Bcr-Abl independent activation of ERK1/2 in an imatinib resistant cell line

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ABSTRACT

Tyrosine kinase inhibitor (TKI)-based therapy has dramatically enhanced chronic myeloid leukemia (LMC) panorama of treatment response, disease progression and overall survival. In spite of its effectiveness and the development of more potent TKIs, resistance to treatment is still an issue. The recurrence of patients whose resistance is unrelated to BCR-ABL mutations points out to the importance of studying other resistance mechanisms. In this context, our group developed an imatinib (IM)-resistant cell line named K-IM through culturing K562 cells in gradually increasing IM concentrations. Since this cell line harbors no BCR-ABL kinase domain mutation, it is a perfect model for the study of Bcr-Abl unrelated resistance mechanisms. K-IM cells displayed an increase in BCR-ABL mRNA levels, but neither an increase in Bcr-Abl activity nor an impaired inhibition by IM were observed. Since ABC transporters are determinants for the multidrug resistance phenotype, P-glycoprotein (Pgp) expression as well as Pgp and breast cancer resistance protein activity was evaluated, however, K-IM cells showed neither Pgp expression nor transporter activity. Another important mechanism is the deregulation of apoptosis pathways and the inhibitor of apoptosis proteins XIAP and survivin have been extensively studied as putative targets for cancer treatment. K-IM cells presented mRNA levels of XIAP similar to its parental cell line K562 and higher mRNA levels of survivin. Protein analysis confirmed an increase in survivin expression, suggesting that this protein could contribute to the cell line's resistance. The search for signaling pathways that could promote survivin overexpression led to the observation of higher phosphorylation levels of Erk 1/2 that persisted even during Bcr-Abl inhibition in the resistant cell line K-IM. This suggests that the MAPK/Erk signaling pathway participates in a Bcr-Abl-independent resistance mechanism. There was a reduction in survivin levels after IM treatment, indicating its regulation is dependent on Bcr-Abl and not MAPK/Erk. One of the proteins that could evoke MAPK/Erk activation is the Shp2 phosphatase that interacts with adaptor proteins leading to Ras activation. However, IM treatment also impaired Shp2 phosphorylation, suggesting it is not the responsible mechanism for MAPK/Erk activation. Our data suggests that different resistance mechanisms that promote resistance may occur simultaneously and that K-IM cell line is an intriguing model for the study of resistance mechanisms and putative drug targets.

Keywords: Chronic myeloid leukemia, imatinib, resistance mechanisms

RESULTS

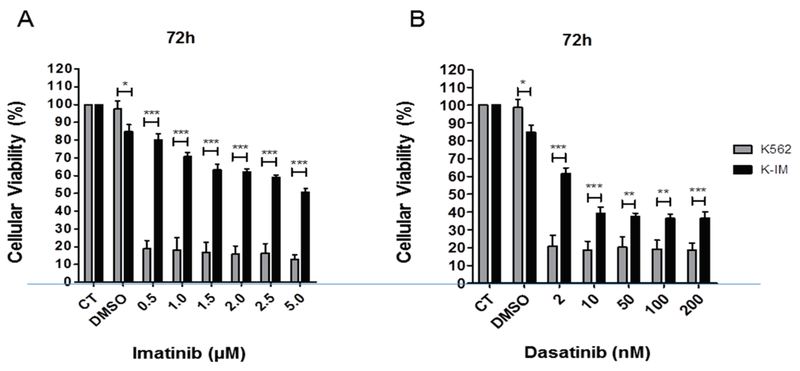

Figure 1: Cellular response to tyrosine kinase inhibitors. K562 and K-IM cells were incubated with different concentrations of imatinib (0.5 to 5.0 μM) (A) or dasatinib (2 to 200 nM) (B) for 72 hours and cell viability was assessed using the MTT assay. Data corresponds to 3 independent experiments bars represent standard error. Statistical analysis was performed using the two-way ANOVA test with Bonferroni post-test in which * corresponds to p<0,05; ** corresponds to p<0,01 and *** corresponds to p<0,001.

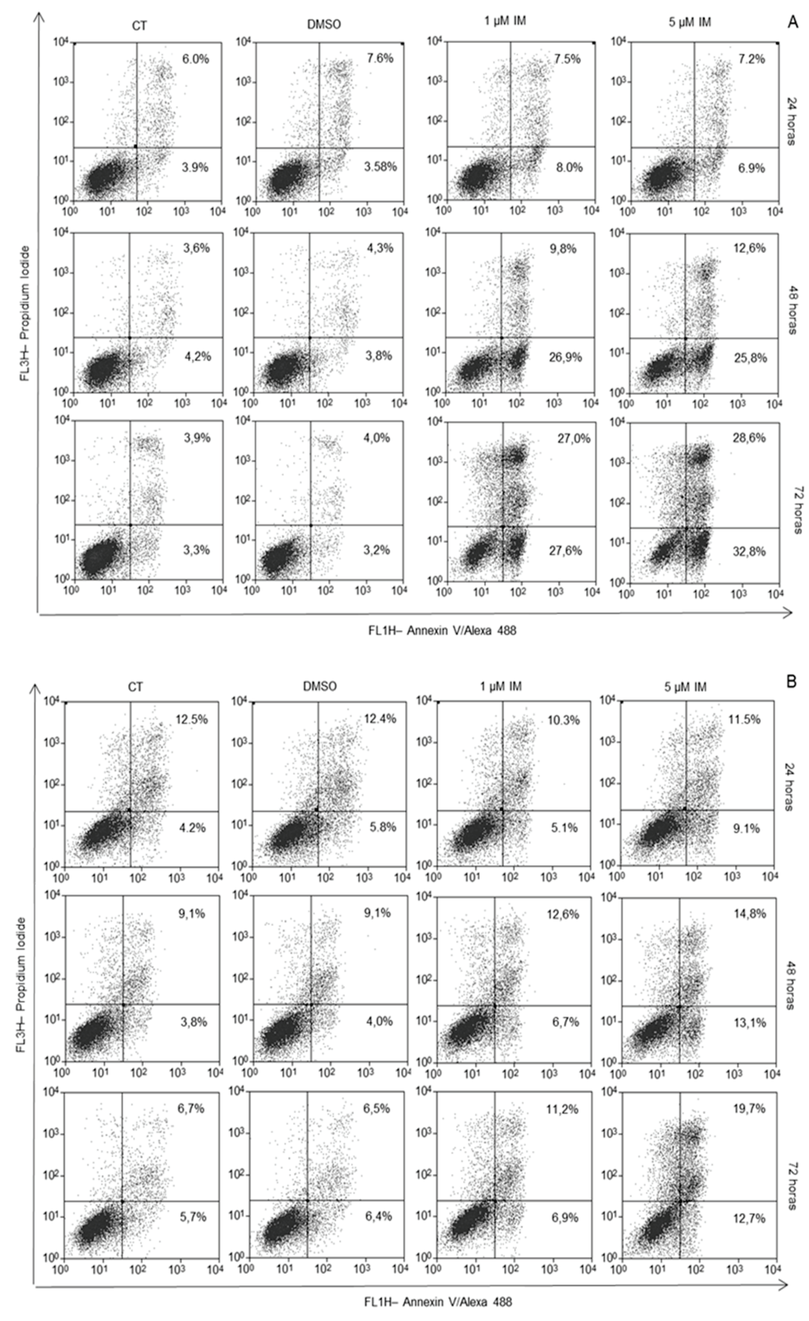

Figure 2: Cell death induction by imatinib. K562 (A) and K-IM (B) cells were treated with 1 or 5 μM imatinib or the vehicle DMSO for 24, 48 or 72 hours. Cell death induction was assessed by annexin V and propidium iodide double-staining. C) Statistical analysis was performed using the one-way ANOVA test with Dunnett post-test in which * corresponds to p<0,05; ** corresponds to p<0,01 and *** corresponds to p<0,001.

Figure 3: DNA fragmentation induced by imatinib. K562 (A) and K-IM (B) cells were treated with 1 or 5 μM imatinibe or the vehicle DMSO for 24, 48 or 72 hours. DNA fragmentation was assessed as the number of events with propidium iodide fluorescence less intense than the fluorescence of the events in the G0/G1 cell cycle phases (SUBG0/G1). Statistical analysis was performed using the one-way ANOVA test with Dunnett post-test in which * corresponds to p<0,05; ** corresponds to p<0,01 and *** corresponds to p<0,001.

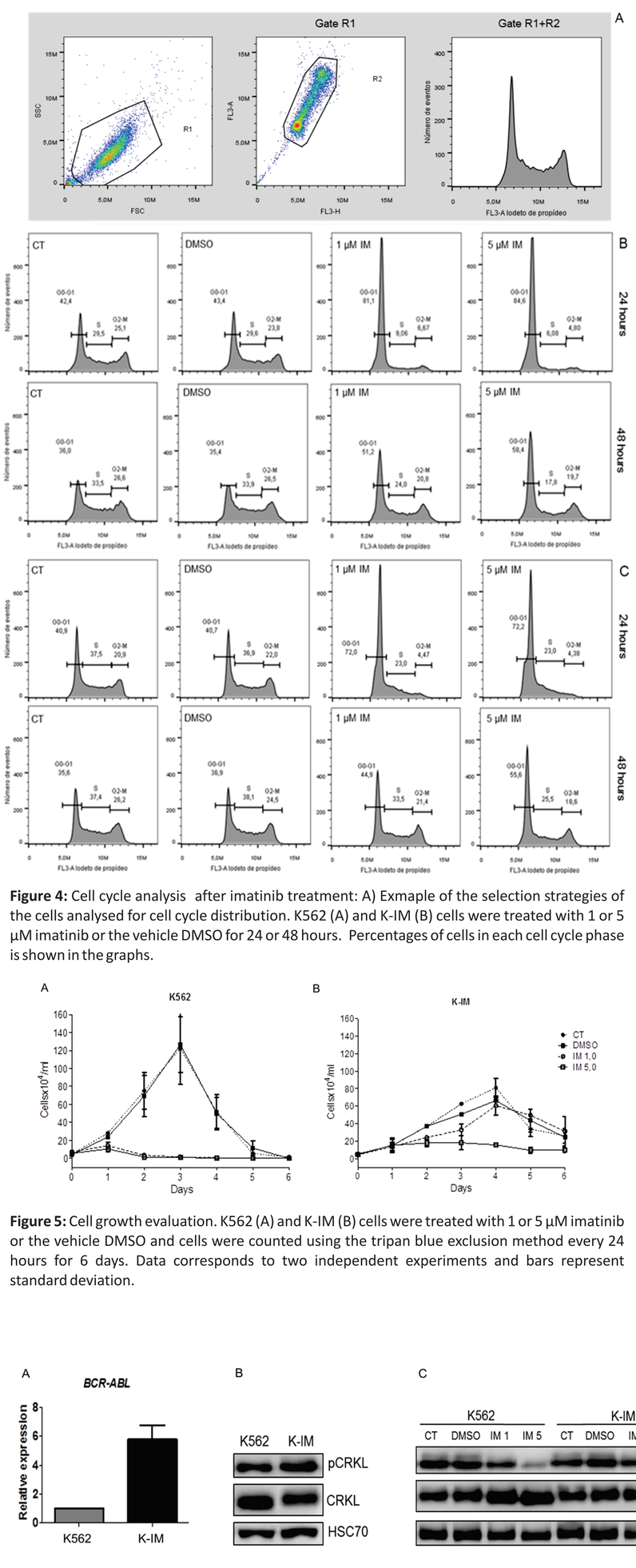

Figure 4: Cell cycle analysis after imatinib treatment: A) Exmaple of the selection strategies of the cells analysed for cell cycle distribution. K562 (A) and K-IM (B) cells were treated with 1 or 5 μM imatinib or the vehicle DMSO for 24 or 48 hours. Percentages of cells in each cell cycle phase is shown in the graphs.

Figure 5: Cell growth evaluation. K562 (A) and K-IM (B) cells were treated with 1 or 5 μM imatinib or the vehicle DMSO and cells were counted using the tripan blue exclusion method every 24 hours for 6 days. Data corresponds to two independent experiments and bars represent standard deviation.

Figure 6: Evaluation of Bcr-Abl status: A) mRNA levels were analyzed using RT-qPCR in the K-IM cell line relative to K562 cell line. β-glucuronidase (GUSB) gene was used as endogenous control. B) Bcr-Abl activity was indirectly evaluated thourgh the CRKL phosphorylation levels, total CRKL and HSC70 protein levels were evaluated as control. C) For the assessment of Bcr-Abl inhibition by imatinib treatment, K562 and K-IM cells were treated with 1 or 5 μM of imatinib or the vehicle DMSO for 24 hours. The protein content and phosphorylation levels of CRKL protein were assessed using Western Blot. HSC70 protein was used as sample loading control.

Figure 7: Evaluation of efflux transport proteins. A) K562 and K-IM cells were incubated with anti-P-glycoprotein (Pgp) antibody conjugated to ficoeritrin and analyzed in the flow cytometer. B) Pgp and breast cancer resistance protein (BCRP) activity was evaluated. Cells were incubated with the fluorescent substrate Rhodamine 123 (Rho) in the presence or absence of the Pgp modulator verapamil (VRP) and analized in the flow cytometer for Pgp activity. Cells were incubated with the fluorescent substrate Pheophorbide A (PhA) in the presence or absence of the BCRP modulator Fumitremorgin C (FTC) and analized in the flow cytometer for BCRP activity. Images are representative of 3 independent experiments.

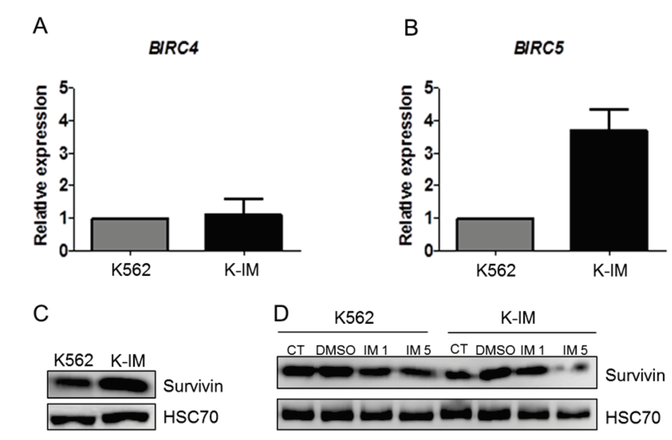

Figure 8: Evaluation of the inhibitor of apoptosis proteins. BIRC 4 (XIAP) (A) and BIRC 5 (survivin) (B) mRNA levels were assessed using RT-qPCR. β-glucuronidase (GUSB) gene was used as endogenous control. C) Survivin protein levels were assessed by western blot and HSC70 protein was used as control. D) K562 and K-IM cells were treated with 1 or 5 μM imatinibe or the vehicle DMSO for 24 hours and survivin protein levels were assessed using western blot. HSC70 protein was used as control.

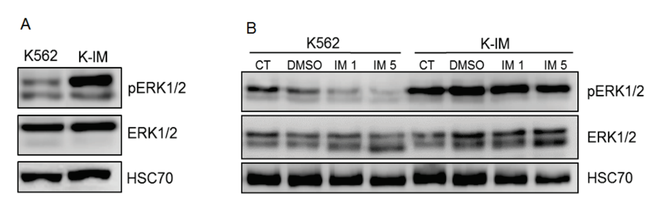

Figure 9: ERK1/2 proteins evaluation. A) ERK1/2 proteins total and phosphorylated levels were evaluated in K562 and K-IM cells. HSC70 protein was used as control. B) K562 and K-IM cells were treated with 1 or 5 μM imatinibe or the vehicle DMSO for 24 hours and ERK1/2 proteins total and phosphorylated levels were assessed using western blot. HSC70 protein was used as control.

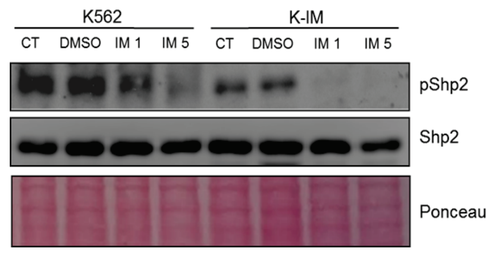

Figure 10: Evaluation of Shp2 levels after imatinib treatment. K562 and K-IM cells were treated with 1 or 5 μM imatinib or the vehicle DMSO for 24 hours and Shp2 total protein and phosphorylation levels were assessed using western blot. HSC70 protein was used as control.

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