Brief report

SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations

*Daniel Nowak,1,2 *Seishi Ogawa,3,6 Markus Müschen,6 Motohiro Kato,3,6 Norihiko Kawamata,1 Antonie Meixel,1 Verena Nowak,1,2 Han S. Kim,1 Sharon Kang,1 Ronald Paquette,2 Mi-Sook Chang,6 Nils H. Thoennissen,1 Max Miossnner,2 Wolf-Karsten Hofmann,2 Alexander Kohlmann,8 Tamara Weiss,8 Torsten Haferlach,8 †Claudia Haferlach,8 and †H. Phillip Koeffler1,9

1Division of Hematology and Oncology, Cedars-Sinai Medical Center, University of California–Los Angeles School of Medicine; 2Department of Hematology and Oncology, University Hospital Mannheim, Mannheim, Germany; 3Department of Hematology and Oncology and 4Department of Cell Therapy and Transplantation Medicine and the 21st Century COE Program, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; 5Core Research for Evolutional Science and Technology, Japan Science and Technology Agency, Tokyo, Japan; 6Leukemia and Lymphoma Program, Norris Comprehensive Cancer Center, University of Southern California, Los Angeles; 7Ronald Reagan University of California–Los Angeles Medical Center; 8Munich Leukemia Laboratory, Munich, Germany; and 9National University of Singapore, Singapore

To elucidate whether tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia is associated with characteristic genomic alterations, we analyzed DNA samples from 45 TKI-resistant chronic myeloid leukemia patients with 250K single nucleotide polymorphism arrays. From 20 patients, matched serial samples of pretreatment and TKI resistance time points were available. Eleven of the 45 TKI-resistant patients had mutations of BCR-ABL1, including 2 T315I mutations. Besides known TKI resistance-associated genomic lesions, such as duplication of the BCR-ABL1 gene (n = 8) and trisomy 8 (n = 3), recurrent submicroscopic alterations, including acquired uniparental disomy, were detectable on chromosomes 1, 8, 9, 17, 19, and 22. On chromosome 22, newly acquired and recurrent deletions of the IGLC1 locus were detected in 3 patients, who had previously presented with lymphoid or myeloid blast crisis. This may support a hypothesis of TKI-induced selection of subclones differentiating into immature B-cell progenitors as a mechanism of disease progression and evasion of TKI sensitivity. (Blood. 2010;115:1049-1053)

Introduction

Chronic myeloid leukemia (CML) patients can develop secondary resistance in the course of treatment with tyrosine kinase inhibitors (TKIs), such as imatinib, nilotinib, or dasatinib.1,2 The main causes for resistance are mutations or overexpression of the BCR-ABL1 fusion protein, reduced bioavailability of the drugs, and activation of compensatory molecular pathways.3,4 CML cells exhibit increased genomic instability, which could lead to genomic lesions harboring additional mechanisms of resistance. Use of high-density single nucleotide polymorphism (SNP) arrays in combination with a new computational algorithm termed “molecular allelokaryotyping” allows robust and detailed detection of cryptic micro-deletions, micro-amplifications, and loss of heterozygosity (LOH), including acquired uniparental disomy.4,5 We performed a genomic DNA profiling of 45 TKI-resistant CML patients with 250K SNP arrays to elucidate genomic alterations, which could be associated with TKI resistance.

Methods

Patients and DNA samples

We studied 45 cases of TKI-resistant CML. From 25 patients, DNA was extracted upon development of clinical resistance to imatinib, dasatinib, or nilotinib. From 20 patients, sequential DNA samples were available for comparison between diagnosis and development of resistance against at least 1 of the aforementioned TKIs. All patients were confirmed positive for BCR-ABL1 by polymerase chain reaction (PCR) and fluorescence in situ hybridization. Eleven resistant patient samples had BCR-ABL1 mutations, including 2 T315I mutations. The anonymized DNA samples were obtained from patients referred to the Munich Leukemia Laboratory, Munich, Germany. Genomic DNA was isolated from mononuclear cells from bone marrow aspirates or leukemic peripheral blood. The acquisition and analysis of DNA samples from the patients were with approval of the ethical committee of all participating institutions. A detailed summary of patient information is given in supplemental Table 1 (available on the Blood website; see the Supplemental Materials link at the top of the online article).

High-density SNP array analysis

High-quality genomic DNA was processed according to the genomic mapping 250K NspI protocol and hybridized to 250K NspI SNP arrays according to the manufacturer’s instructions (Affymetrix). Data analysis of deletions, amplifications, and uniparental disomy was carried out using the CNAG software with nonmatched references as previously presented.
described.5,6 The SNP array data are publicly available at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/projects/geo/) under accession number GSE18964.

Mutation analysis and validation of SNP array results

Mutation analysis of candidate genes was carried out by standard genomic PCR of all exons and subsequent direct sequencing of the amplified and purified PCR products with the BigDye Terminator, Version 3.1 Cycle Sequencing Kit (Applied Biosystems). Validation of copy number results and uniparental disomy (UPD) were carried out as previously described,7 and results are shown in supplemental Figures 1 and 2.

Results and discussion

After exclusion of genomic copy number polymorphisms by comparison of the data with recorded copy number polymorphisms
Table 3. Copy number neutral LOH detected in resistant CML samples

Table 2. Duplications present in TKI-resistant CML samples

Table 3. Copy number neutral LOH detected in resistant CML samples
abnormality, we found no gene disrupting mutations in the remaining alleles of these genes.

Chromosome 17 was most heavily affected by secondary genomic alterations on development of TKI resistance. Four of the 20 serial samples showed newly acquired genomic alterations on chromosome 17. Changes composed either large deletions of chromosome 17p or large duplications or UPD of chromosome 17q (Tables 1-3; supplemental Figure 4). Genomic disruptions occurring on chromosome 17 are one of the most common known changes arising during disease progression.19 In some cases, deletions of chromosome 17p have been found to contain inactivating mutations of p53.10 Of note, in samples 42-R and 45-R, the breakpoints leading to duplication of chromosome 17q lie in close proximity to the STAT3 and STAT5A/B genes.

On chromosome 19q, 2 patients displayed a common region of acquired UPD (19q13.32-19q13.43; Table 3).

On chromosomes 9 and 22, deletions flanking the ABL1 and BCR genes were found in 5 patients. These are deletions of the reciprocal ABL1-BCR fusion product, which are known to occur in up to 10% to 17% of CML cases and have an effect on prognosis of patients treated with either hydroxyurea or interferon-α11 but not with imatinib.12 These deletions have been characterized with conventional cytogenetic methods13 showing that the size of the deletions affects prognosis. However, a tumor suppressor gene has not been identified. In 3 of our 5 patients, the deletions on chromosome 9 spanned a common 1.9-Mb region centromeric to ABL1. One of the genes in the vicinity to the common breakpoint in this deleted region was protein phosphatase 2A activator, regulatory subunit 4 (PPP2R4), an activator of protein phosphatase 2A (PP2A), which was recently shown to be suppressed in imatinib-resistant CML.14 PPP2R4 therefore appeared as a candidate tumor suppressor gene, and we sequenced all exons of this gene in the patients with 9q deletions. However, no alteration from the reference sequence was detected in the remaining allele. On chromosome 22q11, the boundaries of the reciprocal deletions were heterogeneous; and in 2 cases, they began clearly telomeric to the BCR gene (Table 1). These SNP array results can be explained by duplication and insertion of the BCR-ABL1 fusion gene in a situation of a deleted reciprocal ABL1-BCR fusion product as evidenced by cytogenetic analysis (supplemental Table 1). Because the deletions of the reciprocal ABL1-BCR fusions are already detectable in the diagnostic samples, they are probably not associated with secondary resistance against TKIs.

Another accumulation of common lesions detectable on chromosome 22q11 were heterozygous deletions in the immunoglobulin lambda constant 1 (IGLC1) locus. These deletions were detectable in 2 resistant samples without a paired diagnostic sample and in 1 serial resistant sample on development of TKI resistance (Table 1; supplemental Figure 5). Therefore, the acquisition of these deletions is probably associated with TKI resistance or disease progression. Deletions of the IGLC1 locus are occasionally observed in B-cell acute lymphoblastic leukemia,15 resulting from λ light chain rearrangements. Prior investigators have shown that lymphoid blast crisis of CML displays a similar genomic profile to de novo Philadelphia chromosome-positive acute lymphoblastic leukemia.16 Furthermore, recent data suggested that transition from chronic phase CML to lymphoid blast crisis and drug resistance involved activation of the lymphoid transcriptional programs, such as up-regulation of the lymphoid transcription factor PAX5 and the activation-induced cytidine deaminase (AID).17 This uncovered a causative role of AID in the acquisition of BCR-ABL1 mutations and increased genomic instability in the progression of CML. Most importantly, imatinib treatment of PAX5-transduced CML cells led to the selection and outgrowth of CD19+ CML subclones, which showed evidence of de novo immunoglobulin rearrangement indicating RAG1/RAG2 activity. Therefore, finding IGLC1 deletions on development of TKI resistance in our CML samples corroborates these findings and fits well with the clinical observation of lymphoid blast crisis, and interestingly, also myeloid blast crisis with concomitant TKI resistance in these patients.

In conclusion, our high-density SNP array analysis identified new submicroscopic genomic lesions in 26 of 45 TKI-resistant CML patients. The resulting mean of 1.68 copy number alterations per TKI-resistant patient is slightly higher than in SNP array data from chronic phase CML samples demonstrated by Mullighan et al16,18 and less than the frequency of genomic lesions detected by higher-density SNP arrays carried out by Khorashad et al.19 We did not observe a new unequivocal recurrent genomic lesion associated with TKI resistance. Nevertheless, in individual cases, our data identified interesting candidate genes in the context of TKI resistance. Moreover, the observation of acquired IGLC1 deletions on TKI resistance corroborates recent findings37 of a causative role of B-lymphoid transcriptional programs in the disease progression and acquisition of resistance against TKI therapy.

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Authorship


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Correspondence: Seishi Ogawa, Department of Regeneration Medicine, National Institutes of Health grants (5R01CA026038-31). D.N. was supported by a research grant from the Deutsche Forschungsgemeinschaft (DFG, NO 817/1-1). H.P.K. holds the Mark Goodson Chair in Oncology Research at Cedars-Sinai Medical Center and is a member of the Jonsson Cancer Center and the Molecular Biology Institute of University of California–Los Angeles.

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