Genome-Wide Array-Based Comparative Genomic Hybridization of Diffuse Large B-Cell Lymphoma: Comparison between CD5-Positive and CD5-Negative Cases

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ABSTRACT

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin’s lymphoma and exhibits aggressive and heterogeneous clinical behavior. To genetically characterize DLBCL, we established our own array-based comparative genomic hybridization and analyzed a total of 70 cases [26 CD5-positive (CD5+) DLBCL and 44 CD5-negative (CD5-) DLBCL cases]. Regions of genomic aberrations observed in >20% of cases of both the CD5+ and CD5- groups were gains of 1q21-q31, 1q22-q31, 1q24, 1q23-q24, 1q23-q21, 1p36, 3p14, 4q14-q25, 6q27, 2p12, and 17p11-p13. Because CD5 expression marks a subgroup with poor prognosis, we subsequently analyzed genomic gains and losses of CD5+ DLBCL compared with those of CD5-. Although both groups showed similar genomic patterns of gains and losses, gains of 10p14-p15 and 19q13 and losses of 1q43-q44 and 8p23 were found to be characteristic of CD5+ DLBCL. By focusing on the gain of 1q32-q34 and loss of 1p34-p36, we were also able to identify prognostically distinct subgroups among CD5+ DLBCL cases. These results suggest that array-based comparative genomic hybridization analysis provides a platform of genomic aberrations of DLBCL both common and specific to clinically distinct subgroups.

INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) comprises some 30% of non-Hodgkin’s lymphoma cases and is clinically heterogeneous (1, 2). Recent microarray analyses of transcripts of DLBCL specimen have clearly shown biologically distinct subtypes in DLBCL that are also clinically relevant (3, 4). Although several genetic alterations have been identified as etiologically associated with DLBCL (5, 6), genome-wide screening has been insufficient. The recently developed array-based comparative genomic hybridization (array CGH) technique allows high-throughput analysis of copy number changes of a genome at high resolution and accuracy throughout the whole genome. The quantitative measurement of DNA copy number thus obtained may facilitate identification of tumor-related genes (7–9). This article must therefore be hereby marked for advertisement and in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

MATERIALS AND METHODS

Patients. We analyzed DNA samples of 26 cases of de novo CD5+ DLBCL and 44 cases of CD5- DLBCL. These samples were obtained with informed consent from patients at Aichi Cancer Center and collaborating institutions under the approval of the Institutional Review Boards. All patients were reported previously (13, 16, 17). The median age was 61 years and 56 years for the CD5+ and CD5- cases, respectively. Among the CD5+ cases, 68% were female, 80% were at advanced stages (II to IV), 72% had elevated lactate dehydrogenase, 24% had a poor performance status, and 28% had extranodal site(s) of involvement. In the CD5- cases, 41% were female, 52% had advanced stages (III to IV), 45% had elevated lactate dehydrogenase, 10% had a poor performance status, and 38% had extranodal site(s) of involvement. All of the samples were obtained from tumors at diagnosis before any treatment was given.

DNA Samples. DNA was extracted using a standard phenol chloroform method from lymphoma specimens from a total of 70 DLBCL cases: 26 cases of CD5+ DLBCL and 44 cases of CD5- DLBCL. Normal DNA was prepared from peripheral-blood lymphocytes of healthy male donors.

Malignant Lymphoma Cell Line. Cell line used in this study was OCI-LY13.2 (DLBCL, kindly provided by Dr. Ricardo Dalla-Favera of Columbia University, New York, NY; ref. 18). OCI-LY13.2 was maintained in RPMI 1640 supplemented with 10% fetal bovine serum at 37°C in 5% CO2 ~95% air.

Selection of BAC/PAC Clones (BAC/PACs) for Array CGH. The array consisted of 2088 BAC/PACs, covering whole human genome with ~1.5 Mb of resolution. BACs were derived from RP11 and RP13 libraries, and PACs were derived from RP1, RP3, RP4, and RP5 libraries. BAC/PACs used were selected based on information from National Center for Biotechnology Information and Ensembl Genome Data Resources.7,8 These clones were obtained from the BACPAC Resource Center at the Children’s Hospital (Oakland 7 Internet address: http://www.ncbi.nlm.nih.gov/.
8 Internet address: http://wwwensembl.org/.

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Research Institute, Oakland, CA). Clones were ordered from chromosomes 1 to 22 and X. Within each chromosome, clones were ordered from chromosomes 1 to 22 and X on the basis of Ensembl Genome Data Resources of Sanger Center Institute, January 2004 version. All of the clones used for array CGH were confirmed for their location on chromosomes by fluorescence in situ hybridization. Clone names and their locations on chromosomes are available on request.

DNA Amplification for Spotting on Slides. Ten ng of BAC (or PAC) DNA were used as the template for degenerate oligonucleotide-prime PCR (19) with the 5′-amine-modified degenerate oligonucleotide-primed primer (5′-CCCAGCTCAGN6NNATGTTG-3′ where n = A, C, G, or T) and amplified on a TaKaRa PCR Thermal Cycler MP (TaKaRa, Tokyo, Japan) using Ex Taq polymerase (TaKaRa). A 3-minute, 94°C denaturation step was followed by 25 cycles of 94°C for 1 minute, with a final 7-minute extension at 72°C.

DNA Spotting and Quality Control of Glass Slides. Degenerate oligonucleotide-primed PCR products were ethanol precipitated and dissolved in distilled water, and then an equal volume of DNA spotting solution DSP0050 (Matsunami, Osaka, Japan) was added (1 μg/μL). The resulting DNA samples were robotically spotted by an inkjet technique (NGK, Nagoya, Japan) in duplicate onto CodeLink-activated slides (Amersham Biosciences, Piscataway, NJ). In this study, we used only glass slides on which it had been confirmed that all 2088 clones had been spotted completely and uniformly in duplicate.

Array Hybridization. The array fabrication and hybridization was performed according to the method described by Pinkel et al. (20) and Hodgson et al. (21). One μg of tested (tumor or normal) and of referenced (normal) DNA was digested with DpnII and labeled with the BioPrime DNA labeling system (Invitrogen-Life Technologies, Inc., Tokyo, Japan) using Cy3-dUTP and Cy5-dUTP (Amersham Pharmacia Biotech, Piscataway, NJ) for the tested and referenced DNA, respectively. Unincorporated fluorescent nucleotides were removed by means of Sephadex G-50 spin columns (Amersham Biosciences). Tested and referenced DNA were mixed with 50 μg of human Cot-1 DNA (Invitrogen-Life Technologies, Inc.), precipitated, and resuspended in 45 μL of hybridization mixture consisting of 50% formamide, 10% dextran sulfate, 2× SSC, 4% SDS, and 10 μg/μL yeast tRNA (Invitrogen-Life Technologies, Inc.). The hybridization solution was heated to 73°C for 5 minutes to denature the DNA and then incubated for 45 minutes at 37°C to block repetitive sequences. The glass slides spotted with DNA were denatured in 70% formamide/2× SSC at 73°C for 4 minutes, then dehydrated in cold 70, 85, and 100% ethanol for 5 minutes each and air-dried. Hybridization was performed for 66 to 72 hours in a container on a slowly rocking table with 200 μL of 50% formamide/2× SSC, followed by posthybridization washings in 50% formamide/2× SSC for 15 minutes at 50°C, in 2× SSC/0.1% SDS for 30 minutes at 50°C, and in PB buffer (0.1 mol/L NaHPO4 and 0.1 mol/L Na2HPO4 to attain pH 8 and 0.1% NP40) for 15 minutes at room temperature. The glass slides were then rinsed in 2× SSC at room temperature and finally dehydrated in 70, 85, and 100% ethanol at room temperature for 2 minutes each and air-dried. The slides were scanned with an Agilent Micro Array Scanner (Agilent Technologies, Palo Alto, CA), and the acquired array images were analyzed with GenePix Pro 4.1 (Axon Instruments, Inc., Foster City, CA). After automatic segmentation of the DNA spots and subtraction of the local background, intensities of the signals were determined. Subsequently, ratios of the signal intensity of two dyes (Cy3 intensity/Cy5 intensity) were calculated for each spot, converted into log2 ratios on an Excel sheet in the order of chromosomal positions, and then normalized. For centering the log2 ratio of each single clone between the CD5+ and CD5− groups (1966 tests in total) in the CD5+ group or CD5− group. Data analyses were then carried out for the following purposes: (a) comparison of frequencies of gain or loss of each single clone between the CD5+ and CD5− groups (1966 tests each for gain and loss, 3932 tests in total); (b) comparison of overall survival between cases showing gain or loss of a single clone and cases without respective gain or loss (1966 tests for each gain and loss with or without CD5 expression, in total 7864 tests maximum). Fisher’s exact test for probability was used for the former comparison, and a log-rank test for comparing survival curves of the two groups was used for the latter. P for screening of candidate clones for an analysis was <0.05. When a candidate clone was identified, the clone’s continuity with the subsequent clones was examined. In cases where the nth clone and succeeding k clones (k ≥ 0) were found to be candidate clones, the P value for continual association was calculated as:

\[
\sum_{i=0}^{n+k} p_i
\]

on the assumption that each clone is independent throughout the entire genome. Because multiple tests (11,796 tests maximum) were used, the conventional Bonferroni procedure was applied to define the a-error for the final conclusion. Therefore, we defined a value for the calculation given above as < 0.05/12,000 (= 4.2 × 10−6) as statistically significant (24). All of the statistical analyses were conducted with a statistical package STATA, version 8 (College Station, TX).

RESULTS

Genomic Profiles and Data Analysis for DLBCL Cases. Array CGH analysis was performed to compare genomic alterations in CD5+ and CD5− DLBCL cases. All of the clones on chromosome X (57 clones) were separated analyzed because of sex mismatching. Of the 70 DLBCL cases enrolled, 4 cases (1 of CD5+ DLBCL and 3 of CD5− DLBCL) did not show any genomic aberrations. The remaining 25 cases of CD5+ DLBCL and 41 cases of CD5− DLBCL were then subjected to the data analysis. Fig. 1 shows the entire genomic profiles of two representative CD5+ samples (Fig. 1A and B) and one CD5− sample (Fig. 1C). Copy number changes were easily detectable at a high-resolution genome wide. Regions of high-level gain/amplification (defined as log2 ratio > +1.0), and regions suggestive of homozygous loss/deletion (defined as log2 ratio < −1.0) were also easily detected, as were regions showing low-level gain/amplification.
(defined as log₂ ratio +0.2 to +1.0), as well as regions suggestive of heterozygous loss/deletion (defined as log₂ ratio −1.0 to −0.2).

The entire tumor set comprised an average copy number gain of 311.1 Mb or 10.9% of the genome (6.8 regions) and an average copy number loss of 174.4 Mb or 6.8% of the genome (5.9 regions). The CD5⁺ group showed a larger average fraction of copy number gain (370.9 Mb, 13.4%) than the CD5⁻ group (311.1 Mb, 10.9%), whereas the former (110.5 Mb, 3.8%) contained a smaller average fraction of copy number loss than the latter (174.4 Mb, 6.8%). The average number of total alterations consisted of 8.1 regions of gain and 5.4 regions of loss in the CD5⁺ group and 6.0 regions of gain and 6.4 regions of loss in the CD5⁻ group.

We defined region of gain or loss as (a) continuously ordered three clones showing gain or loss or (b) clones showing high copy number gain (log₂ ratio > +1.0) or homozygous loss (log₂ ratio < −1.0; e.g., a gain of 2p15, a loss of 3p14.2 and a loss of 9p21). Recurrent region was defined as a region seen in >20% of cases. The most frequently gained or lost BAC/PACs in each of the recurrent regions are listed in Tables 1 and 2.

In the CD5⁺ group (25 cases), recurrent regions of gain were 1q21.2-q32.3, 1q42.2-q42.3, 2p15.1-p22.1, 7p2.3-qtel, 7q1.2-q21.1, 7q21.1-q31.1, 8p11.23, 8q24.13-qtel, 11q22.3-q31.2, 13q34-qtel, 15, 17q, 19q13.43-qtel, 20, and 21; regions of copy number loss: 1p36.21-p36.32, 2p22.3-p25.3, 7p2.3-qtel, 7q1.2-q31.3, 8p12-q21.3, 11q22.3-q23.1, 13q34-qtel, 14q22.2-q23.1, and 17q. B, regions of copy number gain: 3p14.2-q7.1, 7p2.3-q31.2, 12q12.2-qtel, 15q24.3-qtel, and 18; regions of copy number loss: 1p35.1-p35.1, 9p21, 15q13-q21.1, and 17p. C, regions of copy number gain: 1p36, 3, 6p, 7q21.11-qtel, 11q22.3-q32.2, and 18; regions of copy number loss: 3p14.2, 6q22.3-p25.3, 6q14.1-q7.1, 9q22.33, 15q26.2-qtel, and 17p. Log₂ ratio of −2.01 for the single BAC, RP11-48E21, suggests homozygous loss at 3p14.2 locus.

Fig. 1. Representative array CGH profiles of individual tumors and genome-wide frequencies of copy number alterations. Whole genomic profiles are shown for three representative cases of DLBCL (A and B, CD5⁺; C, CD5⁻). Log₂ ratios were plotted for all clones based on their chromosome position, with vertical dotted bars representing the separation of chromosomes. Clones are ordered from chromosome 1 to 22 and X within each chromosome on the basis of the Sanger Center Mapping Position, February 2004 version. A, regions of copy number gain: 1q21.2-q24.3, 2p15.1-p22.1, 7p2.3-qtel, 7p11.2-p21.1, 7q21.1-q31.1, 8p11.23, 8q24.13-qtel, 11q22.3-q31.2, 13q34-qtel, 15, 17q, 19q13.43-qtel, 20, and 21; regions of copy number loss: 1p36.21-p36.32, 2p22.3-p25.3, 4p15.1-p35.1, 6q22.3-p25.3, 7p2.3-qtel, 7q1.2-q31.3, 8p12-q21.3, 11q22.3-q23.1, 13q34-qtel, 14q22.2-q23.1, and 17q. B, regions of copy number gain: 3p14.2-q7.1, 7p2.3-q31.2, 12q12.2-qtel, 15q24.3-qtel, and 18; regions of copy number loss: 1p35.1-p35.1, 9p21, 15q13-q21.1, and 17p. C, regions of copy number gain: 1p36, 3, 6p, 7q21.11-qtel, 11q22.3-q32.2, and 18; regions of copy number loss: 3p14.2, 6q22.3-p25.3, 6q14.1-q7.1, 9q22.33, 15q26.2-qtel, and 17p. Log₂ ratio of −2.01 for the single BAC, RP11-48E21, suggests homozygous loss at 3p14.2 locus.
Table 1 Most frequently gained clones of CD5+ and/or CD5− DLBCL

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<th>Clone name</th>
<th>Cytogenetic position</th>
<th>Genes*</th>
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<th>CD5− (n = 41)</th>
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* Genes contained in clones.
† % of cases with copy number gain.
‡ Most frequently gained clones of recurrent regions in CD5+ DLBCL.
§ Most frequently gained clones of recurrent regions in CD5− DLBCL.

Table 2 Most frequently lost clones of CD5+ and/or CD5− DLBCL

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<th>Clone name</th>
<th>Cytogenetic position</th>
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* Genes contained in clones.
† % of cases with copy number loss.
‡ Most frequently lost clones of recurrent regions in CD5+ DLBCL.
§ Most frequently lost clones of recurrent regions in CD5− DLBCL.


Among the recurrent regions, the gain of 2p15 (BAC, RP11-17D23), loss of 3p14.2 (BAC, RP11-48E21), and loss of 9p21.1 (BAC, RP11-149I2) showed genomic aberrations by single or two continuous clones. Among 13 cases with gain of 2p15 showed high-level copy number gain (log2 ratio > +1.0) at a restricted position of the genome (~2 Mb resolution level) encompassing two continuously ordered BACs, RP11-17D23 (500 kb centromeric to the REL gene) and RP11-51111 (1.8 Mb centromeric to BAC, RP11-17D23).

Eighteen of the 66 cases (28%) showed loss of 3p14.2. Among the clones contained in 3p14.2, BAC, RP11-48E21 (including FHIT) only was lost in 13 of the 18 cases (7 cases of CD5+ and 11 cases of CD5− DLBCL), with no surrounding BACs showing any obvious copy number losses (Fig. 2A). Among the 13 cases with a single loss, 2 cases showed homozygous loss at BAC, RP11-48E21 (log2 ratio < −1.0). 3p14.2 was also deleted in the cell line OCI-LY13.2 established from a patient with aggressive malignant lymphoma (18). Fluorescence in situ hybridization analyses results were consistent with those of the array CGH (Fig. 2, B and C). Similarly, the BAC clone RP11-149I2, including INK4aARF, only was lost in 9 of the 32 cases that showed loss of 9p21 among a total of 66 DLBCL cases, and 2 of the 9 cases showed homozygous loss.

Finally, we analyzed X chromosomes for male patients only (10 cases in the CD5+ group and 25 cases in the CD5− group). Two CD5+ cases and three CD5− cases showed low-grade copy number gains throughout the entire X chromosome but no high-grade amplification. Heterozygous losses were found at Xq21 in two cases in the CD5+ group, whereas no homozygous loss region was found at chromosome X.
Genomic Copy Number Changes Characteristic of CD5⁺ DL-BCL. We next compared the frequency of gain and loss of clones in the CD5⁺ and CD5⁻ groups. Screening on a single-clone basis for candidate clones showed that 48 clones were more frequently (P < 0.05) gained or lost in the CD5⁺ than in the CD5⁻ group. Among these 48 clones, 6 of 10p14-p15.3, 3 of 12p12, 3 of 16p12, and 9 of 19q13.32-q13.43 were continuous clones in terms of the whole genome mapping position according to the Ensemble Genome Data Resources of the Sanger Center Institute (January 2004 version). The remaining 27 clones showed individual P < 0.05, i.e., with no neighboring clones showing such significance.

Twenty clones were identified as being lost significantly more frequently in the CD5⁺ group than in the CD5⁻ group. Among these 20 clones, 3 clones of 1q43, 6 of 1q43-q44, 2 of 8p23.3, and 5 of 8p23.1-p23.2 were continuous, and the remaining 4 clones showed individual P < 0.05 with no neighboring clones showing such significance. In the CD5⁻ group, 10 gained clones and 6 lost clones showed individual P < 0.05 with no neighboring clones showing such significance.

Clones screened on a single-clone basis as described above were subsequently subjected to multiple comparison corrections to find clones statistically relevant in terms of differences in frequency between the CD5⁺ and the CD5⁻ groups. Because

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\prod_{i = n}^{n+k} p_i
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values of continuous clones with gains of 10p14-p15.3 and 19q13.32-q13.43 and losses of 1q43-q44 and 8p23.1-p23.2 were <4.2 × 10⁻6, these regions of gain and loss were determined to be characteristic of the CD5⁺ group. No significantly frequently occurring region of gain or loss was found in the CD5⁻ group after multiple comparison corrections had been performed. Regions characteristic of CD5⁺ DL-BCL and the BACs they contained are listed in Table 3.

Genomic Copy Number Changes Affecting Prognosis of DL-BCL. The next step was the analysis, using the Kaplan-Meier method and log-rank test, of the probabilities of survival of the cases that had been stratified according to the presence or absence of one of the specific genomic gains or losses. All clones that showed aberrant copy number changes in either the CD5⁺ or the CD5⁻ group were sub-
The remaining 218 gained and lost clones fell short of statistical comparison corrections indicated they had favorable impacts on survival of CD5+ cases of 5p. Among the 34 clones, 19 clones at 5p14.2-q31.3 and 15 at 13q31.3-q34 were continuous and were identified to have deleterious effects on survival. Forty-four of the 44 clones, 29 at 13q21.1-q34, showed significantly inferior survival than did CD5+ cases without such gain, but a gain of 5p did not affect overall survival (Fig. 3C). In contrast, gain at the corresponding region did not affect survival of CD5- DLBCL cases. Similarly, CD5+ DLBCL cases with loss of 1p34.3-p36.21 showed significantly inferior survival than did CD5- cases without such loss (Fig. 3B), whereas loss at the corresponding region did not affect survival of CD5- DLBCL cases. Conversely, CD5- DLBCL cases with a gain of 5p showed superior overall survival (Fig. 3C) to those without such gain, but a gain of 5p had no impact on survival of CD5+ DLBCL cases. A list of BAC/PACs associating with prognosis of CD5+ or CD5- DLBCL and prognostic data can be found in Supplementary Data 1.

### DISCUSSION

Array CGH methods have been successfully used for the high-resolution analysis of genomic alterations not only in a variety of solid tumors (7–9, 25, 26) but also in hematological malignancy (27–30). In this study, we used our own array CGH (10) to analyze 70 cases of DLBCL and identified regions of genomic gain and loss of DLBCLs that were relevant to clinical subtypes and patient survival.

Array CGH detected aberrations of several loci that were undetectable by chromosomal CGH (also known as conventional CGH). Loss of 3p14.2 was one such aberration. This lost locus was detected by array CGH in 18 of 66 DLBCL cases but in none by chromosomal CGH. The responsible regions for 13 of these 18 cases were covered by a single BAC, RP11-48E21, which included the FHIT tumor suppressor gene. This clearly demonstrates that array CGH is more sensitive than chromosomal CGH. These findings also suggest that array CGH is a useful tool for identifying and narrowing down to the target genes. It should be noted, however, that the efficacy of array CGH is limited to identification of copy number changes of genome, and it can therefore not detect chromosomal translocations, mutations, and epigenetic events that could affect gene expressions. The use of the array CGH method in combination with other newly developed technologies such as microarray analysis of transcripts and SKY analysis of chromosomes may further facilitate our understanding of molecular events underlying DLBCL.

We reported previously that chromosomal CGH analyses of patient samples identified six recurrent regions of gain (3q, 6p, 11q21-q24, 12q, 13q22-q32, and 18q) and four recurrent regions of loss (1p, 6q, 17p, and 19p; ref. 17). Array CGH analysis of the same set of patient samples found several novel recurrent regions of gain such as 1q32, 5p13, 7p22-q31, 8q24.13-q24.21, and 16p13, as well as recurrent
regions of loss such as 3p14.2 and 9p21. However, loss of 19p detected by chromosomal CGH could not be confirmed in array analysis. The reasons for this apparent discrepancy between chromosomal CGH and array CGH are not yet entirely clear. One possibility is that chromosome 19 contains blocks of heterochromatin that are difficult to assess with chromosomal CGH. It is therefore possible that the discrepancy is due to the unreliable results obtained with chromosomal CGH for these regions. Other researchers reported that the use of chromosomal CGH methods resulted in the identification of 1q21-q23, 2p12-p16, 3q26-q27, 7q11, 8q24, 9q34, 11cen-q23, 12p, 12cen-q13, 13q32, 16p12, 16q21, 18q21-q22, and 22q12 as recurrent regions of gain and loss at 1p36.13-p36.12.

Our array CGH analysis has been applied by Martinez-Climent et al. (29) to follicular lymphoma and DLBCL transformed from follicular lymphoma. They reported that 14 regions of gain and 9 regions of loss were acquired as a result of the transformation. Among these 23 regions, gains of 4p12-pter, 9q13-q31, and 17q21 and losses of 4q21-q23, 5q21-q23, 9q31-qter, 11q24-q25, and 13q14-q21 were not found as recurrent genomic aberrations in our study of de novo DLBCL. Conversely, we found genomic alterations such as gains of 11q24, 13q13, and loss of 10p14 in our study of de novo DLBCL but not in the reported cases of transformed DLBCL. These findings suggest de novo DLBCL and transformed DLBCL are different in terms of the genes responsible for lymphomagenesis.

Array CGH analysis identified largely identical genomic aberration patterns in the CD5+/H11001 and CD5+/H11002 groups. However, gains of 10p14-p15.3 and 19q13.32-q13.43 and losses of 1q43-q44 and 8p23.1-p23.2 were found to be characteristic of CD5+ DLBCL. These findings, in addition to characteristic clinical behavior, indicate that CD5+ DLBCL is a distinct entity. It has not been demonstrated yet that genes included in the regions of 10p14-p15.3 and 1q43-q44 are linked to malignancy, but the 19q13.2-q13.43 region includes tumor-related genes such as BAX, PEG3, CD37, and IL4R1, with the last one having been identified as a gene responsible for primary mediastinal diffuse large B-cell lymphoma (35). Genomic loss of 8p23 has frequently been found in leukemic MCL (36), and this deletion may be linked with leukemic dissemination and poor prognosis for patients with MCL. Given that, in addition to MCL, 8p23 is recurrently lost in CD5+ DLBCL, one could speculate that this locus may contain tumor suppressor genes accounting for poor prognosis of patients with both CD5+ DLBCL and leukemic MCL.

It was also found that although gain of 13q21.1-q34 and loss of
1p34.3-p36.21 had deleterious impacts on the survival of CD5+ cases, these regions had no such impact on CD5- cases. We are currently analyzing expressions of genes that are included in the loci specifically gained or lost in CD5+ DLBCL to gain additional insights into their roles in lymphoma development. One possible candidate gene for 13q31-q32 amplification is C13orf25, which has been found in the case of gain of 13q31-q32 in B-cell lymphoma. C13orf25 contains several microRNAs (miR-17, miR-18, miR-19a, miR19b-1, miR-20, and miR-92-1) that might be associated with lymphomagenesis (10, 37). There have been no reports, however, on tumor suppressor gene(s) of 1p34.3-p36.21.

In contrast to the CD5- cases, we were not able to find genomic regions, either gained or lost, which were characteristic of CD5- DLBCL, although gain of 5p was found to be associated with a favorable survival. More cases need to be analyzed, however, to clarify the prognostic significance of this gain.

To summarize, we subjected DLBCL cases to array CGH analysis and found genomic regions recurrently altered in DLBCL. By comparing CD5+ and CD5- cases, we were able to identify genomic alterations specific to the CD5+ DLBCL group. Array CGH analysis can thus be expected to provide new insights into the genetic background of lymphomagenesis.

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