Potential Roles of microRNA-29 family in the Pathogenesis of Burkitt Lymphoma: Therapeutic implications

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

ABSTRACTS

Introduction: Aberrant microRNAs expression has been reported in several cancers. Recently, we described reduced expression levels of miR29a/b/c in Burkitt lymphoma (BL) tumors. BL is an aggressive B-cell lymphoma characterized by MYC oncogene translocations. MYC was shown to inhibit miR-29 at the promoter gene. Potential targets of mir-29s contributing to the malignant transformation include the cell cycle regulator CDK6, the anti-apoptotic protein MCL1, and TCL1, a protein critical in the transduction of antiapoptotic signals in B and T cells. Moreover, miR-29s target epigenetic regulators as DNA methyltransferases (DNMTs). We investigated the role of miR-29a/b/c and their targets in the BL pathogenesis. **Methods and Results:** Mature sequences of miR-29a/b/c were transfected to the BL cell lines BL41 and Raji, and evaluated for DNMT3B, MCL1, BIM, CDK6, AKT and TCL1 protein expression as well as for MCL-1 and CDK6 mRNA expression. BL cells were treated with 5-aza-2'-deoxicitidine (decitabine) and evaluated for miR29 expressions and methylation status. DNMT3B inhibition was performed by DNMT3B siRNA. miR-29 a/b1 and miR-29b2/c gene CpG sequences were identified and analysed using *Methylation-specific* PCR.

The mir-29s mimetics modulated the levels of AKT and phosphorylated-AKT proteins, as well as TCL1. Furthermore, decreased CDK6, DNMT3B, MCL1, and increased BIM protein expressions levels were observed. Additionally, decitabine induced *p16* mRNA and protein expression followed by reduction of DNMT1/3B, MYC and CDK6 protein expression in BL cells, and increase of miR-29 expression. However, lower decitabine concentrations did not reduce MYC expression, but increased *p16* mRNA and miR-29 levels and decreased DNMT1. Methylation of CpG sequences at the miR-29b2/c gene promoter and miR-29a/b1 enhancer region was confirmed and decitabine altered the methylation profile. Moreover, DNMT3B siRNA modulated miR-29 expression. Notably, the *MYC* mRNA expression levels were lower in MYC (-) cases in comparison with those cases with *MYC* translocations as well as miR-29s expression levels between MYC-positive and negative cases. **Conclusions:** Our data suggest a role of miRs-29 in the BL pathogenesis and a potential therapeutic effect of epigenetic drugs in the BL treatment. Besides, the study also suggests that miR-29s regulation may involve DNMT-mediated CpG sequence methylation.

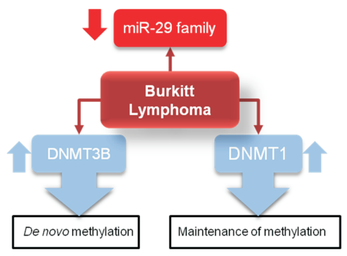
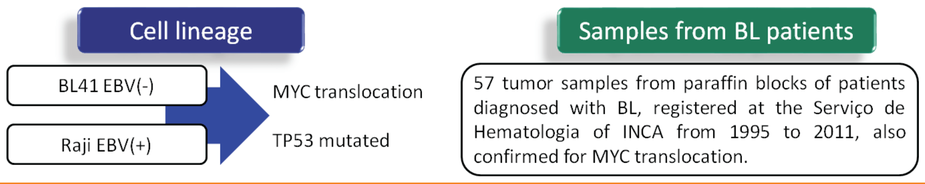


Figure 1. Scheme of the hot spots found in our previously published work with BL tumor samples.

METHODOLOGY



RESULTS

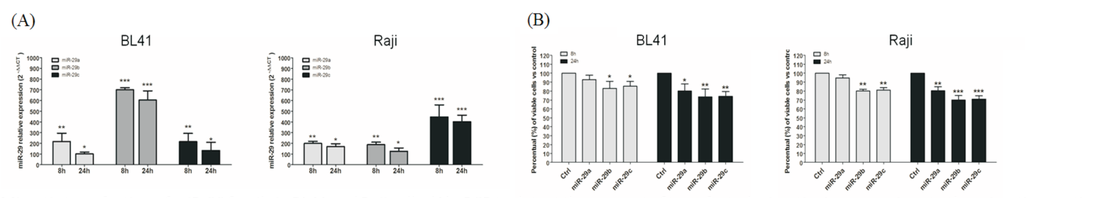


Figure 2. Mimetic transfection of miR-29 family in BL41 and Raji cells. (A) qPCR to detect the amount of transfected mimetics in comparison to the mimetic negative control after 8 and 24h. (B) Cell viability in comparison with the mimetic negative control after 8 and 24h pos transfection evaluated by Trypan blue. * p< 0.05; ** p <0.01; *** p< 0.001.

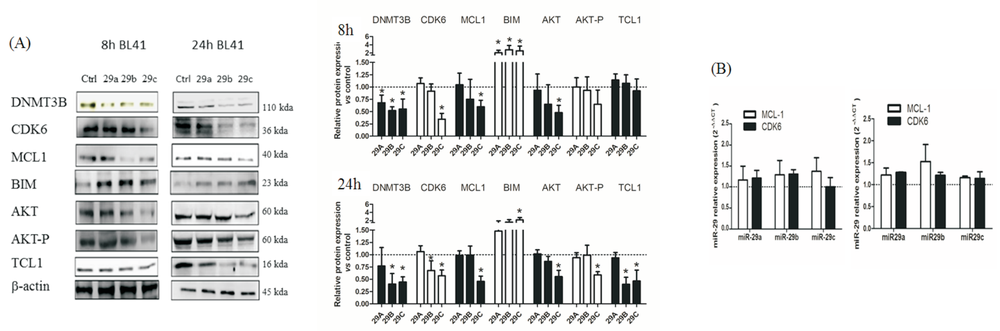


Figure 3. Detection of MCL1, CDK6, AKT full and phosphorylated, TCL1, DNMT3B and BIM protein expression and MCL1 and CDK6 mRNA production after 8 and 24h of miR-29's mimetics transfection. (A) Protein expression after 8 and 24h of 50 nM mimetic transfection in BL41 cells with the densitometry analysis of Western blot results, where the dot line represents the mimetic negative control. (B) The effect of the mimetics on *CDK6* and *MCL1* mRNA after 8h pos mimetic transfection.

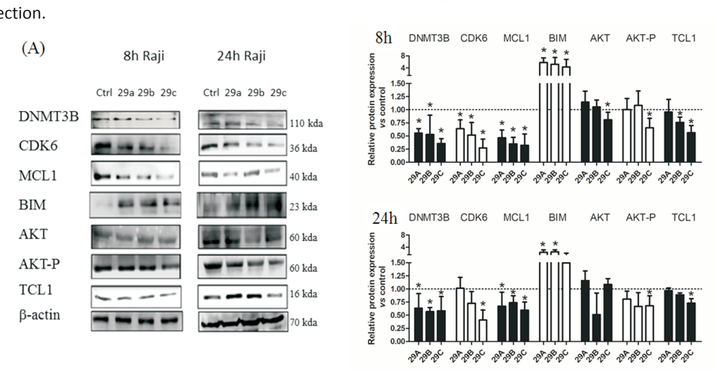


Figure 4. Detection of MCL1, CDK6, AKT full and phosphorylated, TCL1, DNMT3B and BIM protein expression and MCL1 and CDK6 mRNA production after 8 and 24h of miR-29's mimetics transfection. (A) Protein expression after 8 and 24h of 50 nM mimetic transfection in Raji cells with the densitometry analysis of Western blot results, where the dot line represents the mimetic negative control. (B) The effect of the mimetics on *CDK6* and *MCL1* mRNA after 8h pos mimetic transfection.

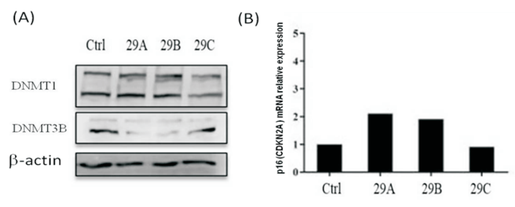


Figure 5. Inhibition of DNMT3B protein expression and its effects on p16 mRNA. (A) DNMT3B and DNMT1 protein expression after 24h post transfection in BL41. (B) *p16* mRNA relative expression evaluated by qPCR after DNMT3B reduction, whereas the bars represent the relative expression in comparison to the negative control.

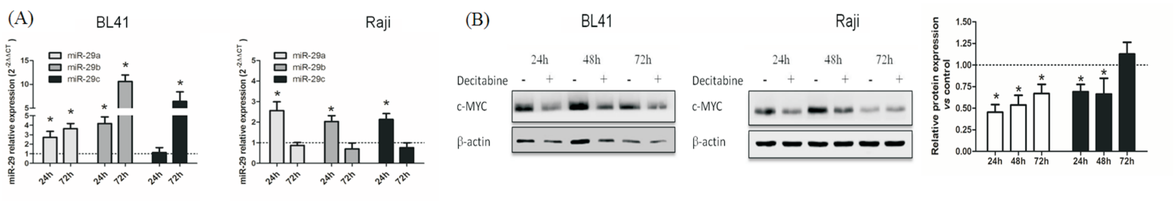


Figure 6. Induction of miR-29s expression after decitabine treatment. (A) BL41 and Raji cells were treated with 1 mM of Decitabine and evaluated by qPCR for miR-29s expression. (B) Decitabine effect was also observed in BL41 and Raji cells, whereas the drug reduced MYC protein expression in which the densitometry analysis shows the media of three independent experiments. The dot line represents the negative control. * p<0,05.

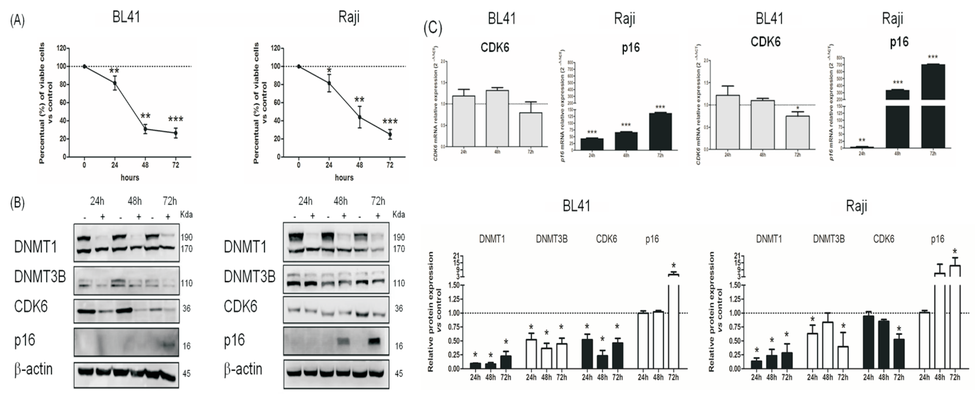


Figure 7. The effect of decitabine in BL41 and Raji cells. (A) Cell viability was evaluated by Trypan blue after decitabine treatment (1 mM) at the corresponding time course and plotted relatively to the negative control. (B) DNMT1, DNMT3B, CDK6 and p16 protein expression, whereas b-actin is the endogenous control, followed by relative protein expression vs control densitometry analysis of Western blot. (C) qPCR of *p16* and *CDK6* mRNA relative expression. The dot line represents the control. * p<0.05; **p<0,01; **p<0,001

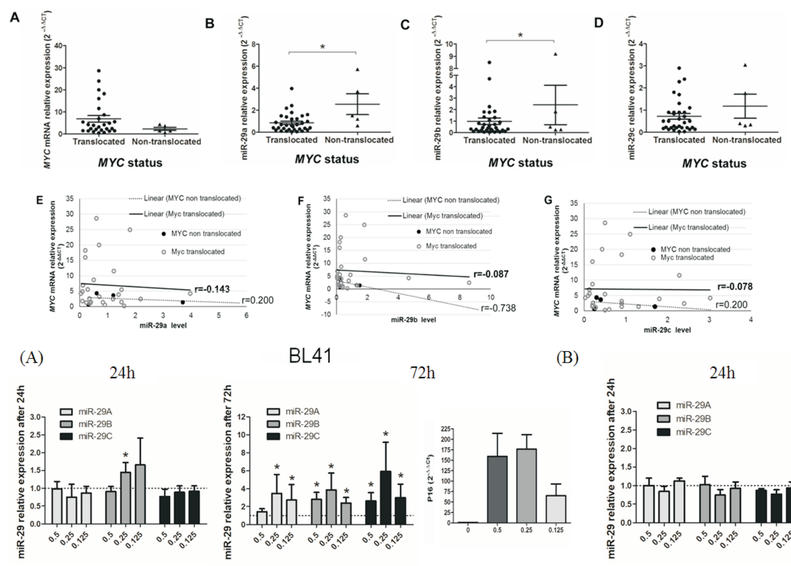


Figure 8. *MYC* mRNA and miR-29a/b/c expression levels in Burkitt lymphoma tumour samples. *MYC* expression levels in BL cases that were positive for the *MYC* translocation *vs* non-translocated cases (A). Expression of miR-29 family members between translocated *vs* non-translocated tumour samples (B, C, D). The results represent the median between the tumour samples. *p<0.05 (Man-Whitney test). Correlation between *MYC* RNA levels and miR-29a/b/c levels in translocated and non-translocated cases using Spearman test.

Figure 9. The effect of 0.5, 0.25 and 0.125 mM of decitabine on miR-29 and *p16* mRNAexpression in BL41 and Raji cells. (A) BL41 cells were evaluated by qPCR for mir-29 expression after 24h and 72h, along with *p16* mRNA expression. (B) Raji cells were harvested and evaluated by qPCR for miR-29 expression after 24 and 72h, also evaluated for *p16* mRNA expression after 72h. The dot line represents the negative control of the experiment. *p<0,05.

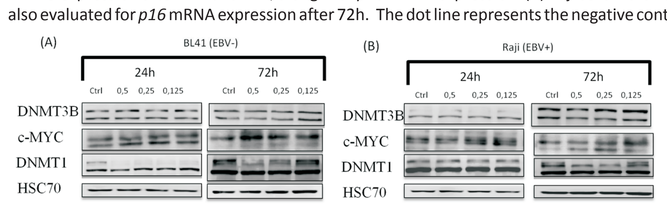


Figure 10. DNMT3B, DNMT1 and MYC protein expression after 0.5, 0.25 and 0.125 mM of decitabine. (A) BL41 and (B) Raji cells were treated with the above mentioned concentrations of decitabine and evaluated after 24 and 72h for DNMT3B, DNMT1 and MYC protein expression. HSC70 was used as the endogenous control.

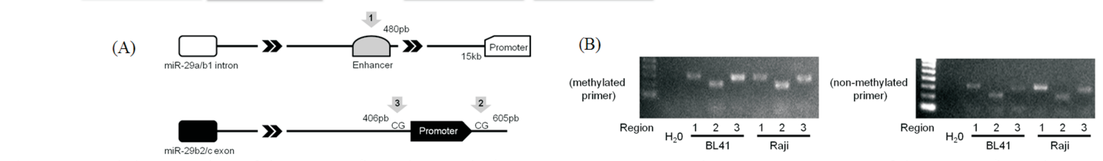


Figure 11. Methylation analysis of the *miR-29a/b1* and *miR-29b2/c* gene promoter regions. Schematic representation of the miR-29a/b1 and miR-29b2/c gene promoter regions. The arrow numbers indicate the detected CpG sequences (A). Methylation status analysis of 1, 2, and 3 regions was performed in BL41 and Raji cells. MSP-PCR using a specific primer set that amplified methylated (left panel) or unmethylated DNA (right panel), (B).

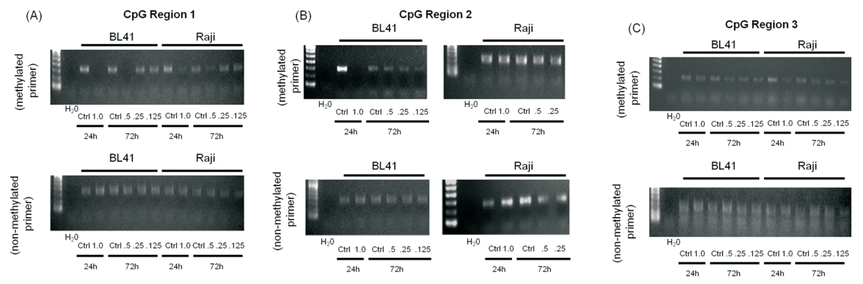


Figure 12. MSP-PCR analysis of the miR-29a/b1 and miR-29b2/c promoter regions in BL cells after decitabine (5-Aza-dC) treatments. BL41 and Raji cells were treated with 1.0, 0.5, 0.25, and 0.125 μM decitabine and evaluated by MSP-PCR for the CpG 1 region (A) and CpG 3 region (B) after 24 h and 72 h. Gel images correspond to methylated (upper panels) and unmethylated (lower panels) DNA.

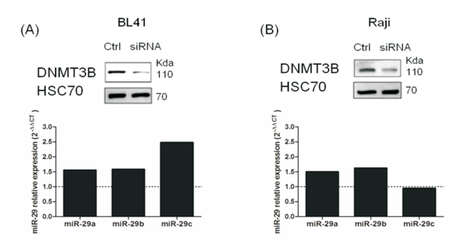
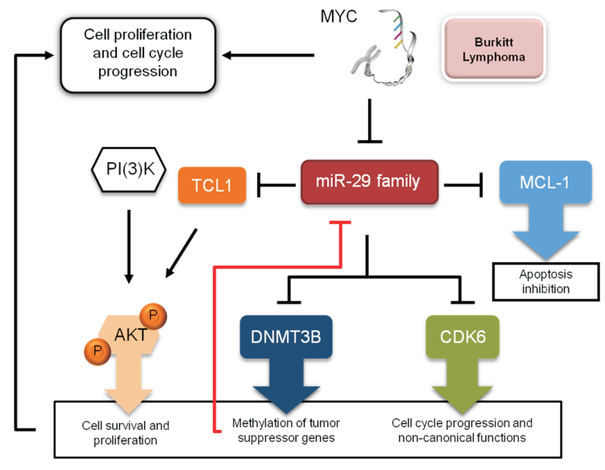


Figure 13. DNMT3B knockdown effects on miR-29a/b/c expression in BL cells. The DNMT3B siRNA transfection efficiency was evaluated 48 h after transfection by Western blot. HSC70 was used as the endogenous control (A). The miR-29 expression levels were measured 48 h after transfection by qRT-PCR, 2^{-CT} method was used to calculate relative expression. (B). The dotted line represents the experimental control (scramble siRNA).

CONCLUSION



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