Case Report

RCSD1-ABL1 Translocation Associated with IKZF1 Gene Deletion in B-Cell Acute Lymphoblastic Leukemia

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The RCSD1 gene has recently been identified as a novel gene fusion partner of the ABL1 gene in cases of B-cell Acute Lymphoblastic Leukemia (B-ALL). The RCSD1 gene is located at 1q23 and ABL1 is located at 9q34, so that the RCSD1-ABL1 fusion typically arises through a rare reciprocal translocation t(1;9)(q23;q34). Only a small number of RCSD1-ABL1 positive cases of B-ALL have been described in the literature, and the full spectrum of clinical, morphological, immunophenotypic, and molecular features associated with this genetic abnormality has not been defined. We describe extensive genetic characterization of a case of B-ALL with RCSD1-ABL1 fusion, by using conventional cytogenetic analysis, Fluorescence In Situ Hybridization (FISH) studies, and Chromosomal Microarray Analysis (CMA). The use of CMA resulted in detection of an approximately 70 kb deletion at 7p12.2, which caused a disruption of the IKZF1 gene. Deletions and mutations of IKZF1 are recurring abnormalities in B-ALL and are associated with a poor prognosis. Our findings highlight the association of the deletion of IKZF1 gene with the t(1;9)(q23;q34) and illustrate the importance of comprehensive cytogenetic and molecular evaluation for accurate prediction of prognosis in patients with B-cell ALL.

1. Introduction

Nonrandom, acquired clonal chromosomal abnormalities are often the primary driver mutations in acute leukemia and are closely, and sometimes uniquely, associated with morphologically and clinically distinct subsets of the disease. Detection of such translocations helps in establishing the accurate diagnosis, prognosis, and risk stratification and monitoring the treatment outcomes. Translocations involving the ABL1 gene at 9q34 are a well-known example of chromosomal abnormalities of major importance for diagnosis, risk stratification, and treatment selection in hematologic malignancies. ABL1 codes for the protein with tyrosine kinase activity. Its major fusion partner is the BCR gene, involved in the t(9;22)(q34;q11) which results in a formation of the so-called Philadelphia chromosome (derivative chromosome 22). The t(9;22)(q34;q11) results in the formation of a chimeric BCR-ABL1 fusion transcript, which encodes a constitutively active ABL1 tyrosine kinase. The BCR/ABL1 fusion is a well-studied entity, present in all the cases of Chronic Myeloid Leukemia (CML) and approximately 20% cases of B-cell ALL. In addition to BCR, six other genes have been reported to form rearrangements with ABL1: ETV6 at 12p13, RCSD1 at 1q24, SFPQ at 1p34, ZMIZ1 at 10q22.3, NUP214 at 9q34.13, FOXP1 at 3p12, SNX2 at 5q23, and EML1 at 14q32 [1–5]. However, the ABL1 translocations with partners other than BCR are rare, and information about the clinical features, morphology, response to therapy, and prognosis for B-cell ALL involving such rearrangements is limited. With the development and use of targeted therapy with specific kinase inhibitors, the outcome for patients with translocations involving the ABL1 gene has dramatically improved. Because of the likely therapeutic implications, recognizing the presence of ABL1 fusions in newly diagnosed cases of ALL is of the highest importance.

We describe a case of B-cell ALL with the occurrence of a rare translocation involving the RCSD1 and ABL1 genes.
ABL1 fusion. Her consolidation course was complicated due to presumed fungal infection treated with Ambisome 500 mg Q 24 hours IV and C. difficile colitis treated with Metronidazole and Vancomycin PO, but she was able to complete the consolidation treatment. The last bone marrow done on November 2014 to see the remission status was hypocellular (20–25% cellularity) with regenerative trilineage hematopoiesis, mild dyserythropoiesis, and no evidence of persistent/recurrent leukemia. The patient had a bone marrow transplant from a sibling in November 2014, but a few weeks after transplant she developed severe sepsis as well as capillary leak syndrome. She remained admitted to the intensive care unit where she unfortunately died on March 12, 2015. Figure 1 illustrates the clinical course of the disease.

2. Materials and Methods

2.1. G-Banding Karyotype Analysis. Karyotypic analyses were performed on unstimulated short-term bone marrow cultures (24 and 48 hours), followed by Giemsa trypsin banding. Cytogenetic findings were described according to the International System for Human Cytogenetic Nomenclature (ISCN2013).

2.2. Florescence In Situ Hybridization (FISH) Analysis. FISH analysis to confirm ABL1 rearrangement was performed with the BCR/ABL1 dual color and dual fusion translocation probe (Abbott Laboratories). Additional FISH testing was done with ETV6/RUNX1-Extra signal, CEP 4-, 10-, and 17-centromeric, and MLL-Break-apart probes (Abbott Laboratories).

2.3. Chromosomal Microarray Analysis (CMA). Genomic DNA was extracted with Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA), according to the manufacturer’s instructions. SNP array testing was performed using the Affymetrix CytoScan HD arrays (Affymetrix Inc., Santa Clara, CA) and following the manufacturer’s recommendations. The data were analyzed with the Chromosome Analysis Suite (ChAS) software from Affymetrix (Affymetrix Inc., Santa Clara, CA).

3. Case Presentation

A 15-year-old female was admitted in June 2014 because of the unusual breakpoint in the ABL1 gene, the RCSD1/ABL1 rearrangements is that the ABL1 gene, the RCSD1/ABL1 fusion relative to other ABL1 rearrangements is that the N-terminal portion of the RCSD1 gene is fused to ABL1 starting from exon 4, while the other ABL1 fusion products also contain ABL1 exons 2 and 3 [7]. As a consequence of the unusual breakpoint in the ABL1 gene, the RCSD1/ABL1 protein retains only a part of the ABL SH2 domain, which would predict association of the RCSD1/ABL1 fusion with ALL rather than CML [10]. Indeed, our patient had B-cell

4. Results

4.1. Cytogenetics. Cytogenetic studies done on the bone marrow sample at the time of diagnosis revealed the karyotype 46,XX,t(1;9)(q24;q34) [10]/46,XX[14] (Figure 1). The second bone marrow sample obtained after induction therapy revealed a normal female karyotype 46,XX[20] (Figure 2).

4.2. Florescent In Situ Hybridization (FISH). FISH analysis at the time of diagnosis revealed a gain of ABL1 signal in 78% of analyzed cells, consistent with the ABL1 rearrangement due to the t(1;9)(q24;q34) (Figure 3). FISH was negative for BCR/ABL1 and ETV6/RUNX1 translocations, MLL gene rearrangement, and trisomy for chromosomes 4, 10, and 17. BCR/ABL1 FISH testing done on bone marrow aspirate after induction therapy revealed no abnormalities. However, day 42 bone marrow aspirate showed rearrangement of the ABL1 locus, consistent with residual disease. Other consecutive samples showed negative FISH results with BCR/ABL1 probe.

4.3. Chromosomal Microarray Analysis. CMA detected an approximately 70 kb copy deletion at 7p12.2 and 90 kb deletion at 13q14.2. The 70kb deletion at 7p12.2 results in a disruption of the IKZF1 gene (Figure 4). The 90 kb deletion at 13q14.2 results in a disruption of the RBL1 gene. The t(1;9)(q24;q34) noted by karyotype analysis was a completely balanced rearrangement and was thus not detected by CMA.

5. Discussion

We describe a patient with B-cell ALL associated with a particularly rare translocation affecting the ABL1 gene, t(1;9)(q24;q34). This translocation involves the RCSD1 gene at 1q21 and has previously been described in only four patients and confirmed by cytogenetic or molecular methods only in two patients [6–9]. The unusual feature of the RCSD1/ABL1 fusion relative to other ABL1 rearrangements is that the fusion products include the ABL1 C-terminal portion of the RCSD1 gene is fused to ABL1 starting from exon 4, while the other ABL1 fusion products also contain ABL1 exons 2 and 3 [7]. As a consequence of the unusual breakpoint in the ABL1 gene, the RCSD1/ABL1 protein retains only a part of the ABL SH2 domain, which would predict association of the RCSD1/ABL1 fusion with ALL rather than CML [10]. Indeed, our patient had B-cell...
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Illustrative diagram of clinical course

| Date       | Event Description                                                                 |
|------------|-------------------------------------------------------------------------------------|
| Day 0      | Diagnosis                                                                          |
| Day 29     | Induction (AALL-1131)                                                              |
| Day 42     | Reinduction with high-risk control arm of AALL-1131                                  |
| Day 52     | Bone marrow transplant done in November 2014                                         |
| Day 105    | Death due to severe sepsis                                                          |

Figure 1: Illustrative diagram showing clinical course of the patient.

Figure 2: G-banded karyotype showing 46,XX, t(1;9)(q24;q34).
ALL, and the same was true for the two previously reported individuals with the t(1;9). The RCSD1 gene codes for a protein kinase substrate CapZIP (CapZ-interacting protein), which is highly expressed in skeletal muscle and more weakly expressed in cardiac muscle. It has been shown that stress-induced phosphorylation of CapZIP may regulate the ability of CapZ to remodel actin filament assembly which is an important step in mitosis [11]. As ABL1 protein has a tyrosine kinase activity and its fusion proteins act as constitutively activated tyrosine kinases, the formation of the RCSD1-ABL1 fusion gene could therefore result in CapZIP phosphorylation with loss of interaction between CapZIP and CapZ, leading to an alteration of the cellular function by affecting the cytoskeleton regulation, which could be an important step in leukemogenesis. It is important to note that B-ALL with the RCSD1-ABL1 fusion has recently been included into a specific molecular subtype of high-risk B-ALL, known as Philadelphia- (Ph-) like ALL, which is characterized by an expression signature resembling that of Philadelphia-positive ALL [12]. Similar to Ph-positive cases, these “Ph-like” leukemia cases have a high probability of relapse and carry poor prognosis, but it has been postulated that they may
respond to treatment with tyrosine kinase inhibitors (TKIs), since they are characterized by activated kinase signature. Indeed, in vitro and in vivo studies, as well as increasing number of case reports, have documented efficacy of using targeted TKIs for Ph-like leukemia cases [12–14], but the prognostic significance of this fusion is still unclear. The patient described by De Braekeleer et al. [2] responded well to initial therapy and achieved a complete remission but relapsed 2 years after the initial treatment.

The patient reported by Mustjoki et al. [7] received a modified CVAD induction therapy and, after two induction courses, still had a residual disease. However, the patient achieved morphological remission after addition of a wide-spectrum TKI dasatinib. Similarly, the patient described by Inokuchi et al. did not achieve complete remission with hyper-CVAD combination chemotherapy but achieved transient clinical effects with TKIs, imatinib, and dasatinib, coadministered with dexamethasone. The patient described in this report did not respond to the standard induction chemotherapy and only achieved full morphologic and cytogenetic remission with continued chemotherapy per the very high-risk control arm of AALL-1131 with the addition of dasatinib. Long-term follow-up information is unfortunately not available, but our case supports previous observations that B-ALL with the RCSD1-ABL1 fusion may be associated with increased risk of induction failure and is also characterized by susceptibility to TKI treatment. In addition to induction failure, a high risk of relapse has also been described in ALL with RCSD1-ABL1 fusion. In the case reported by Inokuchi et al., after short remission on TKIs, the leukemic cells rapidly became refractory to the TKI treatment and subsequently developed three additional reciprocal chromosomal translocations, t(5;16)(q33;q24), dic(18;20)(p11.2;q11.2), and t(10;19)(q24;p13.3). This led the authors to hypothesize that the B-ALL with the RCSD1-ABL1 fusion may be characterized by high genomic instability and propensity for clonal evolution and development of resistance.

Other factors that may modify the disease course and outcome in patients with RCSD1-ABL1 fusion are associated secondary genetic changes, including mutations and deletions of the IKZF1 gene. IKZF codes for a DNA-binding protein Ikaros which displays crucial tumor suppressor functions in the hematopoietic system. Loss of Ikaros function has been linked to the development of lymphoid leukemia, in particular precursor B-ALL, and has been shown to confer a high risk of induction failure and is also characterized by susceptibility to TKI treatment. In addition to induction failure, a high risk of relapse has also been described in ALL with RCSD1-ABL1 fusion. In the case reported by Inokuchi et al., after short remission on TKIs, the leukemic cells rapidly became refractory to the TKI treatment and subsequently developed three additional reciprocal chromosomal translocations, t(5;16)(q33;q24), dic(18;20)(p11.2;q11.2), and t(10;19)(q24;p13.3). This led the authors to hypothesize that the B-ALL with the RCSD1-ABL1 fusion may be characterized by high genomic instability and propensity for clonal evolution and development of resistance.

The findings illustrate the importance of comprehensive genetic evaluation using a variety of assays in order to achieve fully accurate risk stratification for B-ALL patients. Additional cases will have to be identified and studied to ascertain the prognostic significance of the RCSD1-ABL1 fusion with and without cooccurrence of IKZF1 abnormalities. Additionally, the effectiveness of combined treatment with chemotherapy and TKIs for patients with the RCSD1-ABL1 fusion and other genetic lesions associated with Ph-like ALL will have to be explored through large multicenter clinical trials.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**

[1] P. Papadopoulos, S. A. Ridge, C. A. Boucher, C. Stocking, and L. M. Wiedemann, “The novel activation of ABL by fusion to an ets-related gene, TEL,” Cancer Research, vol. 55, no. 1, pp. 34–38, 1995.

[2] E. De Braekeleer, N. Douet-Guilbert, M. J. Le Bris, C. Berthou, F. Morel, and M. De Braekeleer, “A new partner gene fused to ABL1 in a t(1;9)(q24;q34)-associated B-cell acute lymphoblastic leukemia,” Leukemia, vol. 21, no. 10, pp. 2220–2221, 2007.

[3] F. P. Duhoux, N. Auger, S. De Wilde et al., “The (1;9)(p34;q34) fusing ABL1 with SFPQ, a pre-mRNA processing gene, is recurrent in acute lymphoblastic leukemias,” Leukemia Research, vol. 35, no. 7, pp. e114–e117, 2011.

[4] K. De Keersmaecker, C. Graux, M. D. Odero et al., “Fusion of EML1 to ABL1 in T-cell acute lymphoblastic leukemia with cryptic (9;14)(q34;q32),” Blood, vol. 105, no. 12, pp. 4849–4852, 2005.

[5] T. Ernst, J. Score, M. Deininger et al., “Identification of FOXP1 and SNX2 as novel ABL1 fusion partners in acute lymphoblastic leukaemia,” British Journal of Haematology, vol. 153, no. 1, pp. 43–46, 2011.

[6] K. Inokuchi, S. Wakita, T. Hirakawa et al., “RCSD1-ABL1-positive B lymphoblastic leukemia is sensitive to dexamethasone and tyrosine kinase inhibitors and rapidly evolves clonally by chromosomal translocations,” International Journal of Hematology, vol. 94, no. 3, pp. 255–260, 2011.

[7] S. Mustjoki, S. Hernesniemi, A. Rauhala et al., “A novel dasatinib-sensitive RCSD1-ABL1 fusion transcript in chemotherapy-refractory adult pre-B lymphoblastic leukemia with t(1;9)(q24;q34),” Haematologica, vol. 94, no. 10, pp. 1469–1471, 2009.

[8] C. Graux, J. Cools, C. Melotte et al., “Fusion of NUP214 to ABL1 on amplified episomes in T-cell acute lymphoblastic leukemia,” Nature Genetics, vol. 36, no. 10, pp. 1084–1089, 2004.

[9] E. De Braekeleer, N. Douet-Guilbert, P. Guardiola et al., “Acute lymphoblastic leukemia associated with RCSD1-ABL1 novel fusion gene has a distinct gene expression profile from BCR-ABL1 fusion,” Leukemia, vol. 27, no. 6, pp. 1422–1424, 2013.
by BCR/ABL is dependent on intact Src homology (SH)3 and SH2 domains of BCR/ABL and is required for leukemogenesis,” *Journal of Experimental Medicine*, vol. 189, no. 8, pp. 1229–1242, 1999.

[11] C. E. Eyers, H. McNeill, A. Knebel et al., ”The phosphorylation of CapZ-interacting protein (CapZIP) by stress-activated protein kinases triggers its dissociation from CapZ,” *Biochemical Journal*, vol. 389, no. 1, pp. 127–135, 2005.

[12] R. C. Harvey, C. G. Mullighan, I.-M. Chen et al., "Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia,” *Blood*, vol. 115, no. 26, pp. 5312–5321, 2010.

[13] C. G. Mullighan, X. Su, J. Zhang et al., “Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia,” *The New England Journal of Medicine*, vol. 360, no. 5, pp. 470–480, 2009.

[14] R. C. Harvey, C. G. Mullighan, X. Wang et al., “Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome,” *Blood*, vol. 116, no. 23, pp. 4874–4884, 2010.