Suggestive role of Erk 1/2 and survivin in imatinib resistance in the chronic myeloid leukemia cell line K-IM

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BACKGROUND AND AIMS

About 25% of Chronic Myeloid Leukemia (CML) patients develop resistance to tyrosine kinase inhibitor (TKI) treatment. Amidst several molecular mechanisms, Bcr-Abl-independent activation of MAPK/Erk signaling pathway and survivin overexpression were shown to be important mechanisms of imatinib (IM) resistance. Still the need to better comprehend the mechanisms that underlie resistance and to develop new therapeutic strategies lingers. Toward studying aspects of drug resistance, the CML cell line resistant to IM, named K-IM, was developed by our group. Thus, the aim of this study is to unravel the resistance mechanisms present in this cell line and to establish it as a tool to new antitumor drugs development.

METHODS

The CML cell lines K562, K562-Lucena (a MDR cell line) and K-IM were used in this study. Cell viability was assessed using the MTT assay. Mutation was evaluated through direct sequencing. Transcriptional levels were assessed using RT-qPCR. Efflux transporters expression and activity were analyzed by flow cytometry. Cell cycle, DNA fragmentation and annexin V/propidium iodide (PI) incorporation were assessed by flow cytometry. Protein levels and phosphorylation status were assessed by Western blotting.

RESULTS AND CONCLUSION

K-IM cells present no mutation in *BCR-ABL* gene (data not shown), no efflux protein expression or activity (Figure 1 and 2, respectively) and increased mRNA levels of *BCR-ABL* and *BIRC5* (survivin) (Figure 3). K-IM cells were more resistant than K562 and even than K562-Lucena to the TKIs IM and dasatinib (Figure 4). Cells accumulated in G0/G1 cell cycle phase when treated with IM in all three cell lines (Figure 5), nonetheless, in K-IM cells apoptosis may not have been trigged since there is no increase in DNA fragmentation (Figure 6) or annexin V/PI incorporation (Figure 7). Furthermore, when cells were counted, K-IM cell number continued to increase after days in culture with 1,0 µM of IM while K562 and Lucena cell number remarkably reduced when cultured in the same IM concentration (Figure 8).IM-induced Bcr-Abl inhibition drastically reduced Erk 1/2 phosphorylation and survivin protein levels in K562 and K562-Lucena, but not in K-IM, suggesting that they may contribute to this cell line resistance (Figure 9). Our results suggest that the CML cell line K-IM resistance to IM involves Bcr-Abl, Erk and survivin proteins and that it is a useful tool to the study of multifactorial resistance to TKIs and new drugs development.

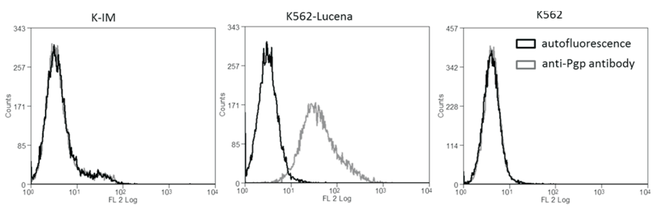

Figure 1: P-glycoprotein (Pgp) expression. Cells of K562, K562-Lucena and K-IM cell lines were incubated with anti-Pgp antibody and then analyzed through flow cytometry. Representative image of 3 independent experiments

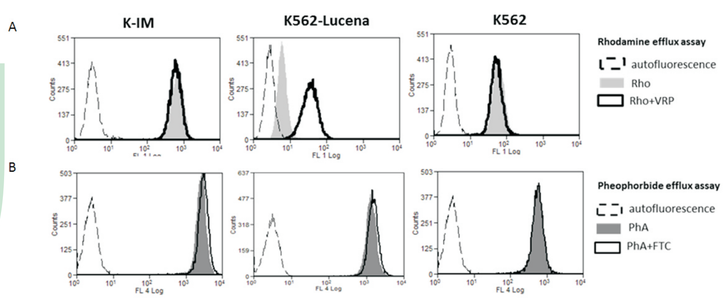

Figure 2: Evaluation of efflux transporters activity. Cells were incubated with the fluorescent substrate rhodamine 123 (Rho) in the presence or absence of the Pgp inhibitor verapamil (VRP) and analyzed in the flow cytometer for the analysis of Pgp activity (A). Cells were incubated with the BCRP fluorescent substrate pheophorbide A (PhA) in the presence or absence of the BCRP inhibitor Fumitremorgin C (FTC) for the analysis of BCRP activity (B). Images representative of 3 independent experiments.

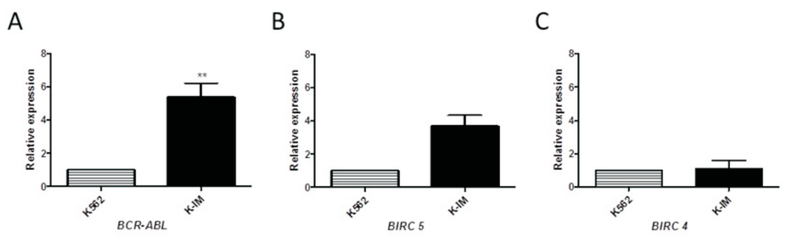

Figure 3: Quantification of mRNA levels. mRNA content of *BCR-ABL*, *BIRC 4* (encoding XIAP) and *BIRC5* (encoding survivin) was determined in the K562 and in K-IM cell lines through quantitative real-time PCR. Statistical analysis was done using the one-way ANOVA test. ** corresponds to p<0.01.

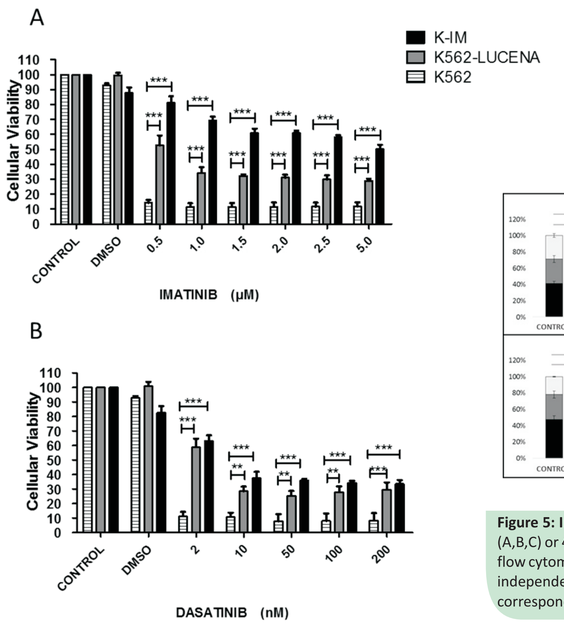

Figure 4: Cellular response to tyrosine kinase inhibitors. Viability of K562, K562-Lucena and K-IM cells was assessed through the MTT assay, after 72 hours of treatment with imatinib (A) or dasatinib (B) in different concentrations. Statistical analysis was made using the two-way ANOVA with Bonferroni post-test. * corresponds to p<0.05, ** corresponds to p<0.01 and *** corresponds to p<0.001.

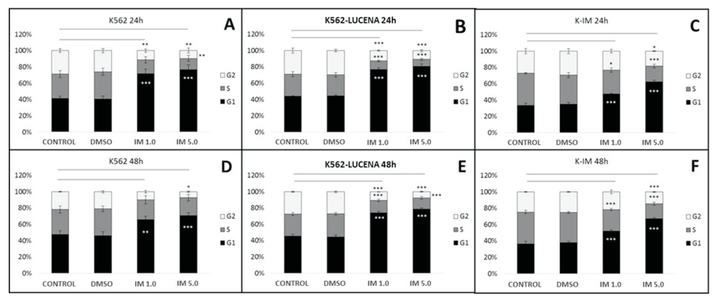

Figure 5: Imatinib treatment affects cell cycle phases distribuition. Cells were treated with imatinib for 24 (A,B,C) or 48 hours (D,E,F) before being stained with propidium iodide (PI). PI fluorescence was detected by a flow cytometer and cells were stratified in the cell cycle phases according to DNA content. Representative of 3 independent experiments. Statistical analysis was done using one-way ANOVA with Bonferroni post-test. * corresponds to p<0.05, ** corresponds to p<0.01 and *** corresponds to p<0.001.

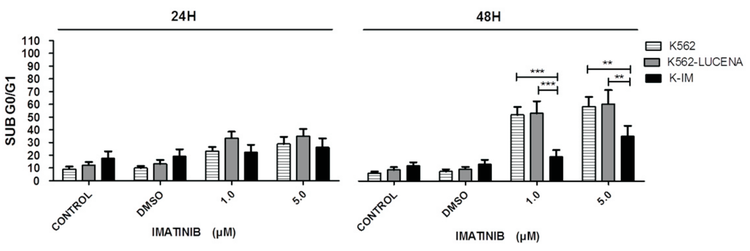

Figure 6: DNA fragmentation evaluation after imatinib treatment. Cells of the K562, K562-Lucena and K-IM cell lines were treated with imatinib for 24 and 48 hours and then stained with propridium iodide (PI) and analyzed in a flow cytometer. Events localized in sub G0/G1 according to DNA content were quantified to determine the percentage of fragmented DNA in the sample. Statistical analysis was made using the two-way ANOVA test with Bonferroni post-test. ** corresponds to p<0.01 and *** corresponds to p<0.001.

Figure 7: Evaluation of death induced by imatinib treatment. Cells of the K562, K562-Lucena and K-IM cell lines were treated with imatinib for 24, 48 or 72 hours and death induction was evaluated in a flow cytometer according to annexin V and propidium iodide stainning. Representative of 3 independent experiments. Statistical analysis was performed using the two-way ANOVA test with Bonferroni post-test. ** corresponds to p<0.01 and *** corresponds to p<0.001.

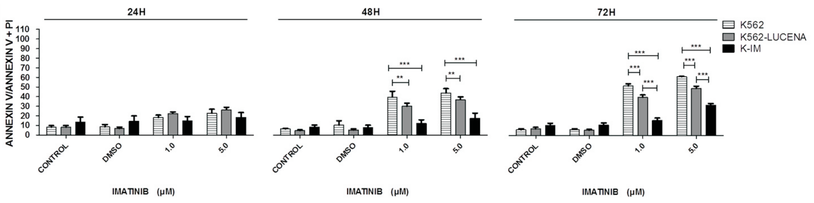

Figure 8: Cell quantification after imatinib treatment. Cells of the K562, K562-Lucena and K-IM were treated with 1.0 µM (IM1) and 5.0 µM (IM5) of imatinib and living cells were quantified every 24 hours through the trypan blue exclusion method.

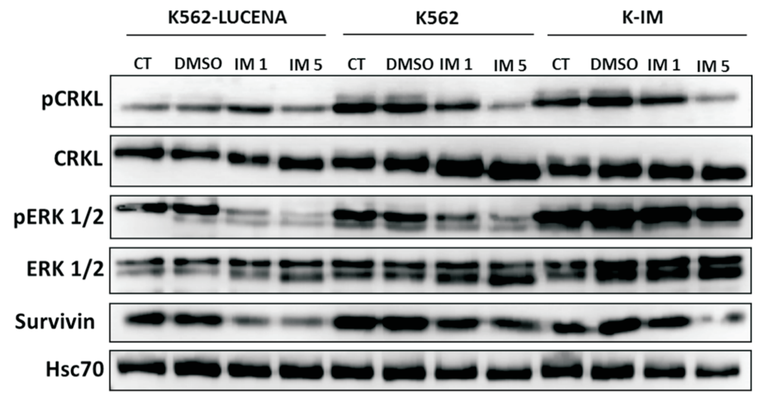

Figure 9: Protein evaluation after imatinib treatment. Cells of the K562, K562-Lucena and K-IM cell lines were treated with 1.0 µM (IM1) and 5.0 µM (IM5) of imatinib for 24 hours and protein content and phosphorylation status of Crkl, Erk and survivin were evaluated by the western blot method. Hsc70 was used as loading control.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

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