# MYC-REGULATED MICRORNAS AND MYC EXPRESSION IN BURKITT LYMPHOMA TUMOURS

**Robaina MCS [1], Soares Lima SC[2], Mazzoccoli L[1], Faccion RS[1], Pinto LW [3], Bacchi CE[4], Klumb CE [1]**

1 PROGRAMA DE PESQUISA EM HEMATO-ONCOLOGIA MOLECULAR, INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO
2 PROGRAMA DE CARCINOGÊNESE MOLECULAR, INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO
3 DIVISÃO DE PATOLOGIA, INSTITUTO NACIONAL DE CÂNCER, RIO DE JANEIRO
4 CONSULTORIA EM PATOLOGIA, BOTUCATU, SÃO PAULO

## INTRODUCTION

Burkitt lymphoma (BL) is an aggressive lymphoma and the most common subtype of B-cell non-Hodgkin lymphoma (B-NHL) in childhood. It is characterized by the reciprocal translocation of *MYC* oncogene with immunoglobulin genes, resulting in MYC protein overexpression. MYC regulates more than 15% of the human transcriptome and a large set of microRNAs (miRNAs) such as the miR-17-92 cluster, miR-29 family, miR-7, miR-9, miR-34a and miR-34b. Recently, BL cases without MYC translocation but with gene expression and pathological characteristics of BL have been identified. In the present study, we aimed to analyze the role of MYC expression on miRNAs regulation in BL tumours.

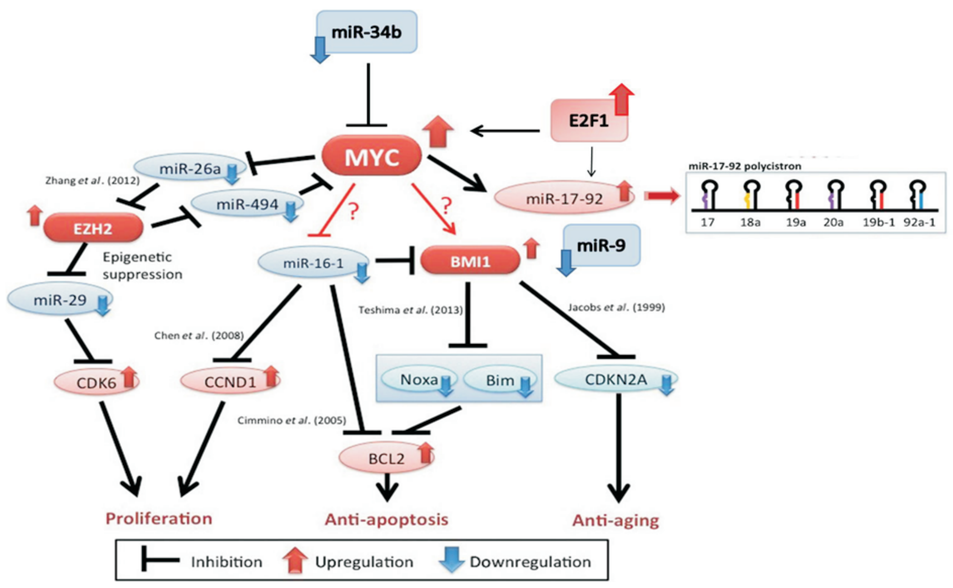


**Figure 1: Representative schematic of miRNA regulated by MYC with importance for BL pathogenesis.** Adapted from Tagawa et al., 2013

## MATERIAL AND METHODS

- 41 formalin-fixed, paraffin-embedded (FFPE) tissue samples of pediatric BL from the Hematology Service of the National Cancer Institute, Brazil;
- RecoverAll™Total Nucleic Acid Isolation Kit for FFPE tissue samples (Ambion®) was used to isolate the miRNAs from BL samples;
- qRT-PCR for miRNA expression analysis was performed using TaqMan® MicroRNA Assays (Applied Biosystems);
- MYC protein expression was analyzed in BL samples by immunohistochemistry and in P493-6 (Burkitt lymphoma model cell line carrying a conditional tetracycline-regulated MYC) by western blotting.

## RESULTS

We observed higher MYC protein expression levels in BL samples harbouring MYC translocation in relation to tumour samples without MYC translocation analyzed by immunohistochemistry (p=0.0180). Given that BL tumour samples showed different MYC protein levels, the next step was to analyze the miRNAs levels regulated by MYC (miR-17-92 cluster, miR-29 family, miR-7, miR-9, miR-34a and miR-34b) and two miRNAs which are not MYC target although they are relevant for apoptosis and proliferation pathways (miR-7 and miR-494). Then, we investigated the expression profile of 13 miRNAs in 41 BL tumour samples with different MYC rearrangement status by quantitative real time PCR. A different miRNA profile was detected between positive and negative MYC-translocated BL tumour samples. We advanced the analysis using P493-6 cell line model to modulate the MYC transcription and it was observed an increase in miR-17, miR-20a, miR-29c and miR-34b expression levels upon MYC inhibition. Conclusion: Our data indicate that MYC expression regulate a set of miRNAs and MYC-miRNA circuitary is a mechanism of sustaining MYC activity in the pathogenesis of BL. Further analyses are need to elucidate the complicated feedback MYC circuitary underlying BL development.

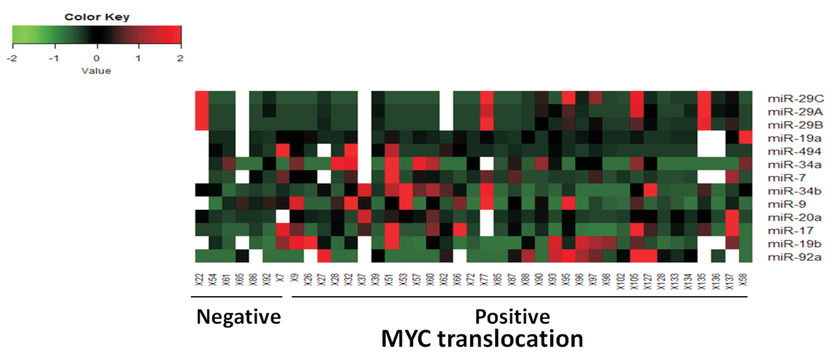


**Figure 2. Heatmap showing the miRNA profile in pediatric Burkitt lymphoma samples.** miRNA expression was evaluated by Quantitative Real time QT-PCR. Expression of each miRNA was normalized to the expression level of RNU6B and reactive lymph node was used as reference.

**Table 1: Patients' characteristics of MYC-negative cases**

| Patient | Gender/Age | Diagnostic | Initial site of disease | Stage | LDH (U/L) | EBV (ISH) | Immunohistochemical profile at diagnosis | Immunohistochemical complementary | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| 22 | M/9 | Burkitt Lymphoma | Cervical mass | I | 315 | Negative | CD20+ | NA | Alive |
| 54 | M4 | Burkitt Lymphoma | Abdomen | III | 570 | Positive | CD20+, CD3- | TDT-, BCL6+, CD10+, BCL2-, Ki67100% | Alive |
| 61 | F7 | Burkitt Lymphoma | Abdomen | III | 1613 | Positive | CD20+ | CD20+, CD10+, BCL2-, Ki67100% | Alive |
| 65 | F/6 | Burkitt Lymphoma | Abdomen | III | 536 | Negative | LCA+, CD99-, EMA-, desmin-, vimentin- | CD10+, CD20+, BCL2-, Ki67>90% | Alive |
| 86 | M/11 | Burkitt Lymphoma | Ileum | II | 385 | Negative | CD20+, Ki67+ | CD10+ BCL6+ | Alive |
| 92 | F/10 | Burkitt Lymphoma | Nasopharyngeal mass | II | 575 | Negative | CD20+, CD10+, Tdt-. CD99- | BCL6+, BCL2-, MUM1-, Ki67>90% | Alive |

LDH lactate dehydrogenase; EBV Epstein-Barr virus; ISH in situ hybridization; NA not available.

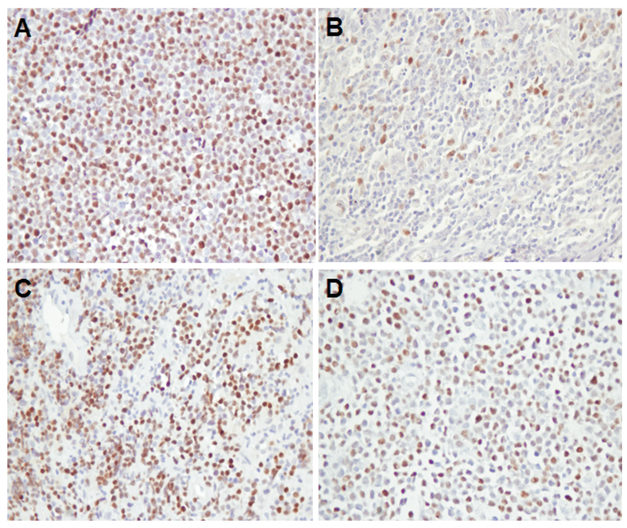


**Figure 3: Representative immunohistochemical detection of MYC protein expression in BL MYC rearrangement negative samples.** A BL sample was used as positive control; B negative expression (less than 20 % positive tumor cells); C positive expression (about 60-70% positive tumor cells) and D positive expression (over 70% positive tumor cells).

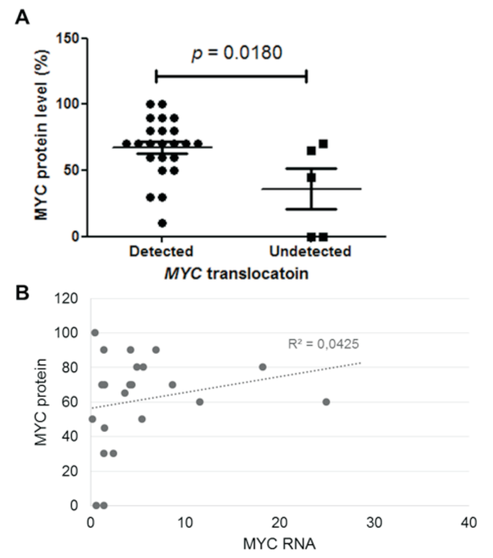


**Figure 4: MYC protein and RNA levels in BL tumour samples**. (A) MYC protein levels in relation to *MYC* gene traslocation. The MYC protein levels is high in BL tumour with MYC translocation than in untranslocated samples. Mann Whitney.Test $p<0.05$ was stastistcally significant. (B) It was observd a positive correlation between MYC protein and RNA levels in BL tumour samples. Sperman Test.

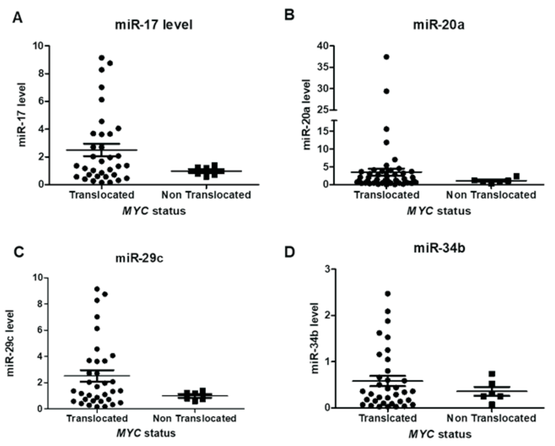


**Figure 5: miRNA expression in different MYC status in BL tumour samples.** miRNA expression level was evaluated by Quantitative Real time QT-PCR. A, B, C and D show the median level of miR-17, miR-20a, miR-29c and miR-34b expression, respectively

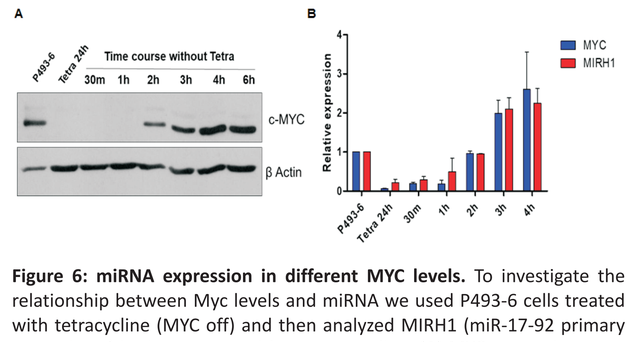


**Figure 6: miRNA expression in different MYC levels.** To investigate the relationship between Myc levels and miRNA we used P493-6 cells treated with tetracycline (MYC off) and then analyzed MIRH1 (miR-17-92 primary transcriptor) in time course without tetracycline. (A) MYC expression were analyzed in cells treated with tretracyclin during 24 hours and 30 minutes, 1, 2, 3, 4 and 6 hours without tetracycline. β-Actin expression served as a protein loading control. (B) MIRH1 levels correlate with mRNA MYC level. miRNA expression was evaluated by Quantitative Real time QT-PCR

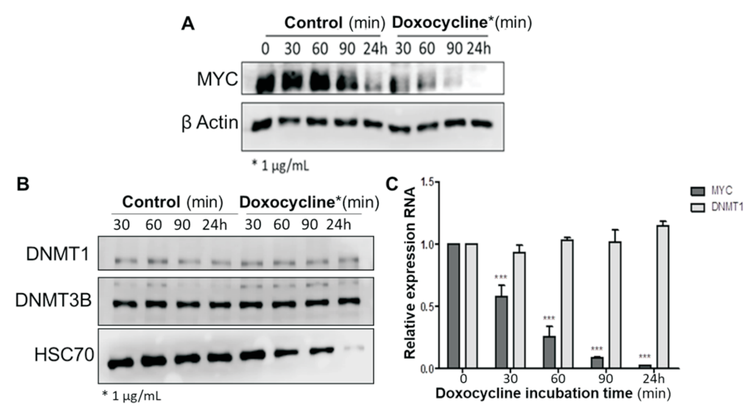


**Figure 7: DNA methyl transferases (DNMT) protein expression in different MYC levels.** To investigate the relationship between Myc levels and DNMTs we used P493-6 cells treated with doxocycline (MYC off). (A) MYC expression were analyzed in cells treated with doxocycline during 30, 60, 90 minutes and 24 hours. β-Actin expression served as a protein loading control. (B) DNMT1 and DNMT3B protein levels in different MYC levels. HSC70 expression served as a protein loading control. (C) It was observed a significant decreased in MYC RNA levels but not in DNMT1 RNA levels in P493-6 cells. RNA expression levels were evaluated by Quantitative Real time QT-PCR

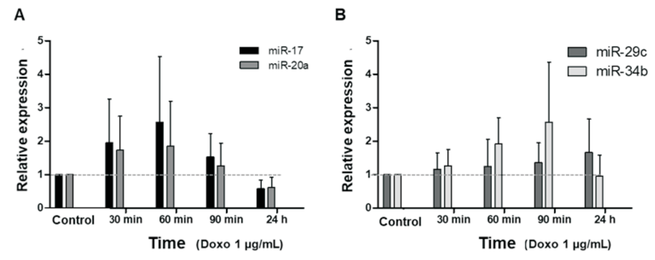


**Figure 8: miRNA expression in different MYC status in P493-6 cells.** miRNA expression level was evaluated by Quantitative Real time QT-PCR. (A,) miR-17 and miR-20a level (B) miR-29c and miR-34b level expression in P493-6 cells treated with doxocycline during 30, 60, 90 minutes and 24 hours. miRNA expression was evaluated by Quantitative Real time QT-PCR.

## CONCLUSION

Our data indicate that MYC expression regulate a set of miRNAs and MYC-miRNA circuitary is a mechanism of sustaining MYC activity in the pathogenesis of BL. Further analyses are need to elucidate the complicated feedback MYC circuitary underlying BL development.

**Funding:** Instituto Nacional de Ciência e Tecnologia (INCT) para Controle do Câncer: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) 573806/2008-0/FAPERJ E26/170.026/2008, Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) E-26/110.238/2014—PPSUS, Programa de Oncobiologia/Fundação do Câncer, and FAPERJ E-26/110.375/2014. SWISS-BRIDGE Foundation, sub-project 1B/2014.

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

MINISTÉRIO DA SAÚDE