The clinical outcomes of chronic myeloid leukemia patients harboring alternatively spliced BCR-ABL variants

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ABSTRACT

Objectives and importance: Tyrosine kinase inhibitors (TKIs) are indispensable for the treatment of chronic myeloid leukemia (CML). However, alternative splicing variants have been recently proposed as mechanisms of TKI resistance, although the clinical significance of these mutations remains controversial. We here present the long-term clinical courses of three CML patients harboring such unique mutations and try to assess their clinical significances. Moreover, the exon 6 frameshift presented here has been rarely reported, which may provide important information on this rare mutation.

Clinical presentation: We report three cases of CML harboring an exon 7 deletion, insertion of 35 intronic nucleotides and an exon 6 frameshift, respectively. Remarkably, all patients obtained better than molecular response following administration of TKIs.

Discussion and conclusion: Three CML cases highlighted an association between such splicing variants and clinical outcomes. The premature termination in the kinase domain due to these mutations likely causes conformational changes and inhibits TKI binding, but it also results in exacerbating kinase activities of CML cells. Thus, the above-mentioned mutants might less affect outcomes of treatment. Noteworthy, clinically available International Scale RT-PCR system cannot distinguish kinase-active mutants from kinase-inactive mutants, which may possibly influence upon interpretation of the treatment efficacy. Clonal quantification on respective mutants could more precisely evaluate CML status in these patients. Therefore, one should realize these important splicing variants and accumulate further experiences.

Introduction

Tyrosine kinase inhibitors (TKIs) have dramatically improved the prognosis of patients with chronic myeloid leukemia (CML) and are currently recognized as essential drugs for the treatment of CML. However, 10–15% of patients show resistance to imatinib through various factors [1,2]. Single nucleotide substitutions in the Abelson murine leukemia viral oncogene homolog (ABL) may mediate drug resistance. Recently, alternative splicing variants such as an exon 7 deletion, insertion of 35 intronic nucleotides at the junction of exon 8/9, and an exon 6 frameshift; i.e. a CAGG transnucleotide insertion at the junction of exon 5/6, have been proposed as mechanisms of TKI resistance [3,4], although the clinical significance of these mutations in the effectiveness of TKIs remains controversial [2,3,5–9].

We report three cases of Japanese patients with CML harboring these mutations, all of whom exhibited favorable outcomes by TKIs (Table 1). These mutants commonly cause breakpoint cluster region (BCR)-ABL truncation by premature translation and conformational changes of the ABL kinase domain, which not only inhibit TKI binding but also impair ABL kinase activity. Therefore, these ‘kinase-inactive’ mutations may not exacerbate CML, but also may not be eradicated by TKI treatment [9]. Since these mutations are not generally scrutinized before administration of TKIs in practical settings, there have been discussions and debates regarding whether these kinase-inactive variants undermine various TKI regimens. We here present the long-term clinical courses of these unique CML patients. Quantification of the proportion of these mutants in residual CML cells has not been tested during their follow-up. However, assuming that these mutants are likely kinase-inactive and thus lose leukemic proliferation, at least our cases suggest that these mutations seemed to less affect outcomes of TKI treatment.

Furthermore, the exon 6 frameshift presented here has been rarely reported and our report provides important information on this rare mutation.

Case presentation

Case #1: A 58-year-old woman was diagnosed with CML and started taking dasatinib 100 mg daily. During the
course, she developed mild liver dysfunction and the dasatinib dose was reduced to 50 mg. Sequence analysis at diagnosis showed insertion of 35 intronic nucleotides at the junction of exon 8/9; she achieved molecular response (MR) 4.5 in three-and-a-quarter years and has maintained MR 5.0 for over one year.

Case #2: A 45-year-old woman was initially treated with dasatinib and achieved major molecular response (MMR) in 7 months. However, she was unable to continue dasatinib because of dasatinib-induced neuropathy [10] and was instead administered 600 mg of nilotinib. The effect of nilotinib was observed within 2 months, exhibiting MMR, although BCR-ABL transcripts kept remaining, ranging 10–40 copy/assay by transcription-mediated amplification (TMA) method. We did not find any mutational changes in ABL at the time. We replaced nilotinib with 800 mg of imatinib, which resulted in poor treatment response. BCR-ABL transcripts were 368 copy/assay measured by TMA method. We did not find any mutational changes in ABL at the time. We replaced imatinib with 800 mg of nilotinib, which resulted in poor treatment response. BCR-ABL transcripts were 368 copy/assay measured by TMA method. We did not find any mutational changes in ABL at the time. We replaced imatinib with 800 mg of nilotinib, which resulted in poor treatment response. BCR-ABL transcripts were yet 132 copy/assay by TMA method. A repeated sequence analysis revealed a frameshift mutation in exon 6, with a CAGG insertion. He was then switched from nilotinib to 100 mg of dasatinib, which elicited an MMR and eventually led to MR 4.0.

Discussion

Previous reports have suggested that deletions and insertions in ABL may confer TKI resistance as these mutations do not necessarily mean rigid TKI resistance. The possible mechanism underlying the favorable outcomes may partly be explained by the reduced kinase activity of the mutant ABL. The prematurity of the activation loop and conformational changes in the ABL kinase domain, which may result in reduced TKI binding, can be extrapolated.

Table 1. Patients' characteristics.

| Case | Age | Gender | AS-BCR/ABL | G-band at diagnosis | Treatments after diagnosis | Latest IS (%) |
|------|-----|--------|------------|--------------------|---------------------------|---------------|
| #1   | 58  | female | Insertion of 35 intronic nucleotides | 46,XX\(i(9;22)(q34;q11.2)\) [19/20] | (1) Das 100 mg/day for about 17 months. (2) Das 75 mg/day for about 4 months (reduced for liver dysfunction) (3) Das 50 mg/day for over 3 years | N.D. |
| #2   | 45  | female | Exon 7 deletion | 46,XX\(i(9;22;15)(q34;q11.2;q11.2)\) [20/20] | (1) Das 100 mg/day for about 7 months (discontinued for polyneuropathy) (2) Nilo 600 mg/day for over 5 years (3) Nilo 400 mg/day for about 22 months (discontinued for drug resistance) (4) Nilo 400 mg/day for about 13 months (discontinued for drug resistance) (5) Das 100 mg/day for over 6 years | 0.0077 |
| #3   | 36  | male   | Exon 6 frameshift | 46,XY\(i(9;22)(q34;q11.2)\) [20/20] | (1) Ima 400 mg/day for about 22 months (discontinued for drug resistance) (2) Ima 400 mg/day for about 13 months (discontinued for drug resistance) (3) Das 100 mg/day for over 3 years | 0.0077 |

Notes: Patients characteristics and treatment history were described, respectively. The international scale was used to monitor the response to treatment by measuring BCR-ABL messenger RNA levels. The latest result of IS (%) in each patient was shown (right column). Das: dasatinib, Nilo: nilotinib, Ima: imatinib, AS-BCR-ABL: alternatively spliced breakpoint cluster region-Abelson murine leukemia viral oncogene homolog, IS: international scale, N.D: not detected.
spanning the BCR-ABL fusion point. Therefore, the IS RT–PCR system cannot distinguish functional BCR-ABL from functionally impaired (kinase-inactive) BCR-ABL, which may not directly be reflective of the treatment efficacy in some cases [9]. Accordingly, in cases with such mutations, residual BCR-ABL detected by IS RT–PCR may represent functionally impaired mutant BCR-ABL and ‘IS-based MMR’ may occasionally be a sign of a resolution equivalent to deeper molecular response. This hypothesis may explain in part why it took 3.5 years for Case #1 to reach MR5.0 without deterioration of CML status. If consistent, Cases #2 and #3 may also achieve deeper molecular response with longer observation periods. However, the mutants observed in our report were not longitudinally tracked and quantified. And, therefore, a further follow-up quantification of the target BCR/ABL clones should be needed. The next generation sequence method is not routinely available outside clinical studies, but it must be useful for characterizing such mutants.

Of note, Case #3 exhibited an infrequent type of mutation and responded less well to nilotinib and better to dasatinib treatment. The respective resistance patterns could have resulted from individual patient factors such as medical history, therapy adherence, amino acid substitutions, or other unidentified mechanisms but it might also result from the structural affinity of each TKI to each mutation [5]. Because of the limited number of clinical reports, it remains uncertain whether the exon 6 frameshift acts differently against dasatinib and nilotinib.

The reason why alternatively spliced BCR-ABL variants are sometimes formed at the specific sites of ABL gene remains poorly understood. In most cases, these spliceosomal errors become evident during TKI treatment; however, a small number of patients exhibit these errors at the time of diagnosis [5,8], which suggests that not only the pharmacological effects of TKIs but also other factors are involved in the pathogenesis.

In summary, we have described three cases of CML with different splicing variants, all of which were considered to be nonfunctional TKI-resistant mutations. The clinical effectiveness of TKIs in patients with such mutations remains unclear, but at least our cases appear to have been well treated by TKIs, although additional discussion is required to determine whether the prognosis of CML could be irrelevant to these mutations. We should be aware of these rare but important splicing variants and extend our knowledge by accumulating basic and clinical data on these mutations.

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