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MDR1, NFKB and PI3K/AKT Pathways Predict Responsiveness to Eribulin in Hematological Malignancies

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Background

Acute leukemias comprises a poor prognosis hematological neoplasms group originating from mutated bone marrow progenitor cells. High mortality and relapse rates related to these diseases make the research for new therapeutic options imperative. Eribulin is a novel microtubule inhibitor currently used in breast cancer therapy, but its effects on acute leukemias have been poorly explored. In addition, understanding eribulin-related resistance mechanisms may help improve treatment response

Objective

To investigate the cellular and molecular effects of eribulin on leukemia phenotype and to evaluate possible biomarkers of response to eribulin, using a molecularly heterogeneous panel of blood cancer cells.

Methodology

A panel containing 13 myeloid neoplasms and 12 lymphoid neoplasms cell lines were used for initial cell viability assays. NB4, NB4-R2, OCI-AML3, MOLM13, Jurkat, and Namalwa cell lines were selected for additional detailed analyzes. Cells were treated with increasing concentrations of eribulin (0-100 nM). Cell viability was assessed by MTT, clonogenicity by colony formation assay, apoptosis by annexin-V/7AAD staining and flow cytometry, cell cycle by propidium iodide staining and flow cytometry, and cell morphology by H&E staining and optical microscopy. Molecular markers of proliferation (STMN1), apoptosis (PARP1), and DNA damage (p-H2AX) were investigated by Western Blot. A correlation was performed between the IC_{50} and gene and protein expression of pathways previously associated with eribulin resistance. Statistical analyzes were performed by ANOVA and Bonferroni post-test and Spearmen test. A p-value <0.05 was considered significant.

Results

Eribulin reduces cell viability of blood cancer cells but not normal leukocytes

Eribulin presented high cytotoxicity in blood cancer cells, only 5 of out 21 cells were considered resistant to the drug (IC_{50}>100 nM). Eribulin-sensitive cells displayed dose and time-dependent cytotoxicity (IC_{50} ranged from 0.13 to 12.12 nM for 72 h).

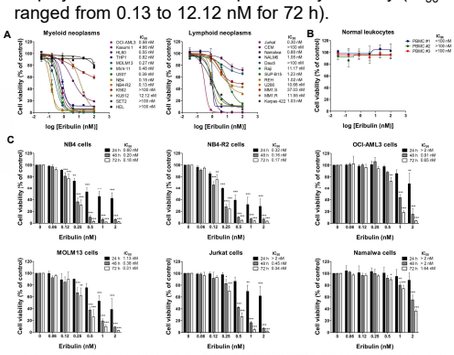

Figure 1 – Eribulin shows time-dependent cytotoxic activity in several cell lines of myeloid and lymphoid neoplasms, but does not affect the cell viability of normal leukocytes. (A) Dose-response cytotoxicity and time-response analyzes using the methylthiazoletrazolium (MTT) assay for a panel of myeloid and lymphoid neoplasm cells treated with increasing concentrations of eribulin (nM) for 72h. **(B)** Dose-response curves for eribulin (0.032 – 100 nM) after 72 hours of exposure in peripheral blood mononuclear cells (PBMC) from three healthy donors. note that at the concentrations tested the IC50 was not achieved (IC50 > 50 µM) in normal leukocytes. **(C)** Bar graphs represent dose and time responses to eribulin (0.06 – 2 nM) after 24, 48 and 72 hours exposure in NB4, NB4-R2, OCI-AML3, MOLM13, Jurkat and Namalwa cells. Values are expressed as the percentage of viable cells for each condition relative to untreated controls. Results are presented as mean ± SD of at least four independent experiments. The p-values and cell lines are indicated in the graphs; *p<0.05, **p<0.01, ***p<0.001; ANOVA test and Bonferroni post-test.

Eribulin reduces clonogenicity, promotes apoptosis and induces cell cycle arrest in acute leukemia cells

In acute leukemia cells, eribulin significantly decreased clonogenicity to long-term exposure, increased apoptosis, induced subG1 cell accumulation, and cell cycle arrest at the G_2/M phase upon 48 h of drug exposure (all p<0.05).

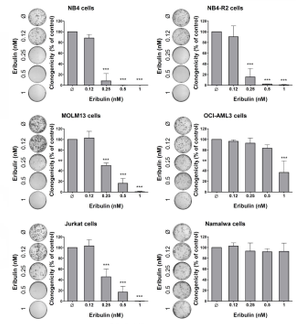

Figure 2 – Eribulin treatment reduces the clonogenicity of NB4, NB4-R2, MOLM13, OCI-AML3 and Jurkat cells. Colonies containing viable cells were detected by adding MTT reagent after 8 days of culturing the cells in question treated with eribulin (0.12, 0.25, 0.5 and 1 nM) in the long term and normalized to the corresponding treated with DMSO controls (Ø). Colony images are shown for one experiment and bar graphs show the mean ± SD of at least three independent experiments. p<0.0001; ANOVA test and Bonferroni post-test.

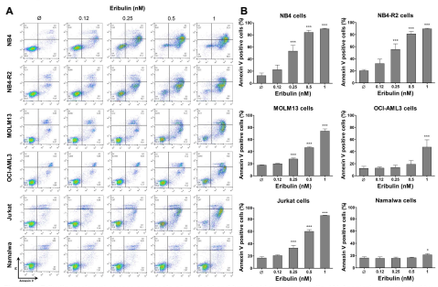

Figure 3– Eribulin treatment causes apoptosis of myeloid and lymphoid neoplasm cells. (A) Apoptosis was detected by flow cytometry in NB4, NB4-R2, MOLM13, OCI-AML3, Jurkat and Namalwa cells treated with graded concentrations of eribulin (0.12, 0.25, 0.5 and 1 nM) for 48 hours using annexin staining method V/7AAD. Representative dot plots are shown for each condition; the upper and lower right quadrants (Q2 + Q3) cumulatively contain the apoptotic population (annexin V+ cells). **(B)** Bar graphs represent the mean ± SD of at least four independent experiments quantifying apoptotic cell death. The p-values and cell lines are indicated in the graphs; *p<0.05, **p<0.01; ***p<0.0001 for eribulin treated cells compared to DMSO treated cells (Ø); ANOVA test and Bonferroni post-test.

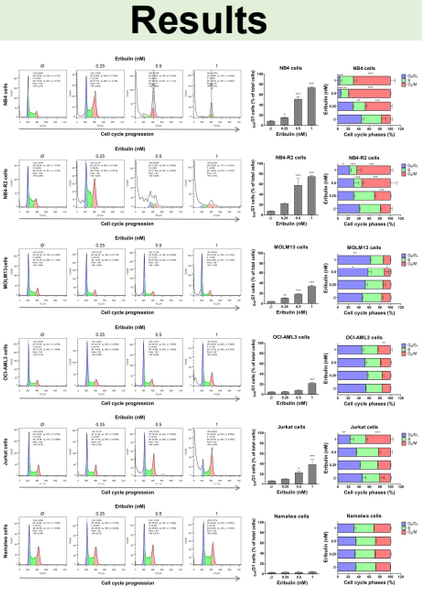

Figure 4 – Eribulin causes cell cycle arrest in leukemic cells in G2/M phases. Cell cycle phases were determined by DNA content analysis by propidium iodide staining and flow cytometry in NB4, NB4-R2, MOLM13, OCI-AML3, Jurkat and Namalwa cells treated with eribulin (0.25, 0. 5 and 1 nM) or vehicle for 48 hours. A representative histogram for each condition is illustrated. The vertical bar graph represents the mean ± SD of the cell percentages in subG1 from at least three independent experiments. The horizontal bar graph represents the mean ± SD of cell distributions that are in the G0/G1, S and G2/M phases of the cell cycle (excluding subG1) from at least three independent experiments. The p values and cell lines are indicated in the graphs; *p < 0.05, **p < 0.01, ***p < 0.001 for DMSO treated cells (Ø) vs. Eribulin; ANOVA and Bonferroni post-test.

Eribulin treatment causes mitotic aberrations in acute leukemia cells

Morphological analysis by H&E staining indicated aberrant mitosis, which corroborates cell cycle findings.

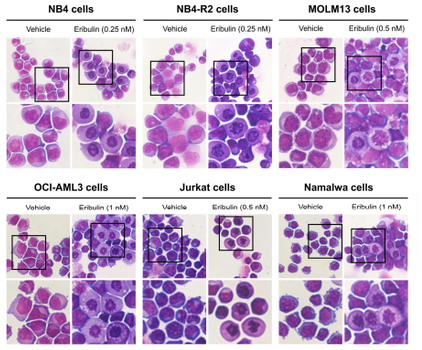

Figure 5. Eribulin treatment generates microtubule instability and aberrant mitoses in acute leukemia cell lines. NB4, NB4-R2, MOLM13, OCI-AML3, Jurkat and Namalwa cells were treated with vehicle or Eribulin for 48 h, fixed and stained with hematoxylin and eosin (H&E). 400× and 1000× magnification images are illustrated.

Eribulin induces molecular markers of DNA damage and apoptosis in acute myeloid and lymphoid leukemia cells

In the molecular scenario, eribulin reduced STMN1 expression and activity, and induced PARP1 and H2AX phosphorylation, indicating a reduction of cell proliferation, apoptosis, and DNA damage (all p<0.05).

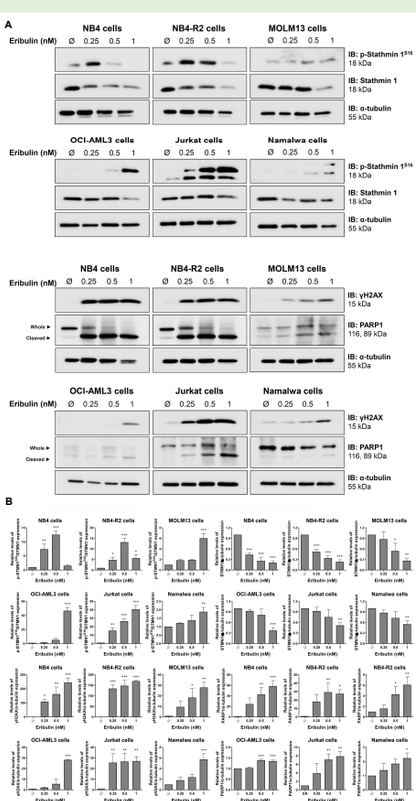

Figure 7 – Eribulin induces molecular markers of DNA damage and apoptosis in acute leukemia cells. (A) Western blot analysis for levels of phospho(p)-stathmin 1S16, STMN1, γH2AX, and PARP1 (total and cleaved) in total cell extracts of Eribulin-treated acute leukemia cells (0.25, 0.5 and 1 nM) or vehicle for 48 hours; Membranes were incubated with the indicated antibodies and developed with the SuperSignal™ West Dura Extended Duration Substrate System and Gel Doc XR+. **(B)** Bar graphs represent the mean ± SD of three independent experiments that quantify the intensities of the indicated protein bands. * p < 0.05, ** p < 0.01, *** p < 0.001; ANOVA and Bonferroni post-test.

MDR1, NFKB and PI3K pathways are related to eribulin resistance in acute leukemia cells

Notably, higher IC50 for eribulin was significantly correlated with high expression of NFkB p65 (total and phosphorylated), MDR1 (ABCB1 and ABCC1), and AKT phosphorylation in blood cancer cells (all p<0.05).

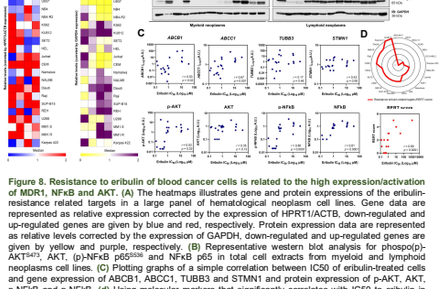

Figure 8. Resistance to eribulin of blood cancer cells is related to the high expression/activation of MDR1, NFkB and AKT. (A) The heatmaps illustrates gene and protein expressions of the eribulin-resistance related targets in a large panel of hematological neoplasm cell lines. Gene data are represented as relative expression corrected by the expression of HPRT1/ACTB, down-regulated and up-regulated genes are given by blue and red, respectively. Protein expression data are represented as relative levels corrected by the expression of GAPDH, down-regulated and up-regulated genes are given by yellow and purple, respectively. **(B)** Representative western blot analysis for phospo(p)-AKT^{S473}, AKT, (p)-NFkB p65^{S536} and NFkB p65 in total cell extracts from myeloid and lymphoid neoplasms cell lines. **(C)** Plotting graphs of a simple correlation between IC50 of eribulin-treated cells and gene expression of ABCB1, ABCC1, TUBB3 and STMN1 and protein expression of p-AKT, AKT, p-NFkB and p-NFkB. **(D)** Using molecular markers that significantly correlates with IC50 to eribulin in hematological neoplasms, a resistance eribulin-related targets (RERT) score was created, in which each cell line receives one point for each gene/protein upregulated (the median was used as the cutoff, maximum of points = 5). The radar graph shows the distribution of points among the analyzed cell lines. Note that cells most resistant to eribulin received the highest scores.

Conclusions

Eribulin reduced the cell viability of acute leukemia cells by disturbing microtubule dynamics and leading to mitotic collapse and cell death, proving to be a potential therapeutic option for blood cancers. Our data indicate that NFkB, MDR1, and PI3K/AKT expression and activation may be useful biomarkers of responsiveness to eribulin in hematological malignancies. Financial support: FAPESP, CNPq and CAPES.

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