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

FERNANDA COSTAS CASAL DE FARIA [1]; MARIA EDUARDA BENTO LEAL[1]; PAULA SABBO BERNARDO[1]; MARCOS BARCELOS PINHO[2], CHAQUIP DAHER NETTO[3], PAULO R. RIBEIRO COSTA[4]; RAQUEL CIUVALSCHI MAIA[1]

1 – Laboratório de Hemato-oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular, Instituto Nacional do Câncer. 2 – Laboratório de Engenharia de Cultivos Celulares – COPPE – UFRJ. 3- Laboratório de Química, Universidade Federal do Rio de Janeiro (UFRJ), Macaé, RJ. 4 - Laboratório de Química Bio-orgânica, Instituto de Pesquisas em Produtos Naturais (IPPN), UFRJ. RJ, Brasil

INTRODUCTION

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 novel compound with anti-tumour effect in two CML cell lines (K562, sensitive and K562-Lucena resistant) whose mechanism of action is being elucidated.

METHODS AND RESULTS

we demonstrate by microarray analysis of CML cells treated with imatinib and LQB-118 several differentially expressed genes. Also, by western blot, that LQB-118 negatively modulates IGF-1R, AKT and mTOR protein levels and alters the expression of all members of miR-29 family, evaluated by qRT-PCR. After IGF-1R silencing by siRNA transfection, we also demonstrate that cellular death induced by LQB-118 was reduced and AKT decreased protein levels was no longer observed. Taken together, we demonstrated that LQB-118 modulates IGF-1R/AKT/mTOR pathway protein expressions and miR–29a/b/c expressions.

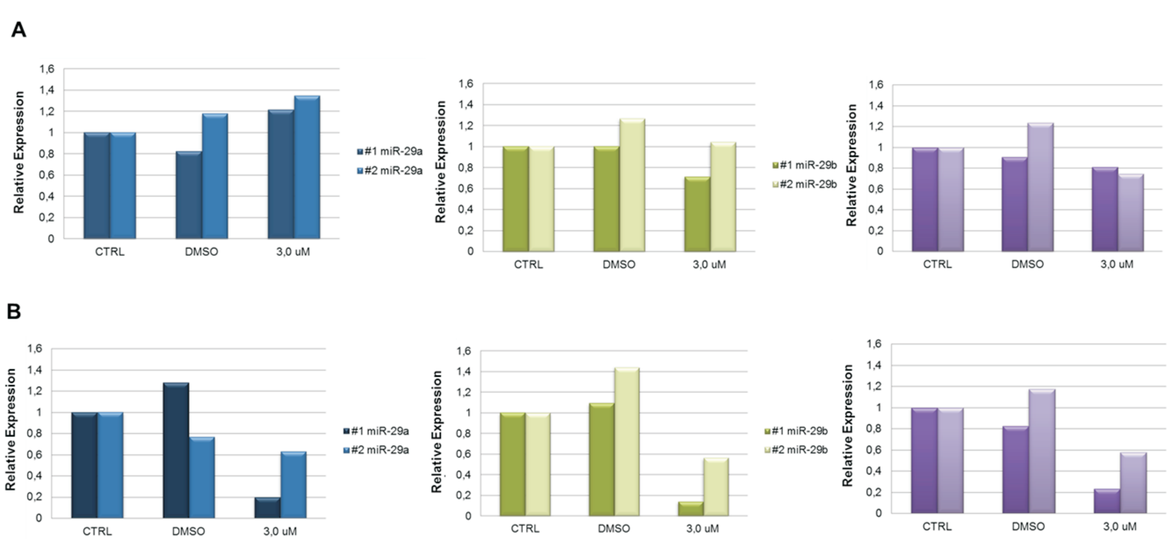


Figure 2: Relative expression levels of miRNAs miR-29a, miR-29b and miR-29c after 24h exposure to LQB-118 in K562 cell line (A) and Lucena cell line (B). miRNAs expressions were normalized by RNU6b. Graphs demonstrate two independent real time PCR experiments (Exp #1 e Exp #2).

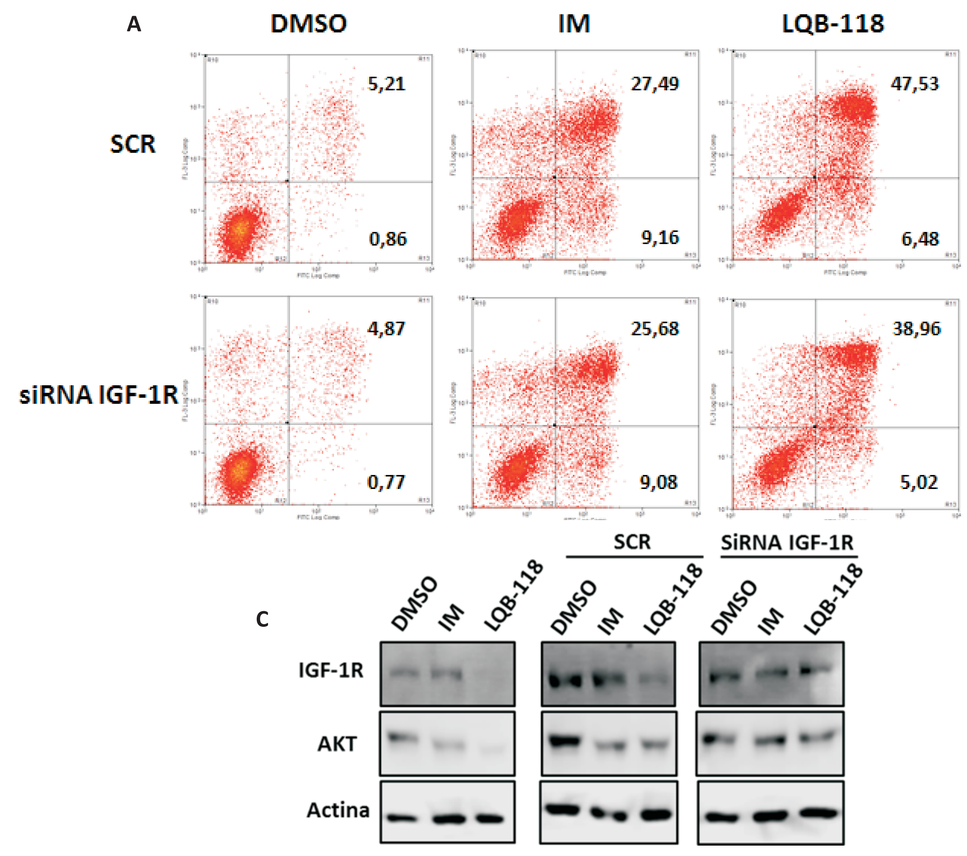


Figure 4: Cell death analysis of Lucena cells after IGF-1R silencing followed by imatinib (IM) or LQB-118 treatment (48h). **A** – Dot plot of Lucena cells silenced for IGF-1R stained by Anexin V and PI after 48h treatment with Imatinib (IM) or LQB-118. **B** – Representative graph of cell death of Lucena cells silenced for IGF-1R after 48h treatment with Imatinib (IM) or LQB-118. **C** –IGF-1R and AKT protein levels evaluated by western blot after 48h treatment with Imatinib (IM) or LQB-118.

OBJECTIVES

Evaluate the molecular mechanism involved in the response of CML cells to LQB-118.

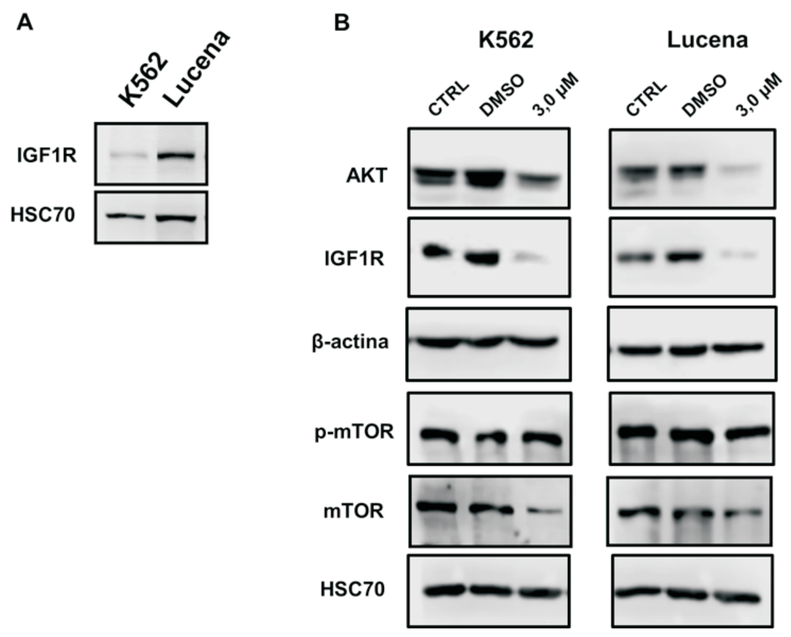


Figure 1: A – IGF1R standard expression levels in CML cell lines K562 and Lucena. **B** – AKT, IGF1R, p-mTOR and mTOR protein levels after 24h treatment with LQB-118 of K562 and Lucena cells.

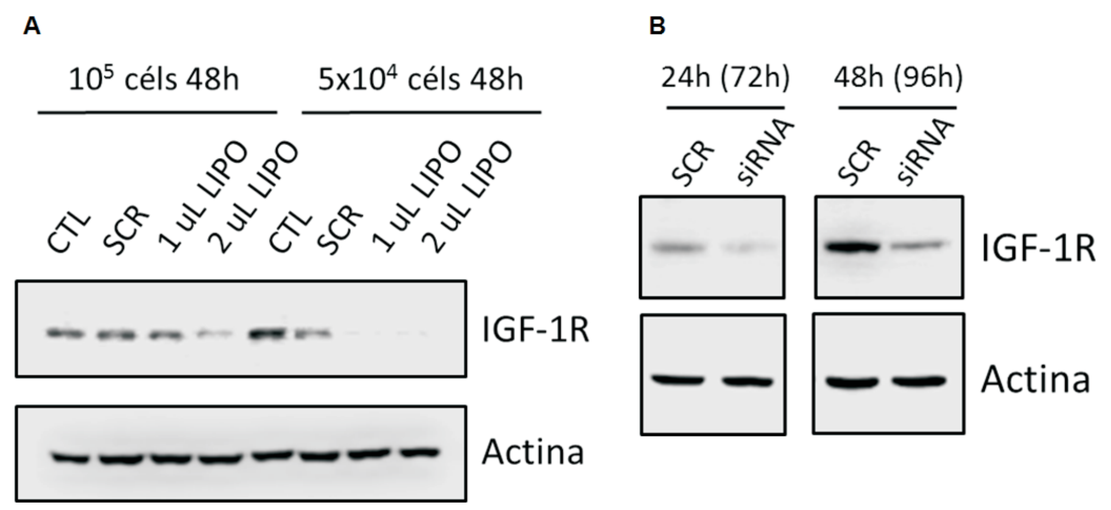
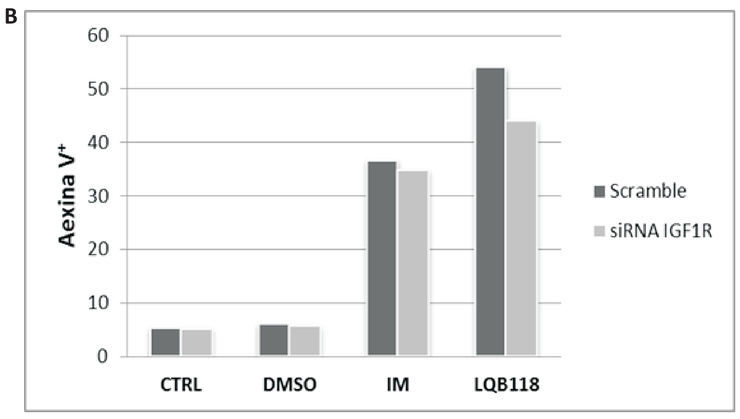


Figure 3: Western Blot analysis of IGF-1R after silencing by siRNA in Lucena cell line after 48h (**A**). Analysis of the maintenance of IGF-1R silencing after 24h (48h) and 48h (96h) of transfection interruption (**B**).



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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