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Case report

Deletion and deletion/insertion mutations in the juxtamembrane domain of the *FLT3* gene in adult acute myeloid leukemia

Kristin K. Deeb [a], Matthew T. Smonskey [a], HanChun DeFedericis [a], George Deeb [c], Sheila N.J. Sait [d], Meir Wetzler [b], Eunice S. Wang [b], Petr Starostik [a,*]

[a] Molecular Diagnostics Laboratory, Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, United States
[b] Leukemia Service, Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263, United States
[c] Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, United States
[d] Cytogenetics Laboratory, Department of Pathology and Laboratory Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, United States

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ABSTRACT

In contrast to *FLT3* ITD mutations, in-frame deletions in the *FLT3* gene have rarely been described in adult acute leukemia. We report two cases of AML with uncommon in-frame mutations in the juxtamembrane domain of the *FLT3* gene: a 3-bp (c.1770_1774delCTACGinsGT; p.F590_V592delinsLF) deletion/insertion and a 12-bp (c.1780_1791delTTCAGAGAATAT; p.F594_Y597del) deletion. We verified by sequencing that the reading frame of the *FLT3* gene was preserved and by cDNA analysis that the mRNA of the mutant allele was expressed in both cases. Given the recent development of *FLT3* inhibitors, our findings may be of therapeutic value for AML patients harboring similar *FLT3* mutations.

1. Introduction

Mutations in the *FLT3* gene have been described in about 25% of acute myeloid leukemia (AML). They are somewhat more common in acute promyelocytic leukemia (APL), and have been associated with an increased risk of relapse, decreased disease-free survival, decreased event-free survival, and decreased overall survival [1]. These mutations result in constitutive activation of the FLT3 protein and are of two types: internal tandem duplication (ITD) mutations in exon 14 resulting from the duplication and tandem insertion of a portion of the juxtamembrane (JM) domain of the *FLT3* gene and missense mutations in exon 20 which alter the aspartic acid residue at position 835 (D835) within the kinase domain of the FLT3 protein. In the case of ITD mutations, the duplicated segment length ranges in size from 3 to several hundred base pairs and is always in-frame and therefore expected to produce a functional protein [2]. Rare deletion and deletion/insertion mutations affecting the *FLT3* juxtamembrane region have been described in childhood acute lymphoblastic leukemia [3,4]. Here, we report two cases of deletion and deletion/insertion mutations in the juxtamembrane domain of *FLT3* in adult AML. Proper identification of these mutations may have prognostic and therapeutic significance for AML patients.

2. Methods

2.1. Patients

2.1.1. Patient #1

A 47 year-old man presented with complaints of shortness of breath, fatigue, and weakness over several days. He had WBC of 42.3×10^9/L and hemoglobin of 4.8 g/dL. Bone marrow morphology showed 95% cellularity with 83% blasts and the case was classified as AML M0 with myelodysplasia-related changes based on the detection of del(5q) by FISH, as the minimal differentiation of the leukemic blasts made the assessment of multilineage dysplasia rather difficult. Molecular diagnostic studies detected wild-type *NPM1* gene and atypically mutated *FLT3* gene. The patient underwent induction with cytarabine and idarubicin-based chemotherapy, but had evidence of primary refractory *FLT3* mutation-positive AML on bone marrow biopsy performed 14 days after initiation of therapy. He then received high-dose cytarabine and mitoxantrone re-induction therapy. Repeat bone

* Corresponding author. Tel.: +1 716 845 8136; fax: +1 716 845 7638.
E-mail address: petr.starostik@roswellpark.org (P. Starostik).

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marrow evaluation upon count recovery revealed remission with 2% blasts and no evidence of *FLT3* mutation. He subsequently underwent allogeneic stem cell transplantation from his sister and remained in remission for five years, after which the AML relapsed with a *FLT3* D835 mutation and del(q5). He died shortly afterwards of infectious complications following re-induction chemotherapy.

2.1.2. Patient #2

A 54 year-old man with a prior medical history of coronary artery disease, diabetes, hypercholesterolemia, and hyperlipidemia presented with new onset of widespread bruising and blood in stool. Physical exam demonstrated scattered ecchymoses. Blood work revealed WBC of 8.6×10^9/L, hemoglobin of 9.8 g/L, and platelet count of 26×10^9/L. Prothrombin time was slightly elevated at 15.6 s (INR 1.25) with normal activated partial thromboplastin and a reduced fibrinogen level of 163 mg/dL. Hematopathologic evaluation of blood and bone marrow confirmed the diagnosis of acute promyelocytic leukemia (APL) with 91% marrow blasts/abnormal promyelocytes. Cytogenetics revealed a reciprocal translocation between the long arms of chromosomes 15 and 17 in 19/20 cells, t(15;17)(q24;q21). Molecular studies demonstrated a high level of the *PMLRARalpha* t(15;17) fusion transcript (164% of control) by quantitative RT-PCR. An atypical *FLT3* mutation was also identified. The patient was initiated on differentiation therapy with oral retinoic acid (ATRA) 45 mg/m^2 and arsenic trioxide 0.15 mg/kg intravenously daily as previously described [5]. Pseudotumor cerebri, scrotal ulcerations, and persistent headaches necessitated ATRA dose reduction. The patient was subsequently found to have CNS involvement by APL and received multiple intrathecal methotrexate injections. He was discharged home with count recovery two months after diagnosis and in complete remission.

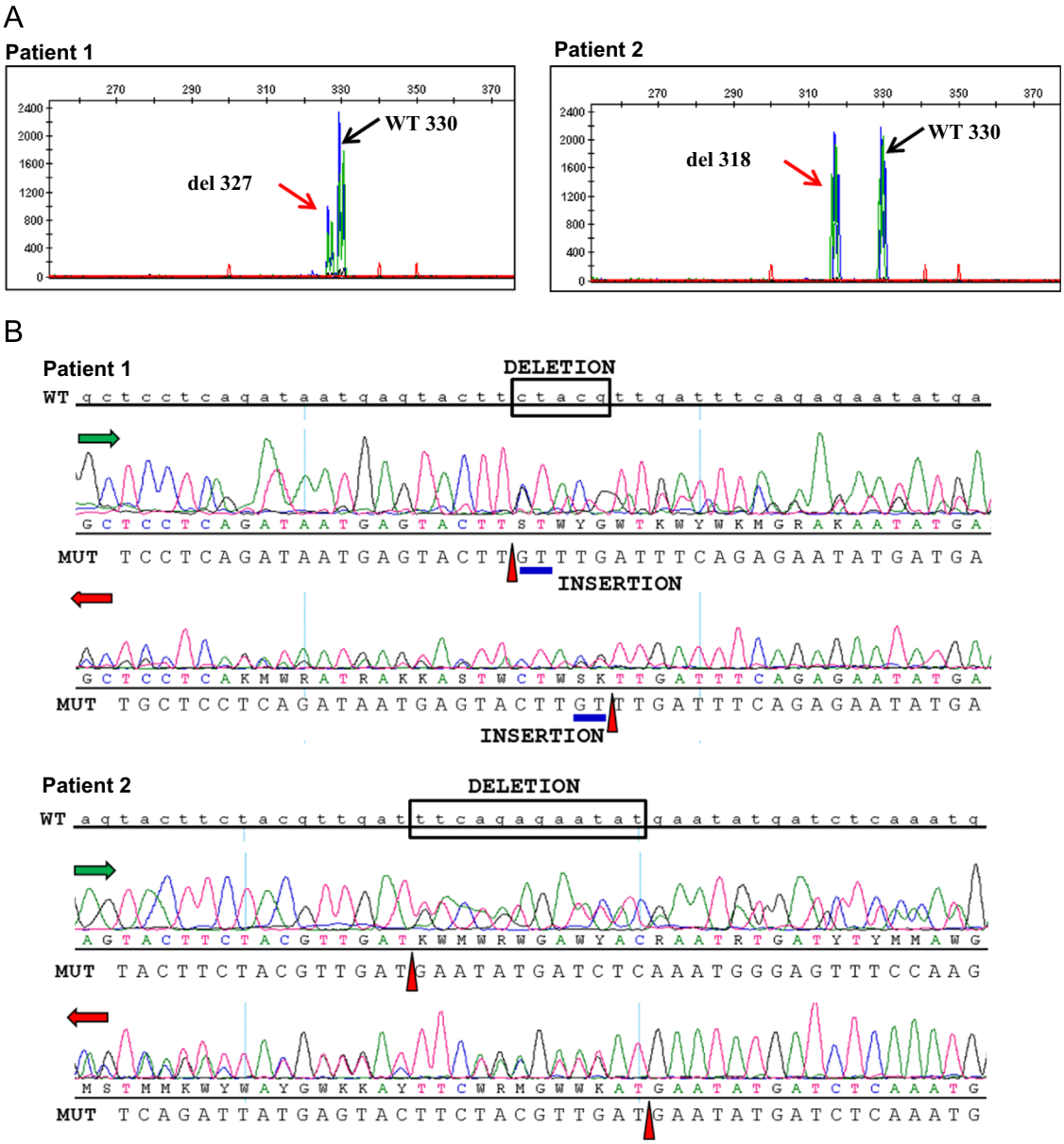

Fig. 1. Deletions in the juxtamembrane domain of the *FLT3* gene. (A) *FLT3* ITD fragment analysis showing wild-type peak (330 bp), and smaller PCR amplification products (327 bp and 318 bp) which correspond to a 3-bp deletion in patient #1 and a 12-bp deletion in patient #2, respectively. (B) Results of Sanger sequencing confirming the deletions. The wild-type (WT) *FLT3* sequence is in lowercase. The sequence of the deletion is boxed. The deletion mutation starting point is depicted by ▲(red) in the respective read directions, and the insertion is indicated by a blue underline. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. *FLT3* ITD and D835 mutation fragment analysis

DNA was extracted from blood or bone marrow samples using the EZ1 DNA Blood Kit (Qiagen, Germantown, MD) on the BioRobot EZ1 system (Qiagen). The *FLT3* PCR-based fragment analysis assay was performed as previously described [6].

2.3. *FLT3* juxtamembrane domain Sanger sequencing

Mutations detected in the juxtamembrane domain of the *FLT3* gene underwent Sanger sequencing in both forward and reverse directions with the Big Dye Terminator v 3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, CA). Results were analyzed in Sequencing Analysis v5.2 software (Life Technologies) and Lasergene SeqMan Pro v10.0 (DNAStar, Madison, WI), and aligned to the *FLT3* reference gene (NCBI RefSeq NM_004119.2).

2.4. *FLT3* mRNA analysis

RNA was extracted from patient samples using the miRNeasy Mini Kit (Qiagen) and converted to cDNA with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Pittsburgh, PA), which was subsequently amplified with primers FLT3F: 5′-6-FAM-GCCAGCTACAGATGGTACAGG-3′ and FLT3R: 5′-TTGCGTTCATCACTTTTCCA-3′. PCR products were analyzed on the ABI 3130*xl* Genetic Analyzer instrument (Life Technologies).

3. Results and discussion

Upon *FLT3* ITD fragment analysis during routine molecular diagnostics work-up at presentation, both patient samples showed an unusual peak in the electropherogram (Fig. 1A). Besides the wild-type allele of 330 bp, a shorter PCR product in the same reaction pointed to the presence of a mutated allele showing a deletion in the PCR-amplified juxtamembrane domain region. Fragments shorter by 3-bp (327-bp) in patient #1 with a mutant allele/wild-type *FLT3* ratio of 0.29, and by 12-bp (318-bp) in patient #2 with a ratio of 0.49 were detected. These fragments were further analyzed by Sanger sequencing to elucidate the nature of the deletions. Compared to the wild-type *FLT3* sequence, patient #1 had a 5-bp deletion (CTACG) mutation combined with a 2-bp (GT) insertion: c.1770_1774delCTACGinsGT mutation (Fig. 1B), giving an overall 3 bp deletion as detected by *FLT3* fragment

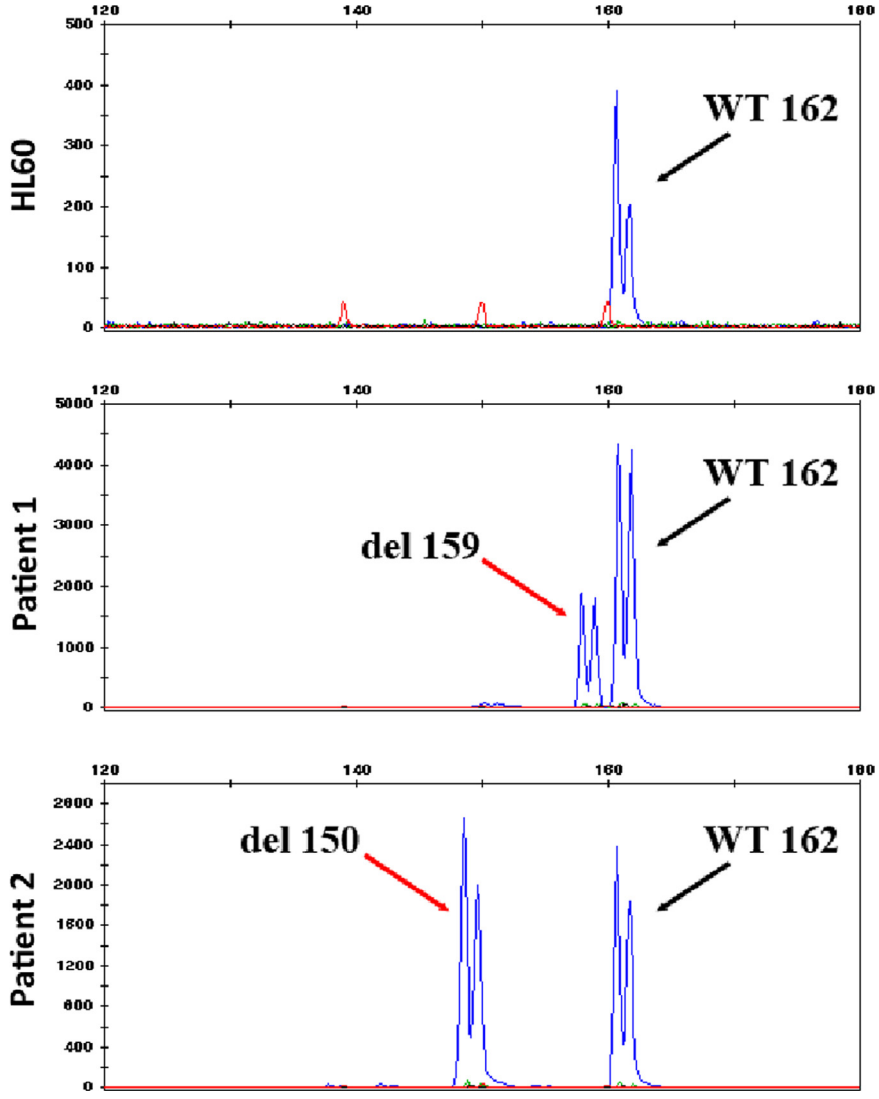

Fig. 2. *FLT3* mutant allele expression. RT-PCR across the *FLT3* juxtamembrane domain containing the deletions shows that all samples (HL60 serves as WT control) express the wild-type allele (162 bp). In addition to that, the patient samples express the mutant alleles (del159 and del150, respectively).

analysis. The deletion resulted in a p.F590_V592delinsLF amino acid change in the juxtamembrane domain. Patient #2 had a c.1780_1791delTTCAGAGAATAT (12-bp deletion) mutation (Fig. 1B) resulting in p.F594_Y597del amino acid deletion in the juxtamembrane domain. Notably, these deletion and deletion/insertion mutations were in-frame and the reading frame of the *FLT3* gene was preserved in both cases. Subsequently, the samples were tested for mutant versus wild type allele expression using cDNA fragment analysis. Both wild-type and mutant alleles were expressed at ratios comparable to the results of the *FLT3* ITD assay (Fig. 2).

FLT3 deletion and deletion/insertion mutations were previously reported in cases of pediatric acute lymphoblastic leukemia [3,4], but seldom described in adult acute leukemia. While the biological significance of this type of *FLT3* mutations is unknown in human disease, a small 10-amino acid (Tyr589 to Tyr599) deletion in the juxtamembrane domain of *FLT3* has been previously shown to lead to constitutive activation of the FLT3 protein in transformed murine IL3-dependent myeloid progenitor 32D cell line [7]. Similar deletion mutations are found in receptor tyrosine kinase *KIT* in gastrointestinal stromal tumors (GIST) [8,9]. An in-frame deletion of 7-amino acids in the juxtamembrane domain of the *KIT* gene resulted in receptor autophosphorylation and malignant transformation of mast cells [10]. These studies and our findings that both patients showed in-frame deletions with mRNA expressed (unfortunately, the samples did not yield enough material for a Western blot) suggest that deletion and deletion/insertion mutations in *FLT3* juxtamembrane domain may lead to receptor activation. Animal models would be a way to prove this hypothesis and show, if inhibition of *FLT3* can be therapeutically exploited in such cases. Whether the presence of these mutations in adult acute leukemia has prognostic significance warrants further investigation of a larger patient cohort.

Conflict of interest

The authors declare no conflicts of interest.

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