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Case Report

Whole-Exome Sequencing in a Pediatric Patient with Relapsed/Refractory Burkitt Leukemia Resistant to Conventional Chemotherapy

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Abstract

Burkitt lymphoma/leukemia (BL) is one of the most frequent B-cell lymphoma types in children. The cure rate for pediatric BL has improved in the last 30 years; long-term event-free survival of patients has reached approximately 90%. However, novel treatment approaches are needed for a group of high-risk patients. Here, we report the case of a pediatric patient with relapsed/refractory BL who died despite undergoing multimodal treatment including clofarabine. To determine the reason for this resistance, we performed whole-exome sequencing using primary, relapsed, and complete remission samples, and identified 34 gene mutations including *TP53*, *ID3*, *ARID1A*, *CCND3*, *DDX3X*, *MYC* and *CHD6*. Relapsed BL seemed to evolve from one of the subclones observed at the initial phase and was accompanied by many additional mutations including the chromatin remodeling mutation that was absent or existed at a lower allele frequency in the diagnostic samples, indicating a multistep process of BL recurrence.

ABBREVIATIONS

BL: Burkitt lymphoma/leukemia; CLO: Clofarabine; SCT: Stem Cell Transplantation; NHL: Non-Hodgkin Lymphoma; CR: Complete Remission; WES: Whole-exome Sequencing; WBC: White Blood Cell Count; CNS: Central Nerve System; R-ICE: Rituximab, Ifosfamide, Carboplatin, and VP-16; CY: Cyclophosphamide; BMT: Bone Marrow Transplantation; B-NHL: B-cell Non-Hodgkin Lymphoma

INTRODUCTION

Burkitt lymphoma/leukemia (BL) is one of the most frequent types of B-cell lymphoma in children. The Epstein-Barr virus (EBV) and *MYC* translocation have been considered to be important players in BL pathogenesis, neither is sufficient to explain the behavior of the tumor [1]. t(8;14) has also been detected in blood cells of healthy individuals, reinforcing the idea that it alone is not sufficient to drive tumor development [2]. The high proliferation of BL cells could be related to dysregulation of the cell cycle induced by the MYC protein, but MYC also activates apoptosis; therefore, BL cells must have mechanisms to overcome this tumor-protective effect [1]. The cure rate for pediatric BL has improved in the last 30 years, and long-term event-free survival of patients has reached approximately 90% [3-6]. However, there remains a group of high-risk patients for whom novel treatment approaches are needed [7]. The efficacy of rituximab combination chemotherapy as a first-line or salvage therapy for various types of CD20-positive non-Hodgkin's lymphoma, including BL, has been described in recent reports [8,9].

Recently, we experienced the case of a relapse/refractory BL patient who was refractory to chemotherapy and died despite undergoing multimodal treatment including rituximab, clofarabine (CLO), and stem cell transplantation (SCT) from HLA 2-locus mismatched related donor. Currently, no standard therapy exists for patients with relapsed and/or refractory non-Hodgkin lymphoma (NHL) who are ineligible for transplantation or who have failed to obtain complete remission (CR) by SCT. To obtain a better understanding of the correlation between gene mutations and clinical course and explore the molecular targets, trio tumor-normal-relapsed DNA specimens were analyzed using whole-exome sequencing (WES).

CASE PRESENTATION

Clinical course

A 9-year-old Japanese boy was admitted to our hospital with complaints of fever, nausea, vomiting, weight loss, and stomachache. He was pale and had petechiae, and a mass around the ileocecum. The results of laboratory testing were as follows: White blood cell (WBC) count, 41.7 × 10^9/L; hemoglobin, 12.7 g/dL; Platelet count, 58.0 × 10^9/L; LDH level, 11,863 U/L; and uric acid level, 13.6mg/dL. Bone marrow smear preparation revealed that more than 90% of the cells were lymphoid blasts with myeloperoxidase negative (Figure 1). Surface marker analysis showed that the leukemic blasts in the bone marrow were positive for CD5 (0.6%), CD10 (34.7%), CD19 (93.3%), CD20 (89.2%), CD22 (37.1%), κ-ch. (1.6%), λ-ch. (96.3%), CD13 (3.6%), CD38 (97.8%), CD56 (0.7%), FMC-7 (76.9%), and HLA-DR (97.2%) antigens. Chromosomal analysis of bone marrow cells revealed 46, XY, +1, der(1;22)(q10;q10), t(8;14)(q24;q32). EBV was not detected in the peripheral blood or bone marrow. Leukemic cells invaded the central nerve system (CNS) (25/ul). The patient was diagnosed with CNS-positive stage IV BL, i.e., Group 4 according to the Japan Pediatric Leukemia/Lymphoma Study Group B-NHL03 clinical study. The treatment schedule was reported previously [10,11]. We safely used rasburicase (0.2 mg/kg) and thrombomodulin (380 U/kg/day) therapy to prevent tumor lysis syndrome progression. Following the initial 4A1 treatment, the patient achieved CR. Although he could maintain CR successfully for 4 months, BL suddenly relapsed after the fourth consolidation therapy (Figure 1). The results of the blood test at relapse were as follows: WBC count, 18.1 × 10^9/L, LDH level of 7,029 U/L, and uric acid level of 7.2 mg/dL. We used rasburicase (0.2 mg/kg) again to prevent tumor lysis syndrome without any anaphylactic reaction, as well as attempted some other regimens, including rituximab, ifosfamide, carboplatin, and VP-16 (R-ICE); however, another CR could not be achieved. Furthermore, the leukemic cells had down-regulated their CD20 expression after six courses of rituximab treatment. To overcome this situation, we tried to use CLO with cyclophosphamide (CY) and VP-16 (CLO 40 mg/m^2, CY 440 mg/m^2, and VP-16 100 mg/m^2 for 5 days) [12]. We safely administered these drugs for 5 days as planned initially and the WBC count was maintained at 0/L for 2 weeks without a blast crisis. No side effects were observed except grade 4 blood toxicity. We continuously performed allo-bone marrow transplantation (BMT) donated from an HLA 2-locus-mismatched related donor. The conditioning regimen consisted of total body irradiation and melphalan. BMT with 3.6 × 10^6 CD34 cells/kg was performed. We could not confirm the engraftment, and a blast crisis occurred concomitantly. The patient died on day 51 after allo-BMT (Figure 2).

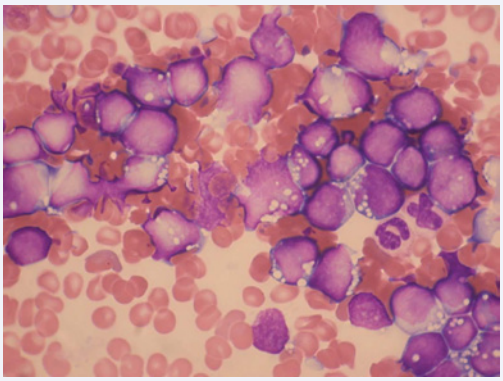

Figure 1 The light microscopic image of the Burkitt leukemia cells. Wright's stain of bone marrow aspiration revealed almost all L3 blast cells.

Whole-exome sequencing analysis

To reveal the clonal origin and major mutational events in relapsed pediatric BL, we performed WES in this patient. Tumour DNA was extracted from the patient's bone marrow mononuclear cells at diagnosis and at the relapse phase, and germline control DNA was obtained from the patient's bone marrow mononuclear cells at CR. Whole-exome capture was accomplished based on liquid phase hybridization of sonicated genomic DNA with a mean length of 150–200 base points (bp) to the bait cRNA library synthesized on magnetic beads (Sure Select Human All Exon 50Mb or V5 kit®, Agilent Technology, CA, USA), according to the manufacture's protocol. Captured targets were subjected to massive sequencing using Illumina HiSeq 2000 with the paired-end 100 bp read option, according to the manufacturer's instructions. WES of three specimens obtained at diagnosis, relapse, and in CR phases were analyzed with a mean coverage of more than ×100, and 95% of the targeted sequences were analyzed at an average depth of more than ×20. Data processing and variant calling were performed as previously described [13]. Candidate somatic mutations were detected using our in-house pipeline Empirical Bayesian mutation Calling (EBCall; see URLs; http://genomon.hgc.jp/exome/) [14]. All candidates were validated by Sanger sequencing. We identified 34 gene alterations including *TP53*, *ID3*, *ARID1A*, *DDX3X, MYC*, and *CHD6* (Figure 3). Of the 34 mutations identified, 10 were specific to relapse (*LRRC36, FAM155B, PHF14, KLF17, AKAP9, CHD6, ZIC1, RHBDF1, HIST1H2BJ,* and *EEA1*), whereas two mutations (*LIM2* and *RFX7*) were specific to the time of diagnosis (Figure 4 and Table 1). Relapsed BL evolved from one of the subclones observed at the initial phase and was accompanied by many additional mutations.

DISCUSSION

We experienced the case of pediatric patient with relapsed/refractory BL who finally died despite undergoing multimodal treatment including CLO. In the present study, a total of 34 somatic mutations were identified, where the number of nonsynonymous mutations was higher in the relapse phase than at the time of diagnosis (24 vs. 32) (Figure 4 and Table 1). Among them, thus

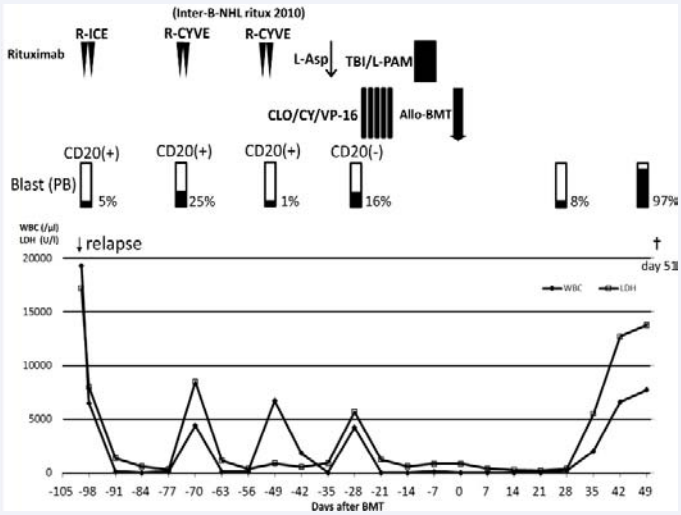

Figure 2 Clinical course of the patient after relapsed. TBI indicates total body irradiation; BMT, bone marrow transplantation; WBC, white blood cell count; PB, peripheral blood; BD, bortezomib and dexamethasone; ICE, ifosfamide, carboplatin, and VP-16; CYVE, Ara-C x2 and VP-16; L-asp, L-asparaginase; L-PAM, melphalan; CLO, clofarabine.

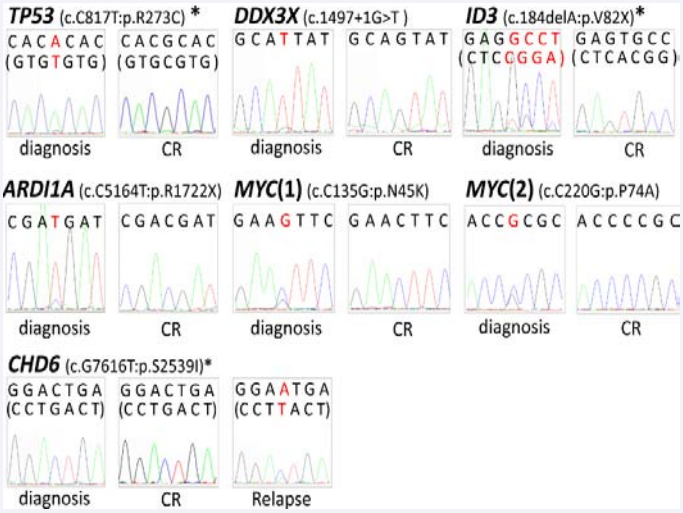

Figure 3 Chromatogram of sequencing with fluorescent-dye chemistry for unfractionated genomic DNA from a Burkitt leukemia patient. *In ID3, TP53, and CHD6, Sanger sequence analysis was performed using their reverse primers. Nucleotide in the parenthesis in each gene shows the coding strand sequence. CR indicates complete remission.

far, *ID3*, *CCND3*, *DDX3X,* and *MYC* were identified as the top four recurrent gene mutations found in four prototypical BL with *IG-MYC* translocation [7]. Love et al. also reported almost similar genetic landscape of mutations in BL using next-generation sequencing [15]. In addition to these mutations, mutations of *TP53* and *ARID1A*, which are known as suppressor genes, were also identified [7,15]. Abate et al. detected lower frequencies of mutations in *MYC*, *ID3*, *TCF3,* and *TP53* and a higher frequency of mutations in *ARID1A* in endemic BL samples [16]. This result indicates dual mechanisms of transformation, i.e., mutation versus virus-driven mechanisms in sporadic and endemic BL [16]. *ID3* mutations occur in 34%–68% of BL and is identified at a greater frequency in adult samples [7]. *ID3* acts a negative transcriptional regulator by sequestering transcription factors with basic helix–loop–helix motifs. Mutated *ID3* attenuates this regulatory interaction [17,18]. *ID3* and its interaction partner *TCF3* are involved in controlling cell cycle progression and survival pathways through tonic B-cell signaling [19,20]. *CCND3* and *ID3* double-hit mutations, as well as 18q21 cytogenetically normal-loss of heterogeneity, have been reported to be associated with poorer outcome [20].

Concerning allele frequencies, intratumoral gene mutations were observed to be heterogeneous, suggesting multiple clonal evolution events during the development of BL (Figure 4). Variant allele frequency of mutated *TP53, ID3, ARID1A,* and *DDX3X* alleles were higher than that of *MYC, IDH3B, PRPF40A,* and *LIM2*. These results suggest that the mutation of *TP53, ID3, ARID1A,* and *DDX3X* occurred at an early stage in leukemic cells, whereas the mutation of *MYC, IDH3B, PRPF40A,* and *LIM2*

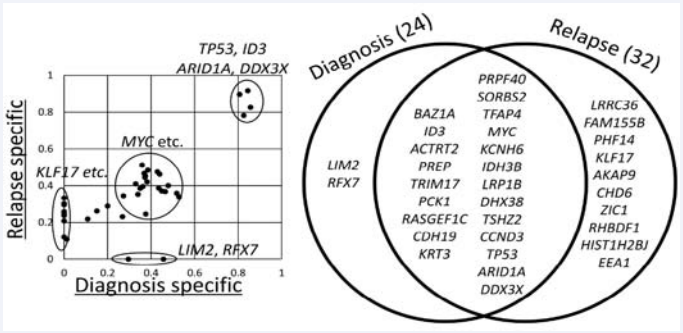

Figure 4 Whole-exome sequencing in a pediatric patient with Burkitt leukemia. Variant allele frequencies of validated gene mutations were shown in a diagonal plot. Thirty-four mutations identified by whole-exome sequencing were shown in Venn diagram.

Table 1: Validated 34 somatic mutations in a pediatric patient with Burkitt leukemia.

Gene	Function	Gene number	Change	Result	Chromosome
LIM2	missense	NM_030657	c.C293A:p.A98E	diagnostic_specific	19
RFX7	missense	NM_022841	c.G816T:p.M272I	diagnostic_specific	15
LRRC36	missense	NM_001161575	c.C1231A:p.L411I	relapse_specific	16
FAM155B	missense	NM_015686	c.C940A:p.Q314K	relapse_specific	X
PHF14	missense	NM_014660	c.G2059A:p.G687S	relapse_specific	7
KLF17	missense	NM_173484	c.A1123G:p.N375D	relapse_specific	1
AKAP9	missense	NM_005751	c.A4373T:p.Q1458L	relapse_specific	7
CHD6	missense	NM_032221	c.G7616T:p.S2539I	relapse_specific	20
ZIC1	missense	NM_003412	c.A533G:p.Y178C	relapse_specific	3
RHBDF1	missense	NM_022450	c.G908A:p.G303D	relapse_specific	16
HIST1H2BJ	missense	NM_021058	c.C120G:p.I40M	relapse_specific	6
EEA1	nonsense	NM_003566	c.G1396T:p.E466X	relapse_specific	12
BAZ1A	frameshift insertion	NM_013448	c.262_263insT:p.P88fs		14
ID3	missense	NM_002167	c.184delA:p.V82X		1
ACTRT2	missense	NM_080431	c.G159C:p.Q53H		1
PREP	missense	NM_002726	c.G1522A:p.G508S		6
TRIM17	missense	NM_001024940	c.G1335C:p.Q445H		1
PCK1	missense	NM_002591	c.A989G:p.N330S		20
RASGEF1C	missense	NM_175062	c.G1144A:p.A382T		5
CDH19	missense	NM_021153	c.A656C:p.E219A		18
KRT3	missense	NM_057088	c.G1126A:p.A376T		12
PRPF40A	nonsense	NM_017892	c.C1735T:p.R579X		2
SORBS2	missense	NM_001145674	c.A2843G:p.E948G		4
TFAP4	missense	NM_003223	c.C172T:p.R58W		16
MYC	missense	NM_002467	c.C135G:p.N45K		8
MYC	missense	NM_002467	c.C220G:p.P74A		8
KCNH6	missense	NM_030779	c.C2681T:p.P894L		17
IDH3B	nonsense	NM_174856	c.499delA:p.I167X		20
LRP1B	missense	NM_018557	c.A10571G:p.N3524S		2
DHX38	missense	NM_014003	c.C1099T:p.R367W		16
TSHZ2	missense	NM_001193421	c.G725A:p.R242H		20
CCND3	nonsense	NM_001136125	c.C622T:p.Q208X		6
TP53	missense	NM_001126115	c.C817T:p.R273C		17
ARID1A	nonsense	NM_006015	c.C5164T:p.R1722X		1
DDX3X	splicing	NM_001193416	c.1497+1G>T		X

occurred as a secondary event. Cells from the founding clone can acquire additional cooperating mutations, yielding subclones that can contribute to disease progression. Notably, we identified mutation of *CHD6* only at the relapsed phase, which is one of the chromatin remodeling genes, as well as *ARID1A* [21]. This results suggested that aberration of chromatin regulation might be associated with disease progression. Relapse may involve dynamic clonal changes following combination chemotherapy and is accompanied by many additional mutations including the chromatin remodeling mutation that were absent or existed at a lower allele frequency in the diagnostic samples, indicating a multistep process of BL recurrence.

Rituximab is a chimeric monoclonal antibody that recognizes the CD20 antigen and is used to treat B-cell NHL (B-NHL) including BL. Although most of the cases benefit from this agent, some cases become resistant to this agent. Few studies have been published which evaluate B-NHL treated with rituximab, and showed that CD20 expression decreased or was lost in many cases of B-NHL persisting after rituximab therapy [22]. In fact, CD20 expression was lost after combination chemotherapy including rituximab in our patient, leading to resistance to conventional chemotherapy. In such severe circumstances, we could safely and effectively use CLO to treat heavily pretreated pediatric patients with relapsed/refractory BL harboring a lot of oncogenic mutations. CLO is tolerated well without significant adverse events, except for grade 4 reversible myelosuppression. We observed that CLO administration provided a favorable response in patients with NHL who have relapsed after receiving multiple therapies and who were refractory to rituximab. Combination therapy is the current standard of front-line treatment for patients with NHL. Therefore, the present case suggests that various CLO-based combination regimens, specifically those with monoclonal antibodies, are worth exploring in refractory patients who are resistant to multiple conventional chemotherapy and rituximab. We consider that this agent effectively reduces leukemic cells when used with cyclophosphamide and VP-16.

In conclusion, from the viewpoint of molecular analysis, WES revealed 34 gene mutations including *TP53*, *ID3*, *ARID1A, CCND3*, *DDX3X, CHD6* and *MYC,* and relapsed BL seemed to evolve from one of the subclones observed at the initial phase and was accompanied by many additional mutations including the chromatin remodelling mutation that were absent or existed at a lower allele frequency in the diagnostic samples, indicating a multistep process of BL recurrence.

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