BCR-ABL Truncation due to Premature Translation Termination as a Mechanism of Resistance to Kinase Inhibitors

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The Philadelphia (Ph) chromosome, a shortened version of chromosome 22, results from a reciprocal translocation between chromosomes 9q34 and 22q11 [1–3]. The Ph translocation positions the *c-ABL* gene of chromosome 9 downstream from the breakpoint cluster region (*BCR*) on chromosome 22; the resulting fusion gene produces a 190- or 210-kDa hybrid protein with constitutive kinase activity associated with chronic myelogenous leukemia (CML). The impressive clinical efficacy of imatinib mesylate, a selective and effective ABL kinase inhibitor, has revolutionized the treatment of CML. However, the development of resistance to imatinib, which occurs over months to years, constitutes a major drawback in the treatment of advanced CML [4, 5]. Mechanisms leading to drug resistance include amplification of the *BCR-ABL* gene, acquired additional genomic alterations, and most importantly, specific mutations within the ABL kinase domain that impede drug binding [2, 3, 6–8].

The ATP-binding site is usually formed between the two lobes of the tyrosine kinase domain. Because the ATP-binding motif is highly conserved, most tyrosine kinase inhibitors generated have been ATP mimetics. Imatinib and other newer agents, such as nilotinib and dasatinib, bind to the ATP-binding cleft within the activation loop (A-loop) of the ABL kinase, establishing extensive contacts with residues lining the cleft and blocking access of ATP to the cleft. Thus, subsequent tyrosine phosphor-

ylation of the substrate is inhibited [5–9]. These inhibitors differ from one another in their molecular structure, how they bind to the BCR-ABL protein, and what other tyrosine kinases they target. These differences lead to different patterns of activity and resistance, resulting in distinct profiles of resistance mutations that are likely to evolve within the kinase domain.

Interrogation of the imatinib database indicates that 136 amino acid changes at 100 different ABL residues have been reported to date, and that the 16 most commonly mutated amino acids account for about 87% of all reported mutations; these include mutations at T315 (12.1%), E255 (10.7%), Y253 (9.3%), M351 (9.2%), and G250 (8.5%) [9]. Other types of mutations, such as deletions and insertions, have only recently been described. Reported in-frame deletions in exon 4 of the ABL kinase include $\Delta 184$ –274 and $\Delta 248$ –274, both of which display a phenotype of inactive kinase, lack of growth factor independence, and increased sensitivity to imatinib, nilotinib, and dasatinib [10–12]. An in-frame deletion skipping the first half of exon 8 has also been documented [13]. In addition, a 35-nt insertion derived from intron 8 was found positioned between the junction of ABL exons 8 and 9 in a patient with chronic CML resistant to imatinib [14]. The resulting truncated BCR-ABL protein lacks the C-terminal nuclear localization, DNA binding, and actin-binding domains.

Fig. 1. Schematic diagram and sequence alignment of wild-type and four mutant Bcr-Abl proteins resulting from premature termination mutations. **a** The organization of wild-type and four mutant protein domains is shown. **b** Amino acid sequences are aligned and labeled in black (wild type), red (exon 7 Del), pink (exon 7 2-nt Del), green (exon 6 997C→T) and blue (exon 6 4-nt Ins). Sequence differences caused by frameshift mutations are underlined. Premature stop codons are indicated by asterisks, and the position of T315 is marked in yellow.

The key structures of the ABL kinase, part of the BCR-ABL leukemogenic molecule, consist of SH3, SH2 and kinase domains, proline-rich regions (P), as well as a nuclear localization signal (NLS), and DNA- and actin-binding (DB and AB) sites (fig. 1a). The core kinase domain is organized into an N-lobe, which carries the highly conserved nucleotide-phosphate-binding site for ATP (the P-loop), and a large carboxyl-terminal C-lobe containing the flexible activation loop (the A-loop), a regulatory subunit for kinase activity [13, 15, 16]. Frequency studies of

BCR-ABL mutations detected in clinical CML samples revealed that mutations mainly cluster in four distinct regions of the kinase domain. Mutations in the P-loop (amino acids 244–255) are most common, followed by the T315I mutation, which causes global conformational changes. M351, the activation loop hinge, interacts with the SH2 domain and participates in autoregulation of kinase activity. The fourth cluster encompasses the A-loop from residues 381–402 [13]. Imatinib resistance is associated with at least 15 single amino acid mutations at 13

Exon 7 deletion

Exon 6, 4-nt frameshift insertion

Exon 6, 997C>T point mutation

Exon 7, 2-nt deletion

Fig. 2. Representative sequence traces of exon 6 and 7 frameshift or point mutations in the *abl* gene. The exon 7 deletion causes a frameshift and a premature termination codon in exon 8. The bases deleted are indicated with question marks. For exon 6 frameshift, a CAGG tetranucleotide insertion at the junction of exons 5 and 6 is indicated by a rectangular box, and a C→T silent mutation is also highlighted. A C→T mutation at nucleotide 997 in exon 6 also results in a termination codon (cag→tag). In exon 7, 2-nt deletion is marked by arrows. The sequences are compared to an Abl kinase domain reference sequence using sequencing analysis software, which aligns the sequences and highlights single or multiple mutations.

distinct positions within the ABL kinase domain; the most frequently involved positions are T315 and E255, known to be crucial for drug binding [2, 13, 17, 18]. P-loop as well as T315 mutations disrupt and shift the conformational equilibrium of the kinase to favor the active state and allosterically prevent imatinib and other kinase inhibitor binding. Accordingly, most of these point mutations confer resistance to imatinib, nilotinib, and dasatinib and are associated with a worse prognosis than are mutations elsewhere [16, 19].

Using a sensitive reverse transcription polymerase chain reaction and DNA sequencing approaches, four previously unrecognized ABL kinase mutations were found in CML and acute lymphoblastic leukemia (ALL) patients with resistance to imatinib, nilotinib, and/or dasatinib: an exon 7 deletion in 3 CML patients, a 4-nt insertion (908insCAGG) near the exon 5/6 junction in 1 CML case, a 2-nt deletion near the end of exon 7 (1088–1089GA) in 1 CML patient, and an exon 6 point mutation (997C→T) in 1 ALL patient. All of these mutations, al-

though through different mechanisms (e.g. deletion, insertion or point mutation), create premature stop codons and cause termination at residues 381, 315, 378, and 333, respectively, leading to two mutants lacking the A-loop (exon 7 Del and exon 7 2-nt Del), one missing T315, M351 and the A-loop (exon 6 4-nt Ins), and one terminated at codon 333, in addition to the lack of the C-terminal region downstream of the kinase domain (fig. 1). These premature termination mutations, along with the previously documented 35-nt insertion in exon 8 (35Ins), may constitute a new class of mutations that (1) cause truncation of the BCR-ABL kinase; (2) abolish the regulatory element in the ABL kinase domain and the downstream C-terminal region, and (3) confer significant drug resistance. Consistent with the 35Ins mutant (terminated at residue 484) which has conformational alterations similar to that in T315I and with comparable drug resistance [14], the four new ABL kinase mutations, with a truncation pattern analogous to that seen in 35Ins, may also display the T315I-like resistance profile.

Although deletions in both the SH3 domain and the C-terminal proline-rich regions have been shown to greatly impair BCR-ABL leukemogenesis in mice [20], deletion of the ABL actin-binding domain or the entire C-terminal region downstream of the kinase domain induces CML-like myeloproliferative disorders in vivo [21]. In addition, the N-terminal portion (encompassing amino acids 1–507) required for homomeric and heteromeric interactions among ABL kinase and its binding partners was found to play an important role in the modulation of kinase activity [22]. Therefore, the truncated proteins resulting from premature translational termination are expected to possess leukemogenic activity and to induce dramatic conformational changes to jeopardize drug binding and cause resistance. It is noteworthy that these newly identified ABL truncation mutations are structurally closely related to the SRC family kinases. The striking similarity between the catalytically active states of the ABL and SRC kinases suggests that these truncated, allosteric mutants could exert effects on drug binding resembling those of SRC kinase or be regulated in an SRC-like manner [23]. Indeed, SRC kinase inhibitors such as PP2, 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo(3,4-*d*)pyrimidine, and PD180970 have been found to effectively induce apoptosis in imatinib-resistant CML cells [24].

Alternative or aberrant splicing in *BCR-ABL* transcripts has been observed in a significant proportion of CML patients and is increasingly recognized as a mechanism for drug resistance [10, 13, 25, 26]. At least three

BCR-ABL splice variants are potentially associated with drug resistance [10, 13, 14]. The deletion spanning the entire exon 7 reported here adds a new member to the list. Given that both normal and aberrantly spliced forms may be present, and many deletion mutations are interpreted as sequencing trace overlays and thus escape diagnosis, extra care should be taken not to disregard these as poor-quality sequence traces (fig. 2). Another potential alternative splice variant frequently detected from imatinib-resistant CML samples in our laboratory is 35Ins, in which a 35-nt insertion derived from intron 8 was found positioned between the junction of *ABL* exons 8 and 9. This insertion also causes a frameshift leading to a premature stop codon following 10 intron-encoded amino acids.

In summary, we describe several novel mutations that result in BCR-ABL truncations of various lengths within the kinase domain, leading to mutants missing the ABL C-terminal NLS, DB, and AB domains. These kinase domain truncations, in addition to the previously reported 35Ins, may emerge as a novel mutation category and a previously undiscovered mechanism associated with drug resistance. The four premature translation termination mutations detected in our study represent the first example linking this class of mutation to multiple drug resistance in CML and ALL patients.

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