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Original contribution

CD79B and *MYD88* mutations in diffuse large B-cell lymphoma☆,☆☆

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Summary Mutations in 2 upstream components of the nuclear factor κB (NF-κB) pathway, *CD79B* and *MYD88*, are important information for new target therapy in malignant lymphoma. We examined the prevalence and clinicopathologic characteristics of *CD79B* and *MYD88* mutation in a cohort of Asian diffuse large B cell lymphoma (DLBCL) patients. *CD79B* and *MYD88* mutations were analyzed by Sanger sequencing in 187 DLBCL tissue samples. *CD79B* immunoreceptor tyrosine-based activation motif spanning exon 5 and 6 and *MYD88* TIR domain spanning exons 3, 4 and 5 were amplified and sequenced. The cell-of-origin was determined based on immunohistochemical stains for CD10, BCL-6 and MUM-1 by Hans' algorithm. *CD79B* was mutated in 16 cases (8.5%), mostly involving the first tyrosine (Y196) of immunoreceptor tyrosine-based activation motif. For *MYD88*, L265P mutation was found in 31 cases (out of 161, 19.3%). In 11 of these, a *CD79B* mutation coexisted, which constituted 69% of *CD79B* mutants and 36% of *MYD88* L265P cases. Clinicopathologic comparison between the mutant and the wild-type group showed that the mean age was older for both *CD79B* (66 versus 58 years) and *MYD88* L265P mutant groups (64 versus 58 years). Survival analyses showed that neither *CD79B* mutation nor *MYD88* L265P was a significant prognostic indicator. In conclusion, *CD79B* and *MYD88* mutations are associated with an older age at onset in DLBCL with a significant overlap, which did not affect the outcome of the disease.

1. Introduction

Aberrant activation of nuclear factor κB (NF-κB) is known largely to promote oncogenic characteristics such as anti-apoptosis, proliferation, and changes in cell adhesion while tumor-suppressing effects have also been demonstrated in certain neoplasms [1]. Oncogenic NF-κB activities have been shown in various solid and hematologic

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malignancies, the latter including diffuse large B cell lymphoma (DLBCL), mucosa-associated lymphoid tissue (MALT) lymphoma, multiple myeloma, Hodgkin lymphoma and some leukemias [2].

DLBCL can be distinguished according to the gene expression profiling into two major subtypes, the germinal center B-cell–like (GCB) type and the activated B-cell–like (ABC) type, and the minor unclassifiable group [3]. Constitutive activation of NF-κB pathway, which is normally transiently activated by antigen-dependent stimulation in B cells, is characteristic of ABC DLBCL [2]. The survival of ABC DLBCL is known to be inferior to that of GCB DLBCL, and the poor response to chemotherapy has been attributed to the anti-apoptotic effect of NF-κB [4,5].

In DLBCL, the mechanism of constitutive NF-κB activity has been traced to somatic genetic alterations in the upstream pathway components such as *CD79A*, *CD79B*, *CARD11*, *A20* and *MYD88* [2]. It has been reported that immunoreceptor tyrosine-based activation motif (ITAM) mutations of *CD79B* and, less frequently, of *CD79A* were present in DLBCL cell lines and biopsy samples, which were largely of the ABC subtype (21% of ABC DLBCL and 3% of GCB DLBCL, for *CD79B*) [6]. *CD79* mutations were shown to increase surface B-cell receptor (BCR) expression and nullify the negative regulation of BCR, which were suggested to support the "chronic active" BCR signaling leading to constitutive NF-κB activation in ABC DLBCL [6].

MYD88 is an adaptor protein of the toll-like receptors and interleukin-1 receptors; through association with interleukin-1 receptor-associated kinases, MYD88 mediates downstream activation of NF-κB and mitogen-activated protein kinases, the outcome of which includes secretion of interleukin-6 (IL-6) [7]. Mutations of *MYD88* that are oncogenic, NF-κB–activating, have been identified in DLBCL by Ngo et al [8]. They showed that the most frequent and most oncogenic form was the L265P mutation of Toll/IL-1 receptor (TIR) domain, which was detected in 29% of ABC DLBCLs as well as in 9% of MALT lymphomas while rare in GCB DLBCLs. Recently, the *MYD88* L265P somatic mutation was demonstrated to be highly recurrent (about 90%) in IgM-secreting lymphoplasmacytic lymphoma (Waldenström's macroglobulinemia; WM) [9]. The use of L265P detection in discriminating WM from a morphologically overlapping B-cell neoplasm, such as marginal zone lymphoma, and in monitoring progression to WM from IgM monoclonal gammopathy of undetermined significance was proposed in subsequent studies [10,11].

With the advent of various targeted therapeutic agents acting on NF-κB–related pathways [12], knowledge on the frequency of individual NF-κB–affecting mutations and the clinicopathologic impact of such mutations is appreciated. We sought to examine the prevalence, clinicopathologic characteristics and possible overlap of the *CD79B* and *MYD88* mutation in a cohort of Asian DLBCL patients.

2. Materials and methods

2.1. Selection of DLBCL cases and categorization

A total of 187 de novo DLBCL cases diagnosed from 1994 to 2005 at Samsung medical center, Seoul, South Korea were chosen based on the availability of the clinical follow-up data and tumor DNA. The diagnosis of DLBCL was made according to the 2008 World Health Organization classification [13], and DLBCL associated with a low grade lymphoma such as MALT lymphoma or an immune-compromised setting was excluded from the selection. All patients underwent chemotherapy with or without other treatment modalities such as surgery and radiation therapy. The first-line chemotherapy regimen was mostly CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone; n = 89) or R-CHOP (Rituximab plus CHOP; n = 86) with the methotrexate-based regimen administered to the 12 primary central nervous system (CNS) DLBCL cases. The "non-GCB" of Hans' algorithm [14] was designated as "ABC" in the present study. Immunohistochemical staining was performed on a 4-μm thick section of formalin-fixed paraffin-embedded tissue processed in an automated system (BOND-MAX, Leica, Wetzlar, Germany) using monoclonal antibodies against CD10 (Leica), BCL-6 (Leica), and MUM-1 (DAKO, Carpinteria, CA, USA). In 72 of 187 cases, the immunohistochemical staining was done in tissue microarray preparations consisting of 700-μm cores of tumor area. Epstein-Barr virus (EBV)–encoded RNA in situ hybridization was carried out using EBV in situ hybridization kit (Leica). Strong reactivity in the majority (>50%) of tumor cells was the criterion for EBV positivity, which resulted in a lower percentage of positive cases (3%) compared with that of our previous report [15].

2.2. DNA isolation and sequencing

Genomic DNA was isolated from a 5-μm thick section of formalin-fixed paraffin-embedded tumor tissue using the QIAamp FFPE DNA Tissue Kit (Qiagen, Germantown, MD, USA). For sequencing *CD79B* immunoreceptor tyrosine-based activation motif (ITAM), polymerase chain reaction was performed to amplify the area spanning exon 5 and 6 using two sets of primers: CD79B-5F (5′-GGGCTGGGGGACACTAACACTC-3′), CD79B-5R (5′-TGGGTGCTCACCTACAGACCAC-3′), CD79B-6F (5′-CGGGGTCAGTGGCCACTATCTG-3′) and CD79B-6R (5′-AGCAGTCACTGAGGCCAGGGAG-3′). Sanger sequencing was done bidirectionally. For amplification of *MYD88* TIR domain spanning exon 3, 4 and 5, primer pairs used were as follows: MYD88-3F (5′-AAGCCTT-CCCATGGAGCTCTGACCAC-3′), MYD88-3R (5′-GCTAGGAGGAGATGCCCAGTATCTG-3′), MYD88-4F (5′-ACTAAGTTGCCACAGGACCTGCAGC-3), MYD88-4R (5′–ATCCAGAGGCCCCACCTACACATTC-3′),