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 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 dual-fusion 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. Case 10 lacked the expression of MYC as determined by 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 chromosomal 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...
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;24),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

**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.
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).4,5,15 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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Table 1. Summary of FISH studies

| Case no. | 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  | R3G2Y2V (Vysis) | 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 (VY5410, Dako, Glostrup, Denmark); MYC-IGH DF probe, Vysis LSI IG/MYC/CEP eight-tricolor 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)(q27q21) was absent by G-banding (Supplementary Figures S1 and S4).
Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)