MicroRNAs as potential biomarkers in the differential diagnosis of Burkitt lymphoma *vs* diffuse large B-cell lymphoma

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INTRODUCTION

Fast and reliable differentiation of Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) is of great clinical importance, as BL and DLBCL patients need to be treated differently, and in BL, once the diagnosis is established, treatment should be initiated promptly. Recently, a category of aggressive B-cell lymphomas that does not meet the criteria of BL or DLBCL has been defined as "high grade B-cell lymphoma, unclassifiable, intermediate between BL and DLBCL"(Intermediate BL/DLBCL). These lymphomas present morphological and genetic features of both BL and DLBCL, but because of their biological and clinical characteristics should neither be classified as BL nor as DLBCL. No efficient treatment is available for patients with aggressive B-cell lymphomas of this group, thus it is important to clearly distinguish the group of "grey zone B-cell lymphomas" from BL and DLBCL.

MicroRNAs, short, non-coding RNA molecules have been implicated in cancer development and progression, and an aberrant expression of numerous microRNAs has been found in multiple human tumor types. miR-155 is over-expressed in a number of non-Hodgkin's lymphomas, including DLBCL. In BL, the expression of miR-155 and of its precursor (*BIC*) is ambiguous and the available data concern mainly BL-derived cell-lines and clinical samples from pediatric BL. The expression of miR-155/BIC in the group of lymphomas classified as intermediate between BL and DLBCL remains unknown.

AIMS:

1/ to investigate the potential of miRNAs, namely miRNA-155 and miR-26a, as biomarkers for the differential diagnosis of BL, DLBCL, and intermediate BL/DLBCL.

2/ to analyze the expression of *BIC* in the clinical samples examined for miRNA-155 expression, in order to learn whether *BIC* contributes to the pathogenesis of BL.

MATERIALS and METHODS

Forty six patients suspected of BL or DLBCL, with atypical histology were examined. The analysis comprised 7 aspirates from lymph nodes and 30 from tumors, 5 surgical biopsies, 2 pleural fluids and 2 blood samples. Immunophenotype was determined by the standard immunohistochemistry (CD3, CD20, Bcl-2, Bcl-6, Ki 67) and by flow cytometry (FCM) of the cells obtained by fine needle aspiration biopsy (FNAB), with antibodies against CD45, HLA-DR, CD19, CD20, CD22, CD23, CD79a, FMC7, CD3, CD5, CD25, CD43, CD56, CD16&CD56, CD11c, CALLA, CD10, CD38, CD44, CD52, CD77, and CD138. BCL2, light and heavy immunoglobulin chains and isotype (control) were also assessed.

The FNAB material was also used to prepare conventional cytological smears (CYT) and to perform cytogenetic and molecular studies.

Karyotyping and FISH were employed to examine the presence of t(8;14)(q24;q32) and alternative *MYC* rearrangements, as well as of *BCL6, BCL2* and *IGH* rearrangements in 26 patients.

The final diagnosis was made in accordance with the recent, 4th edition of the WHO classification (2008).

Morphologic, immunophenotypic, and genetic features that may be useful in distinguishing BL from DLBCL.

CHARACTERISTIC	BL	Intermediate BL/DLBCL	DLBCL
Morphology			
Only small/medium-size cells	Yes	Common	No
Only large cells	No	No	Common
Mixture	No	Sometimes	Rare
Proliferation (Ki67/MIB1)			
>90% and homogeneous	Yes	Common	Rare
<90% or heterogenous	No	Sometimes	Common
BCL2 expression			
Negative / weak	Yes	Sometimes	Sometimes
Strong	No	Sometimes	Sometimes
Genetic features			
MYC **rearrangement**	Yes	Common	Rare
IG-MYC	Yes	Sometimes	Rare
Non *IG-MYC*	No	Sometimes	Rare
BCL2 **but no** *MYC* **rearrangement**	No	Rare	Sometimes
BCL6 **but no** *MYC* **rearrangement**	No	Rare	Sometimes
Double hit	No	Sometimes	Rare
MYC-**Simple karyotype**	Yes	Rare	Rare
MYC-**Complex karyotype**	Rare	Common	Rare

Source: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008.

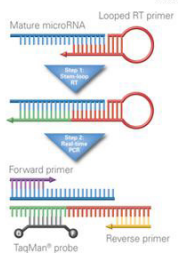
MicroRNA expression assessment

Total RNA was isolated from clinical samples using TriReagent. To quantify the expression of mature miR-155, 10 ng of total RNA were taken and converted to the cDNA by using special loop sequence specific primers. The real-time PCR with sequence-specific primers and TaqMan probe was performed (TaqMan MicroRNA Assays, Applied Biosystems). A small nuclear RNA, snRNA U6, was used as a reference gene.

Along with the miR-155 expression, the level of its precursor *BIC* was evaluated. By using random primers, approximately 1000ng of RNA was reverse transcribed to the cDNA. The *Hprt* gene was used as an endogenous control. For *BIC* and *Hprt* amplification, sequence-specific primers and TaqMan Probe were applied (TaqMan Gene Expression Assays, Applied Biosystems).

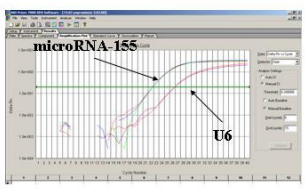
Normal peripheral blood mononuclear cells were used as calibrator samples for the expression assessment.

Schemes of the reverse transcription (RT) and real-time performance

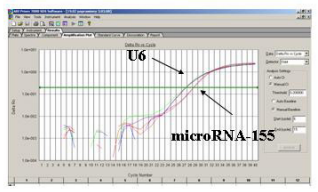


Source: www.appliedbiosystems.com

High expression of microRNA-155



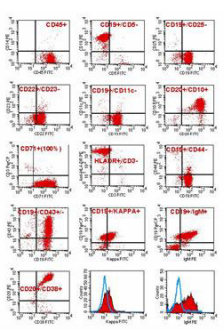
Low expression of microRNA-155



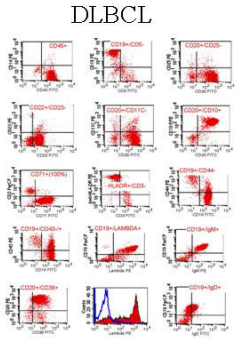
Immunophenotype analysis (FCM)

(Red – B lymphocytes, green – T lymphocytes)

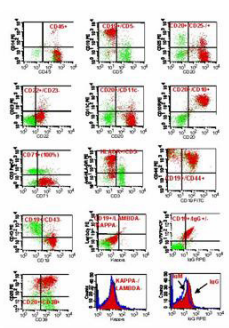
BL



Intermediate BL / DLBCL



DLBCL



BL

CD45+, CD19+ < CD20+, CD22+, CD10+, CD38+, CD43+ CD44-, CD 71(+++), HLADR+, CD5-,CD11c-, CD25-, kappa+, IgM+

Intermediate BL / DLBCL

Immunophenotypic features different from BL:

CD 20+ weaker, CD25 -, CD38+ weaker, CD43-/+, IgM+, IgD+

DLBCL

Immunophenotypic features different from BL:

CD25 -/+, CD38+ the weakest, CD43-, CD44+, HLADR (+) weaker, kappa-, lambda-)

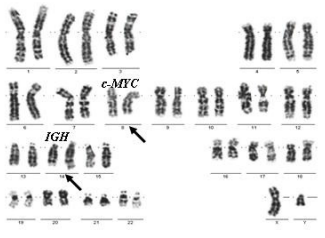
Cytogenetics

BL

MYC-Simple karyotype

Typical t(8;14)(q24;q32)

with *MYC* gene rearrangement

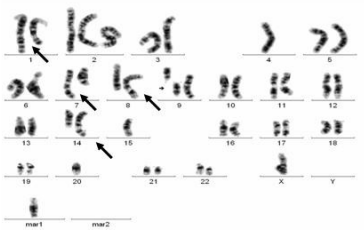


Intermediate BL/DLBCL

MYC-Complex karyotype

Intermediate BL/DLBCL

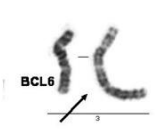
46, X,-Y,t(1;7;8;14)(q32;q22;q24;q32),+ mar

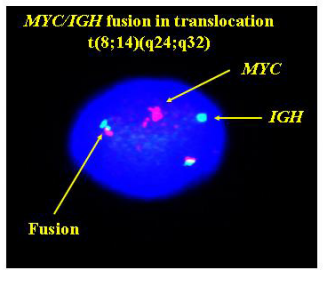


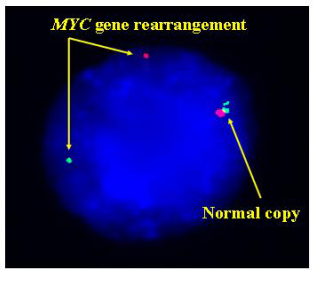
DLBCL

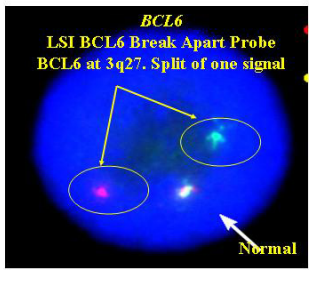
Partial karyotype - aberration of chromosome 3

t(3;6)(q27;q15)

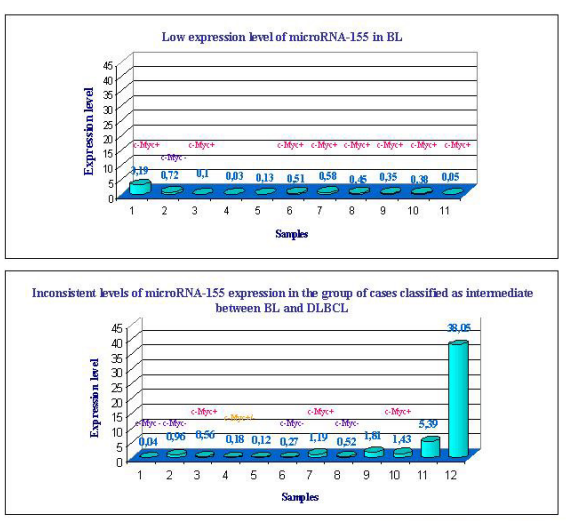


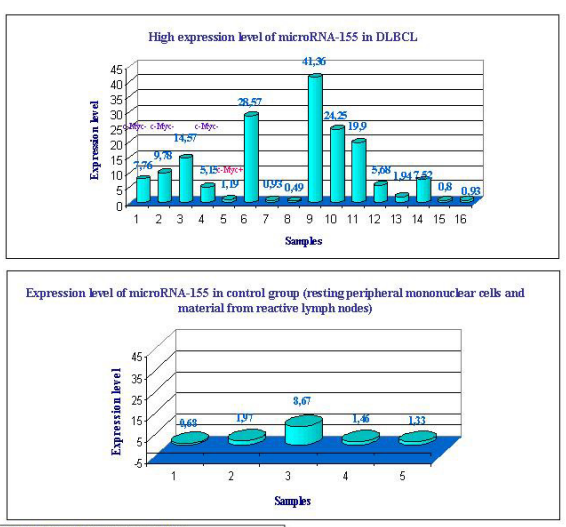


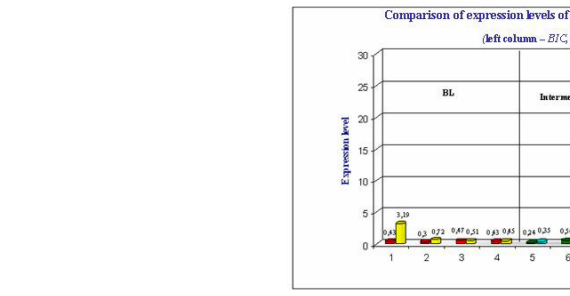


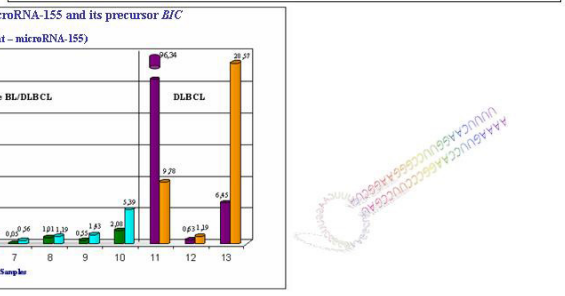


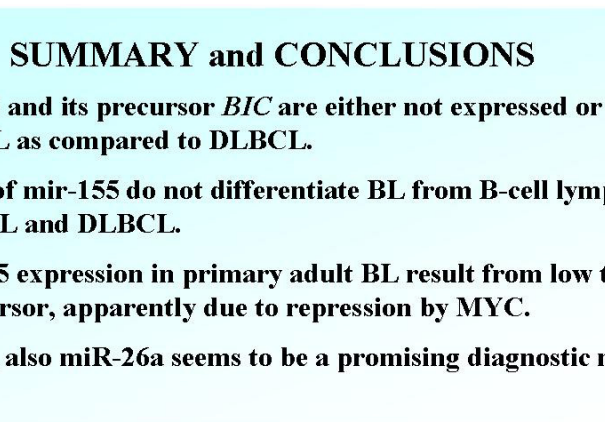
RESULTS











SUMMARY and CONCLUSIONS

1/ In adult BL, miR-155 and its precursor *BIC* are either not expressed or their expression is significantly lower in BL as compared to DLBCL.

2/ The expression level of mir-155 do not differentiate BL from B-cell lymphoma intermediate between BL and DLBCL.

3/ Low levels of miR-155 expression in primary adult BL result from low transcription of *BIC*, the miR-155 precursor, apparently due to repression by MYC.

4/ Apart from miR-155, also miR-26a seems to be a promising diagnostic marker for aggressive lymphomas.