Coexistence of t(2;14;11)(p16.1;q32;q23) and t(14;19)(q32;q13.3) chromosome translocations in a patient with chronic lymphocytic leukemia

A case report

Guangming Liu, MD\textsuperscript{a,c}, Zhongmei Wen, MD, PhD\textsuperscript{b,c}, Xianglan Lu, MD\textsuperscript{d}, Young Mi Kim, PhD\textsuperscript{c}, Xianfu Wang, MD\textsuperscript{c}, Rebecca M. Crew, BS\textsuperscript{c}, Mohamad A. Cherry, MD\textsuperscript{d}, Shibo Li, MD\textsuperscript{c,}\textsuperscript{e}, Yuanyuan Liu, MD, PhD\textsuperscript{a,}\textsuperscript{e}

Abstract

Rationale: With combination of multiple techniques, we have successfully characterized unique, complex chromosomal changes in a patient with chronic lymphocytic leukemia (CLL), a lymphoproliferative disorder.

Diagnoses: The diagnosis was based on white blood cell, flow cytometry, and immunophenotypes and confirmed by karyotype, fluorescence in situ hybridization, and array comparative genomic hybridization from the patient’s blood culture.

Interventions: The patient was given fludarabine, cyclophosphamide and rituximab (FCR) for 6 cycles.

Outcomes: After completion of 6 cycles of FCR, the computed tomography scans of the neck/chest/abdomen/pelvic showed that the patient in CR. During the 10-month follow-up, the patient’s clinical course remained uneventful.

Lessons: The translocation t(14;19) identified in this patient is a recurrent translocation found in patients with chronic B-cell lymphoproliferative disorders and the 3-way translocation involving chromosomes 2, 14, and 11 may play a role as an enhancer.

Abbreviations: array CGH = array comparative genomic hybridization, CLL = Chronic lymphocytic leukemia, CR = complete remission, FISH = fluorescence in situ hybridization, IGH = immunoglobulin heavy chain, MLL = mixed lineage leukemia, MSX1 = muscle segment homeobox homolog 1.

Keywords: array comparative genomic hybridization, chronic lymphocytic leukemia, MSX1 duplication, t(14 ;19), t(2 ;14 ;11)

1. Introduction

B-cell chronic lymphocytic leukemia (CLL) is a genetically heterogeneous neoplasm characterized by the progressive accumulation of CD5+ mature B cells in bone marrow, lymph nodes, and blood. CLL is the most common leukemia in adults in Western countries and the clinical course of disease ranges from a few months of the diagnosis to ≥20 years.\textsuperscript{1,2} The frequently CLL-associated cytogenetic abnormalities include trisomy 12 (10%–20%), del11q22-q23 (5%–20%), and del13q14 (50%). In addition, del17p13 involving deletion of the tumor suppressor gene TP53 occurs in <10% of CLL at diagnosis but up to 30% in refractory cases. Patients with del17p demonstrate aggressive diseases and have very poor prognoses.\textsuperscript{3–7}

It has been reported that chromosomal translocations involving immunoglobulin heavy chain locus (IGH) rearrangement on 14q32 are relatively infrequent in CLL with a frequency of 4%.\textsuperscript{8} However, recent studies have revealed that translocations involving IGH rearrangements occur at a very high incidence rate and significantly affects survival of CLL patients.\textsuperscript{8–10}

The most frequent translocation in CLL is t(14;19) (q32;q13), which juxtaposes IGH and BCL3 resulting in overexpression of BCL3\textsuperscript{11} and is usually associated with unfavorable clinical outcome and trisomy 12.\textsuperscript{12} It was proposed that these 2 changes might cooperate for malignant transformation.\textsuperscript{13} In contrast, patients with del(13q) have a better survival than the patients with other cytogenetic abnormalities.\textsuperscript{4} Furthermore, t(2;14)(p13;q32) is also a recurrent chromosomal change in CLL.\textsuperscript{13}

Chromosomal translocations are regarded as an important prognostic indicator and are always associated with shorter survival in B-CLL patients.\textsuperscript{8} Recently, several alternate translocations, such as t(4;14)(p16q32) to generate FGFR3/IGH, t(11;14)(q13;q32) to form CCND1/IGH, t(14;18)(q32q21) to produce IGH/BCL2 fusion, and t(18;22)(q21;q11) have been identified and provided further insights into the pathogenesis of
CLL. However, complex variant translocations may occur in CLL but have been rarely reported.

In the present study, as confirmed by cytogenetic analysis, we report a patient carrying the classical t(14;19)(q32;q13.3) as well as a novel 3-way translocation t(2;14;11)(p16.1;q32;q23), trisomy 12, and del(13q14). Importantly, we revealed a cryptic gain of chromosome 4p16.2 besides trisomy 12 and del (13q14.11-q21) in this patient. This study was approved by the institutional review board (IRB) at the University of Oklahoma Health Sciences Center (IRB number: 6299; Oklahoma City, OK).

2. Case report

2.1. Clinical characteristics

A 43-year-old female was admitted to the University of Oklahoma Health Sciences Center where she was diagnosed with CLL owing to weight loss and lymphadenopathy. Her hemoglobin and platelet counts were 12.8 g/dL (normal range, 12–16 g/dL) and 158 $\times 10^3$ cells/μL (140–440 $\times 10^3$ cells/μL), respectively. Her white blood cell count was 21.81 $\times 10^3$ cells/μL (4–11 $\times 10^3$ cells/μL) with relative and absolute lymphocytosis of 67% (15%–46%) and 15.7 $\times 10^3$ cells/μL (0.6–5.1 $\times 10^3$ cells/μL), respectively. Flow cytometric analysis found that her 75% monoclonal B-cells showed lambda light chain restriction of moderate intensity; her immunophenotype were as follows: CD5+, CD10-, CD19+, CD20+, CD22(dim), CD23+/-(dim), FMC-7+/-(dim), CD38+.

2.2. Cytogenetics, fluorescence in situ hybridization, and array comparative genomic hybridization analyses

At diagnosis, the patient’s B-cells were subjected to karyotype analyses. The results revealed that 25% (5/20) of the metaphase chromosome displayed a variant translocation among chromosomes 2, 11, and 14, and a translocation between 2 chromosomes 14 and 19 as well as +12 and del(13)(q14.11-q21). The karyotype was designated as 47, XX, t(2;14;11)(p16.1;q32;q23), +12, del (13)(q14.11-q21), t(14;19)(q32;q13.3) (Fig. 1).

Fluorescence in situ hybridization (FISH) analyses were performed in the uncultured and cultured cells using the LSI IGH and LSI MLL dual color break-apart rearrangement probes (Abbott Molecular, Inc., Des Plaines, IL) and LSI BCL11A and LSI BCL3 dual color break-apart rearrangement probes (Empire Genomics, Inc., Buffalo, NY). The uncultured cells were also tested using CLL panel (Abbott Molecular, Inc., Des Plaines, IL). All the experimental procedures followed the manufacturers’ instructions.

On uncultured interphase cells, FISH did not reveal t(11;14)(q13;q32), t(14;18)(q32;q21), del(6)(q23), del(11)(q22), del(17)(p13), but found trisomy 12 in 74% and monoallelic 13q14 deletion in 21% of tested cells. Moreover, variant MLL gene break-apart signals in 16% of 200 examined cells were observed as demonstrated by 1 tiny Spectrum Green signal and 1 normal Spectrum Red signal in Figure 2A, indicating the MLL gene break-apart. Furthermore, 104 of 200 cells (52%) exhibited a biallelic rearrangement of IGH (14q32) as showed by Spectrum Red and Spectrum Green signals when compared to Spectrum Orange signals in the normal IGH (Fig. 2B).
Analyses of karyotyping and FISH results from metaphase cells showed that 3’IGH signals were located on both der(14) and 5’IGH were translocated to der(11) and der(19), respectively (Fig. 3A); 5’BCL3 signal was located on der(19) and 3’BCL3 was translocated to der(14) (Fig. 3B); 5’BCL11A signal was located on der(2) and 3’BCL11A was translocated to der(14) (Fig. 3C); 5’MLL signal was located on der(11) and 3’MLL was translocated to der(2) (Fig. 3D). Taken together, these analyses in metaphase cells confirmed the complex translocations among chromosomes 2, 11, and 14 as well as chromosomes 14 and 19 (Fig. 3E).

Further array comparative genomic hybridization (CGH) analyses on the patient’s DNA sample revealed the presence of an extra chromosome 12 and deletion of 13q14.11-q21, which

Figure 3. Metaphase FISH analysis. (A) Metaphase FISH by DNA-probe LSI IGH (Abbott) using a Spectrum Green–labeled on 5’IGH and Spectrum Red–labeled on 3’IGH indicated biallelic IGH rearrangement. Small translocated segments of 5’IGH is on der 11 and der19. (B) Metaphase FISH by DNA-probe LSI BCL3 (Empire Genomics) using a Spectrum Green–labeled on 3’BCL3 and Spectrum Red–labeled on 5’BCL3 indicated BCL3 rearrangement. Small translocated segment of 3’BCL3 is on der 14. (C) Metaphase FISH by DNA-probe LSI BCL11A (Empire Genomics) using a Spectrum Green–labeled on 5’BCL11A and Spectrum Red–labeled on 3’BCL11A indicated BCL11A rearrangement. Small translocated segment of 3’BCL11A is on der 14. (D) Metaphase FISH by DNA-probe LSI MLL (Abbott) using a Spectrum Green–labeled on 5’MLL and Spectrum Orange–labeled on 3’MLL indicated variant MLL rearrangement. Small translocated segments of 3’MLL (including 1 tiny green signal and 1 normal red signal) is on der 2. (E) Summary of the metaphase by FISH analysis of bone marrow. FISH = fluorescence in situ hybridization.
was consistent with our karyotype analyses. Interestingly, we also found a gain of 4p16.2 (4,788,290–5,227,609 bp) and 13q deletions as a rare recurrent chromosomal changes associated with atypical rearrangement of 14q32 (MYC/IGH) in mantle cell lymphoma (MCL); and t(14;19) (q32;q21) (IGH;BCL2) in follicular lymphoma (FL). The cases with concomitant t(2;14) and t(14;19) translocations have previously been reported,[19] and they are always regarded as a rare recurrent chromosomal changes associated with atypical cytology, trisomy 12, and a progressive disease in CLL.[13,17,18]

The present study is the first report of a CLL case with a complex variant translocation involving 3 chromosomes 2, 11, 14 named t(2;11;14)(p16.1;q32;q23)/BCL11A;IGH;MLL, especially concurrent with t(14;19)(q32;q13)/IGH;BCL3. In contrast to MYC/IGH, CCND1/IGH and IGH/BCL2, the roles for IGH;BCL3 and BCL11A;IGH;MLL fusions in CLL remain poorly understood; however, it is possible the target genes that become overexpressed or gained new functions may be relevant to the poor prognosis of CLL.

The MLL gene rearrangement often occurs in acute myelocytic leukemia (AML), acute lymphoblastic leukemia, and myelodysplastic syndrome. In hematologic malignancies such as CLL, the most common abnormality is the deletion of the MLL(11q23),[15] whereas the MLL gene rearrangement has not been previously observed in CLL. In this study, we revealed this interesting 3-way translocation of the MLL gene rearrangement; whether it contributes to the leukemia progression or even an unfavorable prognosis in CLL warrants further investigation.

It is widely believed that presence of only the IGH rearrangement is not sufficient to induce tumorigenesis, and acquisition of additional genetic aberrations is necessary for malignant transformation.[19] Trisomy 12 observed in this case, is one such genetic anomaly. The cytogenetic abnormality of trisomy 12 associated with intermediate prognosis is observed in up to 50% of IGH/BCL3-positive B-CLLs and was considered to act cooperatively with t(14;19) in leukemogenesis.[20] Interestingly, however, it was reported that patients with 13q deletions as a sole abnormality had the longest estimated survival times compared with other cytogenetic abnormalities.[5,21] Moreover, miR-15a and miR-16–1 locate in this region, and negatively regulate BCL2 expression at a posttranscriptional level.[22]

MSX1 was found to be overexpressed in cell lines derived from MCL and leukemia AML as well as in 3% of patients with MCL and AML.[23] In the present study, array CGH revealed a cryptic gain of MSXI gene besides trisomy 12 and del(13q14.11-q21), which has not been reported previously in CLL. These data suggest an oncogenic role for MSXI in leukemogenesis.

In summary, we reported a rare case of an adult CLL patient with the coexistence of classical IGH/BCL3 translocation and a three-way variant translocation BCL11A/IGH/MLL, as well as trisomy 12 and del(13q). Furthermore, a cryptic genomic alteration involving leukemia-related MSXI gene was found in this case at the level of the array CGH. It is widely believed that presence of only the IGH rearrangement is not sufficient to induce tumorigenesis, and acquisition of additional genetic aberrations is necessary for malignant transformation.[19] Trisomy 12 observed in this case, is one such genetic anomaly. The cytogenetic abnormality of trisomy 12 associated with intermediate prognosis is observed in up to 50% of IGH/BCL3-positive B-CLLs and was considered to act cooperatively with t(14;19) in leukemogenesis.[20] Interestingly, however, it was reported that patients with 13q deletions as a sole abnormality had the longest estimated survival times compared with other cytogenetic abnormalities.[5,21] Moreover, miR-15a and miR-16–1 locate in this region, and negatively regulate BCL2 expression at a posttranscriptional level.[22]

MSXI was found to be overexpressed in cell lines derived from MCL and leukemia AML as well as in 3% of patients with MCL and AML.[23] In the present study, array CGH revealed a cryptic gain of MSXI gene besides trisomy 12 and del(13q14.11-q21), which has not been reported previously in CLL. These data suggest an oncogenic role for MSXI in leukemogenesis.

In summary, we reported a rare case of an adult CLL patient with the coexistence of classical IGH/BCL3 translocation and a three-way variant translocation BCL11A/IGH/MLL, as well as trisomy 12 and del(13q). Furthermore, a cryptic genomic alteration involving leukemia-related MSXI gene was found in this case at the level of the array CGH.

### References

1. Rozman C, Montserrat E. Chronic lymphocytic leukemia. N Engl J Med 1999;333:1052–9.
2. Schwartz G, Klug MG. Incidence rates of chronic lymphocytic leukemia in US states are associated with residential radon levels. Future Oncol 2016;12:163–74.
3. Scarfò L, Ferreri A, Ghia P. Chronic lymphocytic leukaemia. Crit Rev Oncol/Hematol 2016;104:169–82.
4. Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 2000;343:1910–6.
5. Stankovic T, Weber P, Stewart G, et al. Inactivation of ataxia telangiectasia mutated gene in B-cell chronic lymphocytic leukemia. Lancet 1999;353:26–9.
6. Rossi D, Fangazzo M, Rasi S, et al. Disruption of BIRC3 associates with fludarabine chemorefractoriness in TP53 wild-type chronic lymphocytic leukemia. Blood 2012;119:2854–62.
7. Gaidano G, Foà R, Dalla-Favera R. Molecular pathogenesis of chronic lymphocytic leukemia. J Clin Invest 2012;122:3432–8.
8. Martin-Sabero JJ, Ibbotson R, Klapper W, et al. A comprehensive genetic and histopathologic analysis identifies two subgroups of B-cell lymphomas.
malignancies carrying a t(14;19)(q32;q13) or variant BCL3-translocation. Leukemia 2007;21:1532–44.
[9] Mayr C, Speicher MR, Koller DM, et al. Chromosomal translocations are associated with poor prognosis in chronic lymphocytic leukemia. Blood 2006;107:742–51.
[10] Van Den Neste E, Robin V, Franchart J, et al. Chromosomal translocations independently predict treatment failure, treatment-free survival and overall survival in B-cell chronic lymphocytic leukemia patients treated with cladribine. Leukemia 2007;21:1715–22.
[11] Ohno H, Takimoto G, McKeithan TW. The candidate proto-oncogene bcl-3 is related to genes implicated in cell lineage determination and cell cycle control. Cell 1990;60:991–7.
[12] Alpatov R, Carstens B, Harding K, et al. Rare double-hit with two translocations involving IGH both, with BCL2 and BCL3, in a monoclonal B-cell lymphoma/leukemia. Mol Cytogenet 2015; 8:101.
[13] Podgornik H, Pretnar J, Skopec B, et al. Concurrent rearrangements of BCL2, BCL3, and BCL11A genes in atypical chronic lymphocytic leukemia. Hematology 2014;19:45–8.
[14] Geller MD, Pciy, Spurgeon SE, et al. Chronic lymphocytic leukemia with a FGFR3 translocation: case report and literature review of an uncommon cytogenetic event. Cancer Genet 2014;207:340–3.
[15] Nishida Y, Takeuchi K, Tsuda K, et al. Acquisition of t(11;14) in a patient with chronic lymphocytic leukemia carrying both t(14;19)(q32;q13.1) and +12. Eur J Haematol 2013;91:179–82.
[16] Janssens A, Van Roy N, Poppe B, et al. High-risk clonal evolution in chronic B-lymphocytic leukemia: single-center interphase fluorescence in situ hybridization study and review of the literature. Eur J Haematol 2012;89:72–80.
[17] Michaux L, Mecucci C, Stul M, et al. BCL3 rearrangement and t(14;19) (q32;q13) in lymphoproliferative disorders. Genes Chromosomes Cancer 1996;15:38–47.
[18] McKeithan TW, Takimoto GS, Ohno H, et al. BCL3 rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: a molecular and cytogenetic study. Genes Chromosomes Cancer 1997; 20:64–72.
[19] Lee AS, Rudduck-Sivaswaren C, Lie DK, et al. Overlapping deletion regions at 11q23 in myelodysplastic syndrome and chronic lymphocytic leukemia, characterized by a novel BAC probe set. Cancer Genet Cytogenet 2004;153:151–7.
[20] Chapiro E, Radford-Weiss I, Bastard G, et al. The most frequent t(14;19) (q32;q13)-positive B-cell malignancy corresponds to an aggressive subgroup of atypical chronic lymphocytic leukemia. Leukemia 2008; 22:2123–7.
[21] Herholz H, Kern W, Schnittger S, et al. Translocations as a mechanism for homozygous deletion of 13q14 and loss of the ATM gene in a patient with B-cell chronic lymphocytic leukemia. Cancer Genet Cytogenet 2007;174:57–60.
[22] Cimmino A, Calin GA, Fabbri M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005;102: 13944–9.
[23] Nagel S, Ehrentraut S, Meyer C, et al. Oncogenic deregulation of NKL homeobox gene MSX1 in mantle cell lymphoma. Leuk Lymphoma 2014;55:1893–903.