Characterization of 46 patient-specific BCR-ABL1 fusions and detection of SNPs upstream and downstream the breakpoints in chronic myeloid leukemia using next generation sequencing

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Abstract

In chronic myeloid leukemia, the identification of individual BCR-ABL1 fusions is required for the development of personalized medicine approach for minimal residual disease monitoring at the DNA level. Next generation sequencing (NGS) of amplicons larger than 1000 bp simplified and accelerated a process of characterization of patient-specific BCR-ABL1 genomic fusions. NGS of large regions upstream and downstream the individual breakpoints in BCR and ABL1 genes, respectively, also provided information about the sequence variants such are single nucleotide polymorphisms.

Keywords: BCR-ABL, Cancer, MRD, CML, SNP, NGS

Background

BCR-ABL1 transcript level monitoring is a crucial part of therapy response evaluation in patients with chronic myeloid leukemia (CML). Molecular response (MR) in CML is expressed in terms of log reduction in BCR-ABL1 transcript levels from a standardized baseline on an international scale [1]. An increasing number of chronic phase CML patients treated with tyrosine kinase inhibitors (TKIs) achieve sustained deep MR, which has led to the initialization of TKI discontinuation studies [2,3]. Although existing results showing that permanent TKI discontinuation is feasible in a proportion of patients, more than half experience molecular relapse. More sensitive MRD detection might allow to more accurately stratifying patients according to the depth of response, hence to the greater or smaller likelihood of relapse after discontinuation. It was recently shown that MRD could be detected at DNA level despite patients were in stable MR with undetectable BCR-ABL1 transcripts [4,5]. It is supposed that quantification of BCR-ABL1 gene would be useful for MRD monitoring in patients with therapy cessation.

At the genomic level the BCR-ABL1 fusion is unique to each CML patient, since breakpoints are scattered within intron 13 (718 bp) or 14 (2128 bp) of BCR and within intron 1 of ABL1 (140 Kbp). The exact genomic sequence of the BCR-ABL1 fusion must therefore be characterized to design a personalized real-time polymerase chain reaction (PCR) assay. The sensitivity of personalized real-time PCR assay quantifying clone-specific fusions was reported to be down to less than $10^{-5}$ (depending on the amount of DNA entering the reaction) when nested PCR was used [6,7]. As amplification of large regions (>1 Kbp) is needed, the characterization of BCR-ABL1 genomic fusions using conventional sequencing has been a challenging task [8-10]. With the aim to characterize patient-specific BCR-ABL1 fusions, we set up a strategy based on the
generation of large amplicons by multiplex Long-Range PCR (mLR-PCR) and on subsequent ultra-wide sequencing using NGS technology. NGS provided sequences of large regions upstream and downstream the BCR-ABL1 fusions, allowing us to assess the frequency of annotated single nucleotide polymorphisms (SNPs), which could be associated with the fusion gene formation.

Findings

Next generation sequencing of large amplicons carrying BCR-ABL1 fusions

The patient cohort consisted of 24 males and 22 females with CML. Two BCR-ABL1 positive cell lines K562 and JURL-MK were included as controls of BCR-ABL1 fusion identification. Twelve healthy individuals were used as controls for SNP detection in the BCR region from exon 13 to exon 15.

DNA was isolated from total leukocytes of peripheral blood at the time of diagnosis ensuring high amount of BCR-ABL1 DNA present in the sample. Two-round mLR-PCR was performed with HiFi Accuprime Taq DNA polymerase (Invitrogen) enabling amplification of large regions. The amplification strategy is presented in Figure 1. In the first round PCR with one primer in BCR exon 13 and 10 primers in ABL1 intron 1 [9], the long PCR products of 29 patients were obtained. In the second round PCR with one primer in BCR exon 13 and 20 primers in ABL1 intron 1 [10-12] products were obtained in the remaining cases. Median length of long PCR products was 5 Kbp (range 1-12 Kbp). Subsequently, rapid library was prepared for 454 NGS technology according to the manufacturer’s instructions and was run on a GS Junior instrument (Roche Diagnostics). NextGene software (Softgenetics) and reference sequences NG_009244.1 (BCR) and NG_012034.1 (ABL1) were used for sequence analysis.

Patient-specific BCR-ABL1 fusion characterization

Breakpoints in BCR were located in intron 13 in 8/46 patients, carrying the e13a2 mRNA fusion, and in intron 14 in 38/46 patients and in both cell lines, carrying the e14a2 mRNA fusion (Figure 2A). All ABL1 breakpoints were spread within intron 1 (Figure 2A), which is in accordance with a previous study [7]. Sequences of characterized breakpoints are provided in the NCBI Nucleotide database (Accession numbers – KR091980-KR092025).

SNPs detection upstream and downstream from the BCR-ABL1 fusions

Downstream the individual breakpoints in ABL1, 56 annotated SNPs were detected in 15/46 patients (median 3 SNPs/patient, range 1–11). Only 10 of them recurred in two patients, whereas the remaining 46 were detected only once. This heterogeneity in SNP detection was caused by the extreme length of intron 1 (Figure 1, Figure 2A), where the breakpoints were spread, so that the individual ABL1 regions sequenced could not overlap.

Upstream the individual BCR breakpoints, 17 annotated SNPs and 3 novel variants were found altogether in 27/48 cases (56%) (Figure 2B). The dbSNP assigned accessions of newly detected variants (putative SNPs) are as follows:

- NG_009244.1:g.114249A > G|rs527236141|ss1227131697;
- NG_009244.1:g.114278G > T|rs527236142|ss1227132188;
- NG_009244.1:g.115017 T > A|rs527236143|ss1227132282.

The 300 bp region downstream of primers hybridizing to BCR exon 13 was sequenced in all cases (Figure 2B).
Four SNPs were identified within this region altogether in 9% of patients with e13a2 mRNA fusion and in 12% of patients with e14a2 mRNA. Other 16 SNPs were detected almost exclusively in patients with e14a2 mRNA fusion, which is in accordance with the fact, that the BCR region sequenced in these patients was larger than in the patients with e13a2 mRNA. The minor allele frequency among the BCR-ABL1 alleles analyzed corresponds to that found in the 1000 Genome Phase Population and in the cohort, although small, of healthy donors (Figure 2B).

Figure 2 A. Breakpoint traces in BCR intron 13 and intron 14 and in ABL1 intron 1. B. SNPs detected in individual BCR-ABL1 alleles upstream the BCR breakpoints. Dots show detected SNPs; colors of dots correspond to each patient in whom SNPs were detected; colors of BCR breakpoints indicate each patient in whom SNPs were detected; BCR breakpoints shown in black represent patients in whom SNPs were not detected. Twelve healthy donor samples (controls) were used as controls for SNP detection in the BCR region from exon 13 to exon 15. **minor allele in a homozygous form;"
Conclusion

Although increasing number of studies has been published in last few years [6-16], analysis of BCR-ABL1 at the DNA level has been limited and difficult so far. Our mLr-PCR-NGS approach streamlined the laboratory workflow for patient-specific BCR-ABL1 fusion characterization allowing personalizing real-time PCR assays for patients with MRD. The large collection of 46 BCR-ABL1 fusion sequences was obtained in the short time and is at disposal in the NCBI Nucleotide database. Moreover, the NGS enabled to detect SNPs upstream and downstream the patient-specific breakpoints of the BCR-ABL1 gene. NGS of this region in large series of patients has the potential to provide insights on the association between particular SNPs or haplotypes and CML. Although we describe the method on BCR-ABL1 in patients with CML, the strategy can be used in the same manner as a base for DNA-based MRD monitoring in BCR-ABL1 positive acute lymphoblastic leukemia (ALL), where significant differences between MRD levels measured by BCR-ABL1 expression and Ig/TCR DNA-based approach were described [17]. Moreover identical approach can be used for breakpoints localization and sequence features calling in other large fusion genes associated with various cancers, e.g. TEL/ABL1 (atypical CML or ALL) [18], TEL/AML1 (acute lymphoid leukemia) [19], TMPRSS2/ERG (prostate cancer) [20] or EML4/ALK (non-small cell lung cancer) [21].

Competing interests

KMP – Novartis and Bristol Myers-Squibb - research grant and honoraria; SS - Novartis, Bristol Myers-Squibb and Ariad – consultancy; HK - Novartis and Bristol Myers-Squibb - consultancy and honoraria.

Authors’ contributions

JL- has made substantial contributions to analysis and drafting the manuscript; LH- have made substantial contributions to analysis; AB and MJ- have contributed to NGS analysis; SS, JZ and HK - have been involved in drafting the manuscript; KMP, LH- have made substantial contributions to analysis; AB and MJ- have contributed to interpretation of data, contributed to manuscript drafting, design and coordinate the work. All authors gave final approval for submission.

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