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

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 eight 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, ten 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

Table 1. Clinicopathological summary

		Case 1	Case 2	Case 3
Age at onset/gender		38/F	62/M	66/M
Stage (site of involvement)		Stage 4 (axillary LN, inguinal LN, bone marrow)	Stage 1 (inguinal LN)	Stage 1 (retroperitoneal LN)
White blood count (% lymphocyte)		5.6x10⁹/L (44%)*	5.0x10⁹/L (31%)	6.1x10⁹/L (37%)
International prognostic index		Low	Low	Low
Treatment		For primary lesions: rituximab+CHOP followed by rituximab only. At recurrence (8 years after the initial diagnosis): rituximab+fludarabine	No treatment (excisional biopsy only)	rituximab+CVP followed by rituximab only
Outcome		Alive with 2nd CR for 10 years after the initial diagnosis	Alive with CR for 1 year after the diagnosis	Alive with CR for 4.5 years after the diagnosis
FISH	*IRF4* split	+	+	+
	IRF4-IGK fusion	+*	ND	ND
	IRF4-IGL fusion	-*	+	ND
	IGK split	+*	-	NE
	IGL split	-*	+	ND
	IGH split	-*	-	ND
	BCL2 split	-*	-	-
	BCL6 split	-*	-	-
	MYC split	-*	-	-
	CCND1 split	-*	-	-
IHC/ISH	CD5	-	-	-**
	CD10	-	-	-
	CD20	+	+	+
	CD23	+	+	+
	CD43	+	+	+
	CD138	-	-	ND
	BCL2	+	+	+
	BCL6	+*	+	+
	MUM1/IRF4	+	+	+
	IgM	+	+	+
	IgD	-*	-	ND
	EBER	-*	-	-
	TdT	-*	-	ND
	Cyclin D1	-	-	-
	Ki67 labeling index	~10%	~10%	~10%
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)[10]/46XX,t(1;11)(q21;q23)[1]/46XX[9]*	ND	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: fluorescent 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 1L-O) (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*. Case 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

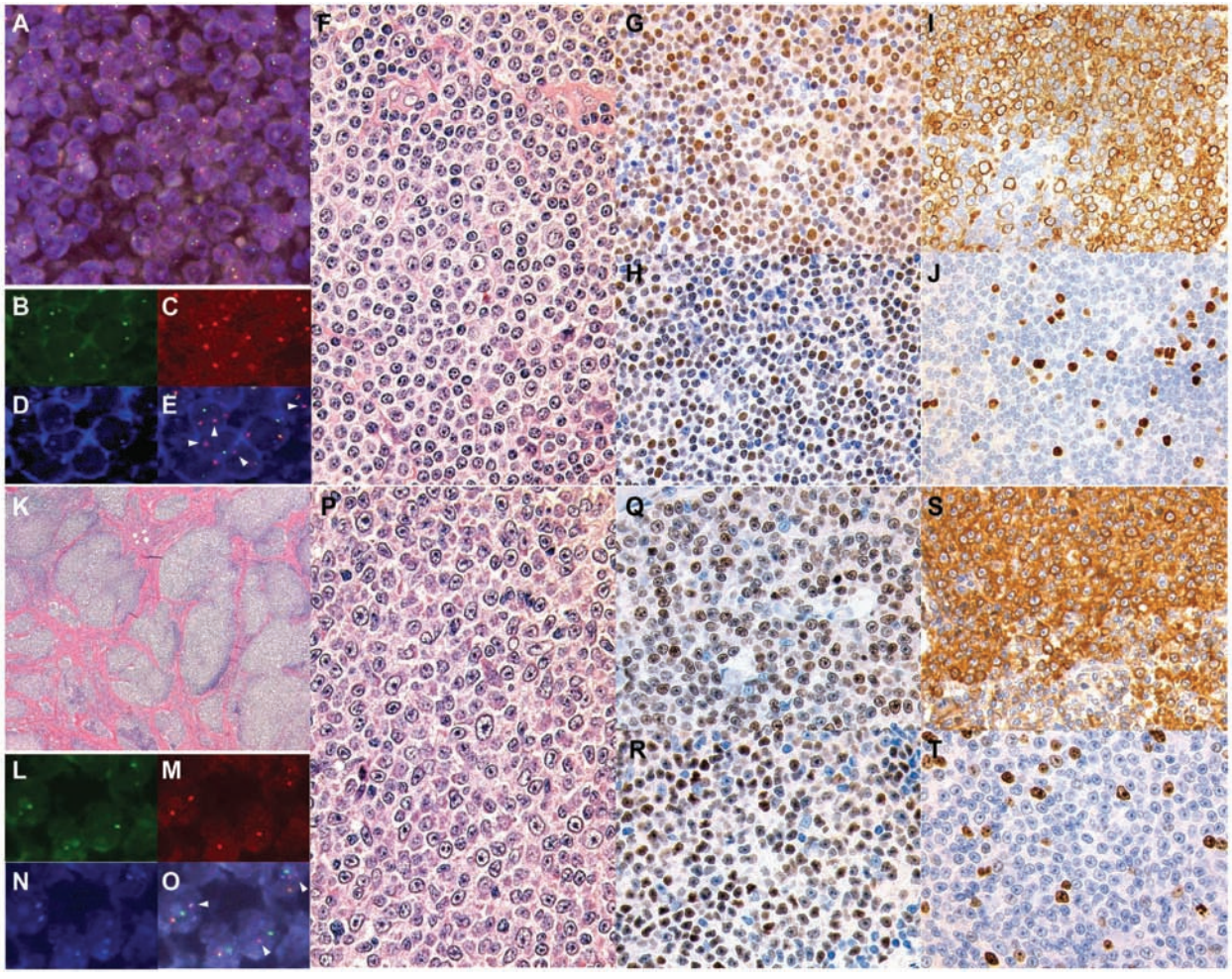

Figure 1. Morphological and histomolecular examination results. Panels A to J and K to T illustrate cases 1 (at recurrence) and 2, respectively. All of the present lesions had positive split FISH assay results for *IRF4*. The lymphoma cells harbored a yellow signal (wild-type *IRF4*) and individual red (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, 4x objective). The infiltrate comprised prolymphocytes and occasional paraimmunoblasts and small lymphocytes (F, 60x objective). In case 2, paraimmunoblasts were prominent and neoplastic small lymphocytes were rare (P, 60x objective). Most of the lymphoma cells expressed MUM1/*IRF4* (G and Q, 40x objective) and BCL6 (H and R, 40x objective) simultaneously. IgM was strongly expressed (I and S, 40x objective). The Ki67-labeling index was low (J and T, 40x objective).

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*$^+$ (Figure 1G and Q), BCL2$^+$, BCL6$^+$ (Figure 1H and R), IgM$^+$ (Figure 1I and S), IgD$^-$, TdT$^-$, and Cyclin D1$^-$. LMO2 was negative in cases 1 and 2 (occasional lymphoma cells were positive in the latter) (*Online Supplementary Figure S1*). The Ki67-labeling index was universally low (approximately 10%; Figure 1J and 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 case 2, but 13q14.3 was deleted and LEF1 was weakly expressed in case 1 (*Online Supplementary Figure S2*), 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 unanswered. Cytogenetic analyses were successful in case 1, 46XX,t(2;6)(p11.2;p25)[10]/46XX,t(1;11)(q21;q23)[1]/46XX[9], and case 3, 46XY,t(2;6)(p12;p25)[1]/46,sl,-Y,-4,-8,-9,-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 to those in the *IRF4*-rearranged high-grade B-cell lymphomas (93.9%-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's 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 clarify the questions presented here, more "PPL" cases should be examined. The prolymphocytic/paraimmunoblastic morphological features and co-expression of BCL6 and MUM1/*IRF4* in most lymphoma cells are key indications for confirmatory FISH for *IRF4* rearrangement.

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