Microarray analysis suggest *TOB2, NCF1* and *IKZF5* as putative targets for LQB-118 and imatinib treatment in chronic myeloid leukemia cells

Faria, F.C.C.[1], Pinho, M.B.[2], Netto, C.D[3], Costa, P.R.R.[4], Maia, R.C.[1]

1 – Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular, INCA.
2 – Laboratório de Engenharia de Cultivos Celulares (LECC), Programa de Engenharia Química, COPPE, UFRJ.
3 – Laboratório de Química, UFRJ – Macaé.
4 – Laboratório de Química Bio-orgânica, Instituto de Pesquisas de Produtos Naturais (IPPN), UFRJ.

INTRODUCTION AND OBJECTIVE

Chronic myeloid leukemia (CML) is a myeloproliferative disorder that is characterized by the BCR-ABL oncoprotein. Treatment of CML is based on tyrosine kinase inhibitors (TKIs), mainly imatinib, and despite its clinical success, one-third of all patients require an alternative therapy. In this context, the development of drugs capable of overcoming TKIs resistance is imperative. The pterocarpanquinone-LQB-118 is a novel compound with anti-tumour effect in CML cells whose mechanism of action is being elucidated. In this context, the aim of this study was to identify and compare the genic expression pattern of CML cell lines after LQB-118 and imatinib treatment.

METHODS

In order to identify potential targets for LQB-118 treatment, CML cell lines K562 and K562-Lucena were used for microarray assays (Affymetrix), followed by real-time PCR for validation of putative targets of the new compound. All experiments were realized in comparison to imatinib treatment and in triplicate.

RESULTS AND CONCLUSIONS

Microarray analysis provided several data, allowing different analysis approaches. First, comparisons between cell lines treated and untreated with the compounds demonstrated 108 and 74 differentially expressed genes in K562 and Lucena cell lines treated with LQB-118 (1.5 µM, 48h), respectively. After imatinib treatment (1.0 µM, 48h), it was observed 4894 and 863 differentially expressed genes in K562 and Lucena cells, respectively. A comparison between treatments of the same cell line was also realized and 62 and 11 genes were differentially expressed in both compounds for K562 and Lucena cell lines, respectively. Among the altered genes observed, *TOB2, TAP2, NCF1* and *IKZF5* were selected for real-time PCR validation. PCR analysis demonstrated that *TOB2* and *NCF-1* were up-regulated in both cell lines after imatinib treatment. After LQB-118 treatment, TOB-2 was up-regulated just in K562 cells and *NCF-1* was not regulated in both cell lines treated with this compound. *IKZF-5* expression was downregulated after both treatments in K562 and Lucena cells. However, TAP2 expression was downregulated only in *K562* cell line treated with imatinib. Taken together, our data suggest that T*OB2, NCF-1* and *IKZF5* may play an important role in the mechanism of action of LQB-118 and imatinib.

A

Comparisons
K562 LQB-118 *vs* K562 CTRL
Lucena LQB-118 *vs* Lucena CTRL
K562 Imatinib *vs* K562 CTRL
Lucena Imatinib *vs* Lucena CTRL

Comparisons
K562 *vs* Lucena – LQB-118
K562 *vs* Lucena – Imatinib (IM)

B

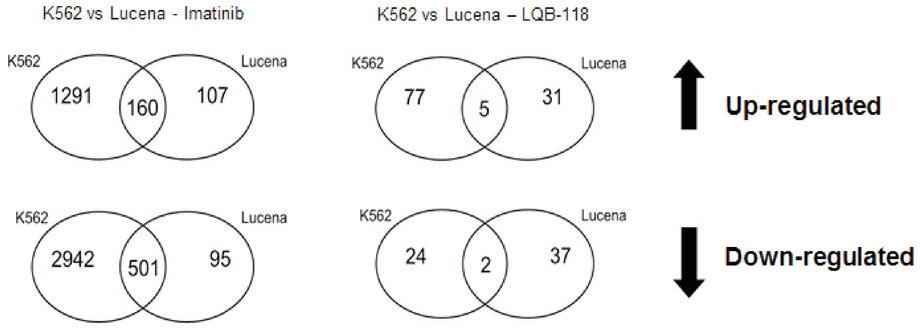

Figure 1: A – Comparisons realized after microarray data analysis for both CML cell lines and compounds. **B** – Number of genes differentially expressed in K562 and Lucena cell lines after treatment with imatinib and LQB-118. Comparisons of gene expression pattern between CML cell lines treated with the same compound (Imatinib or LQB-118).

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Comparisons
K562 x LQB-118 *vs* K562 x Imatinib
Lucena x LQB-118 *vs* Lucena x Imatinib

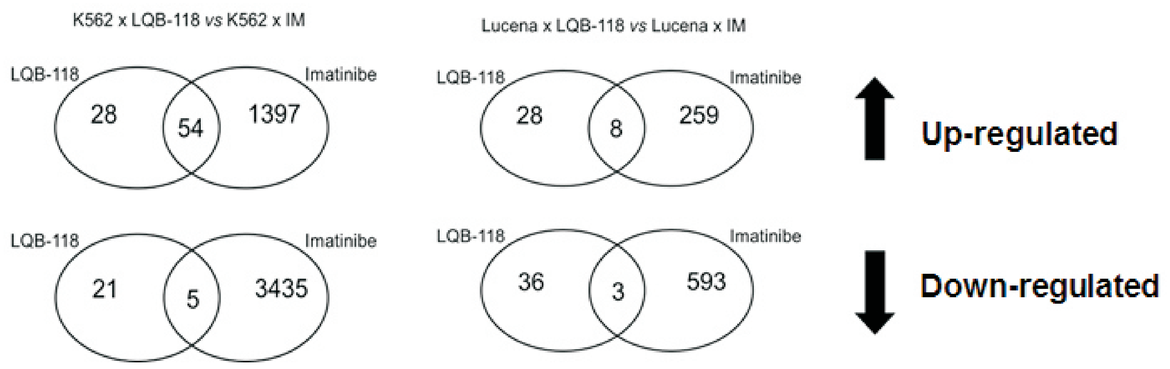

Figure 2: Number of genes differentially expressed in K562 and Lucena cell lines after treatment with imatinib and LQB-118. Comparisons of gene expression pattern between compounds (Imatinib or LQB-118), within the same CML cell line.

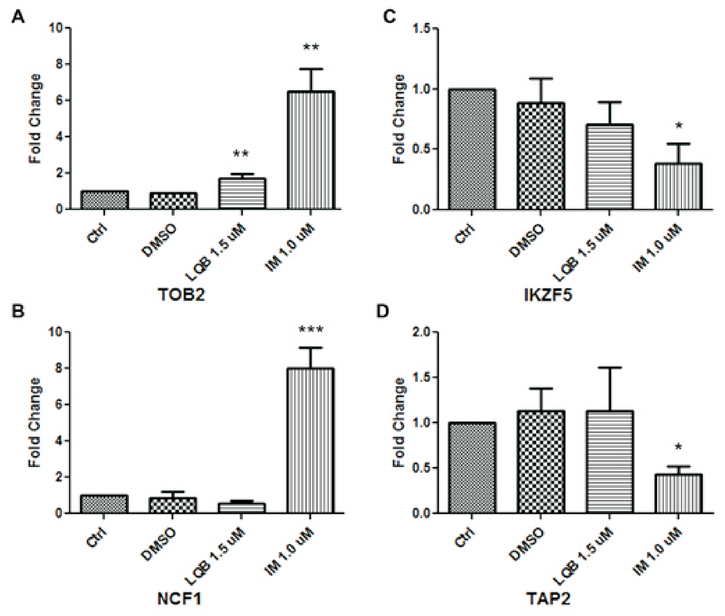

Figure 3: Relative expression mRNA levels of: **A** - *TOB2*; **B** - *NCF1*; **C** – *IKZF5*; **D** - *TAP2*, after 48h treatment with LQB-118 and imatinib K562 cells (**p<0,05*).

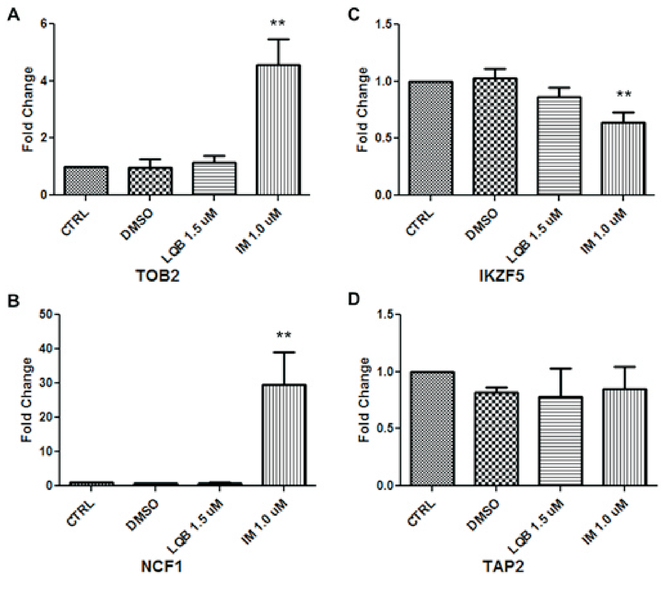

Figure 4: Relative expression mRNA levels of: **A** - *TOB2*; **B** - *NCF1*; **C** – *IKZF5*; **D** - *TAP2*, after 48h treatment with LQB-118 and imatinib Lucena cells (**p<0,05*).

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