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Case Study

Gain of 11q by an Additional Ring Chromosome 11 and Trisomy 18 in CD5-Positive Intravascular Large B-Cell Lymphoma

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Chromosomal abnormalities of intravascular large B-cell lymphoma (IVLBCL), a rare form of extranodal diffuse large B-cell lymphoma, have been described in only a small number of cases. A 59-year-old female presented with pancytopenia and splenomegaly. Bone marrow was normocellular with 30.4% abnormal large lymphoid cells that were positive for CD5, CD19, CD20, HLA-DR and λ chain. Bone marrow biopsy showed intrasinusoidal infiltration of large lymphoid cells. G-banding and spectral karyotyping of the bone marrow cells demonstrated a complex karyotype as follows : 48,XX,−8,+r(11),+12,del(12)(p?)×2,+18,der(19)(19?::p13→qter),der(21)t(8;21)(q11.2;p11.2). Fluorescence *in situ* hybridization on interphase nuclei revealed three signals of *CCND1* at 11q13, but two signals of *BIRC3* at 11q22 and *MLL* at 11q23, indicating that r(11) contained *CCND1*. Together with other reported cases, our results indicate that the gain of 11q as well as trisomy 18 may be among the recurrent chromosomal aberrations in IVLBCL. Furthermore, an additional ring chromosome 11 could be a novel mechanism leading to the gain of 11q. 〔*J Clin Exp Hematop 53(2) : 161-165, 2013*〕

Keywords: intravascular large B-cell lymphoma, chromosomal abnormalities, ring chromosome, gain of 11q, trisomy 18

INTRODUCTION

Intravascular large B-cell lymphoma (IVLBCL) is a rare form of extranodal diffuse large B-cell lymphoma (DLBCL) characterized by the selective growth of lymphoma cells within the lumina of vessels, particularly capillaries.[1-3] Compared with intensive histopathologic and immunophenotypic analyses, chromosomal abnormalities of IVLBCL have been described in only a small number of cases, possibly due to the difficulty of early diagnosis and poor material for cytogenetic analyses. Most of the IVLBCL cases cytogenetically analyzed showed complex karyotypes with multiple numerical and structural changes,[4-12] and gains of 11q and trisomy 18 were included in these abnormalities.[9] Unlike other B-cell lymphomas, rearrangements of immunoglobulin heavy locus (*IGH@*) at 14q32 are uncommon.[10,12] However, specific chromosome aberrations remain to be completely elucidated in IVLBCL. Here, we describe a new case of IVLBCL, which revealed a gain of 11q by an additional ring chromosome 11 and trisomy 18.

CASE REPORT

A 59-year-old woman was admitted to our hospital because of petechiae. Computed tomography scans of the whole body showed splenomegaly but no lymphadenopathy. Peripheral blood values were hemoglobin 96 g/L, platelets 30 × 10^9/L and leukocytes 2.4 × 10^9/L with 41% neutrophils, 16% monocytes, 1% basophils, 23% lymphocytes and 19% abnormal lymphoid cells. Serum levels of lactate dehydrogenase and soluble interleukin-2 receptor were elevated to 1,324 U/L (normal range, 115〜217) and 9,975 U/mL (124〜466), respectively.

Bone marrow was normocellular with 30.4% abnormal large lymphoid cells (Fig. 1a). Immunophenotyping by three-color flow cytometry revealed that these lymphoid cells were positive for CD5, CD19, CD20, HLA-DR and λ chain, but negative for CD10 and CD23. Pathological examination showed intrasinusoidal infiltration of atypical large lymphoid

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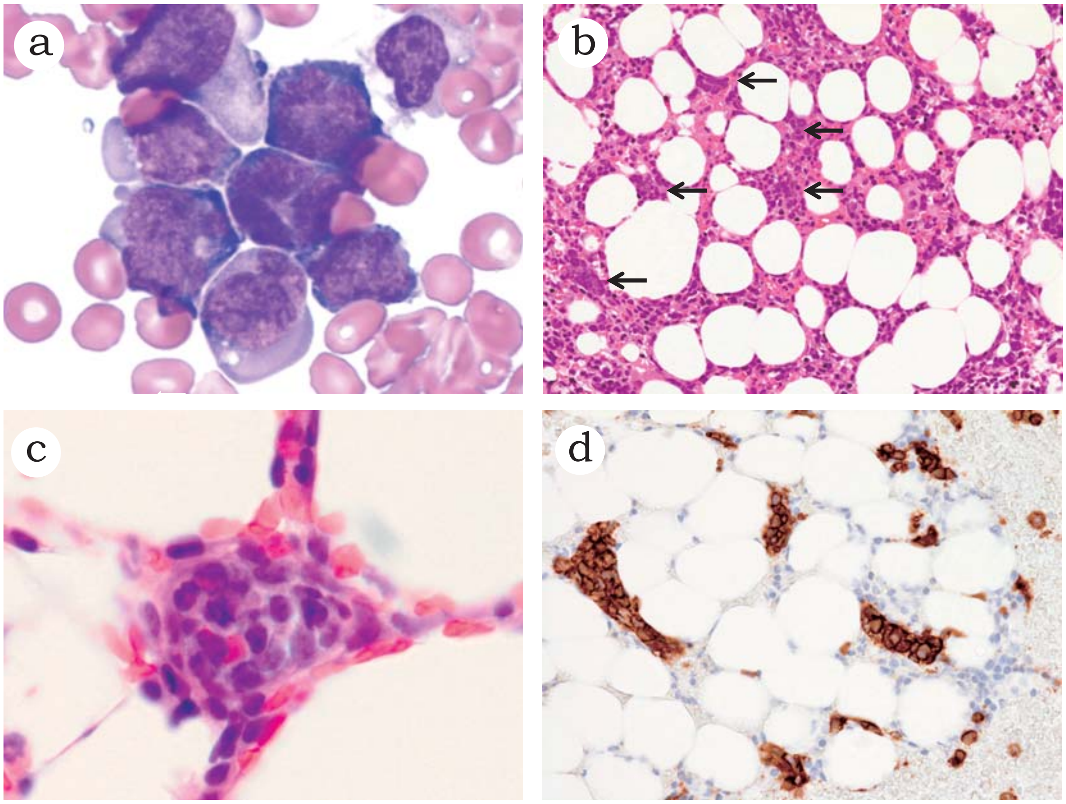

Fig. 1. Morphological findings of the bone marrow cells. (***1a***) Bone marrow smear showing a cluster of large lymphoma cells, which had convoluted nuclei with fine nuclear chromatin and basophilic cytoplasm with vacuoles. May-Grünwald-Giemsa staining, ×1,000. (***1b***) Bone marrow biopsy showing intrasinusoidal infiltration of large lymphoma cells. *Arrows* indicate a cluster of lymphoma cells. H&E stain, ×200. (***1c***) Intrasinusoidal infiltration of large lymphoma cells in the bone marrow. H&E stain, ×400. (***1d***) Immunohistochemical staining shows CD20 positivity for the intrasinusoidal lymphoma cells. ×200.

cells (Fig. 1b & 1c). There was no evidence of hemophagocytosis. Immunohistochemistry confirmed that these lymphoid cells were positive for CD20 and CD79a, but negative for cyclin D1 and myeloperoxidase (Fig. 1d). Lymphoid cells were also positive for MUM1 but negative for CD10 and BCL6, indicating the non-germinal center B-cell phenotype. The MIB-1 index was about 80%. We made a diagnosis of IVLBCL in accordance with the World Health Organization classification.[1]

The patient received systemic chemotherapy with R-CHOP regimen (rituximab 375 mg/m^2 day 1, cyclophosphamide 750 mg/m^2 day 2, doxorubicin 50 mg/m^2 day 2, vincristine 1.4 mg/m^2 day 2 and prednisolone 100 mg/body days 2-6) followed by prophylactic intrathecal injection (15 mg of methotrexate, 40 mg of cytarabine and 3.3 mg of dexamethasone) because the central nervous system is frequently involved in IVLBCL.[3] She achieved complete remission (CR), and received a further seven courses of R-CHOP and three courses of intrathecal injections. She has been in CR for more than 21 months.

G-banding analysis of the bone marrow cells at diagnosis showed 48,XX,−8,+12,del(12)(p?)×2,+18,add(19)(p13),der(21)t(8;21)(q11.2;p11.2),+r1[8]/46,XX[12] (Fig. 2a). Spectral karyotyping (SKY) revised the karyotype as follows : 48,XX,−8,+r(11),+12,del(12)(p?)×2,+18,der(19)(19?::p13→qter),der(21)t(8;21)(q11.2;p11.2)[3]/46,XX[2] (Fig. 2b). That is, an additional ring chromosome was shown to be derived from chromosome 11.

For further characterization of the ring chromosome 11, we next performed fluorescence *in situ* hybridization (FISH) with commercially available chromosome 11- and B-cell lymphoma-associated probes for *CCND1* at 11q13, *BIRC3* at 11q22 and *MLL* at 11q23 on interphase nuclei.[11] FISH with *IGH@/CCND1* showed two *IGH@* and three *CCND1* signals in 9 of 100 cells (Fig. 2c). On the other hand, FISH with *BIRC3/MALT1* showed two *BIRC3* and three *MALT1* signals due to trisomy 18 in 8 of 100 cells (Fig. 2d). Furthermore, FISH confirmed two normal *MLL* signals in all 100 cells (data not shown). These results indicate that the ring chromosome 11 contained *CCND1* at 11q13, resulting in the gain of 11q,

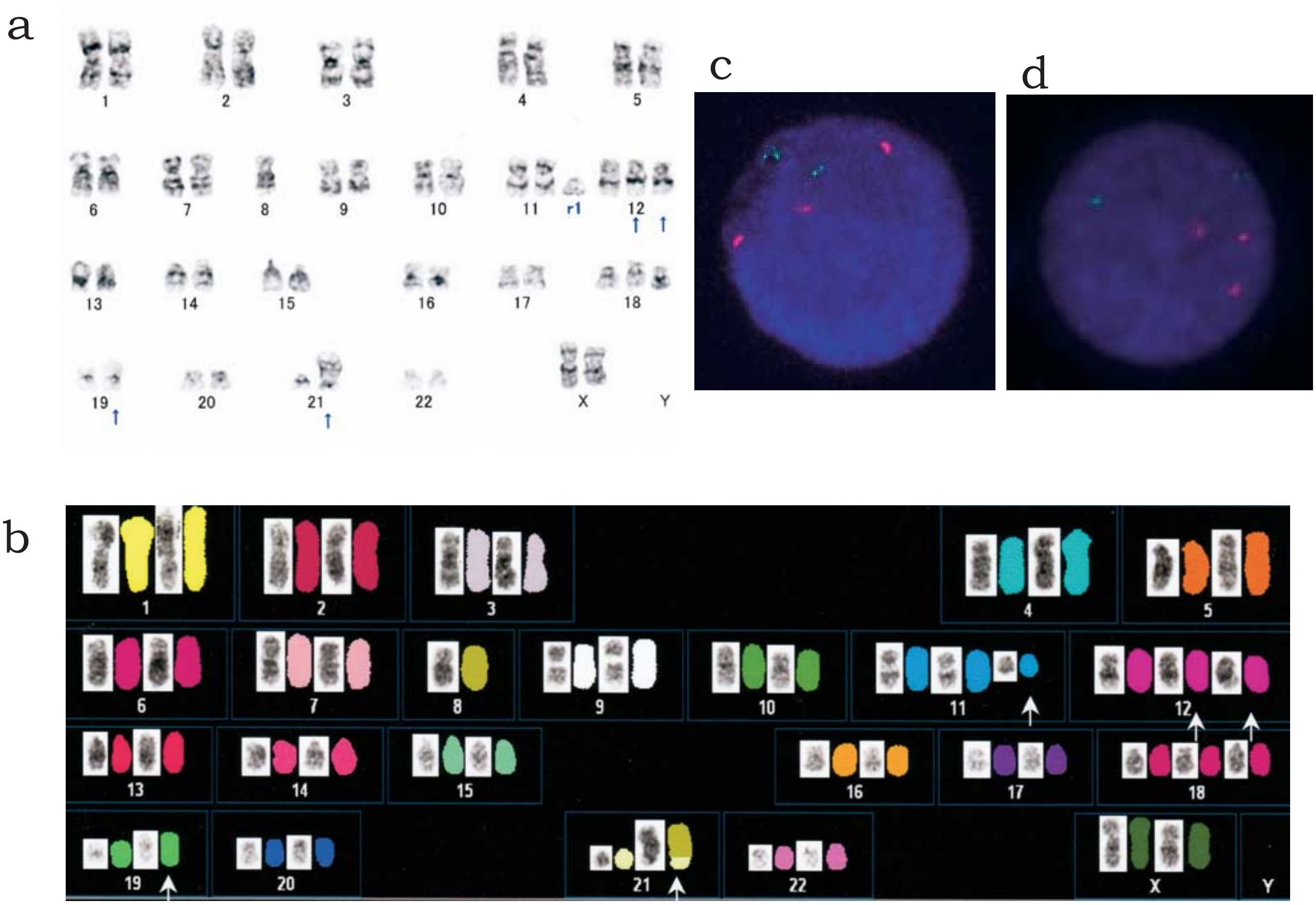

Fig. 2. Cytogenetic findings of the bone marrow cells. (***2a***) G-banded karyotype of the bone marrow cells. The karyotype was initially determined to be 48,XX,−8,+12,del(12)(p?)×2,+18,add(19)(p13),der(21)t(8;21)(q11.2;p11.2),+r1. *Arrows* indicate rearranged chromosomes. (***2b***) Spectral karyotyping (SKY) of the metaphase spread after spectrum-based classification. Chromosomes were assigned a pseudocolor according to the measured spectrum. The grayscale images are inverted from staining with 4', 6-diamidino-2-phenylindole (DAPI) ; the colored images are for SKY. The revised karyotype was as follows : 48,XX,−8,+r(11),+12, del(12)(p?)×2,+18,der(19)(19?::p13→qter),der(21)t(8;21)(q11.2;p11.2). *Arrows* indicate rearranged chromosomes. (***2c***) Fluorescence *in situ* hybridization (FISH) with Vysis IGH/CCND1 DF FISH Probe Kit (Abbott Molecular, Abbott Park, IL, USA) on interphase nuclei. Three *CCND1* signals at 11q13 (*red*) and two *IGH@* signals at 14q32 (*green*) are observed. (***2d***) FISH with Vysis BIRC3/MALT1 DF FISH Probe Kit (Abbott Molecular) on interphase nuclei. Two *BIRC3* signals at 11q22 (*green*) and three *MALT1* signals at 18q21 (*red*) are observed.

but that it did not include *BIRC3* and *MLL*.

DISCUSSION

We have detected a gain of 11q by an additional ring chromosome 11 and trisomy 18 in a patient with IVLBCL by G-banding, SKY and FISH analyses. Khoury *et al*. reviewed cytogenetic findings of 17 published IVLBCL cases, and clarified that the most frequent alterations were −6/del(6q) and +18/dup(18q), which were observed in 59% and 41% of cases, respectively.[9] In addition, commonly duplicated regions that were apparent in > 20% of the karyotypes included all of chromosome 18 (+18) and the 11q13→qter region. Then, we summarized the reported IVLBCL cases with gains of 11q (Table 1) : two cases with an additional whole chromosome 11 (cases 3 and 4) and three cases with duplication or triplication of 11q (cases 5 to 7).[6,7,9,11] Duplicated regions on chromosome 11 seemed to be heterogeneous, and the 11q13 region was involved in case 6.[9] There were also two reported IVLBCL cases with ring chromosomes (cases 1 and 2),[5,8] but their origins were unclear. Thus, our results indicate that the gain of 11q as well as trisomy 18 may be among the

Table 1. Reported cases of intravascular large B-cell lymphoma with ring chromosomes or gains of 11q

Case No.	Age/ Sex	Subtypes	Karyotypes	References
1	63/F	Western	52,X,i(X)(q10),+i(X)(q10)×3,del(1)(q42q44),add(6)(q11),del(6)(q11q27),del(9)(q11q34),−15,**+18,+r**,+2mar[3]/46,XX[27]	Davey *et al.*, 1990 (5)
2	64/F	Western	48,XX,−1,add(1)(p11),der(3)t(1;3)(q21;q21),del(4)(q?),add(6)(q11),+9,del(17)(q11q21),**+18**,add(18)(p11)×2,−19,**+r**,+mar[9]/46,XX[11]	Tsukadaira *et al.*, 2002 (8)
3	76/M	Western	53,XY,+X,t(1;3)(p22;p21),add(3)(q?),+der(5)(q?),i(6)(p10),+7,add(8)(p?),−10,**+11**,+12,del(12)(p?)×2,**+18**,+mar1,+mar2[8]/53,sl,−X,−der(5)(q?),+5,+mar3[2]	Molina *et al.*, 1990 (6)
4	60/M	Asian	73,XXY,+X,+X,+Y,−1,−1,−2,−2,add(2)(p?),−3,−4,−5,−7,+der(9;22)(q10;q10),**+11**,+14,−15,add(15)(p11),−18,−21,−22,−22,−22,+mar1,+mar2,+mar3,+mar4,+mar5,+7mar[1]/75,sl,−X,add(9)(p?),−12,−13,der(16)t(1;16)(q2?1;q2?),+19,+add(21)(p11),−mar4,−mar5,+5mar[1]/75,sl,−X,+add(1)(q11),+5,?8,add(9)(p?),−14,der(14)t(1;14)(q2?;32),+15,der(16)t(1;16)(q2?1;q2?),+19,+add(21)(p11),−mar1,−mar2,−mar3,−mar5,+3mar[1]/46,XY[14]	Murase *et al.*, 2000 (7)
5	61/F	Asian	47,XX,inv(1)(q13q21～25),del(4)(q31),del(5)(q13q33),add(6)(q21),add(8)(p21),−9,**dup(11)(q21q25)**,del(17)(p11),**+18**,−22,+mar1,+mar2,inc[1]/48,sl,add(10)(q22～24),inc[1]/46,XX[18]	Murase *et al.*, 2000 (7)
6	66/M	Asian	47,XY,dup(1)(q32q21),inv(3)(p?q?),del(3)(?q21q24),del(8)(p11),del(10)(?q11q25),der(11)**dup(11)(q13q?21)**t(11;14)(p15;q32),der(14)t(11;14)(p15;q32),+der(14)t(11;14)	Khoury *et al.*, 2003 (9)
7	47/M	Asian	47,XY,add(1)(p36.1),add(1)(q32),add(2)(q31),add(4)(p14),add(9)(p22),**trp(11)(q22q25)**,del(14)(q31q32),add(19)(p13.1),+der(?)t(?;9)(?;q13)[9]/46,XY[11]	Deisch *et al.*, 2009 (11)
8	59/F	Asian ?	48,XX,−8,**+r(11)**,+12,del(12)(p?)×2,**+18**,der(19)(19?::p13→qter),der(21)t(8;21)(q11.2;p11.2)[3]/46,XX[2]	present case

M, male ; F, female ; Western, Western form ; Asian, Asian variant, Ring chromosomes, gains of 11q, and trisomy 18 are described in bold letters.

recurrent chromosome abnormalities in IVLBCL. Furthermore, an additional ring chromosome 11 could be a novel mechanism leading to the gain of 11q.

Ring chromosomes occur when two ends of a chromosome fuse together and form a circular structure.[13] They are found in less than 10% of hematological malignancies, usually as a part of complex karyotypes.[13] Ring chromosome 11 has been reported in 19 cases of acute myeloid leukemia, but in only one case of lymphoid malignancy (acute lymphoblastic leukemia).[4] Therefore, ring chromosome 11 seems to be a very rare abnormality in B-cell lymphomas. In the present case, unfortunately, the ends of the ring chromosome 11 could not be identified by G-banding and SKY. FISH revealed that the ring chromosome 11 contained *CCND1* at 11q13 but did not include *BIRC3* at 11q22, indicating that, at least, the 11q22→qter region was deleted. Genes located on 11q responsible for the pathogenesis of IVLBCL remain to be elucidated.

Trisomy 18 is observed in 15-33% of non-Hodgkin's lymphomas including DLBCL.[14] Considering the relatively high frequency, trisomy 18 may also be involved in the pathogenesis of IVLBCL. Interestingly, as shown in Table 1, all three IVLBCL cases with ring chromosomes (cases 1, 2 and 8) had trisomy 18, implying that the ring chromosome 11 in the present case might be specifically associated with trisomy 18.

Translocations involving 14q32, such as t(14;18)(q32;q21) and t(3;14)(q27;q32), are observed in more than 50% of DLBCL cases,[15] whereas they have hardly been found in IVLBCL.[4] In the present case, FISH revealed no *IGH@* rearrangement as well. Exceptionally, one case with t(11;14)(q13;q32) and one case with t(14;19)(q32;q13) have been reported.[10,12] These findings suggest that IVLBCL might represent a genetically distinct entity from DLBCL. With regard to chromosome gain or loss, gains of chromosome 18 including 18q21-23 have been frequently found,[15,16] while 11q was not included in the common regions of cytogenetic gain in DLBCL.[16] However, in CD5-positive DLBCL, the gain of 11q was shown to be one of the frequently detected abnormalities in addition to gains of chromosomes 3 and 18.[17] Therefore, at present, it is difficult to conclude that the gain of 11q may be specific to IVLBCL.

IVLBCL shows immunophenotypic heterogeneity : $CD5^+$ or $CD5^-CD10^+$, $CD5^-CD10^-$ and $CD5^+CD10^-$. CD5 positivity was associated with a higher prevalence of marrow/blood involvement and thrombocytopenia and a lower frequency of neurologic abnormalities among patients with CD10- IVLBCL.[3] These findings were also applicable to the present case with $CD5^+CD10^-$ phenotype. Currently, the association between immunophenotypic heterogeneity and chromosome abnormalities remains unclear. A detailed and extensive cytogenetic study for more cases would contribute to understand the pathogenesis of IVLBCL.

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