Characterization of acute myeloid leukemia cell line HL60R, resistant to cytarabine, and the antitumor effect of compounds LQB-118 and LQB-223

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BACKGROUND

Acute myeloid leukemia (AML) is a clonal disease characterized by the presence of myeloid cells that lost the ability to differentiate and have high proliferation rate. The standard treatment for AML patients have been the same for the past 40 years, the association of anthracyclines with cytarabine (ara-c). Although this schema had increased survival rate, patients often develop resistance to these drugs, one of the biggest hurdles in the treatment of AML. Previous data of our group showed that the pterocarpanoquinone LQB-118 and the aza-pterocarpanoquinone LQB-223 promoted low toxicity for lymphocytes isolated from healthy individuals. Thus, our purpose is: a) To characterize the molecular mechanisms associated with resistance to AML treatment, and b) To access cytotoxic activity of compounds LQB-118 and LQB-223 in HL60R (resistant to 50 µM ara-c) cell line.

METHODS

The HL60 cell line (parental) was treated with increasing concentrations of ara-c until resistance to 50µM (HL60R). Cell viability and death was accessed via MTT and Annexin V/PI assays, respectively. For further characterization of molecular targets involved in apoptosis and cell proliferation in HL60R, the Western blotting was applied. An array of phosphorylated proteins was carried out for identification of dysregulated pathways due to acquired resistance. Subcellular localization of transcription factor Nrf2 was accessed by immunofluorescence microscopy and HL60R was treated with the compounds to access cellular viability via MTT assay.

RESULTS

HL60R treated with ara-c had a maximum reduction of cell viability of 15% with 200 µM of ara-c in 72 hours. Ara-c did not induce cell death in HL60R when cells were stained with Annexin V/PI. HL60R presented an increase of Bcl-2 and Xiap protein levels, but no alteration was observed in pro-apoptotic proteins Bax and Bak-1 levels. Subcellular localization of Nrf-2 transcription factor was mainly nuclear in both cell lines (HL60 and HL60R), presenting a bigger focus in HL60R. The array of phosphorylated proteins revealed alterations in several signaling pathways. These changes in some pathways, not described before, may be related to acquired resistance to ara-c. The compounds LQB-118 and LQB-223 reduced HL60R cell viability from 3 µM and 5 µM, respectively, in 24 hours.

CONCLUSION

Our data validates the resistance to ara-c in HL60R cell line and suggest important signaling pathways associated with acquired resistance to this drug. LQB-118 and LQB-223 caused a potent antitumor effect in AML resistant cell line HL60R.

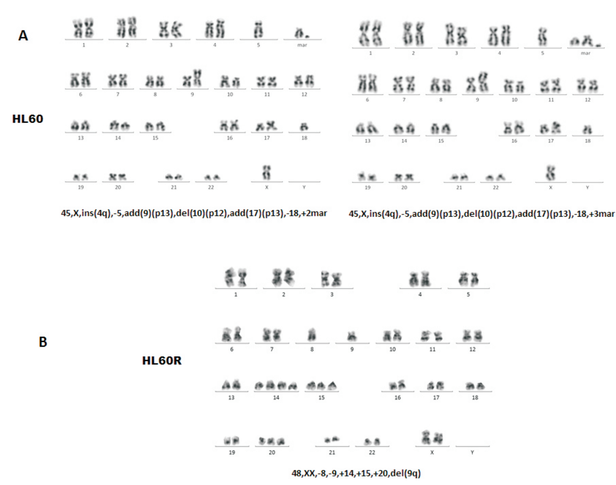

Figure 5: Analysis by classical cytogenetics (GTG banding) showing the chromosomal changes found in HL60 and HL60R cell lines.

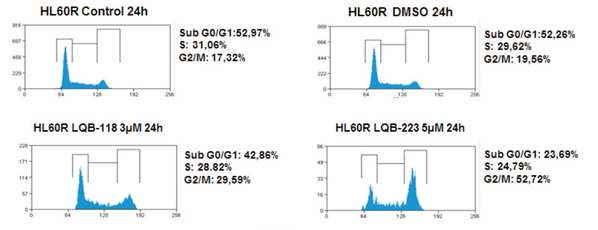

Figure 6: Protein expression in the cell lines HL60 and HL60R by Western blotting. The baseline evaluation of pro-apoptotic proteins (Bax and Bak-1), anti-apoptotic (Bcl-2, and XIAP), proteins involved with proliferation (Survivin), cell survival (AKT) and NRF2 transcription factor, inHL60 and HL60R cell lines. For this result were considered three independent experiments.

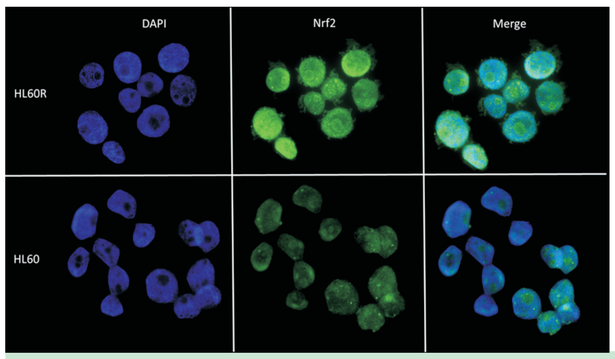

Figure 7: Evaluation of basal subcellular localization of the transcription factor NRF2 in HL60 HL60R cell lines. For this test were performed two independent experiments.

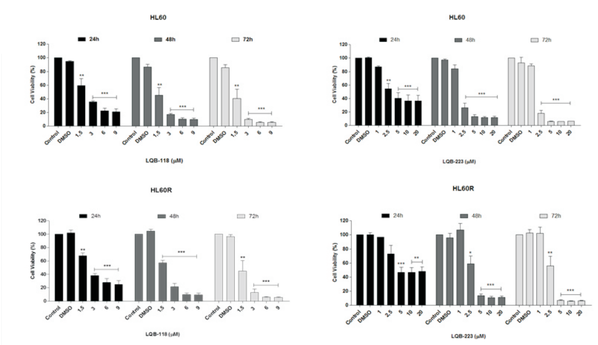

Figure 8: Evaluation of cell viability in HL60 (A and C) and HL60R (B and D) cell lines treated with different concentrations of LQB-118 and LQB-223 after 24h, 48h and 72h. Cell lines were compared with their control of cells trated with DMSO. The graphs correspond to the mean ± standard deviation of three independent experiments. ANOVA with post-test Bonferroni * p <0.05, ** p <0.01, *** p <0.001.

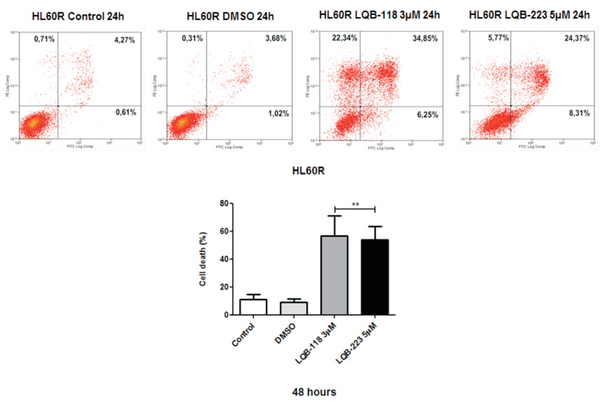

Figure 9: Evaluation of cell death in HL60 (A) and HL60R (B) cell lines treated with new compouds LQB-118 and LQB-223 after 24h. Statistical analyzes of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD. Student's t-test * p <0.05, ** p <0.01, *** p <0.001 compared the DMSO.

Figure 10: Evaluation of cell cycle profile in HL60R cell line treated with new compouds LQB-118 and LQB-223 after 24h. For these results were considered three independent experiments.

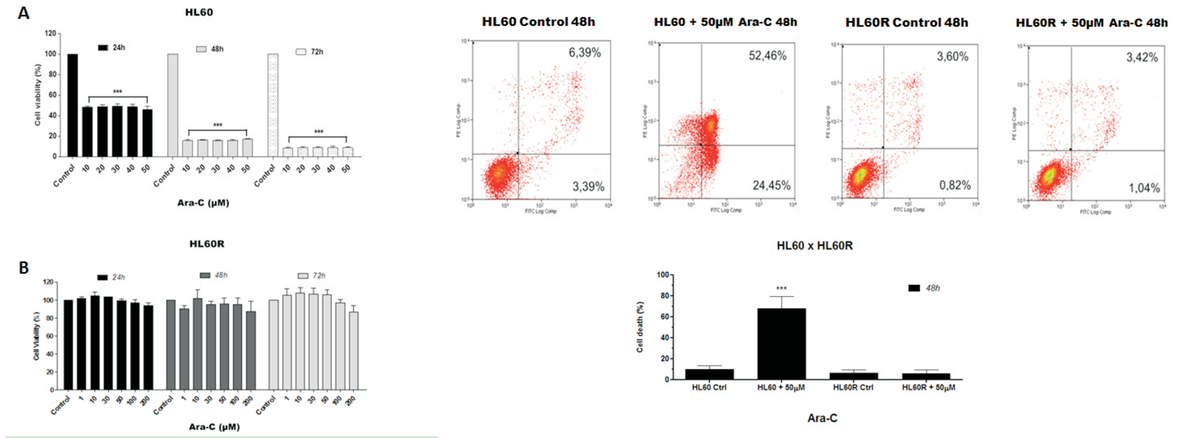

Figure 1: Evaluation of cell viability in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 24h, 48h and 72h. Cell lines were compared with their control (untreated cells). The graphs correspond to the mean ± standard deviation of three independent experiments. ANOVA with post-test Bonferroni * p <0.05, ** p <0.01, *** p <0.001

Figure 2: Evaluation of cell death in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 48h. Statistical analyzes of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD. Student's t-test * p <0.05, ** p <0.01, *** p <0.001 compared the DMSO.

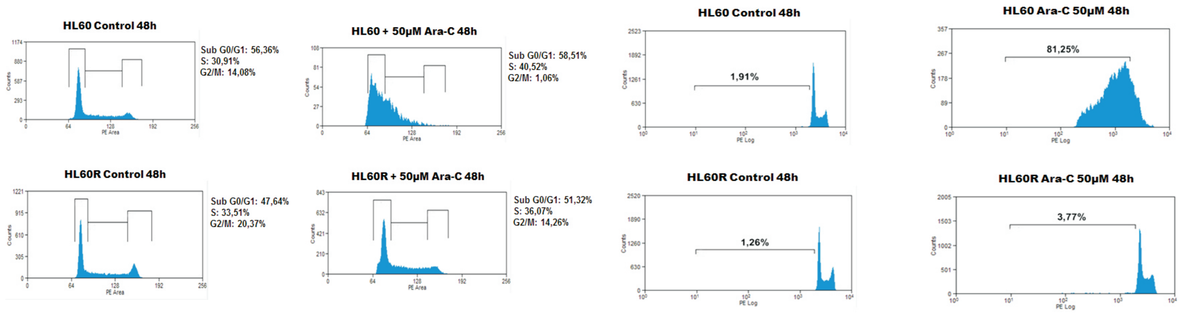

Figure 3: Evaluation of cell cycle profile in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 48h. For these results were considered two independent experiments.

Figure 4: Evaluation of DNA fragmentation in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 48h. For these results were considered two independent experiments.

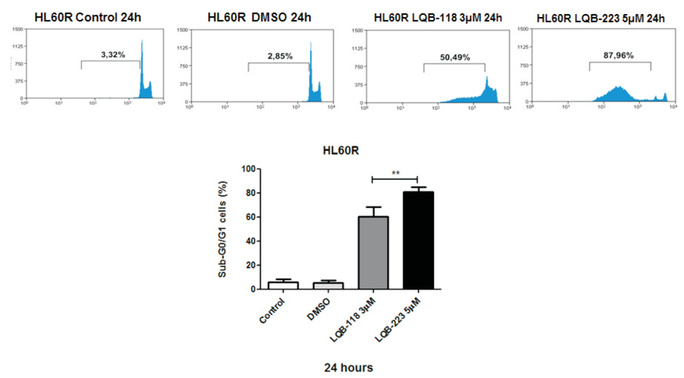

Figure 11: Evaluation of DNA fragmantation in HL60R cell line treated with new compouds LQB-118 and LQB-223 after 24h. Average of five independent experiments ± standard deviation. Student's t-test * p <0.05, ** p <0.01, *** p <0.001 compared to DMSO.

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