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Acquired genomic copy number changes in CML patients with the Philadelphia chromosome (Ph^+)

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Chronic myeloid leukemia (CML) is characterized by the *BCR-ABL1* fusion gene; this fusion gene is usually a consequence of the Philadelphia (Ph^+) chromosome, which results from the t(9;22)(q34;q11.2). Patients newly diagnosed with CML are routinely treated with tyrosine kinase inhibitors; however, the clinical course of the disease can vary, and this variance may be associated with genetic heterogeneity. Array comparative genomic hybridization (CGH) technology is a powerful tool for identifying subtle genomic segmental alterations, which can result from either losses or gains of chromosomal material. These changes may reveal the presence of genes that play important roles in disease initiation or progression or in treatment outcomes. To investigate whether subtle somatic copy number changes (CNCs) are commonly present in CML patients, a pilot study of 19 patients with the Ph^+ chromosome, but who were negative for common secondary chromosomal anomalies [+der(22), +8, i(17q), and +19], was conducted using a high-density whole genomic oligonucleotide array CGH analysis. Four of the 19 cases had somatic segmental CNCs, including the loss of 9q34, 15q25.3, and 15q13 and a gain of 7p21.1–p15.3. The findings demonstrate that subtle genomic changes are relatively common in CML patients with a Ph^+ chromosome and that the clinical significance of these findings, especially the newly discovered regions, must be determined in large patient population studies.

Keywords CML, array CGH, Philadelphia chromosome, *BCR*, *ABL1*

Chronic myeloid leukemia (CML) is characterized by the *BCR-ABL1* fusion gene. This fusion gene is usually a consequence of the Philadelphia (Ph^+) chromosome, which results from the t(9;22)(q34;q11.2). Additionally, the presence of secondary changes at the cytogenetic [+der(22), +8, i(17q), and +19] and molecular (mutations or deletions of *TP53*, *p16*, and *RB1* and amplification of *EVI-1* and *cMYC*) levels, along with a Ph^+ chromosome, contributes to poorer clinical outcomes of CML treatment (1–5). Furthermore, the cryptic deletion at the flanking region of the t(9;22) breakpoint on the derivative chromosome der(9) t(9;22), detected by fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) analyses, has an incidence varying from 15–28% of CML patients (6–10). This cryptic deletion, which accompanies the t(9;22) breakpoint, has been associated with poor prognosis in CML and general resistance to interferon therapy (6,11). A number of molecular mechanisms have been considered to account for this association (12).

In the present study, we investigated whether submicroscopic segmental imbalances of genetic material are involved in the 19 Ph^+ [t(9;22)] CML samples that lack common secondary chromosomal changes, such as +der(22), +8, i(17q), and +19 using high-density whole genomic array CGH.

Materials and methods

Patient samples

A protocol approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center was followed for this study. Retrospectively, a total of 49 Carnoy

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fixative fixed cytogenetic patient samples, which were isolated from the bone marrow or leukemic blood that were diagnosed as CML and were identified as positive for the t(9;22)(q34;q11) by G-banded chromosome or FISH analysis between 2000 and 2010, were collected. We were able to isolate enough DNA from 19 of the 49 samples to perform array CGH (Supplementary Table 1). Sixteen of these cases had only the t(9;22). One case had a variant translocation: t(9;12;22)(q34;p13;q11.2). Another case had loss of chromosomes 7 and 8. The remaining case had an additional balanced translocation: t(5;10)(q13;p15).

DNA isolation and array CGH

DNA was isolated from a Carnoy fixative fixed cytogenetic pellet using the protocol previously described by Grünewald et al. (13). Array CGH was performed on a 385 K oligonucleotide chip (Roche NimbleGen, Inc., Madison, WI) according to the manufacturer's protocol with minor modifications. As an internal hybridization control for each experiment, the patient samples were matched with a pooled normal control DNA sample of the opposite sex (Promega Corporation, Madison, WI). Both the patients' DNA and reference DNA were labeled with either Cyanine 3 (Cy-3) or Cyanine 5 (Cy-5) by random priming (Trilink Biotechnologies, San Diego, CA) and then hybridized to the chip via incubation in the MAUI hybridization system (Biomicro Systems, Inc., Salt Lake City, UT). After 18 hours of hybridization at 42°C, the slides were washed and scanned using an MS200 (Roche NimbleGen, Inc.). Roche NimbleScan version 2.4 and SignalMap version 1.9 were applied for data analysis (Roche NimbleGen, Inc.).

FISH confirmation studies

Subsequent FISH analyses were performed to confirm the copy number changes (CNCs) detected by array CGH. Commercially available FISH probes (Abbott Molecular, Des Plaines, IL) or FISH probes created in-house with BAC/PAC clones (Children's Hospital Oakland Research Institute, Oakland, CA) were used. All of the probes were tested on normal controls in addition to the patient samples.

Results

A total of 19 Ph^+ CML samples in different stages (chronic phase or blast phase) were analyzed for somatic CNCs by array CGH (Supplementary Table 1). The somatic genomic CNCs were detected in four cases (Figure 1), and these imbalances were confirmed by FISH (Supplementary Figure S1); in one case (case 13), the imbalances could not be confirmed due to the lack of material available for FISH confirmation. The sizes of these imbalances ranged from 0.6− 2.5 Mb, and imbalanced regions contained 5 to 47 genes (Table 1).

One case (case 19) had an imbalance of the chromosome 9q34 region, which was associated with the breakpoint of the t(9;22). Case 19 had a loss in the long arm of chromosome 9 at the band q34, which was proximal to the *ABL1* gene on the derivative chromosome 9 (der(9)). Subsequent FISH analysis using the probe LSI ASS-ABL confirmed that 95.5% of cells had this deletion on the der(9). Additionally, array CGH detected a loss of the entire chromosome 7 that was also identified by G-banding analysis. A loss of chromosome 8, observed in 3 of the 20 cells analyzed by G-banding, was not identified by array CGH due to the low percentage (∼15%) of cells that had a loss of chromosome 8.

Three cases had an imbalance in other genomic regions that was not associated with the breakpoints of the t(9;22) (cases 7, 11, and 13). Case 7 had a loss of the long arm of chromosome 15 at band q25.3, which was confirmed by FISH with BAC clone RP11-418F16 (89.5%). Case 11 had a loss in the long arm of chromosome 15 at band q13, which was confirmed by FISH with BAC clone RP11-16E12 (96.0%). Case 13 had a gain of the short arm of chromosome 7 at bands p21.2−p15.3, which could not be confirmed due to the lack of material available to perform an additional FISH analysis; however, the log2 ratio of 7p21.2−p15.3 was ∼0.23, indicating this gain likely resulted from somatic changes rather than constitutional changes, which otherwise have a log2 ratio of ∼0.4 when they are gained.

We excluded the probable constitutional CNCs (Supplementary Figure S2) that were found in our study and normal copy number variations (CNVs) that have been reported in the Database of Genomic Variation (DGV) (http://projects.tcag.ca/variation/) from our data interpretation. The precise pathogenic role of these genomic changes is currently unclear, although a potential association between some of these regions and cancer development has been suggested (14,15).

Discussion

This study represents a genome-wide investigation of genetic imbalances using whole genomic array CGH in a series of 19 patients with Ph^+ CML, who underwent previous cytogenetic studies showing a t(9;22) or a variant of the t(9;22) at initial diagnosis. Array CGH revealed somatic genomic CNCs in 21.1% (4 of 19) of the samples and determined the size of these CNCs. One case had a loss on der(9) adjacent to the breakpoint of the t(9;22). Other imbalance regions included a loss of 15q25.3 and 15q13 and a gain of 7p21.1−p15.3.

Copy number changes accompany the t(9;22) breakpoints

Cryptic deletions of variable sizes on der(9) accompany the t(9;22) breakpoint in approximately 15−28% of the CML patients, as detected by FISH or PCR. These deletions are frequently associated with poor outcomes of CML. Four molecular mechanisms that explain the deletion-associated poor prognosis have been considered: 1) loss of *ABL1-BCR* expression; 2) loss of *BCR-ABL1* at the transcript level; 3) the deletions may be a consequence of underlying genetic instability; and 4) loss of a tumor suppressor (12). Studies have reported various prognostic implications corresponding to the size of the deletion on der(9); patients with larger deletions have worse survival than those with small deletions (6,16), which supports the tumor suppressor gene