit high affinity interaction with ABCG2 in HSCs (44, 9, 12). Although a considerable debate is ongoing about the role played by ABCG2 in mediating imatinib resistance, a recent study shows that ABCG2-transduced K562 cells are protected from imatinib-induced cell death, suggesting that ABCG2 overexpression can indeed trigger resistance to tyrosine kinase inhibitors in CML stem cells (12). In support of this evidence, it has also been shown that overexpression of the ABCG2 transporter promoters were cloned into a luciferase reporter vector and its activity was tested in HL-60 cells as function of c-MYC downregulation. B transcriptional reactivation of ABCG2 following the treatment of HL-60 and K562 cells with 5 μmol/L AZA for 72 hours. ABCG2 mRNA expression was determined by qRT-PCR and compared between control (–AZA) and treatment (†AZA). Results are expressed as fold difference between control and †AZA condition and setting to 1 the expression of ABCG2 in the control condition. C, mChIP was employed in KG-1a, HL-60, and K562 cell lines to determine the methylation status of the ABCG2 promoter in vivo. Position of the putative CpG island is shown schematically with the ABCG2 gene diagram (left). A and B represent a region distal from the transcription start site and the putative CpG island surrounding the transcription start site (TSS), respectively. Relative enrichment obtained with the specific antibody was compared with that obtained with preserum (IgG) which was set to 1 in the graph. Results are the average of 4 independent dual ChIP experiments.
ABCG2 in HEK293 cells results in a reduced intracellular imatinib accumulation (45) and in addition, in vitro studies have confirmed that imatinib is a substrate for transport by ABCG2 (46, 47). It has been previously reported that in human renal carcinoma as well as in multiple myeloma cell lines, alterations in the methylation state of the chromatin-embedded ABCG2 promoter have a dramatic effect on its expression profile (33, 34). Here, we provide a rationale of how epigenetic control of c-MYC-mediated transcription can lead to ABCG2 upregulation in CD34⁺ CML stem cells but not in committed progenitor and mature blood cells. Thus, a combination of transcriptional and epigenetic mechanisms may explain the altered expression of ABCG2, and more in general of ABC transporter genes, hence conferring peculiar and specific drug resistance profiles in different types of leukemia.

Particularly interesting is the finding that a fraction of CML patients displays methylation of the ABCG2 promoter in CD34⁺ progenitor cells, suggesting that these patients may better respond to chemotherapeutic drugs.

Overall, our findings suggest that deregulation of a specific set of ABC transporters may play a crucial role in dictating a drug resistance phenotype and tumor malignancy in CML with numerous implications for CML treatment. Firstly, increased expression of ABC drug transporters by the BCR-ABL/c-MYC network, particularly in the cancer stem cell population, may explain for their self-renewal potential and for less sensitivity to drug treatments. This last observation is consistent with a recent view on how drug resistance can be mediated by stem cells. According to this model, tumors contain a small population of cancer stem cells that produce differentiated offspring that are committed to a particular lineage. Following chemotherapy, the committed cells are killed, but the stem cells, which express a variety of drug resistance mechanisms, survive and can repopulate the tumor (48). Therefore, understanding the molecular bases for drug resistance of HSCs may help devise novel therapeutic interventions aimed at a complete eradication of minimal residual disease. Secondly, upregulation of the ABC genes in the HSC population may predispose cancer cells to developing drug resistance following chemotherapy treatment, particularly when a low dosage of the drug is employed, which may facilitate positive selection and expansion of cells with high drug efflux capacity.

In conclusion, our study unveils novel aspects of the BCR-ABL/c-MYC cross-talk that may have important implications for drug resistance and risk assessment in CML.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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c-MYC Oncoprotein Dictates Transcriptional Profiles of ATP-Binding Cassette Transporter Genes in Chronic Myelogenous Leukemia CD34+ Hematopoietic Progenitor Cells

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