# Decitabine modulates microRNA-34b expression and potentiates chemotherapy-induced apoptosis in Burkitt lymphoma cells

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## BACKGROUND

Burkitt lymphoma (BL) is a B-cell lymphoma frequently diagnosed in children. It is characterized by MYC translocations, which lead to the constitutive expression of the MYC oncogene. MiR-34 family members of microRNAs are direct transcriptional targets of p53 and constitute a part of p53 tumor suppressor network regulating cell-cycle arrest, apoptosis, and senescence. Interestingly, MYC, which is deregulated in the BL, is a direct target of miR-34b. MiR-34s can be silencing by promoter methylation in high number of cancer types. Furthermore, the loss of miR-34 expression has been linked to resistance against apoptosis induced by p53 activating agents used in chemotherapy.

## OBJECTIVES

In this study, we aimed to evaluate the effects of a DNA demethylation agent (decitabine) on miR-34b methylation and expression status in BL cell lines. Additionally, we analyzed the effect of decitabine alone or combined with etoposide (VP-16) on viability, proliferation and cell death of BL cells.

## METHODS

BL cell lines (Ramos, Raji and P493-6) with different TP53 mutation profile were treated with 5-aza-2'-deoxicitidine (decitabine) and evaluated for miR-34b expression and methylation status. MiR-34b methylation analysis was performed by MSP-PCR. The expression levels of miR-34b were measured using Real-time PCR. Predict miR-34b targets MYC, CDK6 and BCL-2 were evaluated by Western blot assay after decitabine treatment. Cell cycle and cell death analysis were assessed by flow cytometry after combined treatment.

## RESULTS

BL cell lines presented miR-34b promoter methylation in four CpG sites analyzed. Decitabine treatment reduced the methylation on CpG regions and induced miR-34b expression in BL cells. Moreover, a decrease of miR-34b protein targets (MYC, CDK6 and BCL-2) through decitabine treatment was observed. Decitabine treatment also triggered apoptosis, DNA fragmentation and reduction of cell viability. Cell death was enhanced by the combined treatment with Decitabine and VP16. Additionally, we analyzed the miR-34b expression levels in formalin-fixed, pediatric BL tissues from a cohort of patients (n = 41). All the BL cases showed low miR-34b levels suggesting that miR-34b methylation may be a mechanism related to miR-34b regulation in BL tumors.

## CONCLUSION

Epigenetic silencing of miR-34b by DNA methylation may contribute to miR-34b repression in BL cells. Decitabine can improve BL treatment. Besides, the present study provides a starting point for novel therapeutics approaches.

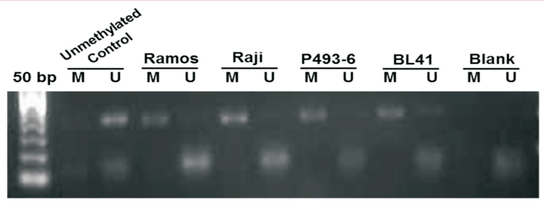



**Figure 1: miR-34b methylation status.** Amplified samples by methylation-specific PCR (MSP) were submitted to electrophoretic run in 2% agarose gel. It is observed an amplification of the specific methylation reactions (M) in all cell lines: Ramos, Raji, P493-6 and BL41.

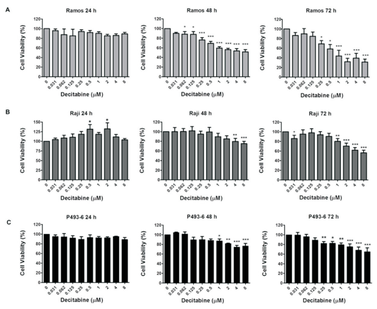



Figure 2: Analysis of the percentage of viable cells after decitabine treatment. The percentage of viable cells (y axis ) of each cell line was analyzed by MTT assay for 24, 48 and 72 hours after treatment with different decitabine concentrations (x axis). Ramos cells (A), Raji cells (B) and P493-6 cells (C). Representative graphics of the mean and standard error of 3 independent experiments were shown.

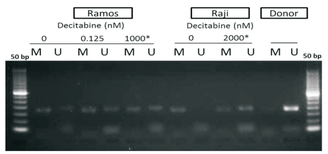
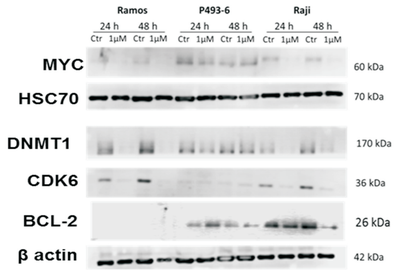



**Figure 3: Analysis of methylation status by MSP after decitabine treatment.** Ramos cell line was treated with 0.125 nM and 1 μM of decitabine and Raji cells was treated with 2 μM. The plasma DNA sample from a healthy donor was used as a positive control of unmethylated reaction. The PCR-MSP products was submitted to electrophoretic run in 2% agarose gel.



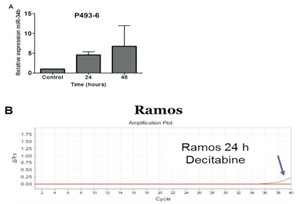




**Figure 4: The miR-34b expression analysis in Ramos and P493-6 cell lines**. Real Time PCR assay was perfomed in P493-6 and Ramos cell lines. (A) The quantitative analysis of miR-34b in P493-6 was performed for 24 and 48 hours with decitabine treatment. (B) The curve shows a increase of miR-34b in Ramos cell line treated with decitabine for 24 hours.

**Figure 5: miR-34b target-proteins expression analysis in BL cells**. It is observed a decreased on MYC, CDK6 and DNMT1 protein levels in treated samples compared to control samples Ramos, P493-6 and Raji cell lines. It was detected a reduction of BLC-2 levels in P493-6 and Raji cells. HSC70 and β-Actin were used as the endogenous control.

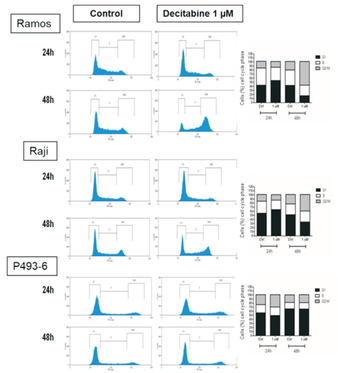



**Figure 6: Cell cycle analysis in Ramos, Raji and P493-6 cell lines after decitabine treatment.** The incubation times with decitabine were 24 and 48 hours. The analyzes was established on 10,000 events. The left panel is a representative cell cycle analysis from one experiment and the right graphics show the percentage of the mean of 3 independent experiments.

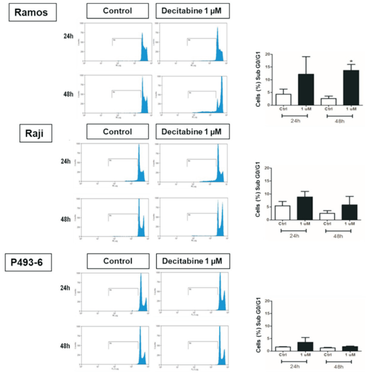



**Figure 7: Cell fragmentation analysis in Ramos, Raji and P493-6 cells treated with 1 μM Decitabine for 24 and 48 hours.** It is observed an increase of cell fragmentation in the treated samples compared to the control samples in Ramos and Raji cell lines. In the left, representative cell fragmentation analysis from one experiment and the graphics represent the mean percentage from 3 independent experiments.

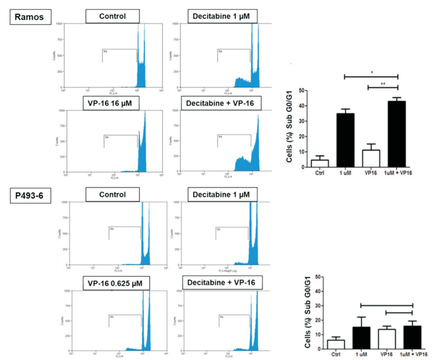



**Figure 8: Cell fragmentation analysis in Ramos and P493-6 cells treated with 1 μM Decitabine and/or VP-16 for 48 hours.** It is observed an increase of cell fragmentation in Ramos cells treated with decitabine alone and in combination with VP-16 compared to the control samples. In the left, representative cell fragmentation analysis from one experiment and the graphics represent the mean percentage from 3 independent experiments. Student T Test * p < 0.05, ** p < 0.01, ***p <0.001.

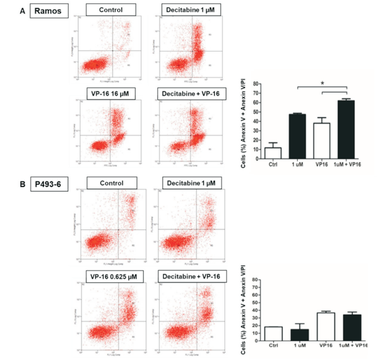



**Figure 9: Cell death analysis by flow cytometry in Ramos and P493-6 cells treated with 1 μM Decitabine and/or VP-16 for 48 hours.** Annexin V/PI staining assay. It is observed an increase of cell death in Ramos cells treated with decitabine in combination with VP-16 compared to decitabine alone. In the left, representative dot plot from one experiment and the graphics represent the mean percentage from 3 independent experiments. Student T Test * p < 0.05, ** p < 0.01, ***p <0.001.

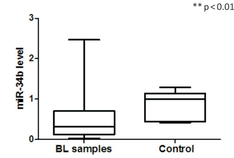



**Figure 10: miR-34b expression in BL tumour samples.** miRNA expression level was evaluated by Quantitative Real time QT-PCR in 41 tumour samples from pediatric BL and 5 samples from reactive lymph node. RNU6B was used as endogenous control

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