Single nucleotide polymorphism microarray analysis of karyotypically normal acute myeloid leukemia reveals frequent copy number neutral loss of heterozygosity

Approximately 40-50% of acute myeloid leukemias (AMLs) show cytogenetically normal karyotypes. These karyotypically normal AMLs are prognostically heterogeneous and show various molecular alterations.[1] In order to explain leukemogenesis or clinical progression of the disease, the genomic characterization of these molecular alterations is needed to find new markers and target genes. Fluorescence *in situ* hybridization (FISH) and chromosomal comparative genomic hybridization (cCGH) studies have mostly been unsuccessful in finding very slight alterations in karyotypically normal AML.[2] However, array-CGH (aCGH), a high-resolution method for genome-wide DNA copy number change detection, has revealed cryptic alterations in 15-59% of karyotypically normal AML.[3,4] Loss of heterozygozity (LOH), identified as allelic imbalance in polymorphic loci, is frequently observed in cancers and is thought to result from genomic losses or gains leading to unequal ratios of parental alleles. However, copy number neutral alteration may result in apparent LOH, i.e., acquired uniparental disomy (UPD), where one of the parental alleles is lost while the other is duplicated. In cancer, UPD has been detected in whole chromosomes as well in telomeric and interstitial areas. The mechanisms resulting in UPD include mitotic recombination, gene conversion, and mitotic nondisjunction with subsequent duplication of the chromosome. Single nucleotide polymorphism (SNP) microarrays have been used to assess simultaneously genome-wide copy number changes and LOH. In karyotypically normal AML, SNP arrays have shown acquired UPD in 10-20% of cases.[5] We analyzed 19 cytogenetically normal AMLs (12 males, 7 females) using GeneChip Human Mapping 50K Array Xba 240 (Affymetrix, Inc., Santa Clara, CA, USA). Patients were diagnosed and treated in Helsinki University Central Hospital. The karyotypes were evaluated by G-banding. The study was approved by the local ethical review board and all participants gave their written consent. DNA was extracted from bone marrow samples using conventional methods. The SNP arrays were hybridized according to the manufacturer's protocols, the arrays were washed and stained using Fluidics station 450, and the images were scanned with GeneChip Scanner 3000 (Affymetrix) and analyzed with GeneChip operating software GCOS (Affymetrix). GTYPE 4.1 software (Affymetrix), using the dynamic model algorithm, was used for genotyping with 95.6% (91.2-98.7%) as the average call rate. DNA copy numbers and LOH were detected using the CNAT4 tool (Affymetrix) and CNAG2.0 software (Affymetrix).[6] The reference genotyping data [46 HapMap CEPH females (Utah residents with ancestry from northern and western Europe)] was downloaded from the international HapMap project's homepage (*http://www.hapmap.org/*). Subsequent LOH analysis was performed using CNAG2.0 software with ten best-fitting reference samples. Regions of LOH were identified as LOH of five continuous SNPs. The copy number ratios (log2 ratios) obtained from GTYPE 4.1 were imported to CGH Analytics software v3.4.27 (Agilent Technologies, Palo Alto, California) to obtain uniform data with previously reported 44K CGH oligonucleotide microarray (Agilent Technologies) results of the same sample set.[4] The SNP experiments were confirmed using the apparent LOH of the X chromosomes in male patients versus the female controls. The *FLT3* mutation analyses had been performed previously.[4] LOH was detected in 12/19 (63%) of the cases (*Online Supplementary Appendix, Table 1*). Recurrent LOH was detected in 7q (2 cases) and 13q (3 cases). One case (#2) showed LOH of whole 13q (Figure 1 A). This patient had an internal tandem duplication (ITD) of FLT3 (*Online Supplementary Appendix, Table 1*) at 13q12 and the relative dosages of the mutated and wild-type *FLT3* alleles were compared.

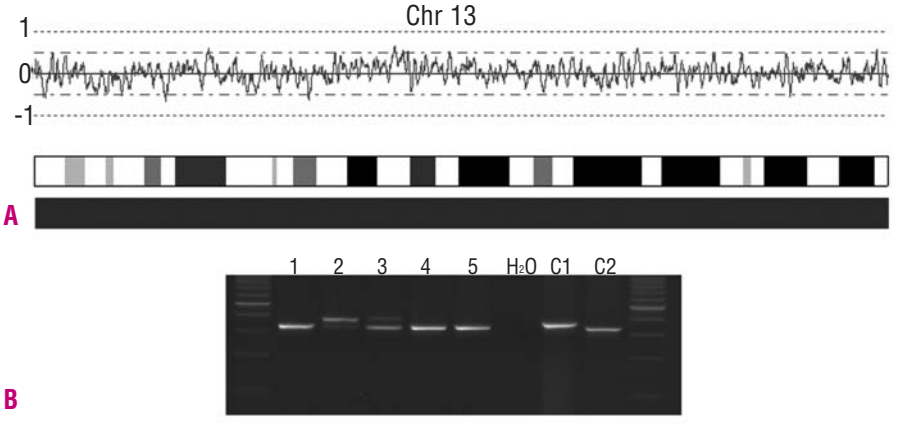

Figure 1. LOH of chromosome 13q and increased dosage of *FLT3*-ITD mutation allele of patient 2. (A) SNP array profile shows normal copy number of chromosome 13q and LOH (blue region at the bottom). (B) Agarose gel results of *FLT3*-ITD mutation analysis. Patient 2 showed more intense upper band, indicating increased dosage of mutant allele (number 2). Patients 1, 4, and 5 have negative *FLT3*-ITD mutation status, patient 3 has positive ITD mutation status with more intense wild type band, C1=clonal control *FLT3*-ITD positive control, C2=polyclonal control (normal control), (FLT3 mutation analysis was performed using an FLT3 Mutation Assay kit for gel detection InVivoScribe Technologies, San Diego, CA,USA).

The prevalence of the mutant allele to the wild-type allele (Figure 1B) confirmed the copy-number neutral LOH. UPD at 13q has often been detected in AML.[5,7] It has been suggested that the ITD mutation of *FLT3* indicates unfavorable prognosis in AML, and an even worse prognosis if the mutation is homozygous.[8] The LOH results obtained by SNP arrays (present study) and aCGH[4] from the same sample set are shown in *Online Supplementary Appendix, Table 1*. SNP arrays did not identify additional copy number alterations compared with aCGH. 4.1 Mb amplification at 8q24.13-q24.21 (#14) and loss at 12p12.3 (#5) previously found by aCGH[4] could be clearly identified by the SNP arrays (*data not shown*). Although the loss at 12p12.3 (#5) could be detected as copy number change in the SNP array, the CNAG2.0 analysis did not recognize it as LOH. SNP arrays failed to detect the smallest alterations (0.4-1.7 Mb) that were identified with aCGH. This discrepancy might result from the different spatial resolution of the SNP array and CGH array probes, the genome complexity reduction step in the SNP array protocol, or lower quality. However, the advantage of SNP arrays over aCGH is LOH detection. In the present study, the SNP arrays revealed LOH and copy number amplification at 8q24.13-q24.22 in one sample (#14), which previous FISH and aCGH studies had proven to harbor an extrachromosomal amplification of 4.1 Mb including *MYC*.[4] LOH of the region indicated that one allele was preferably amplified. The mechanisms of UPD in AML pathogenesis might be associated with genetic or epigenetic alterations of tumor suppressor genes that reside in the region of LOH. As a consequence of LOH, a mutated or epigenetically silenced tumor suppressor allele can be duplicated and the wild-type allele lost leading to tumor suppressor inactivation. Accordingly, homozygous mutations of known AML-associated genes have been detected in regions of acquired UPD.[9] In conclusion, we detected several regions of potential UPD in karyotypically normal AML by using SNP arrays. These regions might harbor tumor suppressors inactivated by mutations or epigenetic alterations. We found SNP arrays to be useful tools for simultaneous analysis of genome-wide LOH and copy number alterations in karyotypically normal AML. The original data will be available at *www.cangem.org*.

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This article has an Online Supplementary Appendix.

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