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, which do not meet the criteria of BL or DLBCL, has been defined as “high-grade B-cell lymphoma, unclassifiable, intermediate between BL and DLBCL” (Interim 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 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 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 microRNAs, namely miR-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 miR-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 parameters from lymph node and 10 from tumors, 5 surgical biopsies, 2 pleural fluids and 2 blood samples. Immunophenotype was determined by the standard immunochemistry (CD5, CD10, CD19, Bcl-2, Bcl-6, KI-67) and by flow cytometry (FCM) of the cells obtained by fine needle aspiration biopsy (FNAB), with antibodies against CD3, CD4, CD8, CD19, CD20, CD22, CD30, CD5, CD25, CD27, CD45, CD10, CD68, CD8, CD45RO, CD45RA, and CD138. B-cell light and heavy immunoglobulin chains and isotype controls (anti-IgM, anti-IgA, anti-IgG) were also assessed.

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

Karyotyping and FISH were employed to examine the presence of BCL11a(18q22)(0.3) and additional/MALT 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).

Immunophenotype analysis (FCM)

<table>
<thead>
<tr>
<th>Cells</th>
<th>Intermediate BL / DLBCL</th>
<th>BL</th>
</tr>
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<tbody>
<tr>
<td>CD5+</td>
<td>31%</td>
<td>31%</td>
</tr>
<tr>
<td>CD10-</td>
<td>70%</td>
<td>70%</td>
</tr>
<tr>
<td>CD19-</td>
<td>25%</td>
<td>25%</td>
</tr>
<tr>
<td>CD20-</td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td>CD22-</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>CD30-</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>CD35+</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>CD45+</td>
<td>99%</td>
<td>99%</td>
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MicroRNA expression assessment

Total RNA was isolated from clinical samples using TRIzol. To quantify the expression of mature miR-155, 10 ng of total RNA were taken and converted to 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, U6, was used as an endogenous control. For BIC and BCL6 amplification, sequence-specific primers and TaqMan Probe were applied (TaqMan Oligo Expression Assays, Applied Biosystems).

Normal peripheral blood nucleosome cells were used as calibrator sample for the expression assessment.

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

High expression of microRNA-155

Low expression of microRNA-155

RESULTS

1/ Low expression of mature miR-155 in intermediate BL/DLBCL

2/ High expression of mature miR-155 in B-cell lymphoma

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.

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.