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LETTER TO THE EDITOR

Cryptic *t*(3;8)(q27;q24) and/or MYC-BCL6 linkage associated with MYC expression by immunohistochemistry is frequent in multiple-hit B-cell lymphomas

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B-cell lymphomas with recurrent cytogenetic rearrangements affecting the chromosomal loci of *MYC* (8q24) and either *BCL2* (18q21) and/or *BCL6* (3q27) are referred to as double- or triple-hit lymphomas.[1] Most frequently, these three oncogenes are rearranged with the immunoglobulin (*IG*) genes, and chromosomal translocations affecting either one of the three *IG* loci as a partner are readily recognized by conventional G-banding. However, fluorescence *in situ* hybridization (FISH) using locus-specific probes with interphase nuclei to detect the rearrangements has become a substitute for time-consuming G-banding analysis. As >90% of *BCL2* rearrangements involve the *IG* heavy chain (*IGH*) gene as the partner and occur downstream of the coding region of *BCL2*,[2] the presence of fusion signals from the nuclei after hybridization with the *BCL2-IGH* dual-color, dual-fusion probe is equivalent to *t*(14;18)(q32;q21) translocation.[3] On the other hand, since *MYC* and *BCL6* involve not only *IG* genes but also diverse non-*IG* partners, and because the breakpoints on both genes distribute within a wide range,[4–6] the presence of break-apart (BA) signals from the nuclei after hybridization with the *MYC* or *BCL6* dual-color BA probe, which hybridizes to opposite sides of *MYC* or *BCL6*, does not necessarily infer equivalent significance to chromosomal rearrangements identified by conventional cytogenetic studies. To determine each partner or identify cryptic or complex translocations involving unexpected chromosomal loci, interphase FISH analysis in combination with G-banding is required.

From the list of patients with B-cell lymphoma diagnosed and treated in our institution between 2008 and 2016, we identified 16 patients who had a lymphoma with recurrent chromosomal breakpoints involving multiple oncogenes, one of which was 8q24/*MYC* (Supplementary Table S1). Of the 16, 4 had no identifiable cytogenetic abnormalities based on G-banding at band q24 of chromosome 8 and band q27 of chromosome 3, despite the presence of BA signals of *MYC* and *BCL6* (Supplementary Figures S1–S4). To complement the cytogenetic studies, we performed a series of interphase FISH studies using commercially available probes. Hybridization with the *MYC* BA probe (Vysis) revealed that red signals representing the 5′ sequences of *MYC* were localized at band q24 of normal-appearing chromosome 8 in cases 10 and 13. In case 14, the signal was localized at the q terminal of the 10q− chromosome [add(10)(q24)]. In case 15, in which the karyotype showed tetraploidy, q24 of two chromosome 8s and the q terminal of a C-group marker were labeled with the 5′ *MYC* signal. On the other hand, green signals representing the 5′ *MYC* were localized at band q27 of normal-appearing chromosome 3 in cases 14 and 15, while the signal was localized at the p terminal of the 2p− chromosome [add(2)(p12)] in case 10, and was lost in case 13 (Figures 1a and b).

Hybridization with the *BCL6* BA probe (Vysis) showed that the red-labeled 5′ *BCL6* signal was localized at 8q24 in cases 10 and 13, the q terminal of 10q− in case 14, and two 8q24s and the q terminal of the C-group marker in case 15. The green-labeled 3′ *BCL6* was localized at 3q27 in cases 10 and 14, while the signal was not identified in cases 13 and 14 (Figures 1a and b). We then hybridized the metaphase spreads sequentially with the *MYC* and *BCL6* BA probes, except for case 15, and confirmed co-localization of the 5′ *MYC* and 5′ *BCL6* signals in cases 10, 13 and 14, and co-localization of the 3′ *MYC* and 3′ *BCL6* signals in case 14 at relevant chromosomal loci (Figure 1a). In case 15, the lack of green signals but presence of four yellow signals suggested that breakage within the *BCL6* occurred at a point included in the region covered by the 5′ *BCL6* probe, and hybridization with another *MYC* BA probe (Dako) showed five yellow and two small red signals, the latter of which were localized at 3q27, indicating that breakage within the *MYC* was close to the 3′ end of the red-labeled 3′ *MYC* probe (Figure 1b).[4,5] In summary, all four cases carried the 5′ *MYC* and 5′ *BCL6* linkage, while two cases lacked the reciprocal 3′ *MYC* and 3′ *BCL6* linkage (Table 1). Case 10 had a *MYC-IGH* and a *BCL6-IGH* linkage independently of the *MYC-BCL6*, and case 13 had a *t*(14;18)(q24:q21)/*BCL2-IGH* translocation.

Clinical and histopathological information on the four cases is summarized in Supplementary Tables S2–S4. Case 10 presented with disseminated disease of Burkitt lymphoma/leukemia and the remaining three developed diffuse large B-cell lymphoma (DLBCL) involving the gastrointestinal tract. Immunohistochemistry of the pathological specimens showed the germinal center B-cell-like immunophenotype in all cases. Case 10 lacked the expression of BCL6 and case 15 was negative for BCL2. Case 10 was negative for the expression of immunoglobulins based on flow cytometry. Immunohistochemistry using the Y69 anti-c-MYC monoclonal antibody (Abcam PLC, Cambridge, UK) demonstrated nuclear staining in >90% of lymphoma cells in all four cases (Supplementary Figure S5). Case 10 showed a rapidly progressive and fatal course, while case 14 responded to conventional chemotherapy and remains in remission >3 years after completing the therapy.

Here, we described four cases of B-cell lymphoma that carried the cryptic *t*(3;8)(q27;q24) and/or *MYC-BCL6* linkage. As the chromosomal materials distal to band q24 of chromosome 8 and those distal to band q27 of chromosome 3 are similar in size and banding appearance, the der(8)*t*(3;8)(q27;q24) and der(3)*t*(3;8)(q27;q24) chromosomes were not reliably recognized by conventional G-banding without the aid of FISH cytogenetics using both *MYC* and *BCL6* probes. On the other hand, we found that the *MYC-BCL6* linkage can occur at not only der(8)*t*(3;8)(q27;q24) but also independent chromosomal loci, suggesting complex translocation involving ⩾3 chromosomal loci or the submicroscopic exchange of chromosome materials. In the current series of multiple-hit lymphoma, the linkage accounted for 25% (4 of 16) of the cases, and *BCL6* was the most frequent non-*IG* partner of 8q24/*MYC* translocation.

t(3;8)(q27;q24) was first described in Burkitt lymphoma/leukemia that developed in an 11-year-old girl who was affected by ataxia telangiectasia.[7] Subsequently, sporadic case reports

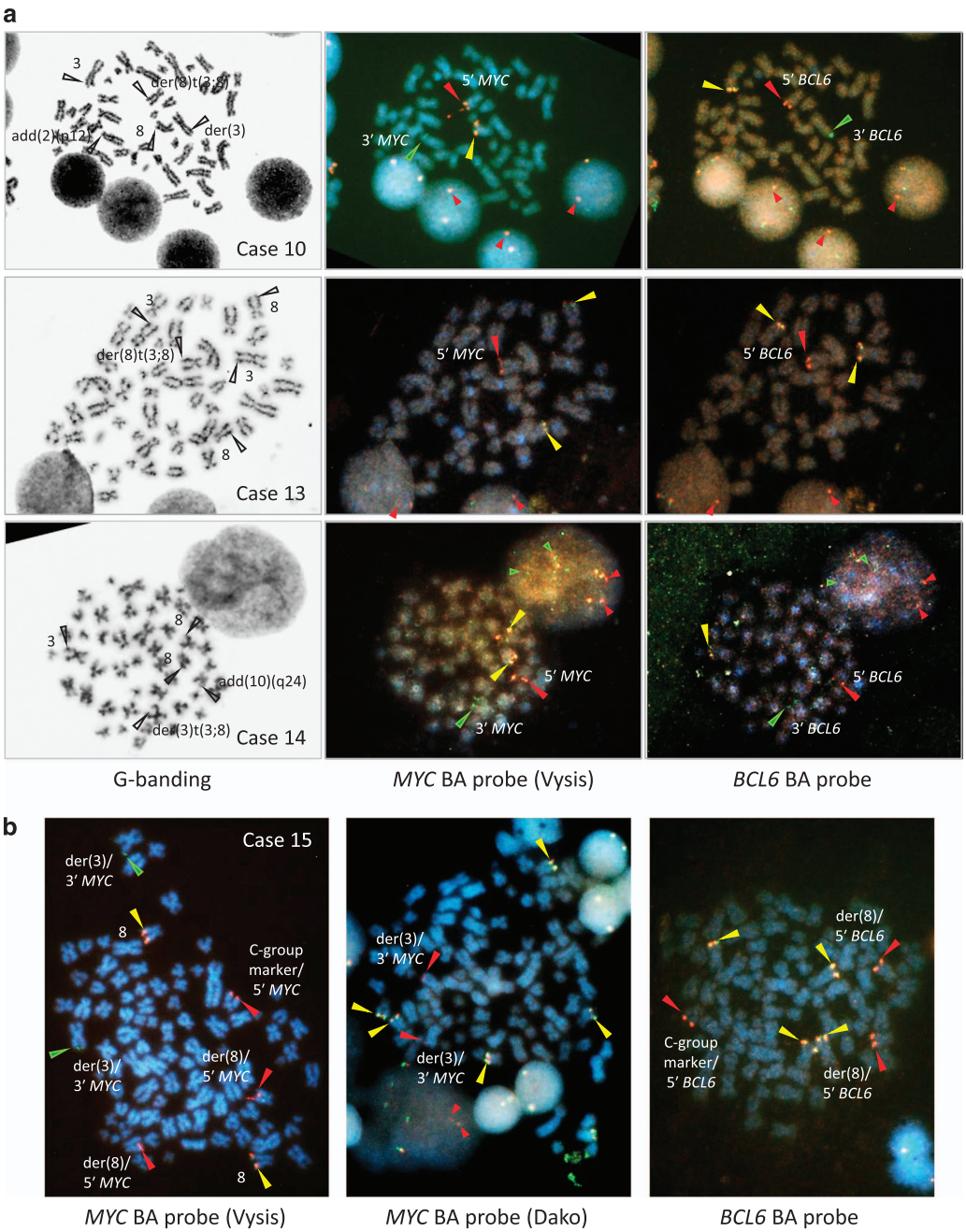

Figure 1. Metaphase FISH. (**a**) Sequential metaphase pictures of cases 10 (top), 13 (middle) and 14 (bottom). G-banding, FISH with *MYC* BA probe (Vysis), consisting of red-labeled 5′ *MYC* and green-labeled 3′ *MYC*, and FISH with *BCL6* BA probe (Vysis), consisting of red-labeled 5′ *BCL6* and green-labeled 3′ *BCL6*, are aligned from left to right. Relevant chromosomes and the FISH signals of each color are indicated by arrowheads. Small arrowheads on the nuclei show co-localization of FISH signals; the nucleus of case 14 shows tetraploidy. (**b**) Metaphase FISH pictures of case 15 with a tetraploid karyotype. FISH with *MYC* BA probe (Vysis), FISH with another *MYC* BA probe (Dako), consisting of green-labeled 5′ *MYC* and red-labeled 3′ *MYC*, and FISH with *BCL6* BA probe (Vysis) are aligned from left to right. Relevant chromosomes and the FISH signals of each color are indicated by arrowheads. Two small arrowheads on the nucleus in middle represent the 3′ *MYC* segment translocated to the *BCL6* locus.

describing B-cell tumors carrying *t*(3;8)(q27;q24) have appeared in the literature; the Mitelman database listed a total of four cases with *t*(3;8)(q27;q24) and additional two cases were found by a literature review.[8–11] In a large series of B-cell tumors focusing upon cytogenetic abnormalities, 1 of 20 cases with DLBCL characterized by multicolor FISH,[12] 2 of 17 cases with 8q24/*MYC*–non-*IG* rearrangement,[4] 3 of 54 cases with FISH-defined *MYC-BCL2* double-hit,[13] and 3 of 10 cases with triple-hit or more were reported to carry *t*(3;8)(q27;q24),[14] respectively. These cases showed Burkitt lymphoma/leukemia, DLBCL, or an intermediate DLBCL/BL histopathology with variable clinical presentations, as observed in our series, ranging from disseminated disease with leukemic manifestation to stage I disease.[4,8–11,14] A single intermediate DLBCL/BL patient with *t*(3;8)(q27;q24) as the sole

Table 1. Summary of FISH studies

Case no.	*Number of FISH signals*				*Co-localization of MYC and BCL6 signals*	
	MYC BA probe	*MYC-IGH DF probe*	*BCL2-IGH DF probe*	*BCL6 BA probe*	*5′ MYC-5′ BCL6*	*3′ MYC-3′ BCL6*
Case 10	R1G1Y1 (Vysis)	R2G3Y1B2	NT*	R1G1Y1	+	−
Case 13	R1G0Y2 (Vysis)	NT	R1G1Y2	R1G0Y2	+	− (Deleted)
Case 14	R1G1Y2 (Vysis)	R4G2Y0B2	R2G2Y0	R1G1Y1	+ (At add(10))	+
Case 15	R3G2Y2 (Vysis) R2G0Y5 (Dako)	R7G4Y0B4	NT*	R3G0Y4	+ (Three copies)	+ (Two copies)

Abbreviations: B, blue (aqua) signal; BA, break-apart; FISH, fluorescence *in situ* hybridization; G, green signal; NT, not tested; R, red signal; Y, yellow (fusion) signal. FISH probes: *MYC* BA probe, Vysis LSI *MYC* dual-color, break-apart rearrangement probe (Abbott Laboratories, Abbott Park, IL, USA) and *MYC* FISH DNA probe, split signal (#Y5410, Dako, Glostrup, Denmark); *MYC-IGH* DF probe, Vysis LSI *IGH/MYC*/CEP eight tri-color dual-fusion probe kit (Abbott Laboratories); and *BCL6* BA probe, Vysis LSI *BCL6* (ABR) dual-color, break-apart rearrangement probe (Abbott Laboratories). **t*(14;18)(q32;q21) was absent by G-banding (Supplementary Figures S1 and S4).

chromosomal abnormality was reported to have achieved >2-year disease-free survival after the initial induction chemotherapy.[10] At present, as the number of reported cases is small, it remains to be determined whether *t*(3;8)(q27;q24) is associated with particular clinical features and the treatment outcome.

In the context of double-hit, it is of special interest whether *t*(3;8)(q27;q24) leads to simultaneous activation of both *BCL6* and *MYC* involved in the translocation. The breakages have been described to occur at the upstream of *BCL6* and downstream of *MYC*, and, as the result of translocation, the 3′ *MYC* links to the *BCL6* in the tail-to-tail orientation on der(3)*t*(3;8)(q27;q24) and the 5′ *BCL6* links to the *MYC* in the head-to-head orientation on der(8)*t*(3;8)(q27;q24), respectively (Supplementary Figure S6).[4,6,15] In a very recent study by Ryan *et al.*[6] using the combination of chromatin immunoprecipitation and next-generation sequencing to map acetylated enhancer elements, the 3′ *MYC* that linked to the *BCL6* on der(3)*t*(3;8)(q27;q24) lacks acetylation, while the 5′ *BCL6* is broadly acetylated, and the 5′ *BCL6-MYC* linkage on der(8)*t*(3;8)(q27;q24) leads to the strong activation of *MYC* by the interaction between the *MYC* promoter and *BCL6* enhancer elements. The authors suggest that *t*(3;8)(q27;q24) does not represent a *MYC-BCL6* double-hit, but is equivalent to a single-hit *MYC*-activating rearrangement, and propose the term 'pseudo double-hit' for this particular translocation.

We showed here that the *MYC* and 5′ *BCL6* linkage was consistently present in four cases, while the reciprocal *BCL6* and 3′ *MYC* linkage was absent in case 10 and the der(3)*t*(3;8)(q27;q24) chromosome was deleted in case 13, suggesting that the *BCL6*-3′ *MYC* linkage on der(3)*t*(3;8)(q27;q24) may not be required for the malignant phenotype of *t*(3;8)(q27;q24)-lymphoma. Taken together with the finding by Ryan *et al.*[6] that the *MYC* mRNA expression level in a *t*(3;8)(q27;q24)-bearing lymphoma was the highest among B-cell lymphomas tested and the uniform expression of MYC protein in >90% of lymphoma cell nuclei in the present four cases, the role of *BCL6* in *t*(3;8)(q27;q24) is to provide its regulatory elements to *MYC*, leading to the enhanced expression of *MYC* mRNA and protein. In other words, *t*(3;8)(q27;q24)/*MYC-BCL6* does not represent a double-hit activating both *MYC* and *BCL6*, but a non-*IG–MYC* single-hit activating solely *MYC*. We need to be aware that nuclear FISH-detected *BCL6* rearrangement does not necessarily indicate the presence of a *BCL6*-activating rearrangement.[6]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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H Ohno[1,2], M Nakagawa[2], C Kishimori[2], K Fukutsuka[2] and G Honjo[3]
[1]*Department of Hematology, Tenri Hospital, Tenri, Japan;*
[2]*Tenri Institute of Medical Research, Tenri, Japan and*
[3]*Department of Diagnostic Pathology, Tenri Hospital, Tenri, Japan*
E-mail: hohno@tenriyorozu.jp

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