MYC-TARGET MICRORNA PROFILE IN PEDIATRIC BURKITT LYMPHOMA TUMORS

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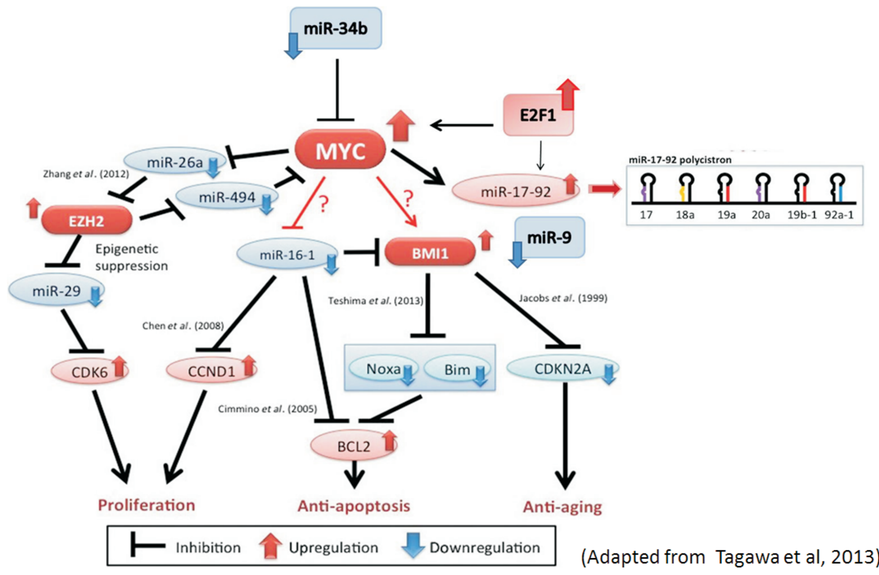
BACKGROUND

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 miR-17-92 cluster. MYC acts regulating several genes involving on cell cycle control, metabolism, and apoptosis. Recently, BL without MYC translocation but with gene expression and pathological characteristics of BL have been identified. In the present study, we aimed to analyze the level of miRNAs regulated by MYC comparing MYC translocation positive and negative BL tumors.



(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;
- MYC translocation was evaluated by fluorescence in situ hybridization (FISH);
- 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) using a StepOne™ System (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

In our previously analysis, about 15% BL cases (6 out of 41) had undetectable MYC translocation when analyzed using FISH and we did not observe a difference among the levels of miR-17-92 members in tumor samples that were negative and positive for MYC translocation. We advanced this analysis including other miRNAs that are regulated by MYC (miR-9, miR-29 family, miR-34a and miR-34b) and two miRNAs which are not MYC target but are relevant for apoptosis and proliferation pathways (miR-7 and miR-494). In summary, we investigated the expression profile of 13 miRNAs in 41 BL tumor samples with different status of MYC rearrangement. It was possible detect a different miRNA profile between positive and negative MYC translocation BL tumor samples. The tumor samples with MYC rearrangement showed miRNA levels higher than the BL cases without MYC rearrangement. In addition, we evaluated MYC protein level by immunohistochemistry in BL MYC-negative cases. High levels of MYC protein were detected in 3 out of 5 cases. It is important to emphasize that the MYC rearrangement negative cases had a histopathological, immunophenotypic and clinical profile of typical BL.

Table 1. Patients baseline characteristics

Characteristic	Number (%)
Diagnostic	
BL	40 (97.6)
BL-HIV	1 (2.4)
Gender	
Female	10 (24.4)
Male	31 (75.6)
Age (years)	
Range	2–18
Mean	7.4
Median	6
Stage (St. Jude)	
I/II	8 (19.5)
III/IV	33 (80.5)
LDH	
>1000	20 (48.8)
≤1000	21 (51.2)
EBV	
Positive	20 (52.6)
Negative	18 (47.4)
MYC rearrangement	
Positive	35 (85.4)
Negative	6 (14.6)
Outcome	
Live	30 (73.2)
Dead	11 (26.8)

LDH lactate dehydrogenase (normal level ≤ 500 U/L), *HIV* human immunodeficiency virus, *EBV* Epstein–Barr virus

Robaina MC et al, 2016

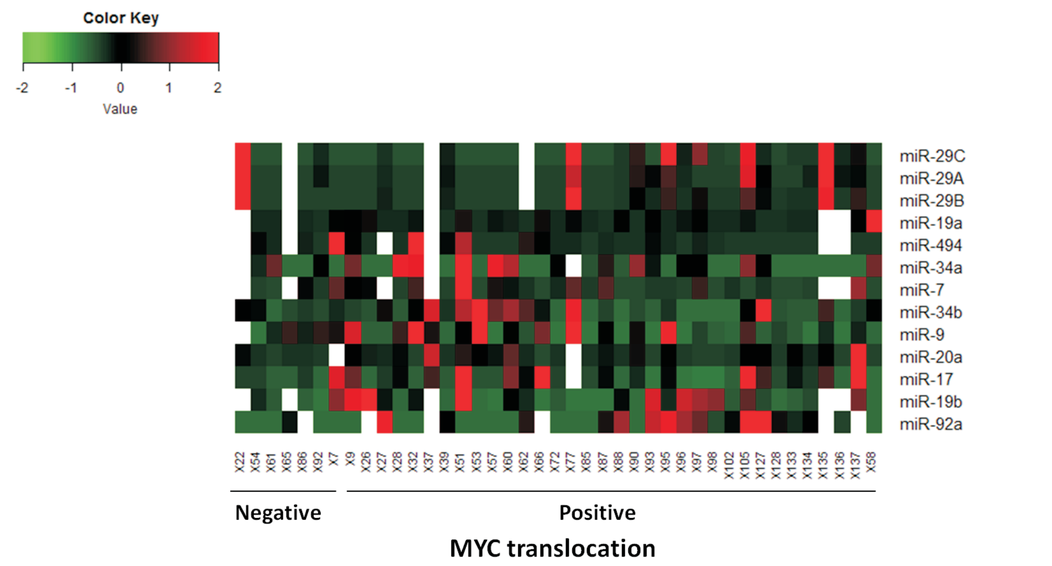


Figure 1. Heatmap showing the miRNA profile in pediatric Burkitt lymphoma samples. miRNA expression was evaluated by Quantitative Real time QT-PCR. Expression of each miRNA was normalized to the expression level of RNU6B and reactive lymph node was used as reference.

Table 2: 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.

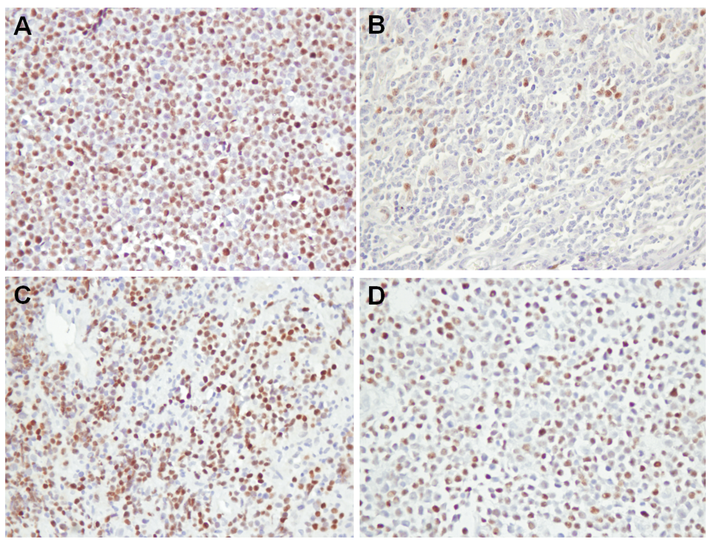


Figure 2: Representative immunohistochemical detection of MYC protein expression in BL MYC rearrangement negative samples. A BL sample was used as positive control; B negative expression (less than 20 % positive tumor cells); C positive expression (about 60-70% positive tumor cells) and D positive expression (over 70% positive tumor cells).

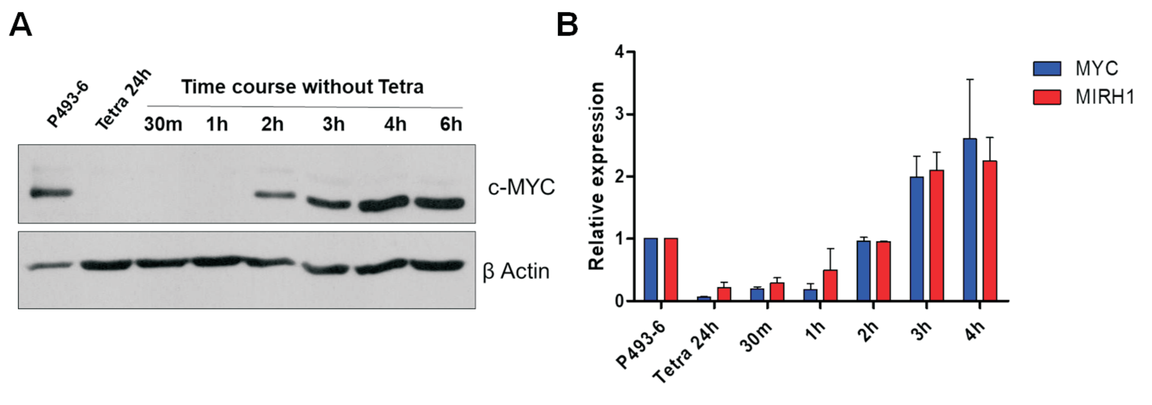


Figure 3: miRNA expression in different MYC levels. To investigate the relationship between Myc levels and miRNA we used P493-6 cells treated with tetracycline (MYC off) and then analyzed MIRH1 (miR-17-92 primary transcriptor) in time course without tetracycline. (A) MYC expression were analyzed in cells 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

CONCLUSION

Our data indicate that MYC expression may be regulated by a set of miRNAs in MYC translocation-negative BL suggesting involvement of MYC-target microRNA in the pathogenesis of specific MYC negative BL tumors.

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