A low-grade B-cell lymphoma with prolymphocytic/paraimmunoblastic proliferation and IRF4 rearrangement

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Haematologica 2013 [Epub ahead of print]

doi:10.3324/haematol.2012.076851

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To the Editor,

Translocations between IRF4 and immunoglobulin genes, present in myeloma(1) and high-grade B-cell lymphomas,(2) have not been reported for low-grade B-cell neoplasms. We report 3 low-grade B-cell lymphoma cases with IRF4 rearrangement showing characteristic morphological features and immunophenotypes.

While checking our laboratory developed FISH probes for IRF4 split assay on paraffin sections, we incidentally found IRF4 rearrangement in case 1 (the sample at recurrence; Figure 1A). The original pathological diagnosis was low-grade B-cell lymphoma, unclassified. The cytogenetic analysis record indicated that this tumor harbored a balanced translocation between the IGK and IRF4 genes, t(2;6)(p11.2;p25). We then performed fusion FISH assays and confirmed IRF4-IGK present and IRF4-IGH absent (Figure 1B-E). The primary lesion (biopsied 8 years prior to the index sample) also displayed IRF4 rearrangement. A case that had been diagnosed as low-grade B-cell lymphoma, unclassified in the original institute was presented in a regional pathology conference, 10 days after the identification of case 1. The cytomorphological feature of the case was reminiscent of that of case 1 although paraimmunoblasts were more predominant (Figure 1P). The morphology prompted us to perform FISH assays for IRF4,
obtaining positive results (Figure 1L–O). We listed it as case 2. Lymphoma samples (784 cases including 232 low-grade B-cell lymphomas) were screened by split FISH assay for IRF4. Case 3 was identified in this process, and the original pathology diagnosis was low-grade B-cell lymphoma, unclassified. The cytogenetic analysis record, t(2;6)(p12;p25), supported the presence of IRF4-IGK. Cases 1 was also in this cohort, and therefore 2 of 232 low-grade B-cell lymphomas (0.86%) harbored IRF4 rearrangement in the cohort.

Histologically, the infiltrate was basically diffuse, and the lymph node architecture was totally effaced. In case 2, however, broad fibrotic bands divided the lymphoma infiltrate into nodules (Figure 1K). The lymphoma cells in the 4 lesions from the 3 patients comprised prolymphocytes, paraimmunoblasts, and small lymphocytes, varying in proportion between lesions. No proliferation centers or lymph follicles were seen, except for the primary lesion in case 1, in which areas of prolymphocytic and paraimmunoblastic infiltration were confluent with a background of neoplastic small lymphocytes and a few regressed primary follicles without follicular colonization were highlighted by the presence of follicular dendritic cell (FDC) network as determined by anti-CD23 immunohistochemistry. In case 1, the recurring lesion showed a diffuse infiltrate of prolymphocytes and occasional paraimmunoblasts (Figure 1F). In case 2, most tumor cells were paraimmunoblasts admixed with occasional prolymphocytes (Figure 1P). On morphological examination only, this cytomorphological feature, together with fibrotic nodularity (Figure 1K), might lead to a differential diagnosis of grade 3 follicular lymphoma (FL). However, these nodules had no FDC networks. Case 3 comprised prolymphocytes and small-to-medium cells with a small central nucleolus in an irregular nucleus [giving an impression of mantle cell lymphoma (MCL)], but it was somewhat indefinite because of admixed T cells and tissue scarcity. The immunohistochemical results for the 3 cases indicated CD5− (dim in case 3 by flow cytometry), CD10−, CD20+, CD23+, CD43+, CD138−, MUM1/IRF4−
LMO2 was negative in cases 1 and 2 (occasional lymphoma cells were positive in the latter) (supplementary Figure 1). The Ki67-labeling index was universally low (approximately 10%; Figure 1J, T). The clinical courses and the results of FISH analyses are summarized in Table 1.

The overall histological impression of the present cases is reminiscent of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) with prominent prolymphocytic/paraimmunoblastic infiltration (the tumor-forming subtype of CLL/SLL(3, 4) or paraimmunoblastic variant of CLL/SLL(5-8)) or tissue involvement of B-cell prolymphocytic leukemia (B-PLL). We examined cases 1 and 2 for 4 chromosomal aberrations, which are commonly observed in CLL/SLL [del(11)(q22.3), +12, del(13)(q14.3) del(17)(p13.1)], by FISH, and LEF1, which is characteristically expressed in CLL/SLL,(9) by immunohistochemistry. All were negative in cases 2, but 13q14.3 was deleted and LEF1 was weakly expressed in case 1 (supplementary Figure 2), implying that case 1 might be more closely related to CLL/SLL.

In CLL/SLL, small lymphocytes are negative or only faintly positive for MUM1/IRF4 and BCL6 expression, whereas prolymphocytes and paraimmunoblasts are moderately positive for MUM1/IRF4 but negative for BCL6.(10, 11) In contrast, most lymphoma cells in the present cases expressed MUM1/IRF4 and BCL6, an uncommon characteristic of well-known low-grade B-cell lymphomas. The cases reported as paraimmunoblastic variants of CLL/SLL were positive for CD5, followed an aggressive clinical course, and harbored IGH-CCND1 (5 of 8 examined cases).(5-8) These features suggest that most of the reported cases represented MCL (especially pleomorphic variant). None of the present cases showed any aggressive clinical behavior and they showed IRF4 rearrangement without other translocations commonly observed in B-cell neoplasms including CCND1 rearrangement. Variable expression of CD5, strong expression of IgM, and absence of IgD in the present cases are not typical in CLL/SLL and may be suggestive
of the immunophenotype of B-PLL. However, none of our patients showed leukemic involvement of the peripheral blood and IRF4 rearrangement has not been reported in B-PLL. Nodal involvement by B-PLL is rare, and previous histological studies probably included MCL.(12) The morphological features, immunophenotype, genetics, and clinical presentation of the present cases seemed incompatible with those of existing subtypes, prompting us to distinguish the present cases as “prolymphocytic/paraimmunoblastic lymphoma (PPL)”. The relationship with other low-grade B-cell neoplasms remains to be clarified.

The question of whether the present cases are “precursors” of the IRF4-rearranged high-grade B-cell lymphomas(2) remains. Cytogenetic analyses were successful in cases 1, 46XX,t(2;6)(p11.2;p25)[10]/46XX,t(1;11)(q21;q23)[1]/46XX[9], and 3, 46XY,t(2;6)(p12;p25)[1]/46,sl,-Y,-4,-8,-9,add(11)(q23),+5mar[1]/46XY[11]. No evidence of rearrangement in BCL2, BCL6, MYC, and CCND1 was obtained in the present cases, whereas in previous cases,(2) 7 and 1 of the 19 IRF4-rearranged high-grade B-cell lymphomas showed BCL6 and MYC rearrangements, respectively. PCR for IGH gene rearrangement was successful in cases 1 and 2. The IGHV usage was V3-11*01 and V1-8*01, and identity to germline sequences from CDR1 to FWR3 regions was 100% and 94.7%, respectively, with intraclonal diversity in the latter. These mutation statuses may contrast those in the IRF4-rearranged high-grade B-cell lymphomas (93.9% to 86.1%).(2) Light-chain genes were the translocation partners of IRF4 in the present cases and IGH in 17 of the 19 IRF4-rearranged high-grade B-cell lymphoma cases studied by Salaverria et al.(2) (P = 0.0065, Fisher exact test). Most patients with high-grade cases were young,(2) indicating that IRF4-rearranged high-grade B-cell lymphomas are likely to be de novo. However, it is interesting that case 15 in the series of Salaverria et al.(2) harbored IGK as its IRF4 partner and was a transformed lymphoma.(2) The present and previous findings indicate that IRF4 rearrangement may be related to the development of low-grade and
primary high-grade lymphomas and that some IRF4-rearranged low-grade lymphomas may progress to high-grade lymphomas.

To elucidate the questions presented herein, more “PPL” cases should be examined. The prolymphocytic/paraimmunoblastic morphological features and coexpression of BCL6 and MUM1/IRF4 in most lymphoma cells are key indications for confirmatory FISH for IRF4 rearrangement.

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Acknowledgments

We thank Drs. Sheng-Tsung Chang, Masahiro Yokoyama, Yasuhiro Terui and the members of the Ganken Ariake Lymphoma Study Group (GALSG) and Tokyo Lymphoma Study Group (TLSG) for their advice. We appreciate Ms. Satoko Baba and Ms. Noriko Matsumoto for their technical assistance. We also thank Ms. Sayuri Sengoku for her administrative assistance. This
study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by grants from the Japan Society for the Promotion of Science; Ministry of Health, Labor, and Welfare of Japan; Vehicle Racing Commemorative Foundation of Japan; and Izumo City Supporting Cancer Research Project.
Authorship and Disclosures

K.T. conceived the disease concept, made diagnoses, identified case 1 by FISH and case 2 by morphology, collected and analyzed the data, and wrote the manuscript. S.S. identified case 3 by FISH, collected and analyzed the data, and contributed to the manuscript. R.A. produced and analyzed the PCR-based data and contributed to the manuscript. N.T. made diagnoses and contributed to the manuscript. A.D. summarized the clinical data and contributed to the manuscript. M.N. contributed to patient care, collected clinical data, and contributed to the manuscript. The authors declare no competing financial interests.
References


Table 1. Clinicopathological summary.

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<th>Age at onset/sex</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<td>38/F</td>
<td>62/M</td>
<td>66/M</td>
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<table>
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<tr>
<th>Stage (site of involvement)</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<tbody>
<tr>
<td>Stage 4 (axillary LN, inguinal LN, bone marrow)</td>
<td>Stage 1 (inguinal LN)</td>
<td>Stage 1 (retroperitoneal LN)</td>
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<table>
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<tr>
<th>White blood count (% lymphocyte)</th>
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<th>Case 2</th>
<th>Case 3</th>
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<tr>
<td>5,600/μl (44%)</td>
<td>5,000/μl (31%)</td>
<td>6,100/μl (37%)</td>
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<th>International prognostic index</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<td>Low</td>
<td>Low</td>
<td>Low</td>
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<th>Treatment</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<td>For primary lesions: rituximab+CHOP followed by rituximab only. At recurrence (8 years after the initial diagnosis): Rituximab+fludarabine</td>
<td>No treatment (excisional biopsy only)</td>
<td>rituximab+CVP followed by rituximab only</td>
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<tr>
<th>Outcome</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
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<td>Alive with 2nd CR for 10 years after the initial diagnosis</td>
<td>Alive with CR for 1 year after the diagnosis</td>
<td>Alive with CR for 4.5 years after the diagnosis</td>
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</table>

**FISH**
- IRF4 split
- IRF4-IGK fusion
- IRF4-IgL fusion
- IGK split
- IGL split
- IGH split
- BCL2 split
- BCL6 split
- MYC split
- CCND1 split

**IHC/ISH**
- CD5
- CD10
- CD20
- CD23
- CD43
- CD138
- BCL2
- BCL6
- MUM1/IRF4
- IgM
- IgD
- EBER
- TdT
- Cyclin D1
- Ki67 labeling

**IGHV usage**
- V3-11*01
- V1-8*01
- NE

**IGHV identity to germline sequence**
- 100%
- 94.7%
- NE

**Cytogenetics**
- 46XX,t(2;6)(p11.2;p25)[1]/46XX,t(1;11)(q21;q23)[1]/46XX[9]*
- 46XY,t(2;6)(p12;p25)[1]/46,sl,Y,-4,-8,-9,-9,add(11)(q23),+5mar[1]/46XY[11]*

LN, lymph node; CHOP, Cyclophosphamide+ Adriamycin+Vincristine+Prednisone; CVP, Cyclophosphamide+Vincristine+Prednisone; CR, complete response; FISH, fluorescein in situ hybridization; IHC/ISH, immunohistochemistry/in situ hybridization; ND, not done; NE, not evaluable.

*Only data at recurrence were available. ** Dim by flow cytometry.
Figure 1. Morphological and histomolecular examination results

Panels A to J and K to T illustrate cases 1 (at recurrence) and 2, respectively. All of the present lesions had positive split FISH assay results for IRF4. The lymphoma cells harbored a yellow signal (wild-type IRF4) and individual red (5’-side of IRF4) and green signals (3’-side of IRF4; A). The split FISH assays for IGK and IGL were positive in cases 1 (B, C, and E) and 2 (L, M, and O), respectively. On the same sections, IRF4 stained blue (D and N) and was observed fusing to the 3’-side of IGK (E, arrowhead) or 3’-side of IGL (O, arrowhead). The infiltration pattern was diffuse, but it was divided by sclerotic bands in case 2 (K, 4× objective). The infiltrate comprised prolymphocytes and occasional paraimmunoblasts and small lymphocytes (F, 60× objective). In case 2, paraimmunoblasts were prominent and neoplastic small lymphocytes were rare (P, 60× objective). Most of the lymphoma cells expressed MUM1/IRF4 (G and Q, 40× objective) and BCL6 (H and R, 40× objective) simultaneously. IgM was strongly expressed (I and S, 40× objective). The Ki67-labeling index was low (J and T, 40× objective).
sFig. 1 LMO2: case 1 (left), case 2 (center and right) 40x objective
sFig 2. LEF1
Figure 2. LEF1

In case 1, the lymphoma cells were weakly positive for LEF1 (A. 40x objective). The staining intensity was lower than that of reactive T cells (B. LEF1: brown; CD20: red. 40x objective). The lymphoma cells in case 2 were negative for LEF1 (C. 40x objective). The cells of small lymphocytic lymphoma (D. Positive control. 40x objective) were positive for LEF1, more intensely in proliferation center cells (upper right).