

東海大学大学院令和三年度博士論文

Double-hit follicular lymphoma and Double-hit
high grade B-cell lymphoma
(Double-hit を有する follicular lymphoma と
high-grade B-cell lymphoma について)

1. Double-hit を有する Follicular lymphoma と High grade B-cell lymphoma の臨床病理学的比較検討
2. Double-hit を有する High grade B-cell lymphoma における
予後不良因子

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This doctoral thesis was written on the basis of the original article published in “Moderna pathology”. I got permissions for the submission of the doctoral thesis to the institutional repository of Tokai University, in addition to reuse of part of my article in my doctoral thesis.

SUMMARY

Diffuse large B-cell lymphoma (DLBCL) with *MYC* rearrangement and *BCL2* and/or *BCL6* rearrangements is called as double-hit lymphoma (DHL) and defined as “High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements” in the World Health Organization 2017 Classification of Tumors of Hematopoietic and Lymphoid Tissues. Most DHL are aggressive B-cell lymphomas. Occasional double-hit follicular lymphomas (DHFL) have been described but the clinicopathological features of DHFL are not well known. To clarify the characteristics of DHFL, I analyzed 10 cases of DHFL, comparing with 15 cases of DHL for clinicopathological and genome-wide copy number alterations profiles. For DHFL, the median age was 67.5 years (range, 48 to 82 years), female/male ratio was 2.3. Eight patients presented with advanced clinical stage. The median follow-up time was 20 months (range, 1 to 132 months). At the end of the follow-up, 8 patients were alive, 2 patients were dead including 1 patient with DLBCL transformation. Rearrangements of *MYC/BCL2*, *MYC/BCL6* and *MYC/BCL2/BCL6* were seen in 8, 1 and 1 cases, respectively. The partner of *MYC* was IGH in 6 cases. There were no cases of histological Grade 1, 4 cases of Grade 2, 5 cases of Grade 3a and 1 case of Grade 3b. Two cases of Grade 3a exhibited immunoblast-like morphology. Immunohistochemistry demonstrated 9 cases with $\geq 50\%$ MYC positive cells. There was significant difference in MYC intensity ($p=0.00004$) and MIB-1 positivity ($p=0.001$) between DHFL and DHL. The genome profile of DHFL was comparable with conventional follicular lymphomas (FL) (GSE67385, $n=198$) with characteristic gains of 2p25.3-p11.1, 7p22.3-q36.3, 12q11-q24.33 and loss of 18q21.32-q23 ($P<0.05$). In comparison with DHL, DHFL had fewer copy number alterations and minimal common region of gain at 2p16.1 (70%), locus also significant against conventional FL ($p=0.0001$).

Second, to clarify relationship between gene expression profile, immunohistochemical feature and clinical outcomes of DHL, I examined 10 cases of DHL (molecular analysis series) with gene expression profile analysis and 22 cases of DHL (immunohistochemical series, including 10 cases of the molecular analysis series) with an immunohistochemical evaluation. The gene expression profile analysis showed that the strong expression of *AICDA* was associated with an adverse prognosis in DHL. I performed immunohistochemistry on AID, the protein product of *AICDA*, for immunohistochemical series of 22 cases and confirmed that its expression was also associated with an adverse prognosis in DHL.

In conclusion, DHFL tended to be high grade histology, high MYC protein expression, high *MYC*/IGH fusion and minimal common region of gain at 2p16.1. DHFL seemed to be a different disease from DHL and have an indolent clinical behavior similar to FL without *MYC* rearrangement.

Overexpression of *AICDA* and AID are poor prognostic factor in DHL.

BACKGROUD AND AIM

MYC as well as *BCL2* and *BCL6* play important roles in the pathogenesis of malignant lymphoma. In aggressive B-cell lymphoma, *MYC* rearrangement (*MYC*-R) is found not only in Burkitt lymphoma, but also in approximately 10% of diffuse large B-cell lymphoma (DLBCL) cases, while *BCL2* and *BCL6* rearrangements (*BCL2*-R and *BCL6*-R) have been detected in 30 and 30-40% of DLBCL cases, respectively. When DLBCL has *MYC*-R and additional *BCL2*-R and/or *BCL6*-R, the lymphoma generally has an adverse prognosis and is named a double-hit lymphoma (DHL). These findings led to the introduction of a new category in the World Health Organization 2017 Classification of Tumors of Hematopoietic and Lymphoid Tissues (WHO classification) called “High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements”.

However, there are some issues that remain unclear about DLBCL with *MYC*-R. The majority of DHL cases are DHL with *BCL2*-R; however, limited information is currently available on clinical differences between DHL with *BCL2*-R and DHL with *BCL6*-R. The prognostic significance of the *MYC* partner gene is also controversial. Some studies demonstrated that a non-IG *MYC* partner was a survival advantage, while others showed no significant difference between cases with an IG or non-IG *MYC* partner. Although DLBCL with *MYC*-R and without *BCL2*-R and *BCL6*-R was initially reported to be associated with an adverse prognosis, Rosenwald et al. recently showed that the negative prognostic impact of *MYC*-R was mainly observed in patients with DLBCL in a 24-month follow-up. Moreover, although DHL was previously considered to have an extremely adverse prognosis, Ennishi et al. and our group recently independently reported that the overall survival (OS) curve of DHL appeared to be similar to that of activated B cell-like (ABC) DLBCL. These findings indicate that some patients with DHL achieved a favorable clinical outcome with R-CHOP therapy, whereas others did not.

Mutational and gene expression profile analyses of DHL have been conducted. A gene expression profile analysis revealed that DHL with *BCL2*-R had a germinal center B cell-like (GCB) profile. However, GCB DLBCL generally showed a favorable prognosis. A “molecular high-grade group” characterized by the gene expression profile may be an adverse prognosis factor in DHL. However, the relationship between genetic features and clinical outcomes remains unclear in DHL.

On the other hand, follicular lymphoma (FL) accounts for 15% to 30% of all lymphoid neoplasms and is the most common histological subtype of indolent non-Hodgkin Lymphoma. The natural history of FL is characterized by recurrent relapses and progressively shorter remissions with a median survival of 10 years. FL usually carries *BCL2*-R and this rearrangement is found in 80% to 90% of cases. Secondary *MYC*-R in FL frequently demonstrates histological transformation to DLBCL with burkitt-like appearance. FL having both *MYC*-R and *BCL2*-R without high grade transformation (double-hit follicular lymphoma; DHFL) is extremely rare and two reports have been published so far. Yoshida M et al. indicated that DHFL may exhibit an indolent clinical course despite *MYC*-R and *BCL2*-R, and Yuan Miao et al.

suggested that DHFL, regardless of low grade morphology, tends to have an aggressive clinical stage. In the latter series of DHFL (7 cases), the male/female ratio was 1:2.5, ranging from 34 to 64 years with a median age of 47 years. Bone marrow involvement was observed in 43%. Grade 3 morphology was found in 86% and immunohistochemical staining of MYC protein positivity ranged from < 5% to 30%. The genetic features of DHFL, however, have not been reported.

In order to compare the characteristics of DHFL and DHL, I analyzed the clinicopathological, immunohistochemical and genome-wide copy number alterations characteristics of 10 cases of DHFL and 15 cases of DHL.

Second, to find prognostic factor of DHL, I prepared a molecular analysis series of 10 cases of DHL for the gene expression profile analysis with clinicopathological data, followed by immunohistochemical series of 22 cases of DHL (including the 10 molecular analysis series cases) for an immunohistochemical evaluation.

DISCUSSION

In this study, I retrospectively analyzed 10 cases of DHFL. Compared to conventional FL cases, so far only 2 reports for DHFL have been reported with 2 cases and 7 cases respectively. DHFL in advanced stage comprised 80% of our study and 86% in the previous report. These frequencies are more than two-thirds of FL with advanced disease (stage III or IV) at diagnosis. Frequency of bone marrow infiltration in DHFL, 43% in the previous report and 20% in our report, was not different from FL (40-70%). Grade 3 in DHFL is a major histological grade and it has a higher incidence than with conventional FL. Transformation of FL into DLBCL is one of the major causes of poor outcome and transformation of FL into DLBCL is reported in 27% of FL. In our study, 1 patient (10%) showed transformation into diffuse large B-cell lymphoma and died after 12 months, 8 patients (80%) were alive in the follow-up period. Although there is not significant difference in overall survival between DHFL and DHL in this study, this is due to sample size. The standard regimen for FL seems to be sufficiently effective for most DHFL. Nevertheless, for both conventional FL and DHFL unknown which intensive chemotherapy is needed for the prevention of transformation.

The role of *MYC* in oncogenesis was first discovered in burkitt lymphoma, in which *MYC* on chromosome 8q24 was juxtaposed to the IGH on chromosome 14q32 or, less commonly, to the IGK (2p12) or IGL (22q11) light chain genes, resulting in deregulated MYC protein expression. When B-cell lymphoma had $\geq 70\%$ MYC positive cells, the lymphoma had *MYC*-R with a high probability. Although it is reported that DHFL had <30% MYC positive cells, 9 cases (90%) of DHFL in our study had $\geq 50\%$ MYC positive cells ($\geq 70\%$, 7 cases). It is emphasized that strongly and weakly positive cells are intermingled with MYC positive cells of DHFL, whereas most of the lymphoma cells in DHL exhibited diffuse and strong expression of MYC ($p=0.000004$). Moreover, 2 cases (20%) of DHFL showed immunoblast-like morphology. Immunoblastic morphology may indicate DLBCL with *MYC*-R.

Immunoblastic morphology is very unusual in FL and correlation with *MYC*-R has been not well known yet.

In our study, 27% (4/15) of DHL cases were triple hit lymphoma (case 3, 8, 9 and 10). Tomita et al. reported that 26% (7/27) of DHL are triple hit lymphoma and all triple hit lymphoma patients died with a median survival of 4 months. In our triple hit lymphoma patients, 1 patient was dead and 3 patients were alive in observation period, with a median survival of 28.5 months. Genomic profile of triple hit lymphoma were similar to that of DHL without *BCL6*-R; of note, gain at 12q12-12q15 was less prominent in triple hit lymphoma.

The *MYC* translocation partner is an important prognostic factor in *MYC* translocation lymphoma. When IG was the *MYC* partner, the patient demonstrated a poorer prognosis than in the *MYC*/non-IG situation. IGH is the *MYC* partner in 33% of DHL. In our study, IGH as the *MYC* partner was found in 60% of DHFL and 42.8% of DHL, respectively. This result may affect the mechanism of pathogenesis, although the reason of indolent clinical course of DHFL is still not fully understood.

Array comparative genomic hybridization data of DHL was already reported by a European group, but to our knowledge, the genomic copy number alterations in DHFL has not been reported. In our study, the genomic profile of DHFL was characterized by gains of 2p24.2-2p12 (40%) with minimal common region at 2p16.1-2p15 (60-70%), 7p22.3-7q36.3 (~ 40%) with minimal common region 7p21.1 (50%) and 12p11.1-12q12 (40%), loss of 1p36.33-1p36.23 (30%), 6q16.1-6q22.31 (20%). Region at 1p36.33-1p36.22 included *TNFRSF14*. Although pattern of copy number alterations of DHFL is similar to that of western conventional FL, there is significant difference in gains of 2p25.3-p11.1 (with minimal common region of 2p16.1) ($p=0.0001$) and 12q11-q24.33 ($p=0.0079$); and loss of 18q21.32-q23 ($p=0.0385$). Therefore, it is expected that those regions with their target genes are related to the lymphoma pathogenesis. In DHFL case 6 which is the only case showed DLBCL transformation, has 6q loss and 8p loss. 6q large loss and 8p loss are the typical alterations acquired in B-cell lymphoma transformation to aggressive phases. The genome profile of DHL was more complex with more copy number alterations. In comparison with DHL, DHFL was characterized by few copy number alterations throughout the whole genome except an minimal common region of gain at 2p16.1 including *BCL11A*. Interestingly, common regions between the two entities were found at 7p21.1 and the target gene of *HDAC9*. I firstly demonstrated differences in copy number alterations between DHL and DHFL. Histological transformation of FL may need more genomic change other than the *MYC* gene.

Second, to find poor prognostic factor of DHL, I evaluated a molecular analysis series of 10 cases of DHL using gene expression profile analysis with clinicopathological correlations, and immunohistochemical series of 22 cases using immunohistochemistry.

In gene expression profile analysis, the stronger expression of *AICDA* could divide favorable and adverse prognosis group in DHL. Therefore, I focused on *AICDA*, and found that the strong expression of *AICDA* and AID correlated with an adverse prognosis in DHL.

AICDA is well known and involved in somatic hypermutation, gene conversion, and class-switch

recombination in B cells. AID, the protein product of *AICDA*, is required for several crucial steps in B-cell terminal differentiation, which is necessary for efficient antibody responses. *AICDA* may also play a role in the epigenetic regulation of gene expression by participating in DNA demethylation. The expression of *AICDA* and AID were previously reported to be stronger in ABC DLBCL than in GCB DLBCL. Kawamura et al. and Arima et al. showed that the expression of AID was associated with an adverse prognosis in DLBCL, NOS. Although the role of AID in the adverse prognosis of DLBCL remains unclear, Kawamura et al. reported AID-induced mutations in tumor suppressor genes and/or in genes responsible for drug sensitivity as one of the direct mechanisms. Error-prone DNA polymerases, which are involved in somatic hypermutation processes together with AID, are also factors potentially contributing to an adverse prognosis in DLBCL. They may play an important role in tumor development by inducing mismatch errors during DNA replication. Liu et al. suggested that AID promoted the insensitivity of tumors to T cell-mediated cytotoxicity. Therefore, accumulated evidence supports the relationship between the expression of *AICDA*/AID and a poor prognosis. Moreover, the overexpression of *AICDA* was associated with Richter's and prolymphocytic transformation of chronic lymphocytic leukemia and the aggressiveness of murine chronic lymphocytic leukemia. Zhang et al. suggested that AID directly induced class-switch recombination in DHL and may result in *MYC* translocation. DHL with the expression of *AICDA*/AID, even *de novo*, may have similarities to the mechanism of transformation, resulting in a poorer clinical outcome. Therefore, *AICDA* and AID may be a pivotal gene and protein, respectively, for an adverse prognosis in DHL.

CONCLUSION

DHFL tended to be histological high grade with high MYC positivity. The genomic profile of DHFL is characterized by few copy number alterations with a profile throughout whole genome and minimal common region of gain at 2p16.1. The clinical behavior of DHFL tends to be less aggressive than DHL. *AICDA* and AID is biomarkers associated with an adverse prognosis in DHL.