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BRIEF COMMUNICATION

Single nucleotide polymorphism array-based karyotyping shows sequential genomic changes from monosomy to copy-neutral loss of heterozygosity of chromosome 7 and 20q deletion within a balanced translocation t(14;20) in AML

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Single nucleotide polymorphism array (SNP-A)-based karyotyping can identify copy-neutral loss of heterozygosity (CN-LOH) as well as cryptic lesions not detected by metaphase cytogenetics. We report serial genetic studies on a patient diagnosed with chronic myelomonocytic leukemia who progressed to acute leukemia. Monosomy 7 was predominantly found at diagnosis, but clones changed to CN-LOH of chromosome 7 with disease progression. Furthermore, subclones with genomic aberrations of 3q gain, 1 p CN-LOH, and trisomy 12 newly appeared, suggesting that they were also involved in the transformation process. Additionally, by SNP-A, a presumably balanced translocation, t(14;20), identified by metaphase cytogenetics, was shown to result in an unbalanced 20q deletion at the breakpoint. The sequential changes identified by SNP-A may provide a better understanding of the mechanism of clonal evolution.

Keywords Chromosome 7, monosomy, copy-neutral LOH, clonal evolution

Chronic myelomonocytic leukemia (CMML) is a clonal stem cell disorder characterized by overlapping myelodysplastic and myeloproliferative features (1). Prognosis is variable and progression to acute leukemia occurs in 15–20% of cases (1). There are no specific cytogenetic abnormalities for CMML. The most frequent recurring abnormalities include +8, −7/del(7q), +21, del(20q), structural abnormalities of 12p, and complex karyotypes (1). However, little is known about the genetic events that determine the progression from CMML to acute leukemia.

Single nucleotide polymorphism array (SNP-A)-based karyotyping can identify copy-neutral loss of heterozygosity (CN-LOH) as well as cryptic lesions not detected by metaphase cytogenetics (MC) (2,3). Somatic CN-LOH results from mitotic homologous recombination events or it may represent an attempt to correct for the loss of chromosomal material, followed by reduplication of the remaining chromosome (4,5). Accordingly, CN-LOH occurs without concurrent copy number change (2–5).

Monosomy 7 is one of the most frequent abnormalities associated with poor prognosis in myeloid malignancies (6). On the other hand, CN-LOH of chromosome 7 identified by SNP-A has been associated with poor prognosis similar to monosomy 7 (7).

This is the first report demonstrating the serial genomic changes from monosomy 7 to CN-LOH during disease progression. In addition, the present study is novel in presenting a serial description of different stages of leukemia in association with cytogenetics and SNP-A, which clarify the mechanisms of clonal evolution.

Materials and methods

Cytogenetics, FISH, and SNP-A

For cytogenetic analysis, unstimulated short-term cultures were set up using bone marrow aspirates, and at least 20

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metaphase cells were analyzed. Interphase fluorescence in situ hybridization (FISH) examinations were done on bone marrow aspirates using commercially available probes according to the manufacturer's instructions: BCR-ABL, CEP7/D7S486(7q31), D20S108(20q12) (Abbott Molecular, Des Plaines, IL).

We applied a genome-wide SNP 6.0 array (Genome-Wide Human SNP array 6.0; Affymetrix, Santa Clara, CA) using genomic DNA according to the manufacturer's instructions. Data were analyzed using the Genotyping Console 3.1 software (Affymetrix). Genomic breakpoints were assigned using hg18/build 36. G-banded karyotype, FISH, and aberrations identified by SNP-A were described according to the ISCN 2009 (8).

Case history

A 58-year-old female presented with dizziness. The initial laboratory findings revealed a leukocyte count of 5.8×10^9/L, hemoglobin 7.6 g/dL, and a platelet count of 120×10^9/L. On a peripheral blood smear, the differential counts of leukocytes showed 35% monocytes, 31% segmented neutrophils, 28% lymphocytes, 3% band forms, 2% myelocytes, and 1% eosinophil. Bone marrow examination revealed a hypercellular marrow and myeloid maturation within the normal limits (myeloblasts 1.4%). Dysplasia was noted in the lineage of myeloid, erythroid, and megakaryocytic cells. Negative *BCR-ABL* rearrangement was confirmed by FISH and mRNA polymerase chain reaction (PCR). The patient was diagnosed as having chronic myelomonocytic leukemia-1 (September 2007) and received azacitidine treatment.

During follow-up, the patient received granulocyte colony-stimulating factor to treat neutropenia. Chromosomal abnormalities were persistently observed. In June 2008, the leukocyte count was 3.2×10^9/L, hemoglobin 10.4 g/dL, and platelet count 29×10^9/L. Differential counts revealed 42% lymphocytes, 39% monocytes, 10% segmented neutrophils, 4% eosinophils, and 5% immature cells. Another bone marrow examination revealed a hypercellular marrow (70%) and increased blasts (15%). Abnormal localization of immature cell clusters was noted on bone marrow biopsy. The patient received decitabine. However, 1 month later, the blast counts increased to 31%. Flow cytometry showed that the blasts were positive for CD13, CD33, CD34, and HLA-DR, and negative for CD14, CD3, CD5, CD19, CD20, CD10, and CD56. Finally, the patient was diagnosed as having acute leukemia transformed from CMML (June 2008). The patient was treated with decitabine until February 2009, but remission was not achieved. Fungal pneumonia developed and the patient died (August 2009).

Results

Sequential genomic changes identified by MC and SNP-A are presented in Table 1 and Figure 1. At diagnosis (September 2007), MC showed monosomy 7 in 100% of metaphase cells analyzed, and SNP-A provided confirmation of the MC findings. Until December 2008, monosomy 7 was persistently observed by MC. In April 2009, two clones were observed by MC. One major clone showed monosomy 7 (90% of metaphase cells), and the other minor clone had two abnormal chromosomes 7 showing addition of material of

Table 1 Follow-up dataset of cytogenetics and SNP-A-based karyotyping

			SNP-A		
Date	Diagnosis	Cytogenetics	Aberrations	Region	Size
Sep 2007	CMML	45,XX,-7, t(14;20)(q24;q13.2)[20]	Loss	7p22.3-q36.3	87 Mb
			Loss	20q13.13-13.13	0.6 Mb
Nov 2007	CMML	45,XX,-7, t(14;20)(q24;q13.2)[18]/46,XX[2]	Not done	Not done	
Jan 2008	CMML	45,XX,-7, t(14;20)(q24;q13.2)[20]	Not done	Not done	
Mar 2008	CMML	45,XX,-7, t(14;20)(q24;q13.2)[20]	Not done	Not done	
June 2008[a]	AML, transformed from CMML	45,XX,-7, t(14;20)(q24;q13.2)[19]/46,idem,+21[2]	Not done	Not done	
Aug 2008	AML, persistent	45,XX,-7, t(14;20)(q24;q13.2)[12]/46,idem,+21[9]/46,XX[1]	Not done	Not done	
Oct 2008	AML, persistent	45,XX,-7, t(14;20)(q24;q13.2)[8]	Not done	Not done	
Dec 2008	AML, persistent	45,XX,-7, t(14;20)(q24;q13.2)[14]/46,idem,+21[6]	Not done	Not done	
April 2009	AML, persistent	45,XX,-7, t(14;20)(q24;q13.2)[18]/46,XX,add(7)(q36)x2, t(14;20)(q24;q13.2)[2]	Loss	7p22.3-q36.3	87 Mb
			Loss	20q13.13-13.13	0.6 Mb
			Gain	3q25.2-q29	46 Mb
			CN-LOH	1p36.33-p34.1	46 Mb
July 2009[b]	AML, persistent	47,XX,add(7)(q36)x2,+12, t(14;20)(q24;q13.2)[20]	CN-LOH	7p22.3-q36.3	87 Mb
			Loss	20q13.13-13.13	0.6 Mb
			Gain	3q25.2-q29	46 Mb
			CN-LOH	1p36.33-p34.1	46 Mb
			Gain	12p13.33-12q24.33	132 Mb
Aug 2009	AML, persistent	47,XX,add(7)(q36)x2,+12, t(14;20)(q24;q13.2)[20]	Not done	Not done	

[a] FISH using D20S108 probe indicated a normal pattern (no 20q12 deletion).
[b] FISH using CEP7/D7S486 indicated a normal pattern (no monosomy 7 or 7q deletion).