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Case report

Identification of a novel *SEPT9-ABL1* fusion gene in a patient with T-cell prolymphocytic leukemia

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ABSTRACT

T-cell prolymphocytic leukemia (T-PLL), a rare type of peripheral T-cell leukemia, is characterized by marked splenomegaly with rapidly progressive lymphocytosis and a poor prognosis. Nine kinds of *ABL1* chimeric genes have been identified in various kinds of hematological malignancies, such as chronic myeloid leukemia and B- or T-lymphoblastic leukemia. However, there have been no reports describing T-PLL cases with *ABL1* rearrangements. We herein report a case of T-PLL with a novel *SEPT9-ABL1* fusion gene which induced strong resistance to tyrosine kinase inhibitors such as imatinib and dasatinib.

1. Introduction

T-cell prolymphocytic leukemia (T-PLL) is a rare type of peripheral T-cell leukemia with a naive T-cell phenotype [1]. The clinical features of T-PLL include marked hepatosplenomegaly and generalized lymphadenopathy with rapidly progressive lymphocytosis. The prognosis is generally poor due to resistance to chemotherapy, with a median survival ranging from 7.5 to 50 months [1–3]. Chromosomal abnormalities such as t(14;14)(q11;q32), inv(14)(q11;q32) and t(X;14)(q28;q11) have been identified in patients with T-PLL, which result in the rearrangement of the *TCL1* or *MTCP1* genes with the T-cell receptor loci, which were thought to contribute to the pathogenesis of T-PLL [3].

We herein report a case of T-PLL with a novel *ABL1* fusion gene which was fused to *SEPT9*, *SEPT9-ABL1*. The case exhibited strong resistance to the tyrosine kinase inhibitors (TKI) used against *BCR-ABL1*. This is the first report of T-PLL with an *ABL1* fusion gene, and additionally, only the second report of a hematological malignancy with an *ABL1* fusion gene that exhibited a poor response to TKI.

2. Case report

A 70-year-old male was admitted to our hospital due to leukocytosis. On a physical examination, lymphadenopathy extending from the bilateral cervical to supraclavicular regions with moderate hepatomegaly was noted. The laboratory data on admission were as follows: white blood cells (WBC), 248×10^9/L with 0% neutrophils, 1% lymphocytes, 1% monocytes, 0% eosinophils, 0% basophils and 98% atypical lymphocytes, which were medium-sized with pale cytoplasm and prominent nucleoli (Fig. 1A); red blood cells (RBC), 4110×10^9/L; hemoglobin (Hb), 12.4 g/dl; and platelets (Plt), 171×10^9/L. Blood biochemistry was normal, except for elevated levels of lactate dehydrogenase and hepatobiliary enzymes. Bone marrow aspirate smears showed marked proliferation of atypical lymphocytes with a similar morphology to that of the peripheral blood cells. Using a cytogenetic analysis, six of six metaphases examined were 46, XY. A flow cytometric analysis showed that the atypical lymphocytes were positive for CD2, CD4, CD5 and CD7. A *BCR-ABL* FISH analysis showed no *BCR-ABL* signals, although 79 of 100 bone marrow cells exhibited atypical signals (*ABL1*: three copies and *BCR*: two copies in each cell) (Fig. 1B). The three *ABL1* signals indicated either simple amplification of the *ABL1* gene or the presence of *ABL1* rearrangement. In order to examine these two possibilities, the 5′-terminal sequence of the *ABL1* gene was analyzed using the 5′ RACE PCR method (SMARTer

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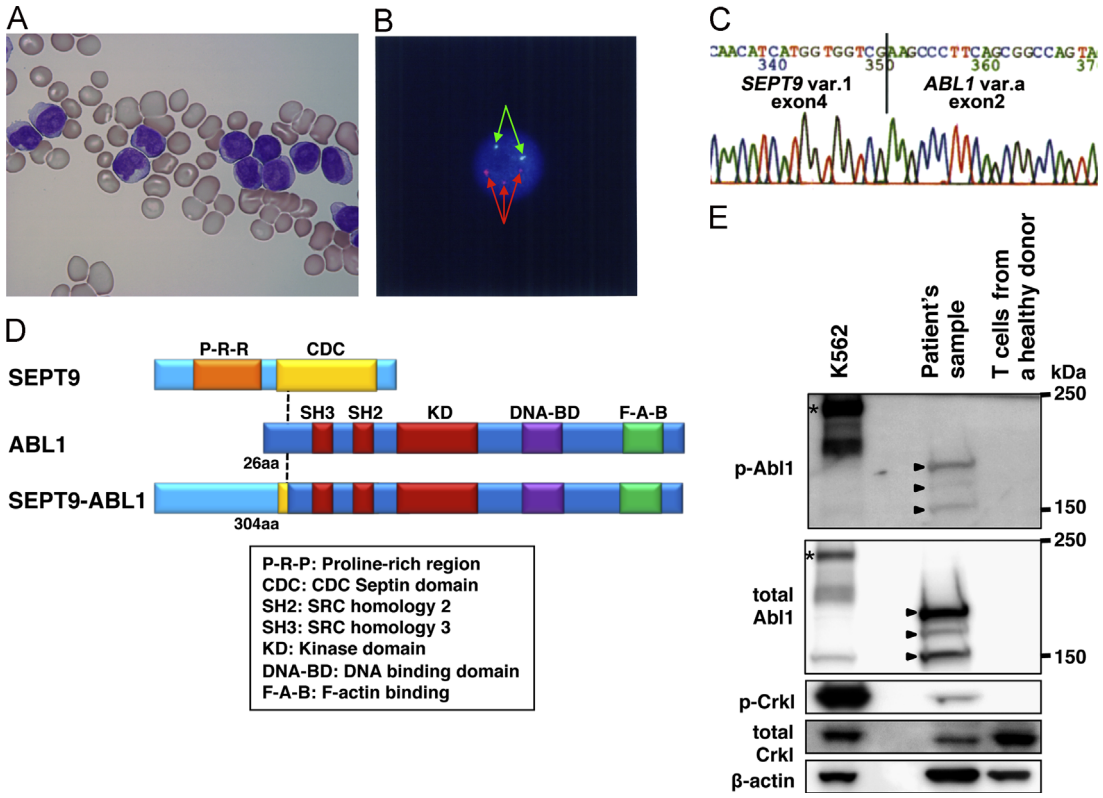

Fig. 1. Clinical and molecular characteristics of T-PLL harboring the *SEPT9-ABL1* fusion gene. (A) Cytology of the leukemic cells in the peripheral blood at diagnosis. The smear underwent Wright–Giemsa staining. (B) A FISH analysis of the bone marrow cells using the *BCR-ABL1* probe. The red signals show three *ABL1* signals, including one normal and two split signals (indicated by the lower arrows), while the green signals show normal biallelic *BCR* signals (indicated by the upper arrows). No *BCR-ABL1* fusion signals were detected. (C) Identification of *SEPT9-ABL1* fusion in the T-PLL cells. The PCR products of 5' RACE PCR using the ABL1 reverse primer were cloned into a cloning vector. Sequencing of the PCR products showed a fusion of exon 4 of *SEPT9* transcript variant 1 (GenBank accession number: NM_001113491.1) to exon 2 of *ABL1* transcript variant a (GenBank accession number: NM_005157.4). (D) The presumed structure of the SEPT9-ABL1 fusion product. (E) The phosphorylation of SEPT9-ABL1 and the downstream target CRKL in the T-PLL cells obtained from the patient. K562 cells and T-cells derived from a healthy donor were used as positive and negative controls for ABL1 fusion. Anti-ABL1 and anti-phosphorylated-ABL1 antibodies detected three SEPT9-ABL1 bands corresponding to 180, 170 and 150 KDa in the patient (arrowheads), as well as 210 KDa BCR-ABL1 in the K562 cells (asterisks), demonstrating the expression and phosphorylation of SEPT9-ABL1. CRKL was phosphorylated only in the cells harboring ABL1 fusion. The anti-phospho-Abl (Tyr412), anti-Abl, anti-phospho-Crkl (Tyr207) and anti-Crkl antibodies were purchased from Cell Signaling, and anti-β-actin was purchased from Sigma-Aldrich.

RACE cDNA Amplification Kit, Takara Bio, Shiga, Japan), according to the manufacturer's protocol. Sequencing of the PCR products demonstrated the fusion of exon 4 of *SEPT9* to exon 2 of *ABL1* (Fig. 1C), suggesting that the *SEPT9-ABL1* fusion gene had the same breakpoint in *ABL1* as that seen in *BCR-ABL1*. The presumed structure of the SEPT9-ABL1 fusion product is shown in Fig. 1D. A Western blot analysis revealed the expression and phosphorylation of SEPT9-ABL1, in addition to the phosphorylation of a downstream target CRKL, in the T-PLL cells obtained from the patient (Fig. 1E). Taking into account the patient's clinical, morphological, immunophenotypic and molecular features, he was diagnosed with T-PLL harboring *SEPT9-ABL1*. This is the first report of a novel *SEPT9-ABL1* fusion gene in a patient with malignancy, as well as T-PLL harboring *ABL1* fusion.

The patient received multiagent chemotherapy using cyclophosphamide, daunorubicin, vincristine, predonisolone and L-asparaginase, and high dose MTX/Ara-C, as well as the single-agent administration of nelarabine, hydroxyurea and tyrosine kinase inhibitors (TKIs) (imatinib and dasatinib). The conventional chemotherapies and cytotoxic agents effectively reduced the WBC count, however, TKIs were unable to do so. He finally died on day 223 after diagnosis (Fig. 2A). An autopsy was performed, and a macroscopic examination showed generalized lymphadenopathy with an enlarged lung, liver, spleen and kidney, while a microscopic examination disclosed leukemic cell infiltration throughout multiple organs (Fig. 2B). These findings suggested the occurrence of multiple organ failure due to a progression of leukemia which eventually caused the patient's death.

3. Discussion

ABL1 is a well-known oncogene that is often associated with the formation of fusion genes, such as *BCR-ABL1*, in human leukemia. The wild-type ABL1 product transduces diverse extracellular signals to protein networks that control proliferation, survival, migration and invasion [4]. Additionally, ABL1 modulates development and cytoskeletal remodeling processes in T-cells. To date, nine genes, including *BCR*, have been shown to fuse to *ABL1* [5]. These genes are divided into two groups according to their structure: one group has a breakpoint in exon 2 including the Src Homology (SH) 2 and SH3 domains in the fusion products and is found in various kinds of leukemia, while the other has a breakpoint in exon 4 excluding these domains and is primarily found in patients with B-lymphoblastic leukemia (B-ALL). Both groups share the C-terminus structure, including the SH1 tyrosine kinase domain. The N-terminal of the fusion proteins usually includes a coiled-coil or helix-loop-helix domain from the partner protein, which induces the oligomerization of the proteins, thus resulting in tyrosine kinase activation, cytoskeletal localization and neoplastic transformation [5].

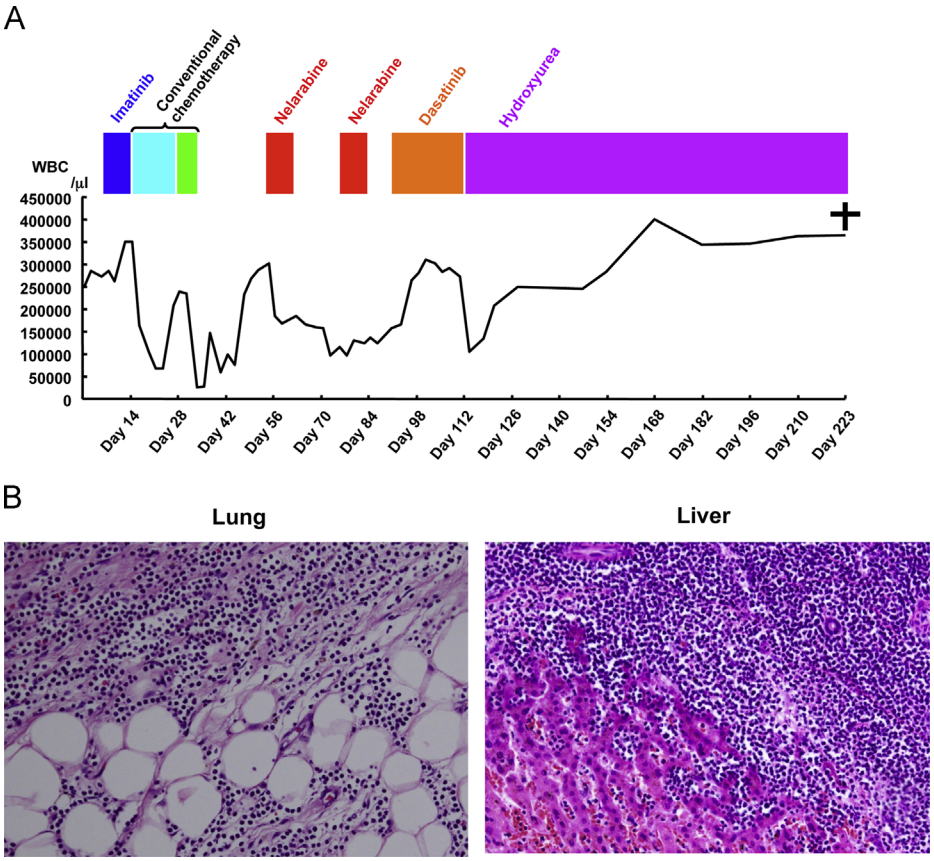

Fig. 2. The refractory clinical course of the patient. (A) The transitional changes in the WBC count in the present case. The administration of imatinib and dasatinib did not reduce the WBC count. (B) The histopathologic examination of the autopsy specimen stained with hematoxylin and eosin showed infiltration of T-PLL cells into the lungs and liver.

Septin proteins belong to a family of proteins that is highly conserved in eukaryotes [6]. These proteins are GTP-binding proteins that form hetero-oligomeric complexes. There are 13 septin genes in humans [6]. The *SEPT9* gene exists at chromosomal location 17q25 and exhibits a ubiquitous expression. SEPT9 plays a role in many cellular mechanisms, such as actin dynamics, axon growth, determination of the cell shape, chromosome segregation, cytokinesis, dendrite formation, DNA repair, membrane trafficking, microtubule regulation and T-cell motility [6]. Furthermore, SEPT9 is deeply associated with the development of various cancers (breast, colon, head, ovarian, neck, leukemia, lymphoma) [6]. In particular, it has been shown that *SEPT9* is a putative proto-oncogene involved in T-cell lymphomagenesis in mice [7].

In patients with hematological malignancies, *MLL* (*KMT2A*)-septin fusion has been repeatedly identified in cells exhibiting myeloid neoplasia in both children and adults. Five different septin genes (*SEPT2*, *SEPT5*, *SEPT6*, *SEPT9* and *SEPT11*) have been identified to be MLL fusion partners [8]. The C-terminal coiled-coil region of septin proteins is preserved in all MLL-septin fusion products, thus indicating that this region contributes to protein–protein interactions and ultimately oncogenesis. In contrast, the N-terminal of SEPT9 is fused to ABL1 in the SEPT9-ABL1 fusion product. It includes the Pro-rich region, which is necessary for binding with SH 3 regions [9]. Because the SH3 domain of ABL1 is preserved in SEPT9-ABL1, this Pro-rich region may contribute to an enhanced chimeric ABL1 kinase potential by promoting interaction with ABL1 SH3 regions, thereby synergistically inducing leukemogenesis.

Regarding the effectiveness of TKIs, some, but not all, patients carrying the *NUP214-ABL1* or *ETV6-ABL1* fusion gene respond to such therapy [5]. A recent report showed that B-ALL with *SNX2-ABL1* responds poorly to dasatinib but partially to imatinib [10,11]. In the present case, SEPT9-ABL1 exhibited a strong resistance to both imatinib and dasatinib. We confirmed that there were no point mutations in the *SEPT9-ABL1* fusion gene. These findings indicate that the TKI response in patients with hematological malignancies associated with various ABL1 fusion products is dependent on the ABL1-partner genes. Further examinations, including conformational analyses of ABL1 fusion products, such as SEPT9-ABL1, and genetic alteration screening of ABL1 fusion-expressing cells, will provide clues uncovering the mechanisms for determining sensitivity to TKI therapy in patients with hematological malignancies harboring *ABL1* fusion products.

Authorship

R.S. performed the experiments, analyzed the data and prepared the manuscript; Hir.M. designed the experiments, performed the experiments, analyzed the data and prepared the manuscript; Hid.K., K.T., Y.O. and Hir.K. analyzed the data; Hid.M. performed the experiments and analyzed the data; K.A. designed the experiments, analyzed the data and prepared the manuscript.

Conflict of interest disclosure

The authors declare no competing financial interests.

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