CD10(-)MUM1(+) follicular lymphoma lacks BCL2 gene translocation and shows characteristic biological and clinical features

Kennosuke Karube MD2,8*, Ying Guo MD1*, Junji Suzumiya MD3, Yasuo Sugita MD2, Yuko Nomura MD5, Kohei Yamamoto MD2, Kei Shimizu MD2, Shirou Yoshida MD2, Hideki Komatani MD2, Morishige Takeshita MD4, Masahiro Kikuchi MD4, Naoya Nakamura MD6, Osamu Takasu MD2, Fumiko Arakawa PhD2, Hiroyuki Tagawa MD7, Masao Seto MD7, Koichi Ohshima MD2

1Department of Pathology, State Key Laboratory of Cancer Biology, Xijing Hospital, Fourth Military Medical University, Xi’an, Shanxi, People’s Republic of China,
2Department of Pathology, School of Medicine, Kurume University, Kurume, Japan
Departments of 3Internal Medicine, 4Pathology and 5Pediatrics, School of Medicine, Fukuoka University, Fukuoka, Japan
6Department of Pathology, Tokai University School of Medicine, Isehara, Japan
7Division of Molecular Medicine and Division of Hematology and Cell Therapy, Aichi Cancer Center Institute, Nagoya, Japan
8JSPS Research Fellow

*Kennosuke Karube and Guo Ying equally contributed to this study.

Running title: CD10(-)MUM1(+) follicular lymphoma

Corresponding author:
Kennonuke Karube, M.D.,
Department of Pathology,
School of Medicine, Kurume University,
Asahimachi 67, Kurume 830-0011, Japan.
Phone: +81-942-31-7547,
Fax: +81-942-31-0342.
E-mail: karube1975@yahoo.co.jp
Summary

CD10 and MUM1 are representative B cell differentiation markers. Follicular lymphoma (FL) is usually positive for CD10 and negative for MUM1. In this study, however, we compared 22 FL with peculiar phenotype; CD10(-)MUM1(+) with 119 typical CD10(+)MUM1(-) FL. All CD10(-)MUM1(+) FL cases exhibited follicular structure with follicular dendritic meshwork, and a high rate of somatic hypermutation and on-going mutation, similar to typical FL. However, CD10(-)MUM1(+) FL were encountered frequently in the elderly compared with CD10(+)MUM1(-) typical FL (67.0 vs 58.7 years, p<0.01), showed high grade (Grade 3A or 3B) morphology (91% vs 17%, p<0.00001), diffuse proliferation (59% vs 19%, p<0.0001), and lacked BCL2/IgH translocation (5% vs 92.5%, p<0.00001), which is the most characteristic aberration in FL, and 88% showed BCL6 gene abnormalities (translocation or amplification). Our results indicate that CD10(-)MUM1(+) FL is different from typical FL with respect to biological and clinical features.

Key words: Follicular lymphoma, CD10, MUM1, high grade, BCL6
Introduction

Follicular lymphoma (FL) is the most prevalent form of low-grade B-cell lymphoma in adults. Typically, FL cells express CD10, BCL2 and BCL6. CD10 is a marker for germinal center (GC) B-cell, and thus its expression suggests that GC B cells are normal counterpart of FL. However, some reports including our previous study described the existence of CD10-negative FL, especially in high grade (Grade3) FL. However, it is not clear whether CD10 negativity is just aberrant loss or meaningful reflecting specific differentiation stage and affecting clinical features. MUM1 (multiple myeloma oncogene 1)/IRF4 (interferon regulatory factor 4) is a lymphoid-specific member of the interferon regulatory factor family of transcription factors and it is a reliable marker of “late-stage GC”, or “post-GC” B cells. In this study, we clinicopathologically compared CD10(-) MUM(+) and `classical` CD10(+)MUM1(-) FL.

Study design

Biological material

Tissue specimens were obtained from human lymph nodes filed at the Department of Pathology at Fukuoka University and Kurume University. The 147 FL cases have already been reported in our previous publication. Paraffin-embedded tissues were available in almost all cases while frozen tissues and cell-suspensions were available in some cases. Histopathological diagnoses and grading were based on the new WHO
classification and carried out by four pathologists (GY, KK, KM and OK)\textsuperscript{1}. Clinical information was obtained by reviewing the tumor registry records and/or patients’ medical charts.

\textit{Immunohistochemistry}

Paraffin sections from each sample were immunostained with monoclonal antibodies against CD10 (Novocastra, Newcastle, UK), Bcl2 (DAKO, Glostrup, Denmark), MUM1 (DAKO), CD21 (DAKO), CD138 (Novocastra) and Bcl6 (Novocastra) following the method described previously\textsuperscript{5}. The following two categories were defined: negative (<30% positively-stained tumor cells) and positive (≥30% positively-stained tumor cells).

\textit{Fluorescence in situ hybridization}

We used 39 cell suspensions fixed with methanol/acetic acid (3:1) and 4 paraffin-embedded tissue blocks. We used LSI IgH Spectrum Green/LSI Bcl2 Spectrum Orange Dual-Fusion Translocation Probe (Vysis, Downers Grove, IL) and the LSI Bcl6 Dual Color breakpoint probe (Vysis). Analyses were performed using the methods described previously\textsuperscript{5}. We were able to evaluate gene translocation as well as amplification in cases fixed with methanol/acetic acid (3:1). However, we could not evaluate gene amplification in paraffin-embedded tissues because morphological detection of single cells was difficult in these samples.
Somatic mutation analysis of VH genes

DNA was prepared from whole sections of 10 paraffin-embedded tissues. Somatic mutations and ongoing mutations in immunoglobulin VH region were identified by using primer sets (FR2B, SJHa and SJHb) and methods described previously.10

Statistical analysis

We used Student t-test and chi-square test to compare clinical and pathological findings among different groups. A P value less than 0.05 denoted the presence of a statistically significant difference.

Results and Discussion

Clinical features of CD10(-)MUM1(+) FL

We compared the clinical features of patients with CD10(-)MUM1(+) FL and 131 patients with CD10(+)MUM1(-) FL (Table 1) (see also Supplementary Table 1). CD10(-)MUM1(+) FL were predominantly elderly men compared with CD10(+)MUM1(-) FL (males: 73% vs 41%, age: 67 vs 58.7 years, respectively). The prognosis of patients with CD10(-)MUM1(+) is summarized in supplementary Table S1. Although the follow-up duration was relatively short in this study (6-72 months, median follow-up:13 months), only two patients survived longer than 5 years. Although small number and short duration of follow-up, CD10(-)MUM1(+) FL cases showed relatively poor prognosis in this study. MUM1 is expressed in various B cell lymphomas and its expression is associated with clinical features including prognosis, especially in diffuse large-B-cell lymphoma.11-13 Further clinical studies that include
larger number of samples and longer follow-up period are needed to clarify the impact of MUM1 expression on the prognosis of FL.

**Pathological features of CD10(-)MUM1(+) FL and comparison with CD10(+)MUM1(-) FL (Figure 1)**

Among 21 CD10(-) FL cases that have been analyzed previously, 8 (38%) were MUM1(+) while only one case (1.3%) among 83 CD10(+) cases was MUM1(+). Thus, it is conceivable that MUM1 expression in FL is linked to CD10-negativity. We added 14 CD10(-)MUM1(+) newly diagnosed FL cases, and a total of 22 CD10(-)MUM1(+) FL cases were analyzed and compared with CD10(+)MUM1(-) typical FL (Table 1) (see also Supplementary Table 1). CD10(-)MUM1(+) FL showed high grade (Grade 3A or 3B) morphology compared with CD10(+)MUM1(-) typical FL (91% vs 17%, \( p<0.00001 \)). CD10(-)MUM1(+) FL showed more frequent diffuse proliferation (59% vs 19%, \( p<0.0001 \)), less frequent BCL2 protein expression (59% vs 93.7%, \( p<0.00001 \)) and lacked BCL2/IgH translocation (5% vs 92.5%, \( p<0.00001 \)), compared with typical FL. BCL6 gene abnormalities (translocation and amplification) were identified in 88% (15/17) of CD10(-)MUM1(+) FL. Staining for CD138 was negative, and CD21 staining showed follicular dendritic cells meshwork in all CD10(-)MUM1(+) FL (Figure 1b). There was not clear difference between Grade 3B and 2/3A among CD10(-)MUM1(+) FL (Supplementary Table S1). The frequency of somatic mutation of immunoglobulin heavy chain in CD10(-)MUM1(+) FL ranged from 2.04 to 25.69% with a mean of 11.81%. Intraclonal diversity, which reflects ongoing mutation, was detected in all cases, suggesting definitive germinal center B
cell origin (Supplementary Table S2). Since CD10(+)MUM1(-) FL are mainly of low-grade (Grade 1/2) type, we selected 20 cases of high-grade (Grade 3A/3B) CD10(-)MUM1(+) FL cases and 20 cases of CD10(+)MUM1(-) FL cases to exclude bias based on morphological differences. For analysis of BCL6 gene abnormality, we added 23 new CD10(+)MUM1(-) high grade cases. However, CD10(-)MUM1(+) FL still lacked BCL2/IgH gene translocation and showed BCL6 gene abnormality while BCL2 protein expression was not different relative to typical FL. Furthermore, to validate MUM1 expression as a marker in CD10-negative FL, we compared CD10(-)MUM1(+) with CD10(-)MUM1(-) FL (n=30). In the latter group, only 44% (13/30) showed high grade morphology (Grade3) (p<0.01, vs CD10(-)MUM1(+) FL).

Similarly 50% (13/26) (p<0.01) and 36% (11/30) (p=0.11) of them lacked BCL2 gene rearrangement and combined diffuse proliferation area, respectively. These proportions were lower than CD10(-)MUM1(+) FL. These results suggest that MUM1 expression defines more homogeneous disease entity among CD10 negative FLs.

Until now, several reports have described an “atypical” minority subtype of FL. Although this group is different from typical FL, it is still a heterogeneous disease entity. High-grade FL, especially Grade 3B, has been discussed as a different entity from typical low-grade FL because of the low-rate CD10 expression and BCL2 gene translocation and high-rate BCL6 gene translocation. However, the proportion of CD10(+) cases among Grade 3B FL varied from 37% to 57%. BCL2 and BCL6 gene rearrangements varied from 13% to 43% and from 30% to 44%, respectively. Next, FL without t(14;18) and with/without BCL6 gene rearrangement, were described as a unique FL subtype by some groups, but they showed the...
variability in morphological and immunophenotypic findings (Grade 3: 13-78%, CD10(+): 29-36%). In this study, almost all (about 90%) CD10(-)MUM1(+) FL were high-grade, negative for BCL2 gene translocation and BCL6 gene abnormality (translocation or amplification). For these reasons, we consider CD10-MUM1+ FL is related to previously described atypical FL but we also suggest (on morphologic, phenotypic, and genetic grounds) that it forms a more homogeneous disease entity. Our findings may also carry the clinically interesting implication that a minority of DLBCL localize anatomically to lymphoid follicles and thereby mimic classical FL, when in reality they are unrelated to the latter disorder. Several features of our cases, (e.g. the frequencies of BCL2 and BCL6 gene rearrangement, the poor survival ) are consistent with this hypothesis.

Acknowledgments

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Figure 1

(a) (b)
(c) (d)
(e) (f)
Figure legends

Figure 1. Pathological features of CD10(-)MUM1(+) follicular lymphoma. In H&E sections, the lymphoma cells form definite follicular structures (a). The follicular structures are confirmed by follicular dendritic cells (FDC) meshwork stained for CD21 (b). In this case, almost all lymphoma cells are large cells and their proliferation shows a “sheet-like” pattern (e). This case was diagnosed as Grade 3B. Lymphoma cells are positive for CD20 (c), MUM1 (d) and (f). All figures are from Case 7.

Original magnification: (a)-(d): X10, (e) and (f): X100
Table 1. Comparison between CD10(-)MUM1(+), CD10(+)MUM1(-) and CD10(+)MUM1(-) follicular lymphomas.

<table>
<thead>
<tr>
<th>Pathological features</th>
<th>CD10(-)MUM1(+) FL</th>
<th>CD10(+)MUM1(-) FL</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>n=22</td>
<td>n=119</td>
<td></td>
</tr>
<tr>
<td>Grade 3A/B (%)</td>
<td>91% (20/22)</td>
<td>17% (20/119)</td>
<td>&lt;0.00001 (χ² test)</td>
</tr>
<tr>
<td>with diffuse area (%)</td>
<td>59% (13/22)</td>
<td>19% (23/119)</td>
<td>&lt;0.0001 (χ² test)</td>
</tr>
<tr>
<td>Bcl2 expression (%)</td>
<td>59% (13/22)</td>
<td>93.7% (112/119)</td>
<td>&lt;0.00001 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 expression (%)</td>
<td>54% (12/22)</td>
<td>72% (86/119)</td>
<td>0.1 (χ² test)</td>
</tr>
<tr>
<td>Bcl2 translocation (%)</td>
<td>5% (1/20)</td>
<td>92.5% (110/119)</td>
<td>&lt;0.00001 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 translocation (%)</td>
<td>30% (6/20)*</td>
<td>4.2% (5/119)</td>
<td>&lt;0.0001 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 amplification (%)</td>
<td>62% (10/16)*</td>
<td>26% (6/23)**</td>
<td>&lt;0.05 (χ² test)</td>
</tr>
<tr>
<td>Only cases of Grade 3A/B</td>
<td>n=20</td>
<td>n=20(+23)*</td>
<td></td>
</tr>
<tr>
<td>with diffuse area (%)</td>
<td>55% (11/20)</td>
<td>45% (9/20)</td>
<td>0.44 (χ² test)</td>
</tr>
<tr>
<td>Bcl2 expression (%)</td>
<td>60% (12/20)</td>
<td>80% (16/20)</td>
<td>0.17 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 expression (%)</td>
<td>52% (11/21)</td>
<td>70% (14/20)</td>
<td>0.24 (χ² test)</td>
</tr>
<tr>
<td>Bcl2 translocation (%)</td>
<td>5.3% (1/19)</td>
<td>60% (12/20)</td>
<td>&lt;0.0005 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 translocation (%)</td>
<td>32% (6/19)*</td>
<td>16% (7/43)**</td>
<td>0.17 (χ² test)</td>
</tr>
<tr>
<td>Bcl6 amplification (%)</td>
<td>62% (10/16)*</td>
<td>26% (6/23)**</td>
<td>&lt;0.05 (χ² test)</td>
</tr>
</tbody>
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Clinical features

<table>
<thead>
<tr>
<th>All cases</th>
<th>n=22</th>
<th>n=129</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>16:6</td>
<td>53:66</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.0</td>
<td>58.7</td>
</tr>
<tr>
<td>B symptoms (+:-)</td>
<td>5:12</td>
<td>16:88</td>
</tr>
<tr>
<td>Stage: I+II : III+IV</td>
<td>12:10</td>
<td>57:57</td>
</tr>
<tr>
<td>LDH (U/I)</td>
<td>423</td>
<td>428</td>
</tr>
<tr>
<td>Only cases of Grade 3A/B</td>
<td>n=20</td>
<td>n=25</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>15:05</td>
<td>16:09</td>
</tr>
<tr>
<td>Age (years)</td>
<td>65.3</td>
<td>57.4</td>
</tr>
<tr>
<td>B symptoms (+:-)</td>
<td>5:12</td>
<td>6:13</td>
</tr>
<tr>
<td>Stage I+II : III+IV</td>
<td>10:10</td>
<td>13:08</td>
</tr>
<tr>
<td>LDH (U/I)</td>
<td>496</td>
<td>740</td>
</tr>
</tbody>
</table>

*One case showed both BCL6 gene translocation and amplification. **23 cases of CD10(+)MUM1(-) FL (Grade 3) were added to previous cases for analysis of BCL6 gene abnormality.
References


5. Guo Y, Karube K, Kawano R, Yamaguchi T, Suzumiya J, Huang GS, Ohshima K. Low-grade follicular lymphoma with t(14;18) presents a homogeneous disease entity otherwise the rest comprises minor groups of heterogeneous disease entities with Bcl2 amplification, Bcl6 translocation or other gene aberrances. Leukemia. 2005;19:1058-1063
6. Bosga-Bouwer AG, van den Berg A, Haralambieva E, de Jong D, Boonstra R, Kluin P, van den Berg E, Poppema S. Molecular, cytogenetic, and immunophenotypic characterization of follicular lymphoma grade 3B; a separate entity or part of the spectrum of diffuse large B-cell lymphoma or follicular lymphoma? Hum Pathol. 2006;37:528-533


12. Tagawa H, Suguro M, Tsuzuki S, Matsuo K, Karnan S, Ohshima K, Okamoto M,


15. Katzenberger T, Ott G, Klein T, Kalla J, Muller-Hermelink HK, Ott MM.
Cytogenetic alterations affecting BCL6 are predominantly found in follicular lymphomas grade 3B with a diffuse large B-cell component. Am J Pathol. 2004;165:481-490


Follicular lymphoma without t(14;18) and with BCL-6 rearrangement: a
lymphoma subtype with distinct pathological, molecular and clinical characteristics. Leukemia. 2002;16:2309-2317
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