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Letters to the Editor

MYD88 (L265P) Mutation in Malignant Lymphoma Using Formalin-Fixed Paraffin-Embedded Section

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TO THE EDITOR

Myeloid differentiation primary response gene 88 (MYD88) is a universal adapter protein that mediates most toll-like receptors, except toll-like receptor 3, and receptors for interleukin-1 and interleukin-18 cytokine signals ; it also activates the transcription factor nuclear factor (NF)-κB by transduction of interleukin-1 receptor-associated kinase (IRAK)-tumor necrosis factor receptor-associated factor-6 (TRAF-6).[1-4] MYD88 mutations in lymphomas are oncogenic and gain of function. Recently, it has been postulated that active mutation in MYD88 drives post-germinal center B-cells to lymphomagenesis, especially diffuse large B-cell lymphoma (DLBCL).[5] DLBCL is subdivided into germinal center B-cell (GCB) subtype and activated B-cell-like (ABC) subtype by gene expression profiling, and the constitutive activation of NF-κB is a hallmark of ABC-DLBCL.[6] In ABC-DLBCL, less clinical benefit was shown for both treatment of CHOP (cyclophosphamide, hydroxydaunorubicin, vincristine and prednisolone) and even rituximab-CHOP regimens compared with GCB DLBCL.[7] A single amino acid substitution (L265P) of MYD88 was found in 29% of ABC-DLBCL.[5] Moreover, whole genome sequencing has proved that all cases of Waldenström macroglobulinemia have the same mutation.[8] This may be evidence of the post-germinal center origin of B-cell lymphoma.

We searched for MYD88 (L265P) mutation in various types of lymphoma using routine materials of formalin-fixed paraffin-embedded (FFPE) tissue. A total of 51 cases of malignant lymphoma diagnosed in the Department of Pathology, Tokai University School of Medicine, were chosen for the study. MYD88 gene was amplified using DNA extracted from FFPE sections, with 5'-GTTGAAGACTGGGCTTGTCC-3' and 5'-GTGCAGGGGTTGGTGTAGTC-3' as sense and antisense primers, respectively. We used 1 μL of template DNA at 200 ng/μL. Polymerase chain reaction (PCR)-amplified product of 176 bp in length, including 265th amino acid, was obtained in all cases and direct sequencing of these products was performed. A chimeric nucleotide T/C was identified, namely, codon 265 of the MYD88 gene was changed from wild-type CTG (leucine) to mutant CCG (proline) (Fig. 1). We found chimeric T/C in 11 of 51 cases examined : lymphoplasmacytic lymphoma (LPL), 7/9 (78%) ; follicular lymphoma, 0/5 ; B-chronic lymphocytic leukemia, 0/4 ; DLBCL, 3/8 (27%) ; Burkitt lymphoma, 0/3 ; extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type (MALT lymphoma), 0/5 ; unclassifiable low-grade B-cell lymphoma (U-LGBCL), 1/1 ; peripheral T-cell lymphoma, NOS, 0/6 ; adult T-cell lymphoma, 0/5 ; and classical Hodgkin lymphoma, 0/5 (positive cases/examined cases).

Despite the possibility of small populations of neoplastic cells being present in the section, which could not be detected by PCR, our result of the mutation frequency (78%) of LPL cases with MYD88 (L265P) corresponds to those reported by other groups (70−100%),[9] demonstrating that FFPE-DNA as a PCR template is suitable to detect this mutation. Three cases of DLBCL with MYD88 mutation in our series had the ABC phenotype. MYD88 mutation is present in both high- and LGBCL. In high-grade B-cell lymphoma, it has been found not only in cases with a nodal origin but also in those with an extra-nodal origin, such as central nervous system DLBCL and primary cutaneous DLBCL, leg type.[9-10] In LGBCL, LPL is a striking target for MYD88 mutation,[11] but a small number of MALT lymphomas have been reported to have this type of mutation. The rates of recurrence of mutations were

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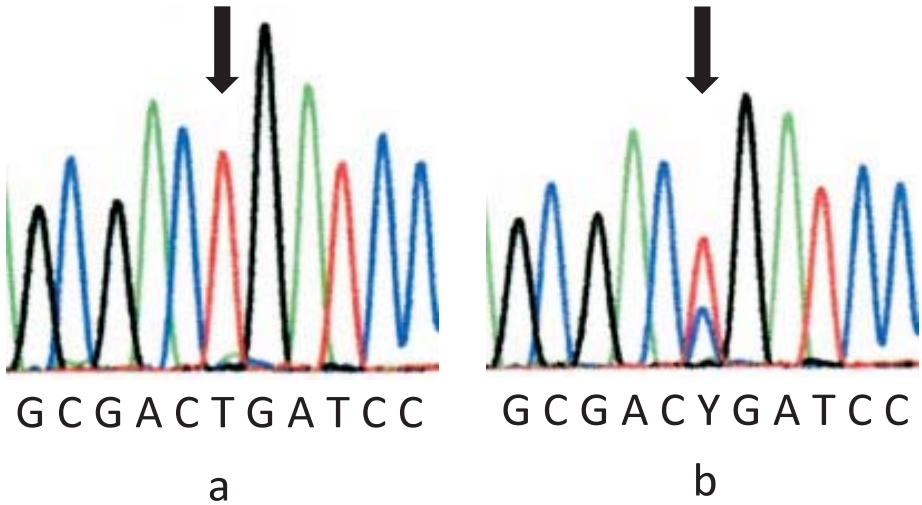

Fig. 1. Chromatograms of direct sequence of polymerase chain reaction-amplified MYD88 gene product of 176 bp in length, including 265th amino acid. *Arrow* indicates T in germline case (*a*) and Y (T/C) in mutation case (*b*).

9% for gastric MALT lymphomas[5] and 6% (3/46) for marginal zone lymphomas, including 5% (1/20) for MALT lymphomas.[11] Li *et al*. also reported 2 cases of MALT lymphoma with orbital adnexa harboring a T978C mutation causing the reported L265P substitution and 1 case of salivary gland MALT with trisomies of chromosomes 3 and 18 harboring an interstitial 27 bp deletion.[12] Recently, Choi *et al*. reported that immunohistochemical high expression of MYD88 protein was significantly associated with tumor recurrence and shortened disease-free survival in DLBCL, but MYD88 L265P mutation was not associated with MYD88 expression.[13]

Interestingly, we found a case of U-LGBCL with MYD88 mutation, in a 67-year-old Japanese patient with bone marrow lymphoma showing diffuse infiltration of small to medium-sized lymphocytes. The immunophenotype of lymphoma cells was $CD3^-$, $CD5^-$, $CD10^-$, $CD20^+$, $BCL\text{-}2^+$, $BCL\text{-}6^-$ and $MUM\text{-}1^-$, without plasma cell differentiation. U-LGBCL is comprised of 0.5−1.2% malignant lymphoma[14] and is a B-cell lymphoma that has no evidence of well-known LGBCC entities. This case may be regarded as a variant of LPL.

In conclusion, MYD88 (L265P) mutation using FFPE can be one of the useful tools for routine diagnosis of B-cell lymphoma.

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