Coexistence of Tetrasomy 8 and Trisomy 8 in Acute Promyelocytic Leukemia (AML-M₃) with t(15;17) (q22;q12)

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Abstract This study was purposely characterized to recognize the first case of acute promyelocytic leukemia (AML-M₃) with t(15;17), trisomy 8 and tetrasomy 8, and explore its characteristics of morphology, cytogenetics, molecular biology, immunology and clinical features. Morphological changes of peripheral blood and bone marrow smears were observed under microscope. Chromosome specimen was prepared by 24h short-term culture of bone marrow cell, RHG banding technique was used for karyotypic analysis. PML-RARA fusion gene transcript was detected by nested reverse transcription-polymerase chain reaction (nested RT-PCR). Interphase fluorescence in situ hybridization (FISH) using chromosome 8 centromere specific probe were carried out to detect abnormal numbers of chromosome 8. Immunophenotypic analysis was performed by flow cytometry. The results showed that peripheral blood smear revealed 65% promyelocyte, and bone marrow aspirate was hypercellular with 72.4% promyelocyte, moderately basophilic cytoplasm with numerous azurophilic granules. Karyotype analysis demonstrated 48,XY, +8, +8, t(15;17) (q22;q12) 16/47,XY, +8,t (15;17) (q22;q12) 3/46,XY, t (15;17) (q22;q12) 1. RT-PCR assay revealed PML-RARA fusion gene transcript (+). FISH showed that the percentages of cells exhibiting 1,2,3,4,5,6 green fluorescence signals were 0.5,7,19,55,18 and 0.5, respectively. This confirmed the presence of tetrasomy 8 and trisomy 8 and also revealed a low percentage of a pentasomy 8 clone. Immunophenotypes of the blasts displayed that CD13 (96.2%), CD33 (55.9%), CYMPO (93.5%) were positive. All the lymphoid markers tested were negative. The patient survival time was just 10 days. It is concluded that tetrasomy 8 is secondary cytogenetic event after t(15;17) in this case. It may be a consequence of clonal evolution of trisomy 8. t(15;17) AML-M₃ with tetrasomy 8 heralds a poor prognosis.

Key words: leukemia, acute promyelocytic leukemia; karyotypic analysis; chromosome aberration; tetrasomy 8; trisomy 8

Trisomy 8 is a common numerical chromosomal abnormality in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). In contrast to trisomy 8, tetrasomy 8 is very rare and, to our knowledge, only 34 cases out of patients with hematological malignancies have been reported: 2 of acute lymphocytic leukemia (ALL), 26 of AML, 4 of MDS, 1 of polycythemia vera (PV) and 1 of myelofibrosis (MF). Among the 26 cases of AML, there were one M0, one M1, seven M2, four M4, twelve M5 and one M7, according to the FAB criteria. Until now, tetrasomy 8 has not been associated with subtype FAB M3 and has not been accompanied by characteristic chromosome translocation for subtype FAB. However, in this report, we describe a male patient of mid-age diagnosed as acute promyelocytic leukemia (AML-M3a) with tetrasomy 8 and t(15;17) (q22;q12).

Materials and Methods

Case description

In November 2001, a 45-year-old male with no particular past history was admitted to the Department of Hematology, The Second Hospital of Shanxi Medical University in China complaining toothache, fever and cough that had lasted for 9 days. Physical examination showed pale, some small petechiae on his skin, but no tenderness and organomegaly. Peripheral blood count was as follows: Hb 81 g/L, Plt 30×10^9/L, WBC 16.9×10^9/L with 65 % of promyelocytes. The bone marrow aspirate appeared hypercellular with 72.4 % of promyelocytes, moderately basophilic cytoplasm with numerous azurophilic granules, and lack of megakaryocytes and platelets. Karyotype analysis showed 48,XY,+8,+8,t(15;17)(q22;q12) 16/47,XY,+8,t(15;17)(q22;q12) 3/46,XY,t(15;17)(q22;q12) 1. RT-PCR assay revealed PML-RARA fusion gene transcript (+). Immunophenotypic analysis of the blasts showed that CD13 (96.2 %), CD33 (55.9 %), CYMPO (93.5 %) were positive, and all the lymphoid markers tested (CD5, CD7, CD10, CD19, CD34, HLA-DR, CYCD3, CYCD79a) were negative. DIC test showed: PTL 19.2×10^9/L, APTT 52.8 s, 3P (+), D-dimer (+). He was only treated with blood transfusion and antibiotics without chemotherapy and died of kidney, heart, and lung failure 10 days after diagnosis.

Cytogenetic analysis

Cytogenetic study was performed at diagnosis. Bone marrow cells were incubated at 37 °C for 24 hours in PRMI 1640 medium supplemented with 20 % fetal calf serum, 2 % penicillin/streptomycin and 5 % L-glutamine. Cell harvest and chromosome preparation were done according to standard procedures. Metaphases were R-banded and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN) 1995.

PML-RARA fusion gene transcript detection

PML-RARA fusion gene transcript was detected by nested reverse transcription-polymerase chain reaction (nested RT-PCR). Total cellular RNA was extracted by standard laboratory procedures from fresh bone marrow cells. Nested RT-PCR was performed in two systems: the first system for detection of L type products using outside primers (CTCACA-GCGCTGACCCCA7/GTCA-TAGAAATGAGGTT-CTT) for the first round of PCR and inside primers (AGCCTGAGGACTTTGTCCCTGA/AACAGCAACCACGTTGGCCA-G) for the second round of PCR, and the second system for detection of S type products using outside primers (CTCACA-GCGCTGACCCCA7/GTCA-TAGAAATGAGGTT-CTT) for the first round of PCR and inside primers (AGCCTGAGGACTTTGTCCCTGA/TCTAAAGTGCGCCTGCA-G) for the second round of PCR. The products of nested RT-PCR were separated by agarose gel electrophoresis.

Fluorescence in situ hybridization (FISH) studies

FISH was performed according to standard procedures with some modification by using chromo 7 some enumeration probes specific for chromosome 8 (CEP 8, Vysis, Inc). Briefly, chromosome DNA was immersed in 2×SSC at 37 °C for 30 minutes, then rinsed through ethanol series (70 %, 85 % and 100 %) for 2 minutes each at room temperature and air-dried, then denatured in 70 % formamide/2×SSC at 73 °C for 5 minutes, dehydrated sequentially in ice-cold ethanol series for 2 minutes each and air-dried. The probe mixture (10μl) was denatured in a water bath at 73 °C for 5 minutes. Then the probe mixture was added to the slide, and a 22 mm×22 mm coverslip was placed on the slide and sealed with rubber cement. Slide was hybridized for 12-16 hours at 42 °C in a humidified chamber. After the coverslip was removed, slide was washed for 2 minutes in 0.4×SSC/0.3 % Triton 100 at 73 °C and then in 2×SSC/0.1 % Triton 100 for 1 minute at ambient temperature. Air dry slide then apply 10 μl 4',6-
diamidino-2-phenylindole (DAPI) II counterstain to the slide and apply coverslip for 20 minutes at ambient temperature. The sample was examined with a fluorescence microscope (Nikon E600, EX450-490).

Results

Cytogenetic analysis
A total of 20 metaphases were analyzed for karyotypic examination. 16 metaphases presented with tetrasomy 8 (Figure 1), 3 metaphases with trisomy 8 and 1 metaphase with normal diploidy. All of 20 metaphases analyzed presented with t(15;17) (q22;q12). The karyotype was 48,XY,+8,+8,t(15;17)(q22;q12) 16/47,XY,+8,t(15;17)(q22;q12) 3/46,XY,t(15;17)(q22;q12) 1.

Figure 1. RHG banded karyotype of bone marrow cell showing 48,XY,+8,+8,t(15;17)(q22;q12)

PML-RARα fusion gene transcript detection
RT-PCR assay revealed that the samples from this patient and positive control had 163 bp band showing S type PML-RARα fusion gene transcript (+). Negative control showed PML-RARα fusion gene transcript (-) (Figure 2).

Fluorescence in situ hybridization (FISH)
A total of 200 interphase nuclei were evaluated for each sample. Normal control showed 98.5% of interphase nuclei with double spots, 1% with one spot, 0.5% with three spots, while this patient revealed 0.5%, 7%, 19%, 55%, 18% and 0.5% of nuclei with one, two, three, four, five and six spots, respectively, indicating the presence of three abnormal clones besides one minor normal clone with disomy 8: a major clone with tetrasomy 8, a minor clone with trisomy 8 and another minor clone with pentasomy 8. Whereas the disomy and trisomy cells had relatively small nuclei, most of the tetrasomy and pentasomy cells had large nuclei with diffuse chromatin (Figure 3).

Discussion
Tetrasomy 8 is a very rare but non-random chromo-
some numerical abnormality and mostly associated with myeloid malignancies. In the 34 reported cases of hematological disorders with tetrasomy 8, 32 cases belong to myeloid disorders, and most of them are AML (26/32) with different subtypes such as M0, M1, M2, M3, M5 and M7. Until now, tetrasomy 8 has not been associated with M3 reported. In our case, peripheral blood smear revealed 65% promyelocytes, and bone marrow aspirate was hypercellular with 72.4% promyelocytes, moderately basophilic cytoplasm with numerous azurophilic granules, POX was 100% positive. Karyotype analysis found t(15; 17) (q22;q12), specific translocation for M3. Nested RT-PCR assay revealed PML-RARA fusion gene transcript (+). Immunophenotypes of the blasts showed that CD13 (96.2%), CD33 (55.9%), CYMPO (93.5%) were positive. The patient can be diagnosed as AML-M3a. To the best of our knowledge, this is the first case of t(15; 17) acute promyelocytic leukemia (AML-M3a) with tetrasomy 8. This case and 1 case of M0, 1 case of M1, 7 cases of M2, 1 case of M7 in the literature suggesting that tetrasomy 8 is associated with a heterogeneous group of myeloid leukemia. It seems to have no real specificity for monoblastic leukemia as formerly claimed.

Tetrasomy of chromosome 8 can occur either by two consecutive events of single nondisjunction of chromosome 8, or by a single event of double nondisjunction of chromosome 8. Because tetrasomy 8 was almost always accompanied by trisomy 8 clone, and Kameoka et al. reported a case of clonal evolution from trisomy into tetrasomy of chromosome 8 associated with the development of AML from MDS, the first mechanism has been thought as many to be more likely. The cytogenetic analysis showed coexistence of disomy 8, trisomy 8 and tetrasomy 8 in our case. This supports the first mechanism proposing a stepwise clonal evolution from normal karyotype through the intermediate stage of trisomy 8 and eventually to tetrasomy 8. However, the relatively rarity of tetrasomy 8 in contrast to the high frequency of trisomy 8 suggests that tetrasomy 8 is not a mandatory consequence of trisomy 8 defect.

In this case, a minor pentasomy 8 clone was found in interphase by the FISH technique. Like tetrasomy 8, the pentasomy 8 might be preceded by tetrasomy 8 and trisomy 8. Because tetrasomy 8 and pentasomy 8 cells had higher proliferative activity with the evidence that these cells had much larger nuclei with more diffuse chromatin than did the trisomy 8 and disomy 8 cells, it is possible that the abnormal clone started with the somatic mutation of trisomy 8 by nondisjunction and gained the advantage of proliferation. During proliferation, one of the trisomy cells had another nondisjunction event involving chromosome 8, giving rise to the tetrasomy 8 and subsequently showed higher proliferative activity than that of trisomy cells and gradually replaced the trisomy 8. When the pentasomy 8 occurred by another single nondisjunction event of chromosome 8 in tetrasomy 8 cells, these cells had much higher proliferation and would gradually replaced the tetrasomy 8. Our patient was in phase of coexistence of disomy 8, trisomy 8, tetrasomy 8 and pentasomy 8 with advantage of proportion of tetrasomy 8.

T(15;17) is specific chromosomal translocation for AML-M3a. Zhang et al. reported a case of AML with tetrasomy 8 and t(15;17) (q22;q21), but it was diagnosed as M3a, not M3, and they thought that t(15;17) occurred after tetrasomy 8. The other chromosomal abnormalities associated with tetrasomy 8 include der(12)t(3;12)⁴, der(22)t(1;11)⁶ and 3, 6, 19, 20 etc. numerical chromosomal abnormality⁷,⁸, and those not belonging to characteristic chromosomal abnormalities are related to FAB subtype of AML. To the best of our knowledge, this is the first case of tetrasomy 8 accompanied by characteristic chromosomal abnormality in AML. Chromosome analysis of bone marrow revealed t(15;17) (q22;q12) in all 20 metaphases examined, and one of these showed disomy 8, 3 of trisomy 8, 16 of tetrasomy 8, suggested that numerical abnormality of chromosome 8 was the second cytogenetic event occurred after t(15;17).

Tetrasomy 8 is an indicator of poor prognosis with survival ranging from 6 days to 2 years (median 20 weeks)⁹. Our patient died 10 days after initial diagnosis without chemotherapy. This case provides further evidence to suggest that tetrasomy 8 is an adverse prognostic finding in AML.

The pathological significance of the acquisition of additional chromosome 8 in myelocytic malignancies remains unclear. Possible mechanisms involved in the transformation process include dosage effects of genes mapping to chromosome 8 and presence of specific mutations or cryptic fusion genes on the duplicated chromosome. The leukemia-associated genes located on chromosome 8 include c-mic, c-mos, MOZ, ETO, MYC FGFR1.
Cryptic changes were detected in association with AML, MDS and chronic myeloproliferative disorders with numerical abnormalities of chromosome 8\(^\text{10,11}\). Additional investigation is needed to determine the pathological significance of numerical chromosomal abnormalities, including trisomy 8 or tetrasomy 8, in the future.

**References**


