CASE REPORT

Mutation profiles of diffuse large B-cell lymphoma transformation of splenic B-cell lymphoma/leukemia, unclassifiable on whole-exome sequencing

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Abstract
A 58-year-old male was diagnosed with splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U). The lymphoma transformed into diffuse large B-cell lymphoma (DLBCL), and multidrug chemotherapy and autologous stem cell transplantation achieved complete remission. Two years later, the lymphoma relapsed as SPLL-U. Serial whole-exome sequencing indicated that the mutation profiles were similar between the onset and relapsed samples while those in DLBCL were partially distinctive, which was in line with the clinical course. Hierarchical clustering revealed that an IGLL5 mutation was the founder mutation proceeding the development of the diseases and suggested that KRAS and other mutations might contribute to the transformation.

KEYWORDS
diffuse large B-cell lymphoma transformation, splenic B-cell lymphoma/leukemia, unclassifiable, whole-exome sequencing

Splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U) is a lymphoproliferative disorder of the spleen involving small B-cell clones which do not meet the diagnostic criteria for any other subtypes of mature B-cell neoplasms in the WHO classification [1]. SPLL-U includes splenic diffuse red pulp small B-cell lymphoma (SDRPL), hairy cell leukemia-variant (HCL-v), and narrow sense SPLL-U that are not classifiable as SDRPL or HCL-v and a limited number of patients with SPLL-U suffer from aggressive clinical courses. Recently published reports explored driver mutations in transformed B-cell lymphoid malignancies [2–6]. However, to date, the data on SPLL-U are scarce...
Herein, we reported a unique case of SPLL-U which transformed into DLBCL. This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan.

A 58-year-old male patient presented with leukocytosis. The bone marrow (BM) was slightly hypocellular with 78.8% atypical lymphocytes. Conventional cytogenetic analyses of the BM aspirate revealed 46,XY in all metaphases. The BM biopsy revealed nodular and diffuse infiltration of lymphoid cells positive for CD20, focally and weakly positive for CD23, weakly positive for CD25, and negative for CD3, CD5, CD10, CD123, BCL-6, Ki-67, c-MYC, and cyclin D1 on immunohistochemical staining (Figure 1A). These findings were not specific to any type of mature B-cell neoplasm. The patient declined a splenectomy. The diagnosis of SPLL-U was made (onset phase, Figure 2), and one cycle of cladribine was administered, resulting in a modest reduction of the atypical lymphocytes.

One year later, computed tomography revealed multiple low-density lesions throughout the liver. A liver biopsy revealed destruction of the hepatic lobule with diffuse infiltration of tumor cells positive for CD20, BCL-6, and Ki-67, and focally positive for c-MYC (Figure 1B), which was consistent with DLBCL, not otherwise specified, germinal center B-cell type (DLBCL phase, Figure 2). DLBCL cells were also found in a BM smear (0.6%) while the atypical lymphocytes had decreased (2.8%). Conventional cytogenetic analyses of the BM aspirate revealed 46,XY in all the metaphases. One cycle of cladribine and four cycles of rituximab were administered. Ten months later, pancytopenia and a liver nodule developed. BM aspirate revealed hypocellular marrow with CD20-negative atypical lymphocytes, and a liver biopsy demonstrated SPLL-U recurrence (second relapse phase, Figure 2). Ibrutinib was started, and the cytopenia gradually improved. The patient has continued receiving ibrutinib and has experienced no further recurrences.

We performed whole-exome sequencing (WES) using DNA obtained from BM at the onset phase, the hepatic lesion at the DLBCL phase, and BM at the first and second relapse phase. Genomic DNA was extracted from each sample using the All Prep kit (Qiagen; Hilden, Germany), and WES was performed using Ion AmpliSeq Exome RDY Kit (Thermo Fisher Scientific, Waltham, MA, USA) on the Ion GeneStudio S5 system (Thermo Fisher Scientific) according to manufacturer’s instructions. Sufficient amount sequence reads (mean 44 million, range: 11.9–80.9 million) were obtained and analyzed using Ion Reporter software to detect pathogenic mutations in the leukemic cells. Mean coverage depth was 135 (range: 25.86–252.6) and mean uniformity was 91% (range: 88.91–92.43) suggesting that WES was successfully worked. Detected mutations were clustered using the group average method. Curated pathogenic variants are shown in Table 1.

In line with the clinical findings, the mutation patterns were generally similar between the onset relapse phase while the mutation profiles in the DLBCL phase partially differed from the others (Figures 3A,B). In the hierarchical clustering, an IGLL5 mutation was detected in all the samples, suggesting that these founder mutations preceded the development of the disease. IGLL5 mutations, which are critical for B-cell development, were frequently detected in various B-cell lymphoid malignancies [10, 11]. These mutations were reported...
| Time Point | Chromosome | Coordinate | Genotype | Type  | Genes | Transcript | Transcript Change | Amino Acid Change | VAF   | Coverage |
|------------|------------|------------|----------|-------|-------|------------|-------------------|------------------|--------|----------|
| Onset      | chr12      | 49416372   | C/A      | SNV   | KMT2D | NM_003482.3 | c.16338+1G > T   | p.?              | 43.23  | 229      |
| Onset      | chr22      | 23230373   | G/T      | SNV   | IGLL5 | NM_001256296.1 | c.34G > T      | p.Ala12Ser       | 40.08  | 237      |
| Onset      | chr3       | 75714935   | C/A      | SNV   | FRG2C | NM_001124759.3 | c.592C > A     | p.Gln198Lys      | 7.54   | 557      |
| Onset      | chr3       | 75714950   | C/A      | SNV   | FRG2C | NM_001124759.3 | c.607C > A     | p.Leu203Met      | 22.28  | 395      |
| Onset      | chr1       | 161047249  | C/T      | SNV   | NECTIN4 | NM_0030916.2 | c.724G > A    | p.Val242Met      | 9.8    | 51       |
| Onset      | chr2       | 107156504  | GT/G     | INDEL | TBCK  | NM_0011163435.2 | c.1370delA    | p.Asn457ThrfsTer15 | 35     | 20       |
| Onset      | chr11      | 118376778  | C/T      | SNV   | KMT2A | NM_001119704.1 | c.10171C > T  | p.Gln3391Ter     | 14.81  | 27       |
| Onset      | chr12      | 25380275   | T/G      | SNV   | KRAS  | NM_0033360.3  | c.183A > C    | p.Gln61His       | 16.67  | 26       |
| Onset      | chr22      | 23230355   | C/T      | SNV   | IGLL5 | NM_001256296.1 | c.16C > T     | p.Gln6Ter        | 22.73  | 22       |
| Onset      | chr22      | 23230379   | A/C      | SNV   | IGLL5 | NM_001256296.1 | c.40A > C     | p.Thr14Pro       | 23.81  | 21       |
| Onset      | chr12      | 49431625   | G/C/G    | INDEL | KMT2D | NM_0033482.3  | c.9513delG    | p.Pro3172HisfsTer25 | 29.03  | 31       |
| Onset      | chr3       | 48680471   | G/A      | SNV   | CERS3 | NM_001407.2  | c.8335C > T   | p.Arg2779Trp     | 7.55   | 53       |
| Onset      | chr4       | 106157167  | C/T      | SNV   | TET2  | NM_001127208.2 | c.2068C > T  | p.Gln690Ter      | 7.41   | 54       |
| Onset      | chr4       | 185018473  | C/T      | SNV   | ENPP6 | NM_153343.3  | c.1042G > A   | p.Gly348Ser      | 8.51   | 47       |
| Onset      | chr5       | 40692160   | C/T      | SNV   | PTGER4 | NM_000958.2 | c.1147C > T  | p.Arg383Trp      | 7.84   | 51       |
| Onset      | chr6       | 54805306   | G/A      | SNV   | FAM83B | NM_001010872.2 | c.1537G > A  | p.Gly513Arg      | 13.95  | 43       |
| Onset      | chr7       | 150846025  | G/A      | SNV   | GBX1  | NM_001098834.2 | c.743C > T   | p.Ala248Val      | 11.43  | 35       |
| Onset      | chr10      | 5929864    | G/A      | SNV   | ANKRD16 | NM_019046.2 | c.481C > T  | p.Pro1615Ser     | 6.94   | 72       |
| Onset      | chr12      | 25380275   | T/G      | SNV   | KRAS  | NM_0033360.3  | c.183A > C    | p.Gln61His       | 16.67  | 36       |
| Onset      | chr12      | 70824288   | G/A      | SNV   | KCNMB4 | NM_014505.5 | c.489G > A   | p.Arg163His      | 13.89  | 36       |
| Onset      | chr15      | 42985912   | G/A      | SNV   | STARD9 | NM_020759.2 | c.12136G > A  | p.Gly404Ser      | 17.14  | 35       |
| Onset      | chr17      | 7579340    | G/A      | SNV   | TP53  | NM_000546.5  | c.347C > T   | p.Ser116Phe      | 11.11  | 36       |

(Continues)
| Time Point     | Chromosome | Coordinate | Genotype | Type   | Genes   | Transcript | Transcript Change | Amino Acid Change | VAF | Coverage |
|---------------|------------|------------|----------|--------|---------|------------|-----------------|------------------|-----|----------|
| DLBCL         | chr17      | 20916179   | C/T      | SNV    | USP22   | NM_0015276.1| c.908G > A      | p.Gly303Asp       | 9.43| 53       |
| DLBCL         | chr19      | 38828038   | G/T      | SNV    | CATSPERG| NM_021185.4| c.164G > T      | p.Arg55Met        | 18.18| 22       |
| DLBCL         | chr20      | 56188345   | G/A      | SNV    | ZBP1    | NM_030776.2| c.544C > T      | p.Gln182Ter       | 8   | 50       |
| DLBCL         | chr22      | 23230373   | G/T      | SNV    | IGLL5   | NM_001256296.1| .34G > T     | p.Ala12Ser       | 15  | 20       |
| First relapse | chr12      | 49416372   | C/A      | SNV    | KMT2D   | NM_003482.3| c.16338+1G > T | p.?              | 34.01| 441      |
| First relapse | chr22      | 23230373   | G/T      | SNV    | IGLL5   | NM_001256296.1| .34G > T     | p.Ala12Ser       | 43.1 | 536      |
| First relapse | chr3       | 75714950   | C/A      | SNV    | FRG2C   | NM_00124759.3| .607G > A    | p.Leu203Met      | 16.55| 840      |
| First relapse | chr3       | 75714950   | C/A      | SNV    | FRG2C   | NM_00124759.3| .607G > A    | p.Leu203Met      | 16.55| 840      |
| First relapse | chr12      | 122359408  | G/A      | SNV    | WDR66   | NM_144668.5| .197G > A    | p.Gly66Glu       | 11.76| 51       |
| First relapse | chr21      | 14982716   | T/C      | SNV    | POTED   | NM_174981.3| .167T > C   | p.Met56Thr       | 6.48 | 108      |
| Second relapse| chr12      | 49416372   | C/A      | SNV    | KMT2D   | NM_003482.3| c.16338+1G > T | p.?              | 15.63| 435      |
| Second relapse| chr22      | 23230373   | G/T      | SNV    | IGLL5   | NM_001256296.1| .34G > T     | p.Ala12Ser       | 18.76| 453      |
| Second relapse| chr3       | 75714935   | C/A      | SNV    | FRG2C   | NM_00124759.3| .592G > A   | p.Gln198Lys      | 5.68 | 1092     |
| Second relapse| chr3       | 75714950   | C/A      | SNV    | FRG2C   | NM_00124759.3| .607C > A   | p.Leu203Met      | 13.41| 753      |
| Second relapse| chr12      | 49416372   | C/A      | SNV    | KMT2D   | NM_003482.3| c.16338+1G > T | p.?              | 12.87| 272      |

Abbreviation: DLBCL, diffuse large B-cell lymphoma. Detected variants were annotated with transcript and protein location information according to HGVS-nomenclature. And more, information from the 1000G, Exac, cosmic, and CLINVAR databases was also added. To exclude SNPs, variants with a prevalence greater than 1% in a given regional population (using 1000G and Exac) were excluded. Variants which previously reported as myeloid- or lymphoid- associated mutations given by cosmic and CLINVAR were further selected as candidate pathogenic mutations. All annotation was performed using Ion Reporter software (Thermo Fisher Scientific) which contains the above databases. Finally, candidate mutations were manually curated by molecular hematologists.
to be linked to canonical activation induced-cytidine deaminase (AID) activity [11]. Although AID normally contributes to the diversity of antibodies by introducing somatic mutations in immunoglobulin genes, off-target AID activity could induce genomic instability and initiate oncogenesis [12]. The IGLL5 mutations might have played a key role also in the present case.

Additionally, several types of KMT2D mutations were found in all the samples. KMT2D is a histone H3 lysine 4 methyltransferase promoting chromatin opening and transcriptional activation of the targeted genes [13]. It frequently shows mutations in various lymphoid malignancies [10, 13–16]. To our knowledge, the present study is the first to detect KMT2D mutations in SPLL-U although the exact reason why different KMT2D mutations appeared alternately was unclear. Further investigation is warranted to explore the underlying pathogenesis in SPLL-U. Sanger sequencing confirmed the presence of IGLL5 p.Ala12Ser and KMT2D c.16338+1G > T mutations with a lower allelic ratio than that detected from WES, possibly result by PCR bias (Figure 3C).

Several studies explored driver mutations in transformed aggressive B-cell lymphoid malignancies by next-generation sequencing (Table 2) [2–6]. In the present case, KRAS mutation, which drives aggressive cell proliferation, was found at the DLBCL phase and might have contributed to the progression of SPLL-U into DLBCL in a process similar to that reported in Richter syndrome [6].

Our study has some limitations: it was a single case report, and the unavailability of spleen samples analysis prevented the identification of specific SPLL-U types (SDRPL, HCL-v, and narrow sense SPLL-U) and SMZL [7]. However, the WHO classification defines SPLL-U as a provisional entity requiring additional molecular studies [1]. The present case provides an important insight into the pathogenesis of SPLL-U despite the limited evidence available. We also note that the percentage of atypical lymphocytes in the microscopic examination could be lower than VAFs in WES because not only apparently malignant cells harbor mutations, as previously reported [17].

In summary, we described the clinical presentation, immunophenotype, and genetic landscape of a case of DLBCL transformation of SPLL-U. Serial analyses using next-generation sequencing have the potential to provide useful information about the origins and progression of this uncommon disease. More patient data and prospective studies are needed to deepen our understanding of SPLL-U pathophysiology.
FIGURE 3 (A) Hierarchical clustering by whole-exome sequencing. Mutation profiles in each phase were described. The color indicates the variant allele frequency of each mutation as shown in the figure. Mutations were classified into seven groups according to hierarchical cluster analysis. Names of the clusters correspond to the clones in (B). (B) Fish plot showing the clonal transition of tumor cells. BM, bone marrow; DLBCL, diffuse large B-cell lymphoma. (C) Sanger sequencing results of the IGLL5 and KMT2D mutations in the onset sample. Results from HL-60 were used as the non-mutated control. The result of the onset sample was shown in both forward (Onset Fwd) and reverse sequence (Onset Rev).

TABLE 2 Mutations in transformed aggressive B-cell lymphoid malignancies from literature review

| Author, year | Primary disease | No. of serial samples | NGS | Mutations detected in transformed cases |
|--------------|-----------------|-----------------------|-----|----------------------------------------|
| Fabbri et al., 2013 | CLL | 9 | WES | TP53 and NOTCH1 |
| Kiel et al., 2012 | SMZL | 6 | WGS | NOTCH2 |
| Bouska et al., 2017 | FL | 12 | WES | MYC, EBF1, IRF4, RPN1, SOCS1, SYNE1, SGK1, PIM1, EP300, BMP7, ETS1, SARDH, TAF1, FBX011 and HIST1H1E etc. |
| Vogelsberg et al., 2020 | ISFN | 10 | Targeted sequencing | TP53, CD79B, and HIST1H1B etc. |
| Klintman et al., 2021 | CLL | 17 | WGS | TP53, XPO1, NOTCH1, SF3B1, BIRC3, ATM, RFS15, Braf, KRAS, TRAF3, SETD2, PTEN11, MGA, and BAZ2A etc. |

Abbreviations: CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; ISFN, in situ follicular neoplasia; NGS, next-generation sequencing; SMZL, splenic marginal zone lymphoma; WES, whole-exome sequencing; WGS, whole-genome sequencing.
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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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