ANALYSIS OF MICRORNAS EXPRESSION IN PEDIATRIC PATIENTS WITH BURKITT LYMPHOMA/LEUKEMIA ACCORDING TO THE MYC TRANSLOCATION STATUS

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INTRODUCTION

Translocations between MYC oncogene and immunoglobulin genes (*IGH, IGL, IGK*) have been considered for many years the genetic hallmark of Burkitt Lymphoma/Leukemia (BL/L) and, one of the most important parameters for the definition of BL/L at diagnosis. Supporting the idea of *MYC* post-transcriptional deregulation in BL/L, some studies have found differential expression patterns of specific miRNAs, and other factors, in comparison to other Non-Hodgkin Lymphomas (NHL), in which *MYC* plays a role as the central hub of a complex network in the pathogenic model of BL/L. Here, we studied the expression levels of *MYC, BCL2, CD10* and selected miRNAs, in a subset of patients from our pediatric BL/L cohort, aiming to explore associations between miRNA expression and the presence of a translocated *MYC (t-MYC)*.

METHODS AND RESULTS

The expression levels of *MYC, BCL2*, and *CD10* gene, as well as miRNAs -9*, -155, -let7a, -let7b, -let7e, -150, -21, were studied in 10 BL/L tumor samples (9 lymph nodes and 1 bone marrow, BM) with 9 cases harboring the t(8;14)(q24;q32) and 1 without t-*MYC*.

Additionally, 3 BL and 2 Diffuse Large B Cell Lymphoma (DLBCL) cell lines, 3 reactive follicular hyperplasia (RFH) and 2 normal BM samples were included.

RNA was extracted from FFPE BM biopsy, BL/L and RFH lymph node using the MasterPure™ RNA Purification Kit (Epicentre). RNA from BM and cell lines was extracted with Direct-zol™ RNA MiniPrep (Zymo Research).

Relative expressions of CD10 and BCL2 were evaluated by TaqMan® assays, as previously described, using the average of ACTB and B2M reference genes for normalization.

MYC expression was quantified with SYBR green® assays using the average of ACTB and GUSB for normalization.

miRNAs were quantified with stem-loop TaqMan® assays after reverse transcription with MicroRNA Reverse Transcription Kit (Applied Biosystems) for each miRNA and the reference small RNA RNU48. Quantification values were expressed as fold change (2-ddCq) after calibration with the classical BL sample exhibiting the lowest expression level.

Epstein-Barr virus (EBV) was detected by *in situ* Hybridization (ISH) and Polymerase Chain Reaction (PCR).

The BL/L sample without t-*MYC* showed a gene expression pattern alike the other BL/L samples, except for miR155, miRlet7b and CD10 (**Figure 1A-C**), which showed a trend for low expression.

For this reason, the sample without t-*MYC* was included in the BL/L group for subsequent analysis, except for the differentially expressed genes.

BL/L samples showed higher expression levels of *MYC*, and lower expression levels of *BCL2* and miR150 in comparison to RFH (MYC: median 6.33 vs. 2.10; BCL2: 0.60 vs 120.6; miR150: 1.24 vs 6.4; $P<0.05$, Mann-Whitney test).

Hummel and coworkers in 2006, proposed a molecular signature for BL, including cases with lymphomas without t-*MYC*. Among them, *CD10* and *BCL2*, besides *MYC*, were found to be differentially expressed and are now used as classifiers for BL signature. BL and DLBCL cell lines showed a particular pattern of gene expression, different from the primary samples, however, these differences were not statistically significant (**Figure 1A-F**).

Some studies have observed a differential expression of miR9* in cases lacking t-*MYC*, however, we were unable to test this hypothesis due to the small size of our sample.

In the BL/L group, there was a trend for correlations ($p<0.1$) between *MYC* and miR9*, *MYC* and miR150, miRs -21 and -let7a, and miRs -21 and -let7e. There are reports that show downregulation of miR150 in BL/L, and we could also observe that trend for the association between miR9 150 and *MYC*.

Finally, the detection of EBV by RNA-ISH allowed us to detect one case expressing the EBERs transcripts (**Figure 2**).

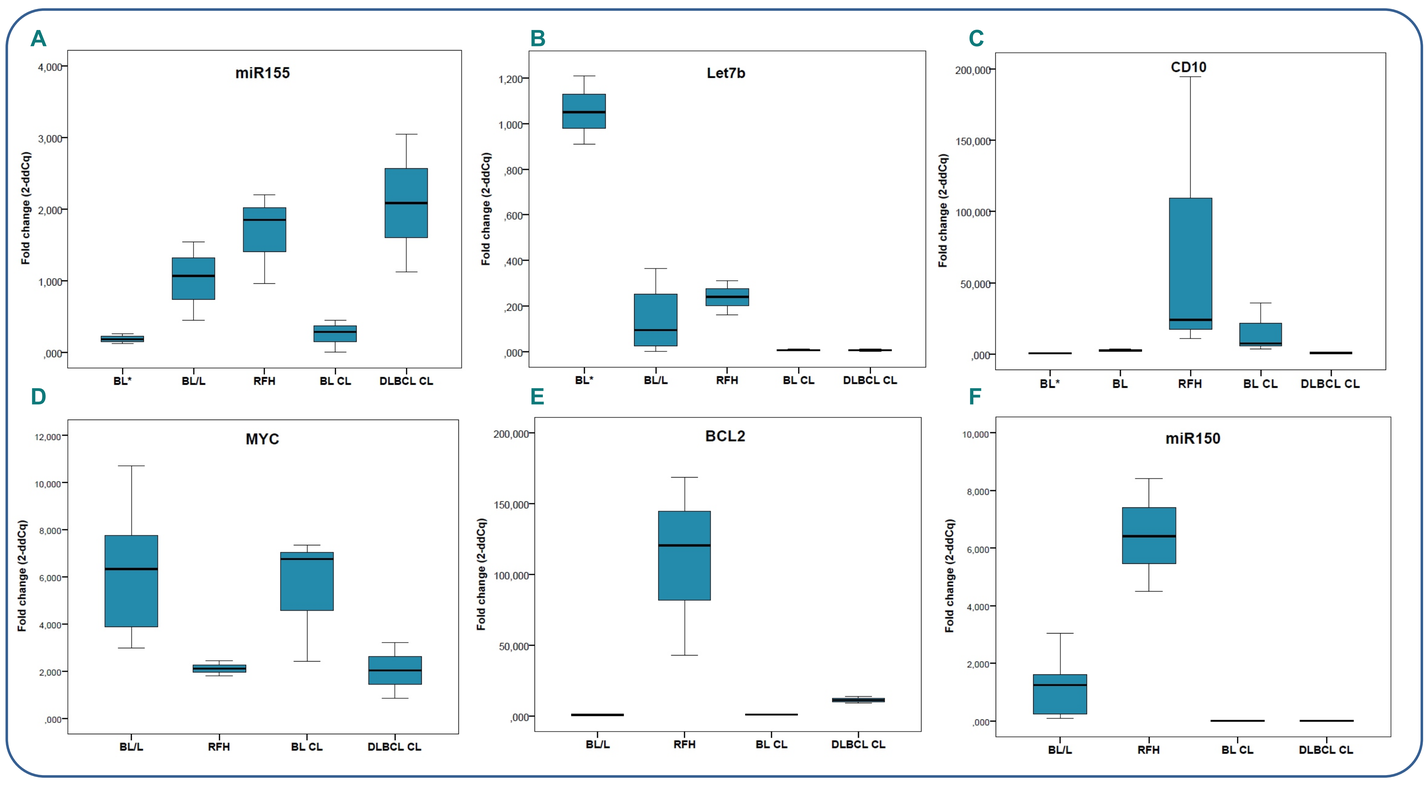

Figure 1: Comparisons of cellular genes and microRNA (miR) expressions among BL/L group, reactive follicular hyperplasias (RFH) and BL- and diffuse large B cell lymphoma (DLBCL)-derived cell lines. A: miR155; **B:** Let7b; **C:** CD10; **D:** MYC; **E:** BCL2; **F**: miR150. BL*: represent the BL/L case without t-*MYC*; BL/L: classical Burkitt lymphoma/leukemia; BL CL represent the mean values of BL-derived cell lines; DLBCL CL represent the mean values of diffuse large B-cell lymphoma (DLBCL)-derived cell lines; RFH: reactive follicular hyperplasia lymph nodes. Bars represent the mean of fold change values in each category, except by BL*, which represents the mean value of fold change ± standard deviation.

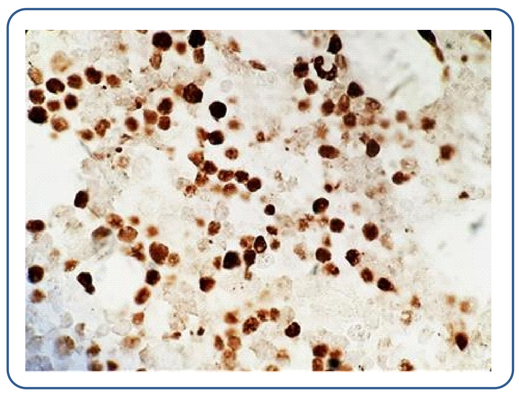

Figure 2: EBV+ Burkitt lymphoma. Cell nuclei exhibit a brown staining due to the reactivity with RNA EBERs probes (100x).

CONCLUSION

Our preliminary data highlight some potential interactions between miRNAs and *MYC* in BL/L, such as downregulation of miR150 in BL/L and a trend for low expression of miR155, miRlet7b and CD10 in the case without t-MYC. These are preliminary results that need to be confirmed in a larger group of cases.

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