INVESTIGATIONAL STUDY OF LQB-118 MECHANISM OF ACTION IN CHRONIC MYELOID LEUKEMIA CELLS

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BACKGROUND

Chronic myeloid leukemia (CML), a myeloproliferative disorder characterized by the BCR-ABL oncoprotein, presents its treatment based on tyrosine kinase inhibitors (TKIs), mainly imatinib. However, despite its clinical success, almost 30% of all CML patients demand alternative therapy. In this context, the development of drugs capable of overcoming TKIs resistance is imperative. The pterocarpanquinone-LQB-118 is a compound with anti-tumour effect in two CML cell lines (K562, sensitive and K562-Lucena resistant) whose mechanism of action is being elucidated.

AIMS

To evaluate the molecular mechanism involved in the response of CML cell lines to LQB-118.

MATERIAL AND METHODS

DNA microarray, Western blot, siRNA for IGF-1R, qRT-PCR, MTT and AnexinV/FITC assay were performed in order to evaluate LQB-118 molecular mechanism.

RESULTS

We demonstrated by microarray analysis of CML cells treated with imatinib and LQB-118 several differentially expressed genes. Also, that LQB-118 negatively modulates IGF-1R, AKT and mTOR protein levels and alters the expression of all members of miR-29 family. After IGF-1R silencing, we also demonstrate that cellular death induced by LQB-118 was reduced and AKT decreased protein levels was no longer observed. In addition, we evaluated the effect of CML cells treated with imatinib in combination withLQB-118. The combined treatment increased cell death rate in both cell lines, in comparison to the isolated treatments. Taken together, we demonstrated that LQB-118 modulates IGF-1R/AKT/mTOR pathway protein expressions and miR–29a/b/c expressions. Besides, it was also demonstrated that LQB-118 combined with imatinib induces cell death in CML cells presenting chemo-sensitive
or chemo-resistant phenotypes.

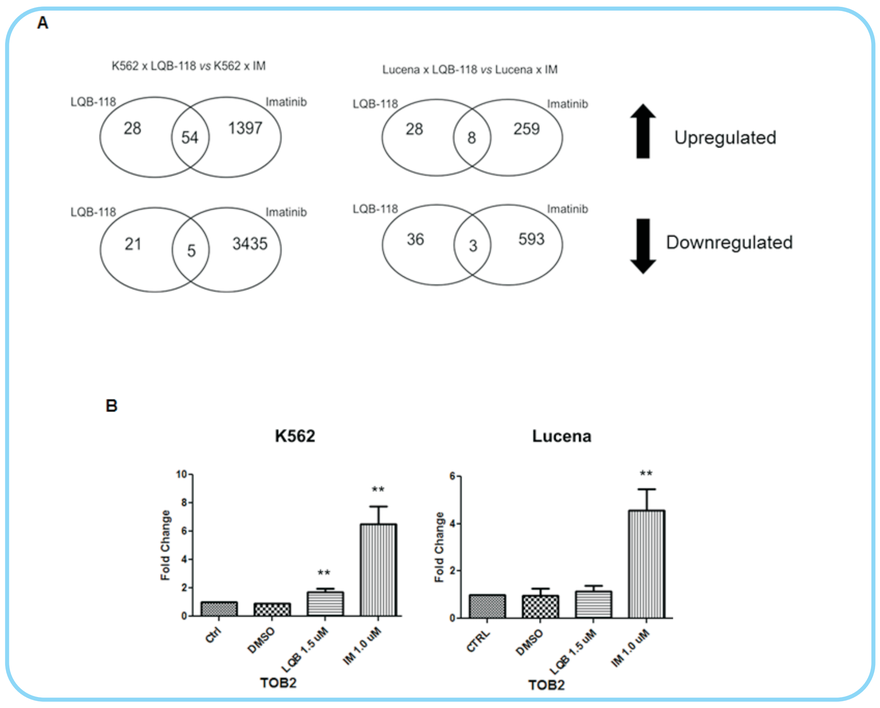

Figure 1: A – Representative scheme of the number of genes diferencially expressed in common in both CML cell lines after comparison between treatments with LQB-118 (1.5 µM) and imatinib (1.0 µM) for 48h. **B** – Real-time PCR relative expression of TOB2 in CML cell lines after 48h treatment with LQB-118 compound and imatinib (**p<0,05*).

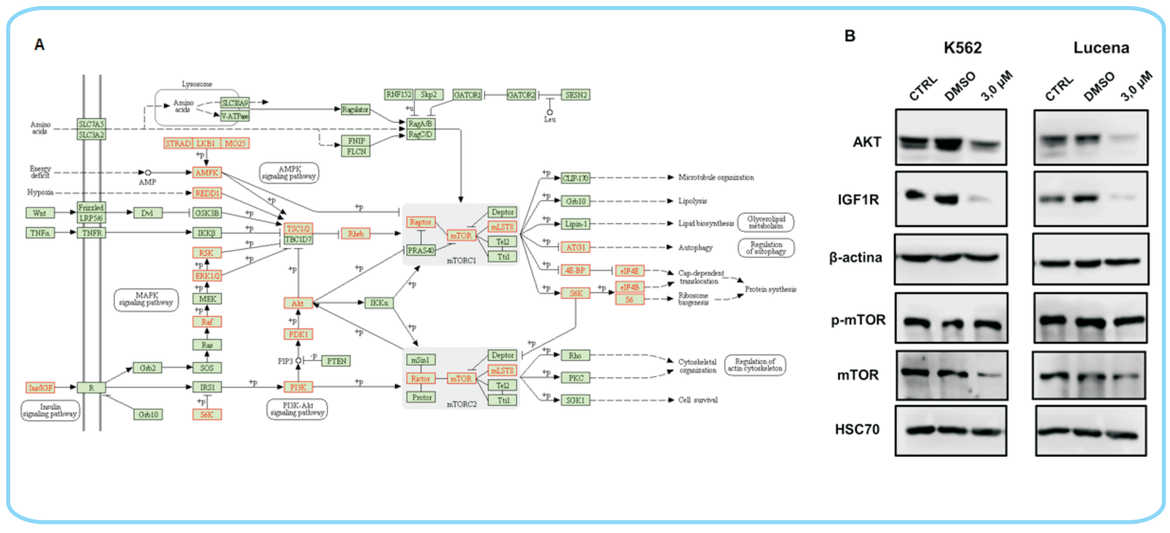

Figure 2: A – Ontologic analysis of important cellular signaling pathways in CML cell lines after treatment with LQB-118 (1.5 M; 48h). In red, the genes diferentially expressed (http://www.genome.jp/). **B** – Protein expression of AKT, IGF1R, p-mTOR e mTOR in K562 and Lucena cell lines after 24h treatment with LQB-118.

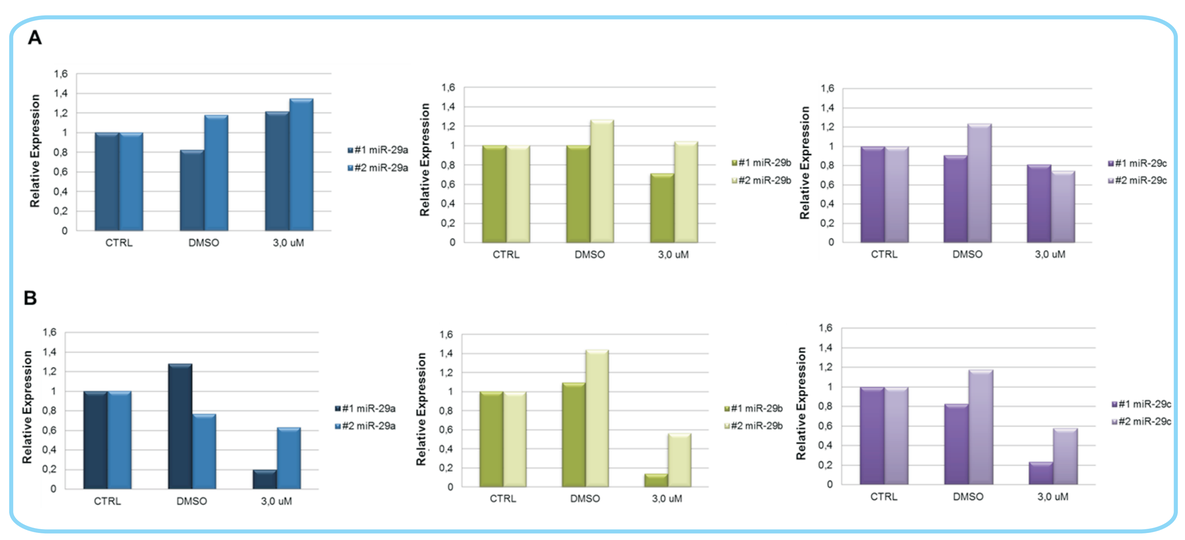

Figure 3: Relative expression levels of miRNAs miR-29a, miR-29b and miR-29c after 24h exposure to LQB-118 in K562 cell line (**A**) and Lucena cell line (**B**). miRNAs expressions were normalized by RNU6b. Graphs demonstrate two independent real time PCR experiments (Exp #1 e Exp #2).

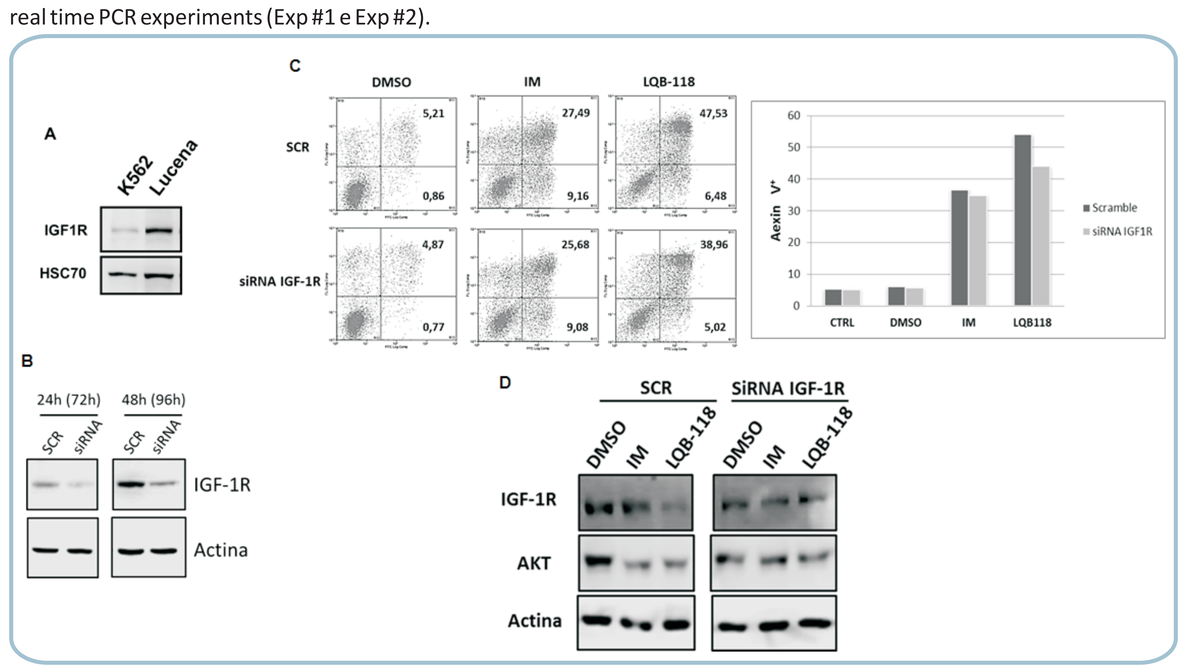

Figure 4: A – IGF-1R standard expression levels in CML cell lines K562 and Lucena. **B** – Analysis of the maintenance of IGF-1R silencing after 24h (48h) and 48h (96h) of transfection interruption. **C** - Representative dot plot and graph of cell death of Lucena cells silenced for IGF-1R after 48h treatment with Imatinib (IM) or LQB-118. **D** - IGF-1R and AKT protein levels evaluated by western blot after 48h treatment with Imatinib (IM) or LQB-118 after IGF-1R inhibition, in Lucena cells.

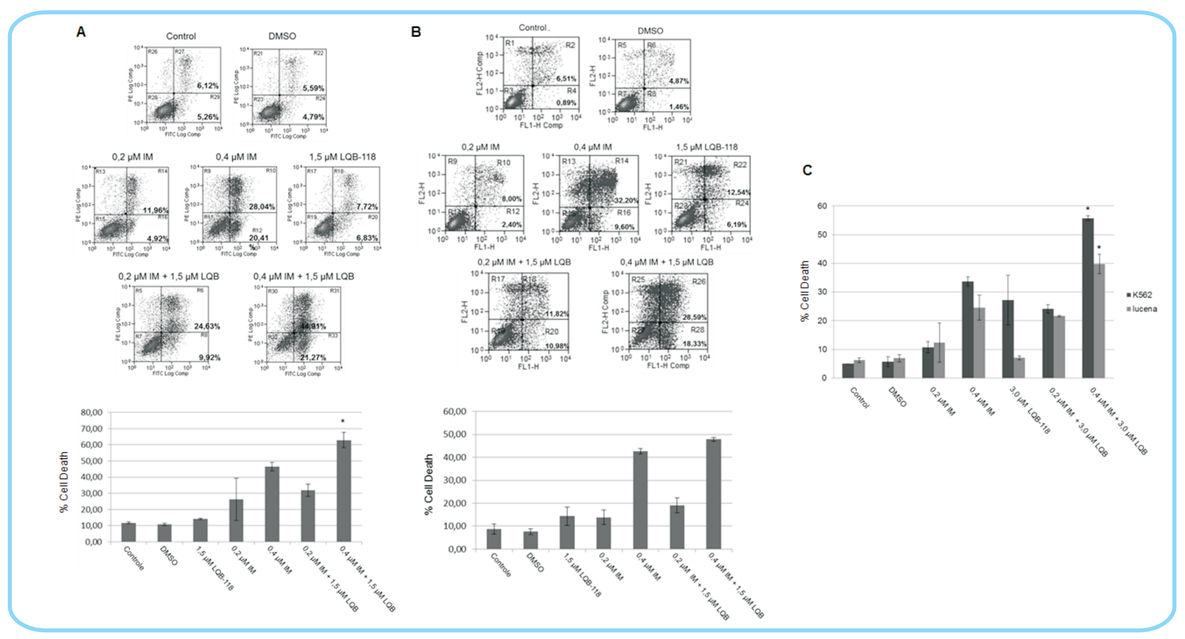

Figure 5: A – Representative dot plot and graph of K562 cells cell death after 48h treatment with Imatinib (IM) or LQB-118 and in combination (IM + LQB118). **B** - Representative dot plot and graph of Lucena cells cell death after 48h treatment with Imatinib (IM) or LQB-118 and in combination (IM + LQB118). **C** - Representative graph of Lucena cells cell death after 48h treatment with Imatinib (IM) or a higher concentration of LQB-118 (3.0 M) and in combination (IM + LQB118).

CONCLUSION

We suggest that IGF-1R pathway may be an important target pathway involved in LQB-118 mechanism in CML cells.

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