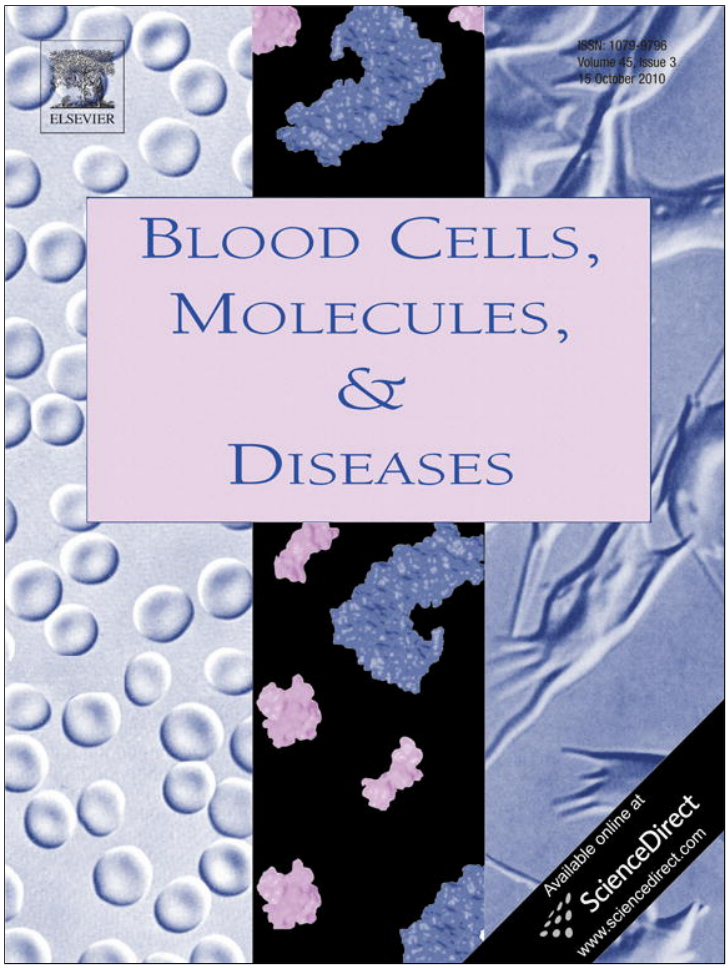

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BAX/*BCL-XL* gene expression ratio inversely correlates with disease progression in chronic myeloid leukemia

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ABSTRACT

BCR-ABL fusion gene is implicated in the pathogenesis of chronic myeloid leukemia (CML), encoding the oncoprotein p210$^{BCR-ABL}$ with anti-apoptotic activity. The inability to undergo apoptosis is an important mechanism of drug resistance and neoplastic evolution in CML. The gene transcript expression of mitochondrial apoptotic related genes *BAX* and *BCL-XL* was evaluated by quantitative Real Time PCR (qPCR) *in vitro* in K562 cells and *in vivo* in peripheral blood of 66 CML patients in different stages of the disease: 13 cases at diagnosis, 34 in chronic phase (CP), 10 in accelerated phase (AP) and 9 in blast crisis (BC).

Our results in K562 cells showed that all treatments with different tyrosine kinase inhibitors (TKIs) induced a decreased expression of the antiapoptotic oncogene *BCL-XL*, whereas the proapoptotic gene BAX remains constant with minor modifications.

A significantly lower *BAX*/*BCL-XL* expression ratio (mean ± SEM) than a group of healthy individuals (4.8 ± 0.59) were observed in CML patients at diagnosis (1.28 ± 0.16), in AP (1.14 ± 0.20), in BC (1.16 ± 0.30) and in 18% of cases of patients in CP (2.71 ± 0.40). Most CP cases (82%) showed a significantly increased ratio (10.03 ± 1.30), indicating that the treatment with TKIs efficiently inhibited the expression of BCL-XL by blocking BCR-ABL oncoprotein.

The *BAX*/*BCL-XL* ratio showed a significant inverse correlation (Spearman $P<0.0001$) with *BCR-ABL*/*ABL* relative expression indicating that low BAX/BCL-XL was associated with disease progression. Accordingly, the follow up of a cohort of eight cases during 6 months from diagnosis showed that while the *BAX*/*BCL-XL* ratio rapidly increased after treatment in seven cases with good evolution, it decreased in the single case that showed rapid evolution and short survival.

Our data suggest that *BAX*/*BCL-XL* expression ratio may be a sensitive monitor of disease progression and an early predictor of TKI therapy responsiveness in CML patients.

Introduction

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the chimeric *BCR-ABL* fusion gene, which encodes for a constitutively activated tyrosine kinase (p210$^{BCR-ABL}$.) with anti-apoptotic activity.

The tumor expansion is directly related to the imbalance between proliferation and cell death [1]. Defects in the apoptotic machinery may contribute to the malignant phenotype in CML.

Apoptosis is regulated by *BCL-2* family members, which consist of antiapoptotic genes such as *BCL-2*, *BCL-XL* and *MCL-1* and proapoptotic genes such as *BAX*, *BAK* and *BAD*. The relative expression of these proteins is essential in the regulation of cytochrome *c* release from the mitochondria, a key step for apoptotic activation [2]. Aberrant expression patterns of these proteins in cancer cells may result in growth and persistence of tumor cells due to apoptosis inhibition. The overexpression of *BCL-2* has been associated with drug resistance in hematological malignancies [3,4]. In acute myeloid leukemia (AML) the upregulation of *BCL2* has been associated with shorter survival [3,5], whereas a high Bax protein expression level is considered a good prognostic indicator in AML [6]. Moreover, CML derived cells expressing p210 showed antileukemic-drug resistance by overexpression of the antiapoptotic gene *BCL-XL* instead of *BCL-2* [7,8]. However, the actual contribution of *BCL-XL* to the transforming phenotype in CML is still unclear, with conflicting published data [9,10].

Taking into account that the inability to undergo apoptosis is an important mechanism of drug resistance and neoplastic evolution, we studied the transcript expression profile of *BAX* and *BCL-XL* genes in

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CML derived cells treated *in vitro* with different tyrosine kinase inhibitors (TKI) and in peripheral blood samples of CML patients in different stages of the disease. Therefore, the aim of this study was to investigate the relationship between the expression of *BAX* and *BCL-XL* genes and the progression to more aggressive phases in CML.

Materials and methods

K562 cell culture

K562 (ATCC catalog No. CCL-243) is a cell line derived from a patient with chronic myeloid leukemia *BCR-ABL* positive [11]. These cells were maintained in 5% CO_2 atmosphere at 37 °C in RPMI 1640 medium supplemented with 10% of fetal bovine serum. K562 cultures were treated with different *in vitro* treatments; Imatinib (IMA) 1, 2.5, 5 and 20 μM, Dasatinib (DAS) 1 μM, Nilotinib (NIL) 1 μM, Homoharringtonina (HHT) 10 μg/ml during 48 h and the quantification of *BAX*, *BCL-XL*, *β-ACTIN* transcript levels were assessed.

Patients and controls

A total of 66 peripheral blood samples from CML patients in different phases of the disease (Table 1) and 10 healthy individuals were studied. All cases had signed a written informed consent and the study was approved by the Ethics Committee of National Academy of Medicine of Buenos Aires. Thirteen patient's samples were taken at diagnosis prior treatment, 34 in chronic phase (CP), 10 in accelerated phase (AP) and 9 in blast crisis (BC), all cases were treated with imatinib mesylate.

Total RNA was extracted and purified from peripheral blood leukocytes by TRIZOL (Invitrogen) method according to manufacturer's instructions. Total unselected cDNA was synthesized from 2 μg of total RNA using random hexamer primers and MMLV reverse transcriptase during 1 h at 37 °C; followed by incubation at 72 °C for 5 min and stored at −20 °C. Aliquots of 5 μl were used for quantitative Real Time PCR amplification analysis.

Quantitative real-time PCR

Quantitative real-time PCR (qPCR) was performed using the LightCycler 2.0 (Roche Diagnostics, Mannheim, Germany), based on the SYBR-Green method. Total volume of 20 μl qPCR reaction mixtures contained 5 μl of sample cDNA, 1× PCR Mix (LC FastStart DNA Master SYBR Green I, Roche), 3.5 mM $MgCl_2$ and 0.25 μM of each primer.

Molecular response assessment was achieved by calculation of the ratio between *BCR-ABL* and *ABL* transcript levels.

Optimal reaction conditions for amplifying both *BCR-ABL* and *ABL* cDNAs were as follows: 50 cycles of a 4-step PCR (95 °C 5 s, 60 °C 3 s, 72 °C 12 s, 80 °C 1 s) after an initial denaturation (95 °C 10 min). *BCR-ABL* and *ABL* forward and reverse primers used in this study were published by Gutierrez et al. [12] and Beillard et al. [13], respectively.

Table 1
Patient's characteristics.

Total patients	66
Males	35
Females	31
Median age (years)	49.03
Range age (years)	19–79
At diagnosis[a]	13
In CP[b]	34
In AP[b]	10
In BC[b]	9

[a] Untreated.
[b] Treated with TKIs.

To determine the expression of apoptosis-related genes we designed oligonucleotides to amplify *BAX*, *BCL-XL* and *β-ACTIN* (control) genes using PrimerSelect (DNA Star Lasergene ver 7.0) and Oligo version 4.1 software.

Optimal reaction conditions for amplifying these genes were as follows: 50 cycles of a 4-step PCR (95 °C 5 s, 62 °C 3 s by 5 cycles and 57 °C 3 s by 45 cycles, 72 °C 15 s, 84 °C 1 s) after an initial denaturation (95 °C 10 min). Designed primers were: *BAX* forward: 5′-GGG ACG AAC TGG ACA GTA ACA-3′, *BAX* reverse: 5′-CCG CCA CAA AGA TGG TCA C-3′, *BCL-XL* forward 5′-ACT GTG CGT GGA AAG CGT AG-3′, *BCL-XL* reverse 5′-GGT TCT CCT GGT GGC AAT G-3′, β-ACTIN forward 5′-ATG TTT GAG ACC TTC AAC ACC CC-3′ and β-ACTIN reverse 5′-GCC ATC TCT TGC TCG AAG TCC AG-3′.

K562 cells were used as positive control and lymphocyte cells from healthy donors as controls for reproducibility and accurateness of the expression levels. Calibration curves were performed to estimate qPCR amplification efficiencies and to quantify *BAX* and *BCL-XL* transcripts within the dynamic range (Supplementary Fig. S1).

Statistical analysis

Statistical analyses were carried out by use of Student's *t*-test. Correlation between *BCR-ABL*/*ABL* and *BAX*/*BCL-XL* ratios was analyzed using Spearman non-parametrical analysis. A one-way ANOVA was also applied. Data analyses were achieved by use of GraphPad version 4.0 software.

Results

In vitro studies

In order to evaluate the dynamic response to treatment of *BAX* and *BCL-XL* gene expressions in CML-derived cells, we conducted qPCR measurements of BAX/BCL-XL ratio in K562 cell line exposed to different TKI during 48 h in three separated experiments as follows: (a) Different concentrations of Imatinib (IMA); 1, 2.5, 5 and 20 μM (Fig. 1a). (b) Different TKI; IMA 1 μM, Dasatinib (DAS) 1 μM, Nilotinib (NIL) 1 μM and Homoharringtonina (HHT) 10 μg/ml (Fig. 1b).

BAX/BCL-XL ratio was significantly lower in the control (mean ± SEM: 0.37 ± 0.04) culture with respect to each treated culture: 1 μM IMA (2.8 ± 0.8), 2.5 μM IMA (4.06 ± 0.6), 5 μM IMA (5.85 ± 0.9), 20 μM IMA (9.7 ± 0.1), 1 μM DAS (3.03 ± 0.04), 1 μM NIL (3.05 ± 0.05) and 10 μg/ml HHT (1.57 ± 0.07). The observed increment in the ratio with the different TKI is due to the downregulation of *BCL-XL* after treatment through BCR-ABL blocking.

In order to determine whether *BAX* or *BCL-XL* (or both) were responsible for the observed increased levels of *BAX*/*BCL-XL* ratio after treatment, each one of these gene transcripts were quantified with respect to β-ACTIN as a control gene. Our results showed that *BCL-XL* gene expression significantly decreased in all treated cultures, whereas *BAX* remained mostly constant with no significant changes (Supplementary Fig. S2). These observations indicate that the differences in the *BAX*/*BCL-XL* expression ratio are directly related to major variations in *BCL-XL* transcript levels.

In vivo studies

CML phase group analysis

All 66 patients were diagnosed as CML and all of them showed the Philadelphia chromosome and *BCR-ABL* molecular rearrangement. All patients were treated with imatinib 400 mg daily, and those cases with treatment failure or suboptimal response were switched to a second generation TKI [14].

The relative expression levels for *BCR-ABL* vs. *ABL* and the apoptosis-related genes, *BAX* vs. *BCL-XL*, are expressed as qPCR ratios. The mean ratio *BAX*/*BCL-XL* in 10 healthy volunteers was 4.82 ± 0.59

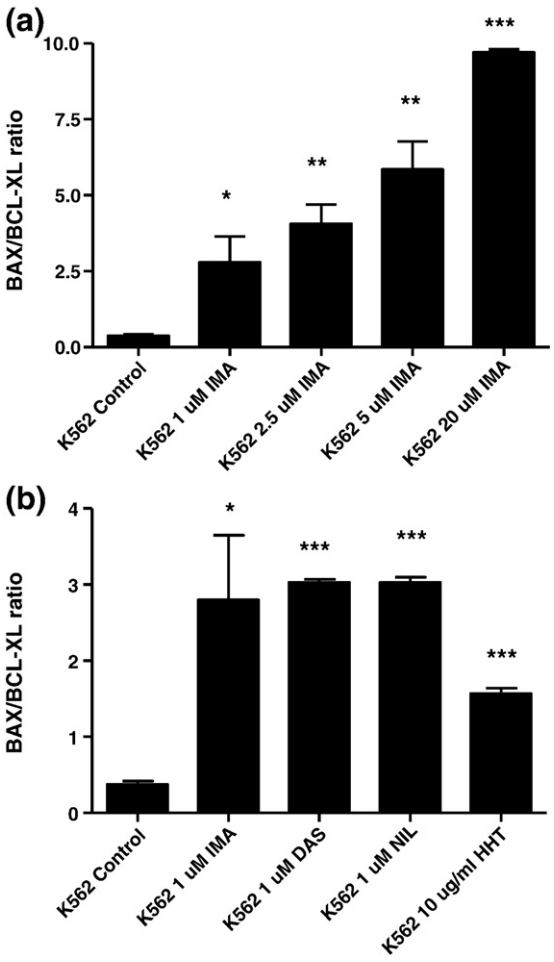

Fig. 1. In vitro analysis of the *BAX/BCL-XL* expression ratio. (a) K-562 cells treated with different concentrations of Imatinib (IMA) (1, 2, 2.5, 5 and 20 μM). (b) K-562 cells treated with effective concentrations of different TKIs: IMA, Dasatinib (DAS), Nilotinib (NIL) and Homoharringtonine (HHT). Student's *t*-test significant limits are indicated as $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

(X ± SEM). This ratio was significantly lower in CML cases at diagnosis, patients in AP and patients in BC. Remarkably, patients in CP showed a mean ratio higher than the control group. This feature may be attributed to the action of the effective treatment with proapoptotic TKI. However, in CP two subgroups can be easily recognized: one with low *BAX/BCL-XL* ratio (6 cases) and the other one with high ratio (28 cases) (Table 2; Fig. 2).

Relative quantification *BCR-ABL/ABL* in each group of CML patients are shown in Table 3 and Fig. 3. The mean BCR-ABL/ABL value at diagnosis (76.62%), in AP (32.11%) and in BC (23.08%) were found to be significantly higher than patients in CP (5.89%) ($p<0.003$). In the

Table 2
BAX/BCL-XL ratios and CML phases.

CML phase	*BAX/BCL-XL* ratio (X ± SEM)
Healthy donors (controls) ($n=10$)	4.82 ± 0.59
Patients at diagnosis ($n=13$)***	1.28 ± 0.16
Patients in chronic phase ($n=34$)**	8.73 ± 1.21
Subgroup high ratio ($n=28$)	10.03 ± 1.30
Subgroup low ratio ($n=6$)	2.71 ± 0.40
Patients in accelerated phase ($n=10$)***	1.14 ± 0.20
Patients in blastic crisis ($n=9$)***	1.16 ± 0.30

Significant differences respect to control group $^{**}p<0.01$, and $^{***}p<0.001$.

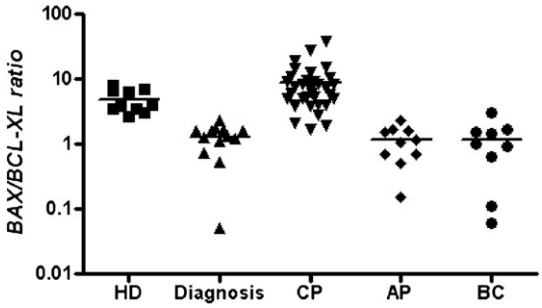

Fig. 2. *BAX/BCL-XL* expression ratio in chronic myeloid leukemia (CML) and controls. Patients with CML and controls classified: Healthy donors (HD), at diagnosis, chronic phase (CP), accelerated phase (AP) and blastic crisis (BC).

group of CP patients, it is noteworthy the dispersion of data indicating a substantial heterogeneity in the molecular outcome and/or response to imatinib therapy. This characteristic may be related to the fact that CP includes cases with complete, major, minor or null molecular response to imatinib therapy.

Correlation analysis between BAX/BCL-XL vs. BCR-ABL/ABL

In order to estimate the utility of *BAX/BCL-XL* ratio, we address an analysis of correlation between *BAX/BCL-XL* vs. *BCR-ABL/ABL* (Fig. 4). As a result we observed a significant inverse correlation (Spearman $p<0.0001$).

While most of CML patients in CP (82%) showed an increase in BAX/BCL-XL ratio, patients at diagnosis , AP, BC and six patients in CP (under the shaded rectangle) showed a significant reduction in BAX/BCL-XL ratio (Fig. 4).

Patients in CP are the most heterogeneous group; in fact they are distributed in three out of the four possible quadrants of *BAX/BCL-XL–BCR-ABL/ABL* combinations (i.e., high–low, low–low and high–high, respectively) but consistently lacks low–high ratios. Interestingly, the last one ratios includes only patients in AP, BC and at diagnosis indicating that low values of *BAX/BCL-XL* and high of *BCR-ABL/ABL* may be used as a marker of more aggressive disease.

These data indicate that *BAX/BCL-XL* ratio can identify a subset of cases in CP with poor response to tyrosine kinase inhibitors although in some cases they showed low level of *BCR-ABL* fusion gene transcripts.

BAX/BCL-XL ratio during follow up of CML patients treated with TKI

The ratio *BAX/BCL-XL* at diagnosis was periodically evaluated during a follow up of 6 month in a group of eight patients. In all cases, the ratio exceeded the mean *BAX/BCL-XL* ratio of healthy donors (4.8 ± 0.59) in the first month of treatment with TKI (Supplementary Fig. S3). In the second and third evaluation after treatment, the BAX/BCL-XL ratio showed some fluctuations with values above 4.8 in seven out of eight cases. All these cases showed good response to treatment. Only one out of the eight cases (case 3 in Supplementary Fig. S3) showed a very low ratio at third month after imatinib treatment. Notably, this latter case developed a progression to an AP and died in BC five months later.

Table 3
BCR-ABL/ABL ratio in CML phases.

CML phase group	BCR-ABL/ABL(%) (X ± SEM)
Patients at diagnosis (untreated) ($n=13$)**	76.62 ± 8.6
Patients in chronic phase ($n=34$)	5.89 ± 1.5
Patients in accelerate phase ($n=10$)**	32.11 ± 9.8
Patients in blastic crisis ($n=9$)**	23.08 ± 9.8

Significant differences respect to patients in chronic phase $^{**}p<0.01$.

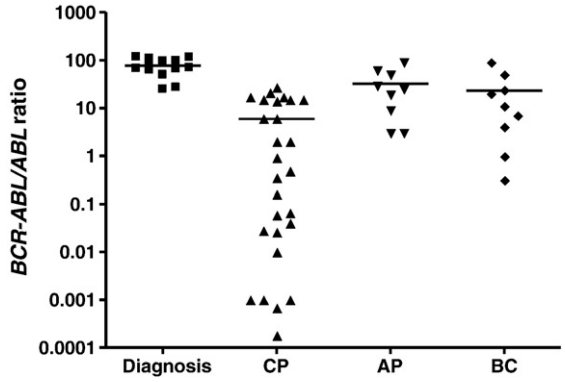

Fig. 3. Typical measurements of *BCR-ABL*/*ABL* relative expression in chronic myeloid leukemia (CML) at different disease stages. Patients are studied at the following stages: at diagnosis, chronic phase (CP), accelerated phase (AP) and blastic crisis (BC).

In order to gain insight into the biology of TKI treatment in CML, *BCR-ABL*/*ABL* expression was assessed in parallel in this cohort of eight cases. The comparison of this classical progression monitor, *BCR-ABL*/*ABL*, with *BAX*/*BCL-XL* expression ratio showed that the latter rendered an immediate augment in response to TKI, whereas the former, a delayed decrease in *BCR-ABL*/*ABL* relative expression following the proapoptotic tendency. These results agree with the theoretical time course of biological events in TKI treated CML cells: (a) TKI induced inhibition of $p210^{BCR\text{-}ABL}$, (b) extensive modification of transcript expression of apoptosis-related genes, such as *BAX* and *BCL-XL*, (c) activation of the apoptotic machinery, (d) cell death of $p210^{BCR\text{-}ABL}$ positive clones resulting ultimately in lower values of *BCR-ABL*/*ABL*.

Discussion

Chronic Myeloid Leukemia (CML) is a malignant disease of the human hematopoietic stem cell characterized by the presence of the Philadelphia chromosome, t(9:22)(q34:q11), resulting in the *BCR-ABL* fusion gene [15]. Patients who develop CML are currently treated with tyrosine kinase inhibitors (TKIs), which inhibit the function of the oncogene *BCR*/*ABL*. Most CML cells undergo apoptosis when *BCR*/*ABL* tyrosine kinase activity is suppressed by TKIs. Cells surviving drug treatment are either stem cells (CML in early phase) or cells with *BCR*/*ABL*-dependent or -independent mechanisms of drug resistance (CML in advanced phase) [16–18]. The presence of *BCR-ABL* rearrangement has been linked to downregulation of pro-apoptotic genes (as *BAX*) and up-regulation of anti-apoptotic genes (as *BCL-XL*) [19,20].

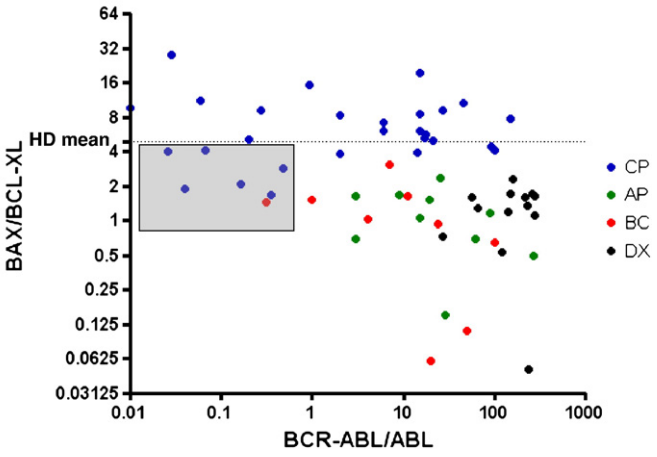

Fig. 4. Correlation between *BAX*/*BCLX-L* and *BCR-ABL*/*ABL* values in chronic myeloid leukemia (CML) at different stages. Spearman correlation test of the entire cohort of CML patients renders $P<0.0001$. The shaded quadrant selects six cases in CP associated with low *BCR-ABL*/*ABL* and low *BAX*/*BCL-XL* ratio. Dotted horizontal line indicates *BAX*/*BCL-XL* ratio mean obtained from healthy donor samples. Patients with CML are classified at different stages of the disease: at diagnosis (DX), chronic phase (CP), accelerated phase (AP) and blastic crisis (BC).

In acute myeloid leukemia (AML), it is known that apoptosis plays a role in response to chemotherapy, suggesting an association between therapy-induced apoptosis and therapeutic efficacy. Consequently, the analysis of mitochondrial apoptotic proteins may represent a significant prognostic tool to predict outcome. In AML, low BAX/BCL2 ratio is correlated with poor cytogenetics risk, whereas high ratios correlates with longer overall survival and disease free survival [21]. However, in CML the contribution of apoptotic genes in the transforming phenotype is still unclear.

In this study we measured transcript expression of *BAX* and *BCL-XL* genes in K562 cell line treated *in vitro* with different TKIs, and also in CML patients in different phases of the disease. Our results in K562 cells showed that all *in vitro* treatments induced a decreased expression of the antiapoptotic oncogene *BCL-XL*, which determines an increase in BAX/BCL-XL ratio after treatment. These results encouraged us to characterize this ratio in CML patients in treatment with different TKIs.

We detected an important variation of *BAX*/*BCL-XL* ratio among patients in different stages of CML. We observed a significant decrease in the *BAX*/*BCL-XL* ratio in all CML patients at diagnosis (1.28 ± 0.16), AP (1.14 ± 0.20), BC (1.16 ± 0.30) and 18% of cases in CP (2.71 ± 0.40), with respect to a group of healthy individuals (4.82 ± 0.59). This low ratio in CP patients is due to high expression of *BCL-XL*, which was not associated with changes at the cellular or clinical level. In the majority of CP cases (82%), this ratio increased significantly (10.03 ± 1.30), indicating that the treatment with TKIs efficiently blocked the oncoprotein $p210^{BCR\text{-}ABL}$, and consequently *BCL-XL* expression was inhibited, and apoptosis occurred. These data show similarities with those reported by other authors [10,22], who detected an increase in BCL-XL during disease evolution. The *BAX*/*BCL-XL* ratio showed an inverse significant correlation (Spearman $P<0.0001$) with the level in *BCR-ABL*/*ABL* transcripts, indicating that lower *BAX*/*BCL-XL* ratio was related to disease progression.

Despite the vast literature on the biochemical signals triggered by *BCR-ABL* very little is known about the mechanism by which *BCR-ABL* inhibits apoptosis. An experimental model developed by Amarante et al. [8] has shown that the ectopic expression of *BCR-ABL* leads to upregulation of *BCL-XL*.

In CML, the expression of *BCL-XL* is induced by BCR-ABL protein kinase through activation of the signal transducer and activator of transcription-5 protein (STAT5) [10]. The proliferation rate has been related to a terminal leukemia clinical stage, blastic crisis, and to high levels of STAT5 activity [22]. Cases of CP with high expression of *BCL-XL* and a lower *BAX*/*BCL-XL* ratio might be therefore considered at high risk. Our results expressed as an index (*BAX*/*BCL-XL*) may be useful, because scores lower than the control value were observed in cases with disease progression. We considered that the study of the expression of apoptosis-related genes by qPCR in CML could be an important test in the follow up of patients because it permits identification of predictive factors of response to treatment.

Our data about the follow up of eight patients showed that the increase of *BAX*/*BCL-XL* ratio represents an immediate marker of the proapoptotic changes, whereas the delayed reduction in *BCR-ABL*/*ABL* relative expression reflects the final fate of the *BCR-ABL* positive clones.

It is interesting to note that the relationship *BAX*/*BCL-XL* can identify a subgroup of patients in chronic phase with low ratio and loss of response to TKIs.

Prospective studies would be necessary to demonstrate the clinical implications of the expression of apoptotic-related genes in CML disease progression.

Although chromosome aberrations other than t[9,22] were detected in CML progression, a specific molecular marker has not been characterized yet.

Our data suggest that *BAX*/*BCL-XL* expression ratio may be a sensitive monitor of disease progression and an early predictor of TKI therapy responsiveness in CML patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcmd.2010.07.011.

References

[1] F. Bresciani, R. Paoluzi, M. Benassi, et al., Cell kinetics and growth of squamous cell carcinomas in man, Cancer Res. 34 (1974) 2405–2415.
[2] A. Van Stijn, N. Feller, A. Kok, et al., Minimal residual disease in acute myeloid leukemia is predicted by an apoptosis-resistant protein profile at diagnosis, Clin. Cancer Res. 11 (2005) 2540–2546.
[3] L. Campos, J. Rouault, O. Sabido, et al., High expression of bcl2 protein in acute myeloid leukemia is associated with poor response to chemotherapy, Blood 81 (1993) 3091–3096.
[4] F. Lauria, D. Raspadori, D. Rondelli, et al., High bcl-2 expression in acute myeloid leukemia cells correlates with CD34 positivity and complete remission rate, Leukemia 11 (1997) 2075–2078.
[5] C. Wuchter, L. Karawajew, V. Ruppert, et al., Clinical significance of CD95, Bcl-2 and Bax expression and CD95 function in adult de novo acute myeloid leukemia in context of P-glycoprotein function maturation stage and cytogenetics, Leukemia 13 (1999) 1943–1953.
[6] Y. Ong, M. McMullin, K. Bailie, A. Invine, et al., High bax expression is a good prognostic indicator in acute myeloid leukaemia, Br. J. Haematol. 111 (2000) 182–189.
[7] A. Benito, M. Silva, D. Grillot, et al., Apoptosis induced by erythroid differentiation of human leukemia cell lines is inhibited by Bcl-XL, Blood 87 (1996) 3837–3843.
[8] G. Amarante-Mendez, A. McGahon, W. Nishioka, et al., BCL-2 independent BCR-ABL mediated resistance to apoptosis protection is correlated with up regulation of BCL-XL, Oncogene 16 (1998) 1383–1390.
[9] Y. Wei, D. Stockelberg, S. Hulberg, et al., Changes in expression of apoptosis-related genes are linked to the molecular response to imatinib treatment in chronic-phase chronic myeloid leukemia patients, Acta Hematol. 117 (2007) 83–90.
[10] S. Gutierrez-Castellanos, M. Cruz, L. Rabelo, et al., Differences in BCL-XL expression and STAT5 phosphorylation in chronic myeloid leukemia patients, Eur. J. Haematol. 72 (2004) 231–238.
[11] C. Lozzio, B. Lozzio, Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome, Blood 45 (1975) 321–326.
[12] M. Gutierrez, G. Timson, A. Siraj, et al., Single monochrome real-time RT-PCR assay for identification, quantification and breakpoint cluster region determination of t (9;22) transcripts, J. Mol. Diagn. 7 (2005) 40–47.
[13] E. Beillard, N. Pallisgaard, V. van der Velden, et al., Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using "real-time" quantitative reverse-transcriptase polymerase chain reaction (RQ_PCR)—a Europe against cancer program, Leukemia 17 (2003) 2318–2357.
[14] M. Baccarani, G. Saglio, J. Goldman, et al., Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European Leukemia Net, Blood 108 (2006) 1809–1817.
[15] S. Faderl, M. Talpaz, Z. Estrov, et al., The biology of chronic myeloid leucemias, N. Engl. J. Med. 341 (1999) 164–172.
[16] P. Salomoni, B. Calabretta, Targeted therapies and autophagy: new insights from chronic myeloid leukemia, Autophagy 5 (7) (Oct 2009) 1050–1051 Epub 2009 Oct 14.
[17] M. Bianchini, C. De Brasi, P. Gargallo, et al., Specifc assessment of bcr-abl transcript overexpression and imatinib resistance in chronic myeloid leukemia patients, Eur. J. Haematol. 82 (2009) 292–300.
[18] P. Gargallo, M. Cuello, P. Negri Aranguren, et al., Amplification of the BCR/ABL fusion gene clustered on a masked PH chromosome in a patient with myelocitic blast crisis of chronic myelocytic leukemia, Cancer Genet. Cytogenet. 143 (2003) 140–144.
[19] G. Brumatti, R. Weinlich, C. Chehab, et al., Comparison of the anti-apoptotic effects of BCR-ABL, BCL2 and BCL-XL following diverse apoptogenic stimuli, FEBS Lett. 541 (2003) 57–63.
[20] F. Ravandi, H. Kantarjian, M. Tapaz, et al., Expression of apoptosis proteins in chronic myelogenous leukemia: associations and significance, Cancer 91 (2001) 1964–1972.
[21] G. Del Poeta, A. Venditi, M. Del Principe, et al., Amount of spontaneous apoptosis detected by Bax/Bcl-2 ratio predicts outcome in acute myeloid leukemia (AML), Blood 101 (2003) 2115–2131.
[22] M. Horita, E. Andreu, A. Benito, et al., Blockade of the BCR-ABL kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of BCL-XL, J. Exp. Med. 191 (2000) 977–984.