Functional role of overexpressed genes in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with intrachromosomal amplification of chromosome 21 (iAMP21)

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INTRODUCTION

Intrachromosomal amplification of chromosome 21 (iAMP21) has been described in 2-3% of B-cell precursor ALL (BCP-ALL) and is associated with unfavorable prognosis due to high relapse risk (Fig. 1).

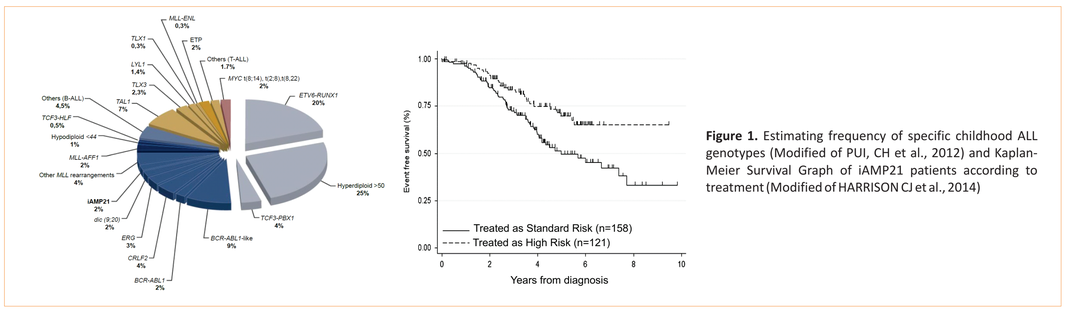


Figure 1. Estimating frequency of specific childhood ALL genotypes (Modified of PUI, CH et al., 2012) and Kaplan-Meier Survival Graph of iAMP21 patients according to treatment (Modified of HARRISON CJ et al., 2014)

A striking feature is that all patients exhibit one common region of amplification (CRA) at chromosome 21 as well as overexpress the *LGMN* gene (Fig. 2). Additionaly, it has been recently shown that *OPN* is differentially expressed in BCP cells at the time of relapse.

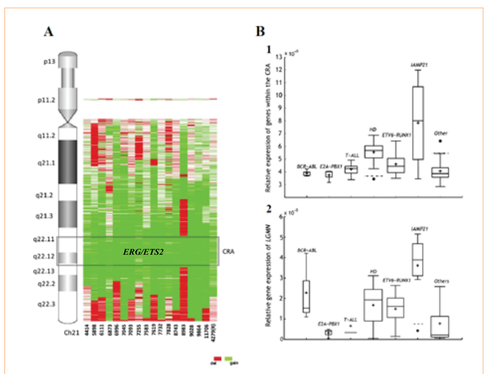


Figure 2. Summary of abnormalities in iAMP21 BCP-ALL. **(A).** Heat map of chromosome 21 abnormalities detailing the regions of deletion (red), gain (green), and normal copy number (white) relative to genomic location. (Modified of Rand V, et al., 2011). **(B)** Box plot diagrams illustrating expression of those genes within the CRA [1] and *LGMN* expression [2]. (Strefford, JC et al. 2006)

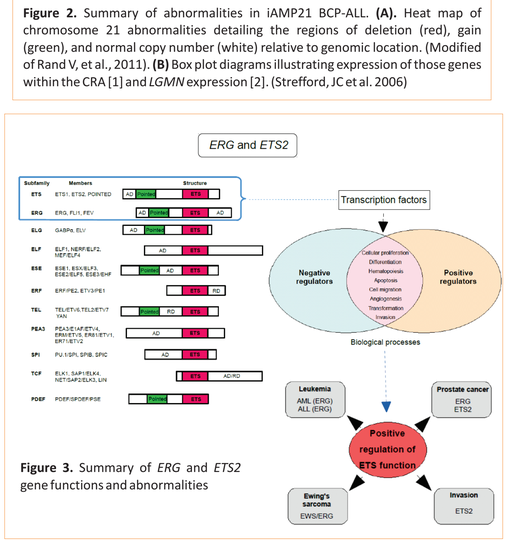


Figure 3. Summary of *ERG* and *ETS2* gene functions and abnormalities

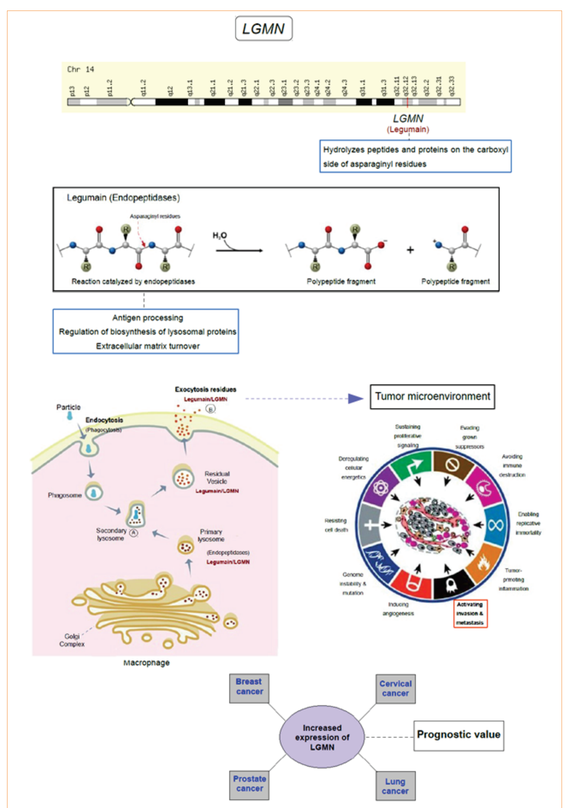


Figure 4. Summary of *LGMN* gene function and abnormalities

HYPOTESIS

Deregulated expression of *ERG, ETS2, LGMN* and *OPN* in iAMP21 BCP-ALL subtype may be correlated with chemotherapeutic resistance.

OBJECTIVE

To evaluate the role of *ERG, ETS2* and *LGMN* in ALL chemotherapeutic resistance.

METHODOLOGY

Experimental design

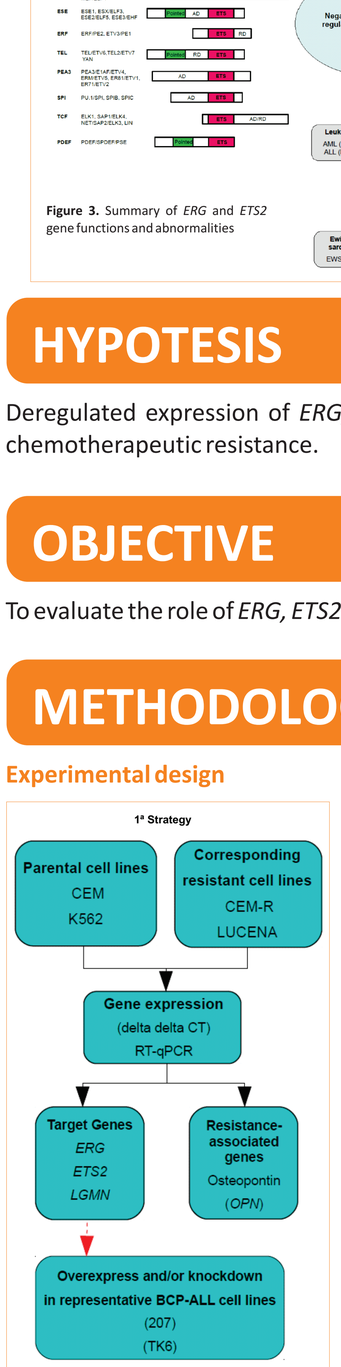


Figure 5. Flowchart of 1st strategy

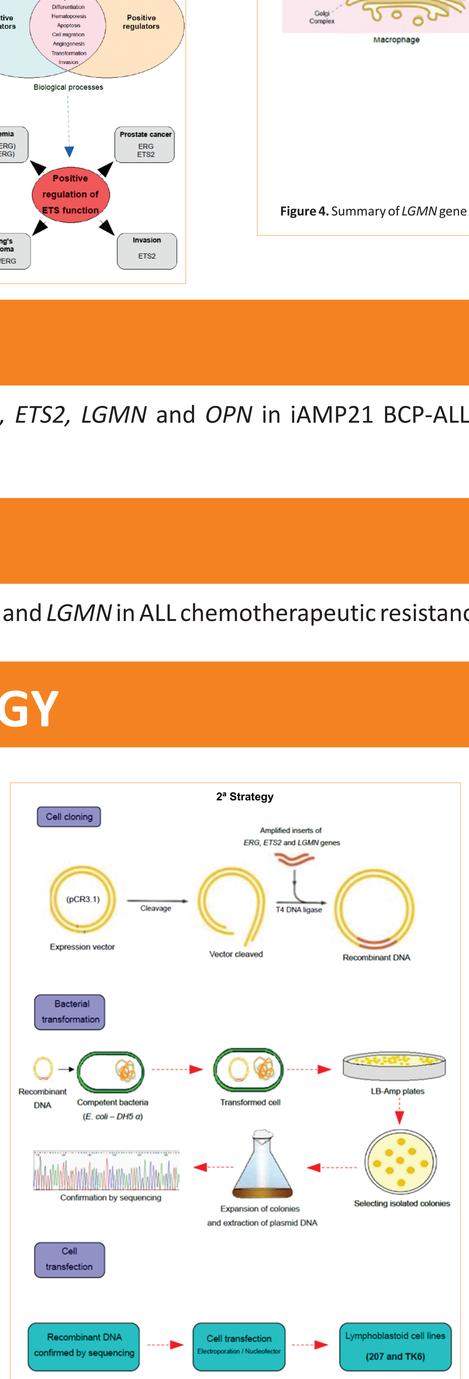


Figure 6. Flowchart of 2st strategy

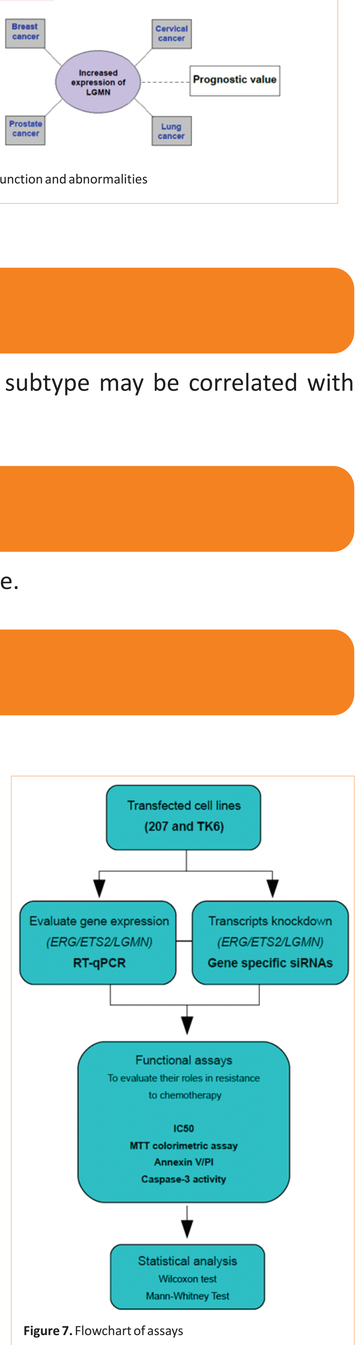
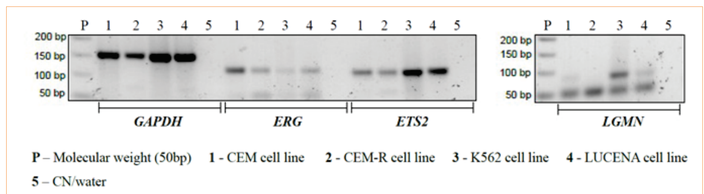


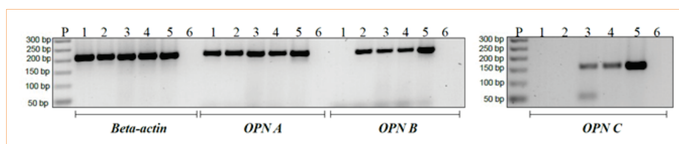
Figure 7. Flowchart of assays

RESULTS



P – Molecular weight (50bp) **1** - CEM cell line **2** - CEM-R cell line **3** - K562 cell line **4** - LUCENA cell line **5** – CN/water

Figure 8. Agarose gel indicating the fragments resulting from amplification with the oligonucleotide primers of the *GAPDH, ERG, ETS2* and *LGMN* genes



P – Molecular weight (50bp) **1** - CEM cell line **2** - CEM-R cell line **3** - K562 cell line **4** - LUCENA cell line **5** – CP/TPCA **6** – CN/ water

Figure 9. Agarose gel indicating the fragments resulting from amplification with the oligonucleotide primers of the Beta-actin, *OPN A*, *OPN B* and *OPN C* genes

Our RT-qPCR results showed that *ERG, ETS2* and *LGMN* transcripts are overexpressed in resistant cell lines CEM-R and LUCENA, when compared to their parental cell lines.

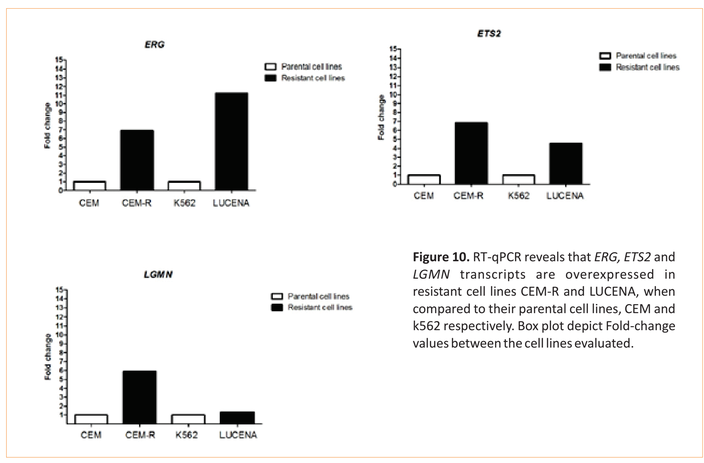


Figure 10. RT-qPCR reveals that *ERG, ETS2* and *LGMN* transcripts are overexpressed in resistant cell lines CEM-R and LUCENA, when compared to their parental cell lines, CEM and k562 respectively. Box plot depict Fold-change values between the cell lines evaluated.

We also evaluated the expression of *OPN* and their splice variants, once these gene products have also been associated to chemoresistance. We found that the three *OPN* splice variants are overexpressed in CEM-R and LUCENA resistant cell lines in relation to their parental cell lines.

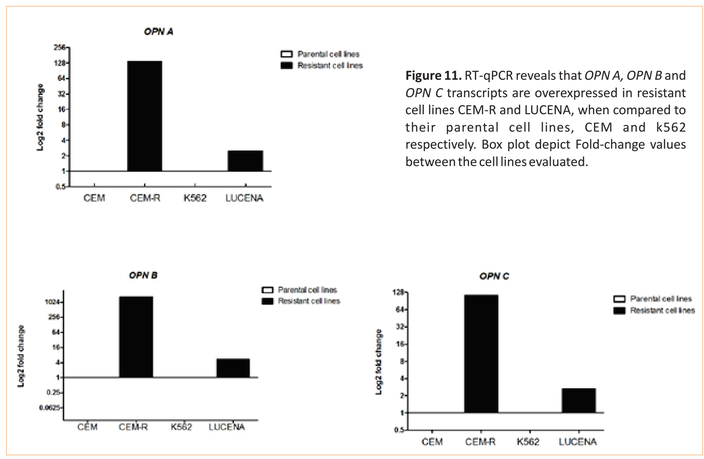


Figure 11. RT-qPCR reveals that *OPN A, OPN B* and *OPN C* transcripts are overexpressed in resistant cell lines CEM-R and LUCENA, when compared to their parental cell lines, CEM and k562 respectively. Box plot depict Fold-change values between the cell lines evaluated.

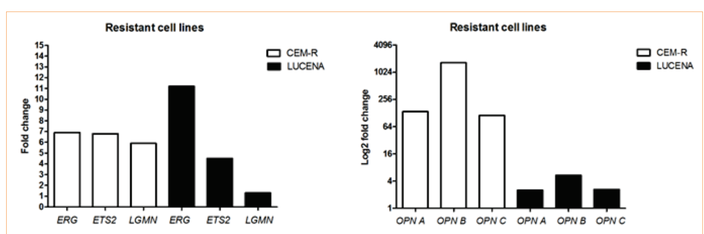


Figure 12. Box plot depict fold-change values differences between the *ERG, ETS2* and *LGMN* transcripts, as for the *OPN A, OPN B* and *OPN C* transcripts in the resistant cell lines evaluated, CEM-R and LUCENA respectively.

CONCLUSION AND PERSPECTIVES

Our preliminary data evidence that overexpression of *ERG, ETS2* and *LGMN* and also of *OPN* splice variants provide early evidence that these gene products are associated to resistance to chemotherapeutic drugs in leukemia cells. Thus, functional assays will be performed to evaluate their roles in resistance to chemotherapy.

Footnote

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