INVESTIGATION OF EFFECTS OF LQB-223 IN AML CELL LINES WITH DIFFERENT RESISTANCE PHENOTYPES

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Introduction

Acute myeloid leukemia (AML) is a clonal disease identified by myeloid precursor cells that lost the capacity to differentiate and increase of cell proliferation. The standard treatment of AML patients involves the association of cytarabine and anthracyclines. However, this treatment has been presenting high death rate and low overall survival after five years for AML. For these reasons, is essential to investigate new strategies to overcome the observed resistance, including new drugs. In this context, the LQB-223 is a new compound that demonstrates a high antitumoral potential in tumoral cell lines including AML. Thus, our objectives are to evaluate and characterize the antitumoral effect in AML cell lines with different resistance phenotypes to standard treatment.

Methods

The AML cell lines U937 (M4/M5 subtype with complex karyotype), HL60 (M2 subtype responsive to standard treatment) and HL60R (HL60 derived and extrinsically resistant to 50 M of Cytarabine) were treated with 5 M of LQB-223 for 24 hours. To investigate the possible DNA damage and modulation of cell cycle regulation pathways, the Western Blotting technique was used after treatment with LQB-223. To elucidate the subcellular localization of transcription factor FOXO3a, we used the immunofluorescence technique after treatment with LQB-223.

Results and Conclusions

Our results showed that LQB-223 increased p-H2AX expression in all cell lines tested. These results indicate an increase of DNA damage by double strand break. Besides that, LQB-223-treated cells demonstrated decreased expression of protein targets involved in cell cycle regulation pathways. The cell lines HL60 and U937 treated with LQB-223 presented decreased expression levels of ERK and p-ERK, but with no alteration of expression in HL60R cells. We also observed decreased levels of AKT and p-AKT in HL60 and of mTOR and p-mTOR in HL60R. The data indicate that LQB-223 treatment induces DNA damage and modulates pathways that regulate cell cycle, such as MAPK and PI3K/AKT/mTOR pathways. However, we have not observed modulation of subcellular localization of transcription factor FOXO3a in U937 cell line. This indicates that LQB-223 does not modulate the subcellular localization of this transcription factor. Taken together, our data suggests that LQB-223 mechanisms of action are related to DNA damage and important cell cycle regulation pathways.

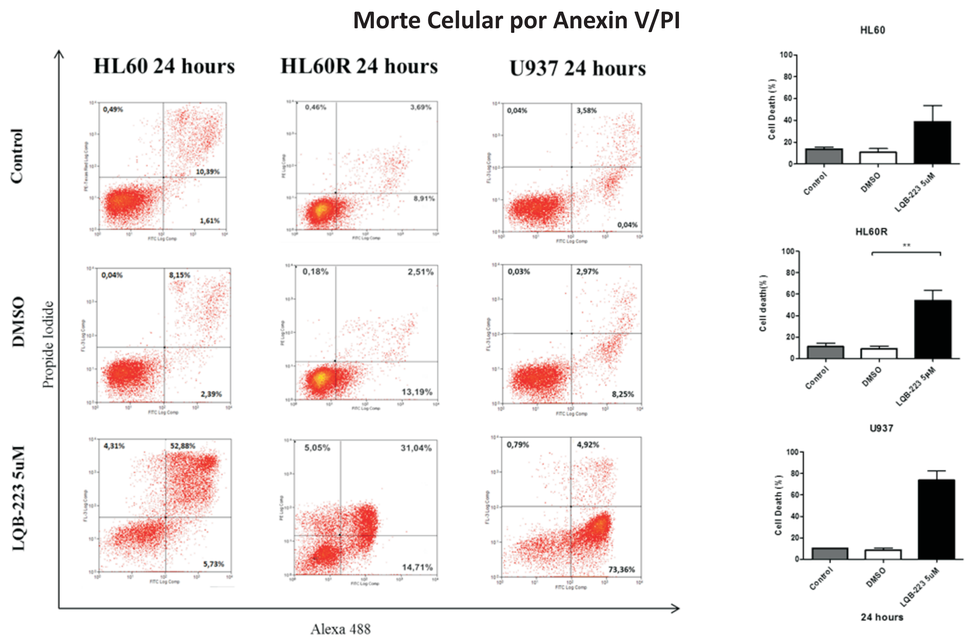

Figure 1: Cell death analysis of AML cell lines treated with LQB-223. Anexin V+ and Anexin V+ + PI cells of HL60, HL60R and U937 cell lines, after 24h of LQB-223 treatment.

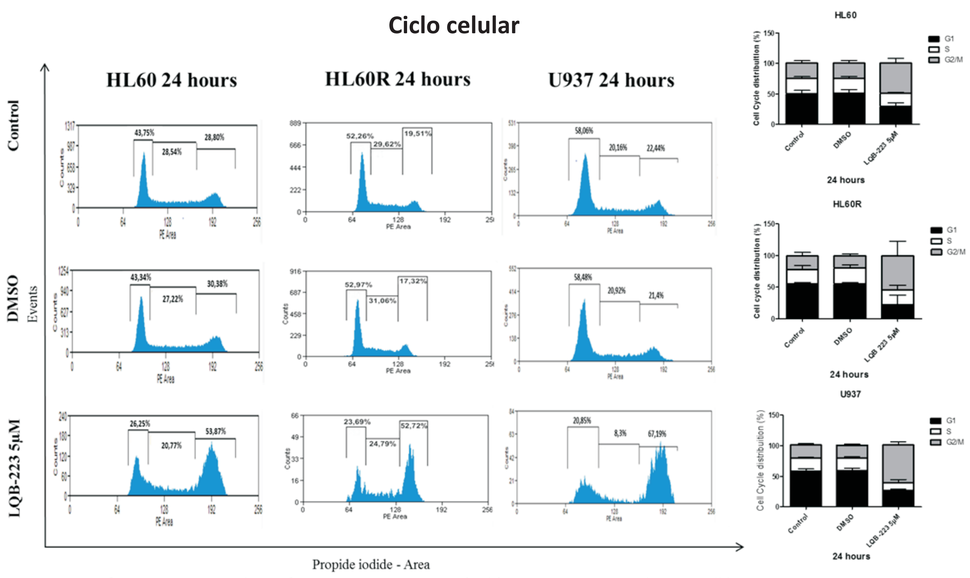

Figure 2: DNA fragmentation analysis after LQB-223 treatment (24h) in HL60, HL60R and U937 cell lines.

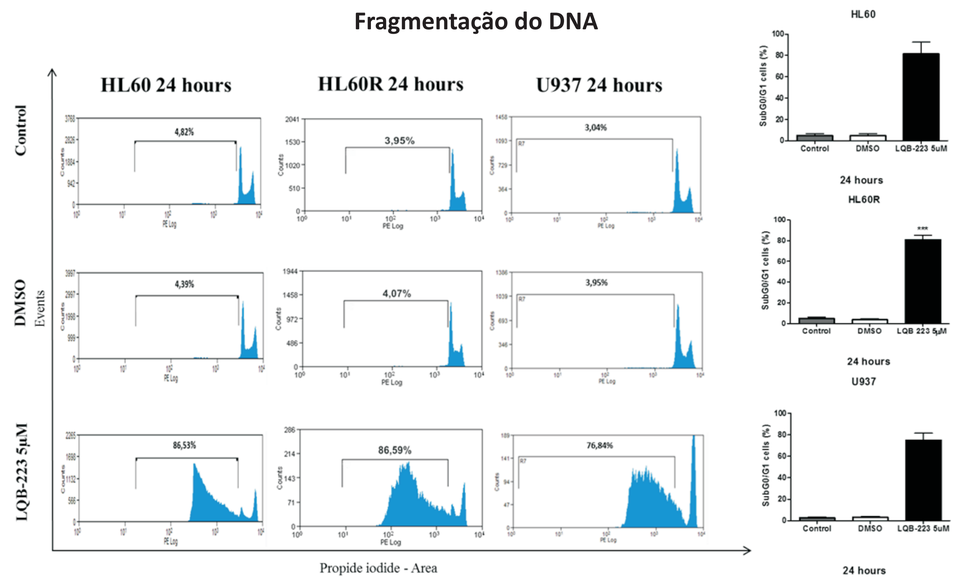

Figure 3: Cell cycle distribution of AML cell lines HL60, HL60R and U937 treated with LQB-223 for 24h.

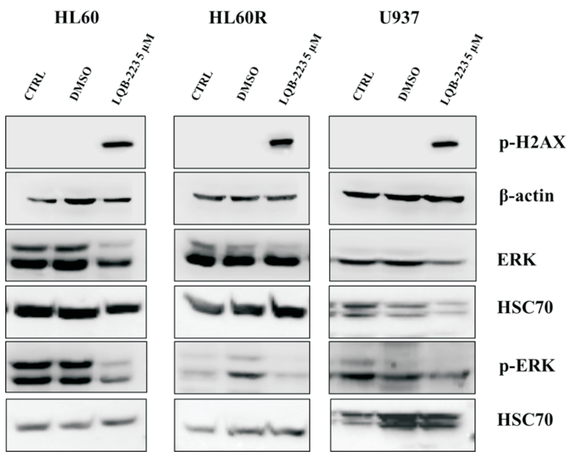

Figure 4: Western blot protein levels of H2AX phosphorylation, ERK and ERK phosphorylation, in AML cell lines HL60 (2N), HL60R (2N) and U937 (1N), after 24h of LQB-223 treatment.

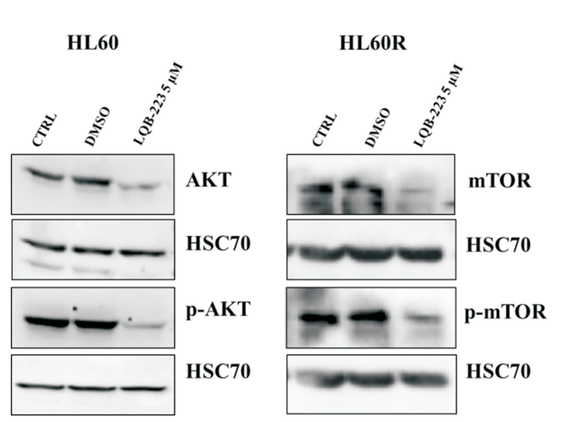

Figure 5: Western blot protein levels of AKT, AKT phosphorylation (HL60, 2N), mTOR and mTOR phosphorylation (HL60R, 2N), after 24h of LQB-223 treatment.

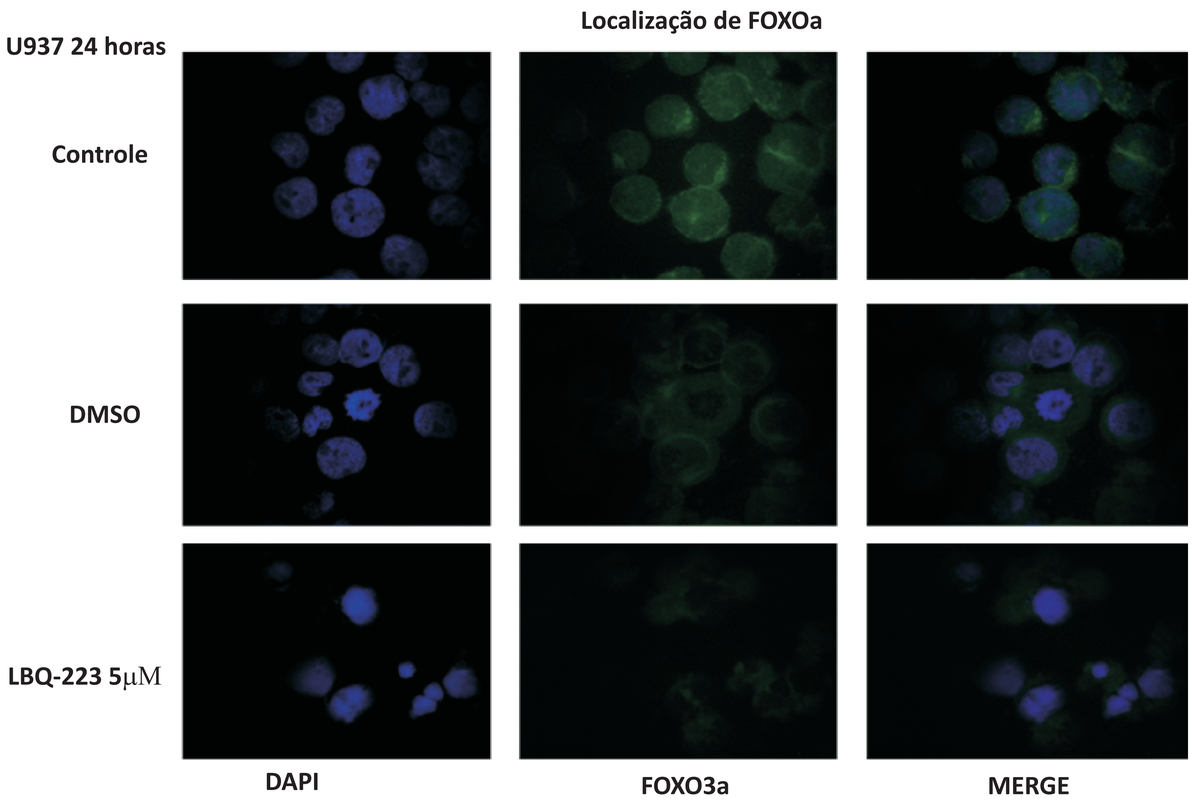

Figure 6: Immunofluorescence subcellular localization of FOXO3a, in AML cell line U937, after 24h of LQB-223 treatment. Representative of three experiments.

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