RELATIONSHIP BETWEEN IKZF1 AND BTG1 DELETIONS AND GLUCOCORTICOID RESISTANCE IN ACUTE LYMPHOBLASTIC LEUKEMIA

AMANDA DE ALBUQUERQUE LOPES MACHADO[1], BRUNO DE ALMEIDA LOPES[2], JÚLIO CÉSAR SANTORO OLIVEIRA ASSIS[1], ETEL RODRIGUES PEREIRA GIMBA[3], MARIANA EMERENCIANO[1]

[1]Molecular Cancer Study Group, Division of Clinical Research, Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil.
[2]Laboratory of Immunology and Molecular Biology, Institute of Health Sciences, Federal University of Bahia (UFBA), Salvador, Brazil.
[3]Cellular Biology Program, Research Coordination, Instituto Nacional de Câncer José Alencar Gomes da Silva (INCA), Rio de Janeiro, Brazil

Introduction and aim: Acute lymphoblastic leukemia (ALL) is characterized by impaired differentiation of lymphoid cells. Despite overall survival improvements in the last decades, treatment of relapsed cases remains a major clinical challenge. Studies have shown that secondary events, such as deletions in genes related to the regulation of lymphoid cell maturation, and progression through the cell cycle (e.g. *IKZF1, CDKN2A, JAK2* and *BTG1*) are strongly related with patient prognosis as well as drug response. Considering the wide use of glucocorticoids in the treatment of ALL, it is necessary to elucidate the mechanisms that result in their resistance process in order to develop methodologies for therapeutic advances.The present study aims to characterize the gene expression profile of genes related with the glucocorticoid response in an ALL cell lineage knocked-down for *BTG1* and *IKZF1* genes. Additionally, we will evaluate the silencing effects in response to glucocorticoid treatment using cell viability assays. **Methods:** The 207 cell line response to glucocorticoids was evaluated by the analysis of half maximal inhibitory concentration of the drug (IC50). Cells were cultured in RPMI 1640 medium supplemented with 20% of fetal bovine serum in a 96 wells plate in 5% carbon dioxide and 37°C. Concentrations ranging 0.25 to 5mM of dexamethasone and prednisolone were used. The viability of cells subjected to pharmacological treatment was measured with the MTT colorimetric test. The knockdown of *BTG1, IKZF1*, or both together was performed by electroporation of specific siRNA in 207 cell line. Silencing efficiency was evaluated by RT-qPCR. Statistical analyses were performed using the software package GraphPad Prism 7, and P-values<0.05 were interpreted as significant. **Results:** First, we could successfully knockdown *BTG1* (P-value = <0.0001) and *IKZF1* (P-value = 0.0005) genes. After silencing each gene separately, both genes, *BTG1* (P-value = <0.0001) and *IKZF1* (P-value = 0.0001), were silenced at the same time. Treatment with 1 mM dexamethasone and 3mM of Prednisolone were able to inhibit 50% of the 207 cell line growth 24h hours after incubation. **Discussion:** We were able to establish the experimental conditions of transfection and silencing of *BTG1* and *IKZF1* genes in an ALL cell line. In addition, we identified the IC50 of dexamethasone and prednisolone in 207 cell line for the first time. Thus, development of cell line models mimicking *BTG1* and/or *IKZF1* deletion consists in an important strategy to understand their impact on glucocorticoid resistance and establish in vitro methodologies to evaluate molecular and proliferative features in response to glucocorticoid stimulation. Next, we aim to assess cell viability in a *BTG1* and *IKZF1* knockdown background. At last, we will evaluate the expression profile of genes associated with glucocorticoid response (*NR3C1, DUSP1, FBXW7* and *SGK1*) in this scenario.

METHODS

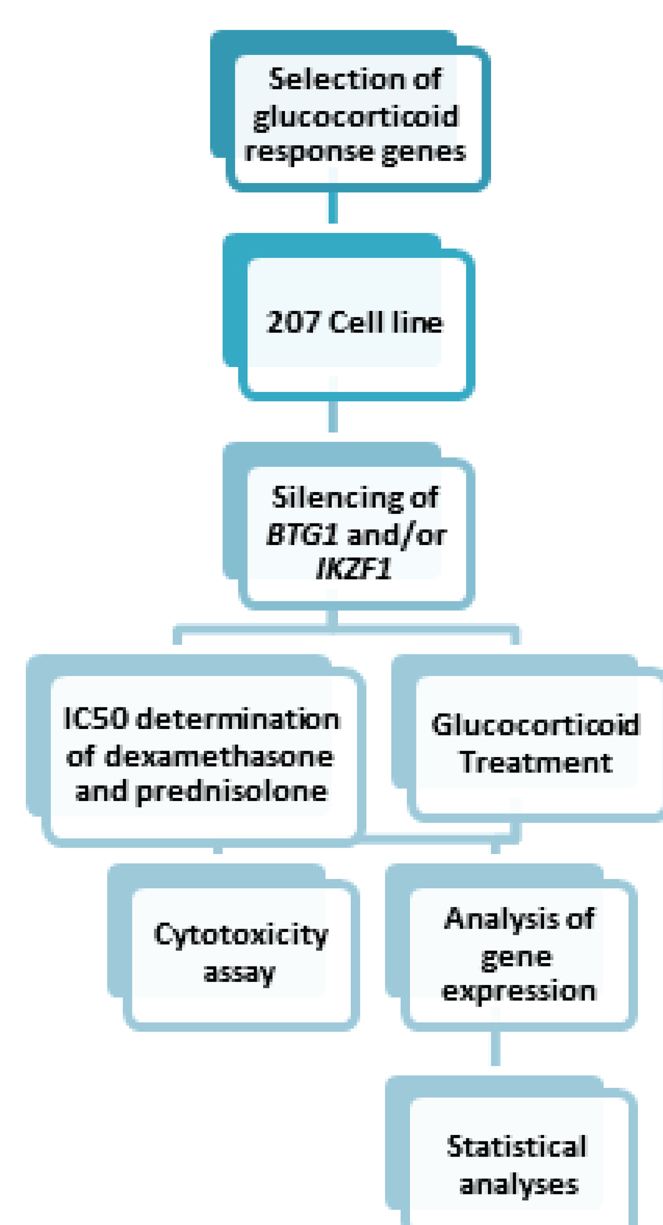

Fig. 1: Flowchart describing the steps of thestudy, including their methodologies.

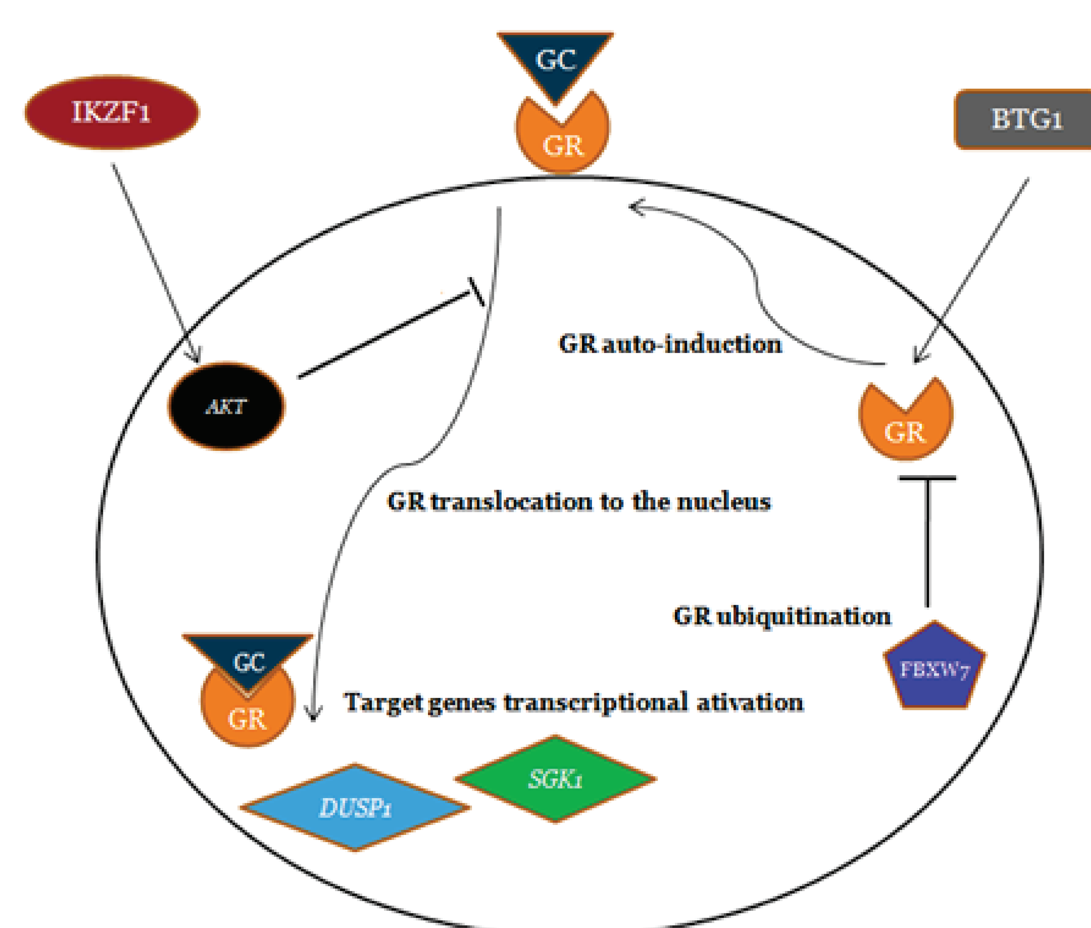

Fig. 2: Scheme illustrating the role of *IKZF1* activity on AKT phosphorilation which modulates glucocorticoid receptor (GR or *NR3C1*) translocation to the nucleus and the importance of *BTG1* in receptor auto induction. Downstream, are highlihted genes related to glucocorticoids response and receptor ubiquitination.

RESULTS

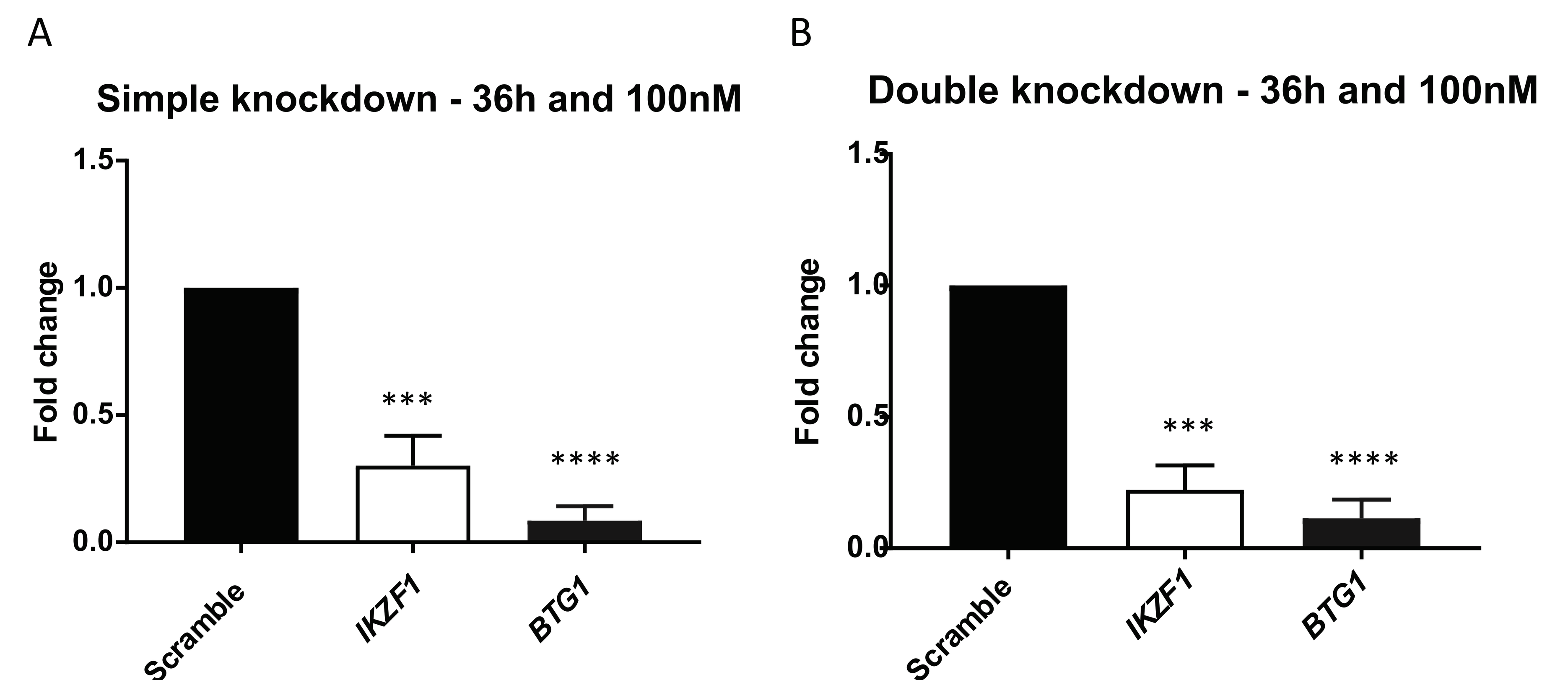

Fig. 3: *BTG1* and *IKZF1* knockdown efficiency after electroporation. Silencing was successfully realized (A) separately *BTG1* (*P*-value = <0.0001) and *IKZF1* (*P*-value = 0.0005) and (B) at the same time *BTG1* (*P*-value = <0.0001) and *IKZF1* (*P*-value = 0.0001). Student *t* test was used to assessed statistical significance ($P < .05$) between scramble and silenced cells.

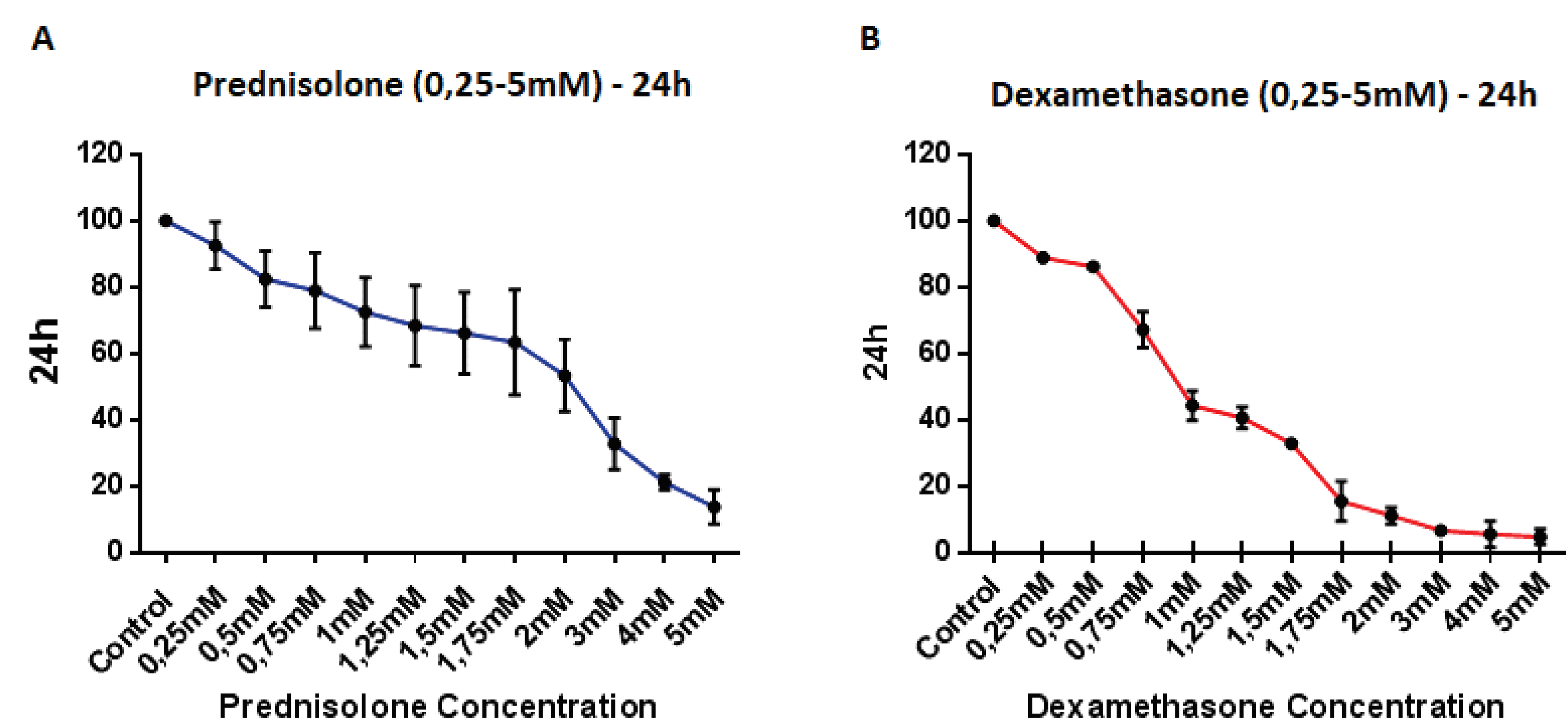

Fig.4: Crescent concentrations, ranging 0.25 to 5mM, of dexamethasone and prednisolone were used for IC50 determination. After 24h of treatment, was determined that (A) 3mM of prednisolone and (B) 1 mM dexamethasone was able to inhibit 50% of the 207 cell line growth 24h hours after incubation.

CONCLUSION

We established the experimental conditions of transfection and silencing of *BTG1* and *IKZF1* genes in an ALL cell line. In addition, it was identified the IC50 of dexamethasone and prednisolone in 207 cell line. The development of cell line models mimicking *BTG1* and/or *IKZF1* deletion consists in an important strategy to understand their impact on glucocorticoid resistance. Next, we aim to assess cell viability and evaluate the expression profile of genes associated with glucocorticoid response (*NR3C1, DUSP1, FBXW7* and *SGK1*) in a *BTG1* and *IKZF1* knockdown background.

KEYWORDS: Leukemia, Glucocorticoid Resistance.

Acknowledgment: CNPq, FAPERJ, CAPES e MINISTÉRIO DA SAÚDE

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA

INCA SUS MINISTÉRIO DA SAÚDE PÁTRIA AMADA BRASIL GOVERNO FEDERAL