Deregulated expression of miR-29 family members in Burkitt lymphoma cells is associated with modulation of targets involved in proliferation, apoptosis inhibition and DNA methylation

L. Mazzoccoli[1], M. Robaina[1], M. Bonamino[2], L.M.M.D. Rezende[3], E. Queiroga[4], C.E. Bacchi[4], C.E. Klumb[1].

1 Programa de Pesquisa em Hemato-Oncologia Molecular, Instituto Nacional de Câncer, Rio de Janeiro, Brasil
2 Programa de Carcinogênese Molecular, Coordenação de Pesquisa, Instituto Nacional de Câncer, Rio de Janeiro, Brasil
3 Divisão de Patologia, Instituto Nacional de Câncer, Rio de Janeiro, Brasil
4 Consultoria em Patologia, Consultoria em Patologia, Botucatu - São Paulo, Brasil

INTRODUCTION AND OBJECTIVES

MicroRNAs are small non-coding regulatory RNAs (19-25 nucleotides) that bind to specific sites of their target genes and regulate post-transcriptional gene expression. Aberrant expression levels of microRNAs have been described in several cancers. Recently, we described that miR29-a, miR29-b, miR29-c expression levels are reduced in Burkitt lymphoma tumors. BL is an aggressive B-cell lymphoma characterized by MYC oncogene translocations which leads to the constitutive expression of the MYC oncogene. c-MYC was shown to contribute to miR-29 repression through E-box MYC binding site located on the miR-29 promoter region. Potential targets of mir-29s contributing to the malignant transformation include the cyclin-depedent kinase 6 cell (CDK6) a cell cycle regulator, the anti-apoptotic protein Mcl-1, and TCL-1, a protein critical in the transduction of antiapoptotic signals in B and T cells. Moreover, miR-29s target epigenetic regulators as DNA methyltransferases (DNMTs). In the present study, we thus aimed to investigate the role of miR-29a/b/c and their targets in the BL pathogenesis.

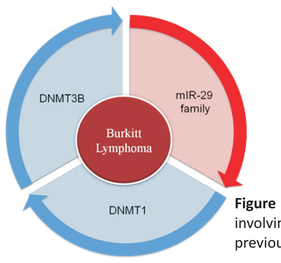
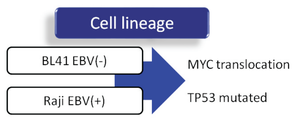


Figure 1. Scheme of the hot spots involving BL pathogenesis found in our previously published work.



Tumor samples from BL patients

57 tumor samples from patients diagnosed with BL, registered at the Serviço de Hematologia, INCA, Rio de Janeiro, Brazil from 1995 to 2011.

RESULTS

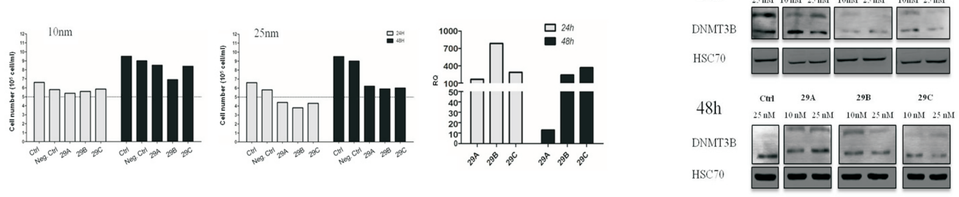


Figure 1. The effect of miR-29 family mimetics on DNMT3B expression and cell viability on BL41 cells. (A) Cell viability after 24h and 48h post transfection. (B) qPCR to detect the expression levels of transfected mimetics in comparison to the control. (C) The effect of miR-29 transfection on DNMT3B protein expression.

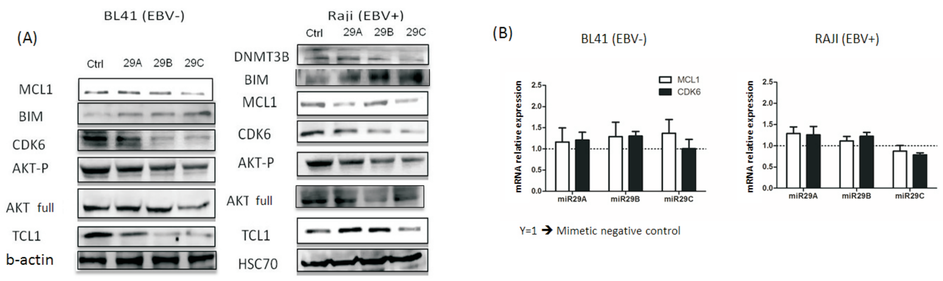


Figure 2. Detection of MCL1, CDK6, total and phosphorylated AKT, TCL1, DNMT3B and BIM protein expression and MCL1 and CDK6 mRNA expression after 24h of miR-29's mimetics transfection. (A) Protein expression 24h after 50 nM of mimetic transfection in BL41 and Raji cells. (B) The effect of the mimetics on CK6 and MCL1 mRNA expression after 24h post mimetic transfection.

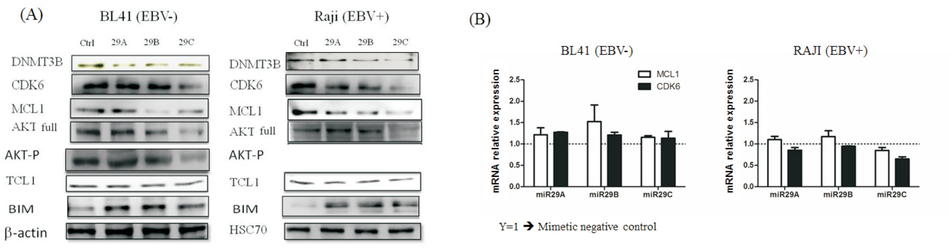


Figure 3. Detection of MCL1, CDK6, total and phosphorylated AKT, TCL1, DNMT3B and BIM protein expression and MCL1 and CDK6 mRNA production after 24h of miR-29's mimetics transfection. (A) Protein expression 8h after 50 nM of mimetic transfection in BL41 and Raji cells. (B) The effect of the mimetics on CK6 and MCL1 mRNA expression after 8h post mimetic transfection.

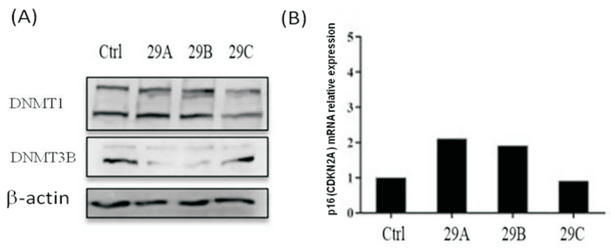


Figure 4. Inhibition of DNMT3B protein expression and its effects on *p16* mRNA. (A) DNMT3B and DNMT1 protein expression after 24h post transfection in BL41. (B) *p16* mRNA relative expression evaluated by qPCR after DNMT3B reduction, whereas the bars represent the relative expression in comparison to the negative control.

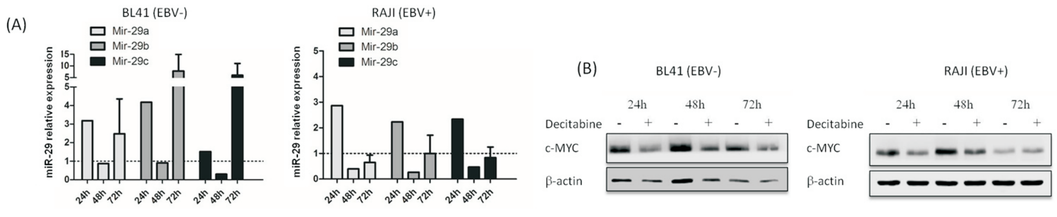


Figure 5. Induction of miR-29s expression after decitabine treatment. (A) BL41 and Raji cells were treated with 1 mM of Decitabine and evaluated for miR-29s expression by qPCR. (B) Decitabine also reduced c-MYC protein expression in both the lineages.

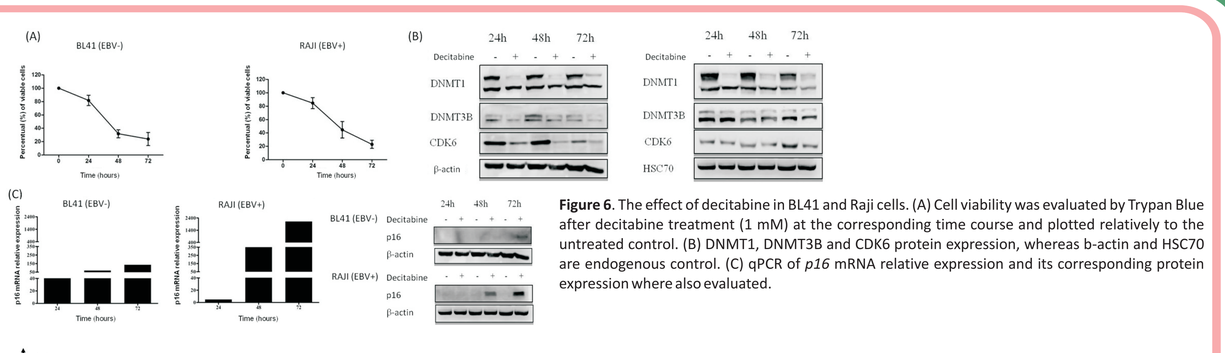


Figure 6. The effect of decitabine in BL41 and Raji cells. (A) Cell viability was evaluated by Trypan Blue after decitabine treatment (1 mM) at the corresponding time course and plotted relatively to the untreated control. (B) DNMT1, DNMT3B and CDK6 protein expression, whereas b-actin and HSC70 are endogenous control. (C) qPCR of *p16* mRNA relative expression and its corresponding protein expression where also evaluated.

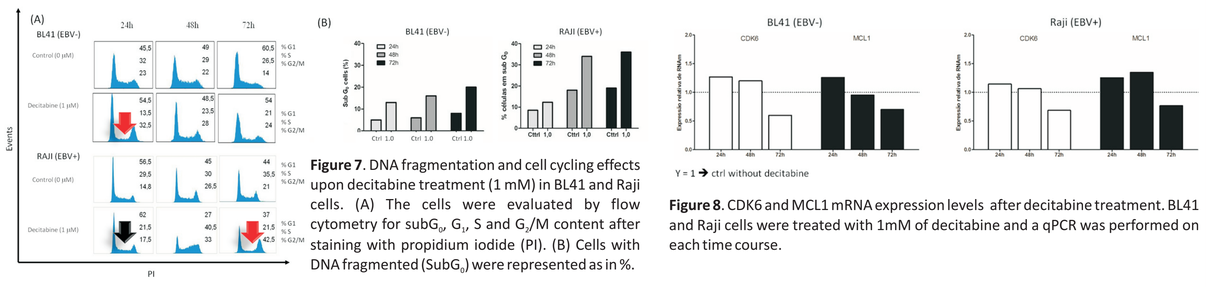
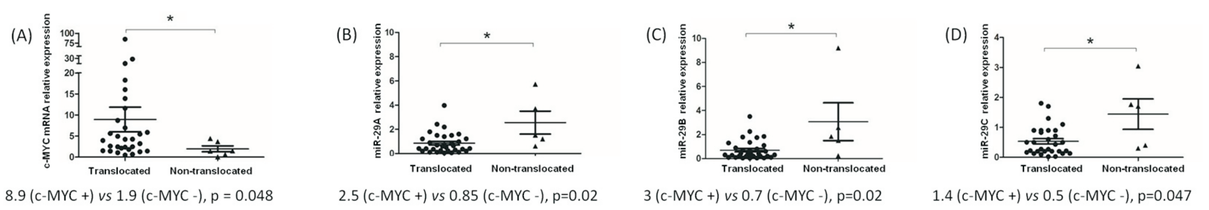


Figure 7. DNA fragmentation and cell cycling effects upon decitabine treatment (1 mM) in BL41 and Raji cells. (A) The cells were evaluated by flow cytometry for subG$_0$, G$_1$, S and G$_2$/M content after staining with propidium iodide (PI). (B) Cells with DNA fragmented (SubG$_0$) were represented as in %.

Figure 8. CDK6 and MCL1 mRNA expression levels after decitabine treatment. BL41 and Raji cells were treated with 1mM of decitabine and a qPCR was performed on each time course.



8.9 (c-MYC +) *vs* 1.9 (c-MYC -), p = 0.048

2.5 (c-MYC +) *vs* 0.85 (c-MYC -), p=0.02

3 (c-MYC +) *vs* 0.7 (c-MYC -), p=0.02

1.4 (c-MYC +) *vs* 0.5 (c-MYC -), p=0.047

Figure 9. c-MYC mRNA and miR-29 expression between translocated vs non-translocated BL tumor samples. (A) BL tumor samples positive for c-MYC translocation vs non-translocated tumor samples. (B) Expression of miR-29 family between translocated vs non-translocated tumor samples. The results represent the median between the samples, (*p<0.05, Man-Whitney test).

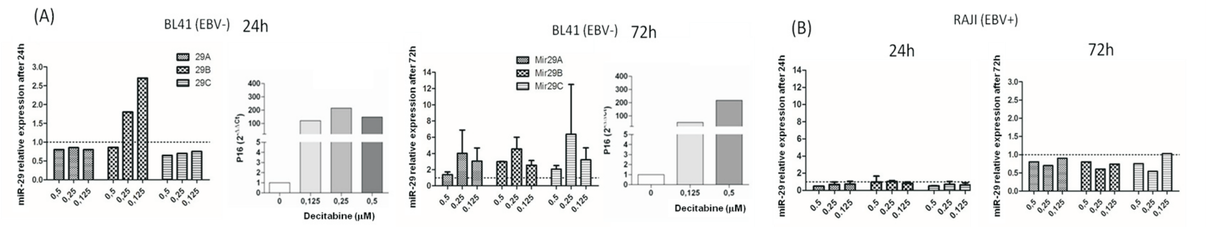


Figure 10. The effect of 0.5, 0.25 and 0.125 mM of decitabine on miR-29 and mRNA p16 expression in BL41 and Raji cells. (A) BL41 cells were evaluated by qPCR for mir-29 expression after 24h and 72h, and for p16 mRNA expression. (B) Raji cells were harvested and evaluated by qPCR for miR-29 expression after 24 and 72h. The dot line represents the negative control of the

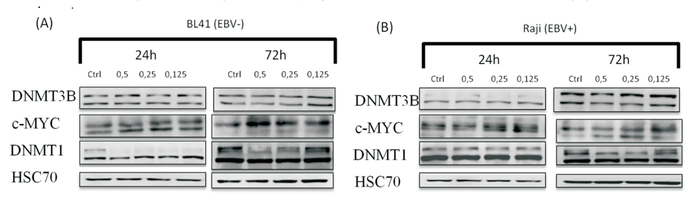


Figure 11. DNMT3B, DNMT1 and c-MYC protein expression after 0.5, 0.25 and 0.125 mM of decitabine. BL41 (A) and Raji cells (B) were treated with decitabine and evaluated after 24 and 72h for DNMT3B, DNMT1 and c-MYC protein expression. HSC70 was used as the endogenous control.

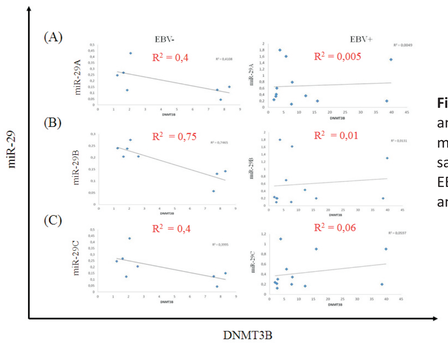
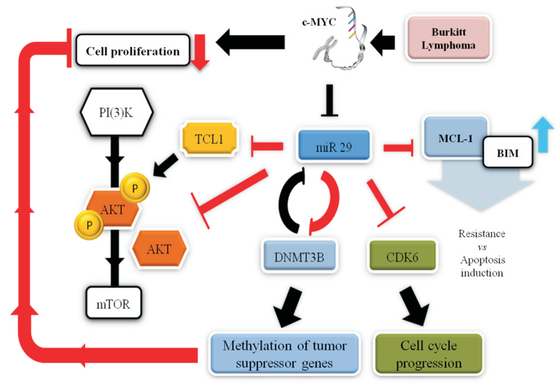


Figure 12. Analysis of miR-29 and DNMT3B mRNA expression in BL tumor samples positive for c-MYC translocation and its relationship with EBV. (A) miR-29A expression, (B) miR-29B and (C) mir-29C were analyzed vs DNMT3B mRNA expression regarding EBV status (EBV- or EBV+ BL samples). R^2 is the correlation coefficient. On EBV- BL samples, there is an inverse correlation between mRNA DNMT3B levels and miR-29s, whereas the counterpart EBV+ the correction is not observed. In summary, the EBV status may add another level of complexity on DNMT3B and miR-29 interaction, contributing to the pathogenesis.

CONCLUSIONS



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