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Case Report

A Novel In-Frame 231bp Deletion Mutation in *ABL1* Kinase Activation Loop

Abstract

Tyrosine kinase domain (TKD) mutation is one of the most common causes for tyrosine kinase inhibitors' resistance in patients with chronic myeloid leukemia (CML). Mutations in the exon 7 of *ABL1* gene are one of the most common TKD mutations, especially in the Indian population, but they are frequently underreported, and their clinical significance is not clear. We are reporting a novel *ABL1* exon 7 mutation in a previously diagnosed and treated patient CML who presented at the blast crisis stage. Cytogenetic studies showed multiple copies of Philadelphia (Ph) chromosome along with isochromosome 17. Kinase domain mutation studies showed a novel 231bp in-frame deletion mutation (p. 372_448del) in the activation loop of *BCR-ABL1* chimeric protein. The given mutation would result in a complete loss of activation loop, including DFG domain-regulating activation status of the catalytic domain. This mutation, along with cytogenetic abnormalities, could have contributed to progression to blast crisis.

Keywords: *ABL1 exon 7 mutation, chronic myeloid leukemia, novel mutation, tyrosine kinase inhibitor resistance*

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Introduction

Tyrosine kinase inhibitors have become an integral part of the standard treatment protocol for chronic myeloid leukemia (CML) and are usually associated with excellent outcomes.[1] However, a subset of patients exhibit imatinib resistance, and *ABL1* kinase domain mutation is one of the most common causes for the same.[2-4] Although point mutations involving different domains of *ABL1* kinase are reported frequently, large deletion mutations involving *ABL1* exon 7 are also common, especially 185bp deletion involving exon 7 (c. 1086_1270del, p.R362fs*21) which has been reported in up to 25% of CML, at different time points.[5,6] Pathogenic potential of these mutations remains unexplained; however, affected domains are critical for *ABL1* kinase activity, and loss of these domains may potentially affect the activity of enzymes and/or interfere with imatinib binding.[6-8] We are reporting a novel 231bp deletion mutation involving exon 7–8 resulting in the loss of the regulatory activation domain.

Case Report

A 38-year-old male patient, previously diagnosed with CML, presented with cough, fever, altered sensorium, and other features suggestive of sepsis. He was diagnosed with CML in 2008 and was treated with imatinib (400 mg BD); however, no records containing documentation of treatment responses were available. Since the last 6 months, imatinib was stopped, and the patient was treated with hydroxyurea. Physical examination revealed pallor and massive splenomegaly. Peripheral blood smear examination revealed leukocytosis (white blood cell count: 242.6 × 10^9/L) with 30% blasts. Chromosomal analysis revealed two clones: smaller clone (two metaphases) showed t(9;22) (Ph chromosome) as the sole anomaly, while larger clone (18 metaphases) showed the presence of additional chromosomal abnormalities: isochromosome 17q and three copies of Ph chromosome. Tyrosine kinase domain mutation analysis was performed using peripheral blood specimen.

RNA extraction was performed with Qiagen RNA blood mini kit (Qiagen, Germany), and cDNA was prepared using high-capacity cDNA reverse

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transcription kit (Applied Biosystems, CA). Complete *ABL1* kinase domain of *BCR-ABL1* fusion transcript was amplified by the nested polymerase chain reaction (PCR) technique as described by Alikian *et al*.[4] PCR products were sequenced using BigDye Terminator v3.1 cycle sequencing reaction, and products were analyzed on ABI3500 Genetic analyzer (Applied Biosystems, Foster City, USA). Sequences were compared with *ABL1* reference sequence (NM_005157).

Sequencing revealed the presence of a 231 bp deletion mutation (CDS: c.1113-1343del231, p. 372-448del), an in-frame mutation causing deletion of 77 amino acids [Figure 1], resulting in a complete loss of activation loop along with the adjoining regions of C-terminal lobe. No other pathogenic variants were identified. This mutation was not reported either in COSMIC database or as an alternative transcript in NCBI or Ensembl databases (as assessed in October 2018).

The deleted sequence was flanked on the either end by GU sequence, the most common splicing acceptor site. The proximal end of the deleted region showed AG sequence, a common splicing donor site (5'-G*GU*/*AG*GGG…. G*GU*/*GU*AU-3'). These findings raised a possibility of alternative splicing as a potential mechanism for this mutation. Common exon 7 mutation del185bp (c. 1086_1270 del185bp) also involves the same region, and it is postulated that the given mutation could be the result of alternative splicing.[5,6] Interestingly, del231bp starts 27bp downstream of del185bp mutation, at the next potential donor splice site (CCACAG/*AG*AUCUUGCUG-CCCGAAACUGCCUG*GU*/*AG*GGG). Hence, we hypothesize that del231bp mutation may have occurred during alternative splicing if the splice site of del185bp was missed and the next available splice site is used.

Discussion

Pathogenic potential of exon 7–8 deletion mutations is controversial, and its clinical significance is not completely understood. Initial studies indicated that the activation loop mutations may not result in imatinib resistance. However, activation loop, especially DFG motif (amino acids: 381–383) and Tyr393 (residue essential for regulation of kinase activity), plays a critical role in the activation of *ABL1* kinase and can influence substrate binding.[7,8] The common del185bp mutation causes frameshift resulting in the formation of a truncated protein with aberrant terminal portion of catalytic loop (amino acids: 350–363) and loss of the activation loop. The resultant truncated protein may have lost biological activity.[5,6,8] In contrast, del231bp mutation (del p. 372-448) is restricted to activation loop and a part of C-terminal lobe while preserving most of the catalytic domain (amino acids: 350–362)[8,9] and the remaining part of C-terminal lobe. Due to loss of the DGF domain and Tyr393, the resultant protein may not able to switch from active (open) and inactive (form) and can have abnormal imatinib-binding site. Furthermore, imatinib can bind only to *ABL1* protein which is in inactive state. del231bp mutation may lead to the loss of switching between active and inactive states and can potentially reduce imatinib binding, thus resulting in imatinib resistance. However, these hypotheses are based on the protein structure prediction models, and it is essential that these findings are confirmed with additional studies.

The presence of this mutation was associated with blastic transformation. However, alteration of treatment and clonal evolution in the form of acquisition of additional poor risk abnormalities (isochromosome 17q and additional copies of Ph chromosome) may have contributed to blastic transformation. TKD mutation analysis was not performed before blastic transformation and hence, it could not be ascertained whether the given mutation was present before or has developed during clonal evolution and was responsible for disease progression.

Although exon 7-8 mutations are common in CML, especially in the Indian population,[6] they are relatively underreported. This is probably due to the inability of the commonly used primer(s) to cover area during testing,[5,6] lack of the understanding regarding the pathogenicity of these mutations, their contribution to imatinib resistance, and prognostic-therapeutic implications of detecting these mutation(s).[9,10] Hence, a large study evaluating prognostic and therapeutic significance of these mutations is warranted.

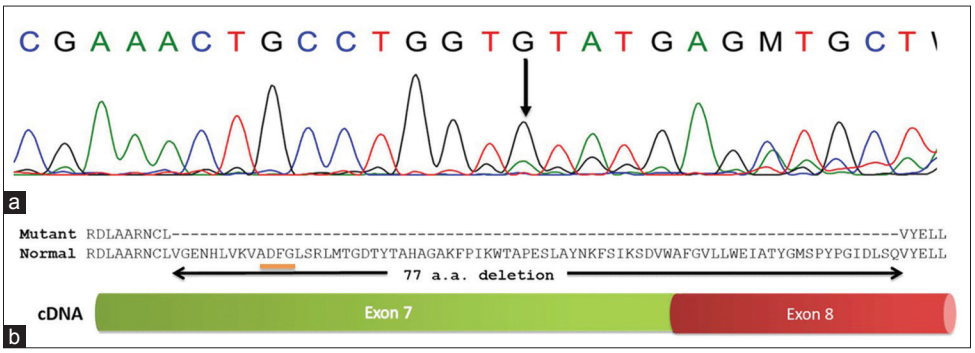

Figure 1: (a) Sanger sequencing showing the presence of deletion mutation. (b) Deletion mutation caused loss of 77 amino acids coded in exons 7 and 8

Conclusion

We are reporting a novel 231bp deletion mutation in the *ABL1* kinase activation loop domain (exons 7 and 8) of *BCR-ABL1* fusion transcript in a patient of CML-blast crisis. This mutation caused in-frame deletion of 77 amino acids, resulting in loss of the activation loop and a part of the C-terminal lobe. This mutation may have arisen due to the alternative splicing, a mechanism similar to a common del185bp mutation.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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