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| Complete List of Authors: | Ernst, Thomas; Universität\-sklinikum Jena, Klinik für Innere Medizin II  
Score, Joannah; University of Southampton, Wessex Regional Genetics Laboratory  
Hidalgo-Curtis, Claire; University of Southampton, Wessex Regional Genetics Laboratory  
Deininger, Michael; Oregon Health and Science University, Hematology  
Lackie, Peter; University of Southampton, Infection Inflammation and Immunity  
Ershler, William; Harbor Hospital, Hematology/Immunology Unit  
Goldman, John; Imperial College London, Haematology  
Cross, Nicholas; University of Southampton, Salisbury District Hospital, Wessex Regional Genetics Laboratory  
Grand, Francis; University of Southampton, Wessex Regional Genetics Laboratory |
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Identification of FOXP1 and SNX2 as novel ABL1 fusion partners in acute lymphoblastic leukaemia

Thomas Ernst¹, Joannah Score², Michael Deininger³, Claire Hidalgo-Curtis², Peter Lackie⁴, William B Ershler⁵, John M Goldman⁶, Nicholas C.P. Cross² and Francis H. Grand²

¹ Universitätsklinikum Jena, Klinik für Innere Medizin II
Jena, Germany

² Wessex Regional Genetics Laboratory, University of Southampton, Salisbury, United Kingdom

³ Oregon Health and Science University, Hematology
Portland, Oregon, United States

⁴ University of Southampton, Infection Inflammation and Immunity
Southampton, United Kingdom

⁵ Harbor Hospital, Hematology/Immunology Unit
Baltimore, Maryland, United States

⁶ Harbor Hospital, Hematology/Immunology Unit
Baltimore, Maryland, United States

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Corresponding author:

Francis H. Grand, PhD
Wessex Regional Genetics Laboratory
University of Southampton
Salisbury SP2 8BJ
United Kingdom

Tel:  +(44) 1722 429080
Fax:  +(44) 1722 338095
E-Mail: F.H.Grand@soton.ac.uk
Summary

We have identified two novel ABL1 fusion genes in two patients with B-cell acute lymphoblastic leukaemia associated with a t(3;9)(p12;q34) and a t(5;9)(q23;q34), respectively. Molecular analysis revealed a FOXP1-ABL1 fusion for the t(3;9) and a SNX2-ABL1 fusion for the t(5;9). The fusions were confirmed by specific amplification of the genomic breakpoints using reverse transcription polymerase chain reaction (RT-PCR). The identification of ALL with rare ABL1 fusion partners is important because the leukaemia may respond to tyrosine kinase inhibitors in the same way as ALL patients with a classical BCR-ABL1 fusion gene.
Introduction

Constitutively activated mutants of the non-receptor tyrosine kinase ABL1 play a central role in the pathogenesis of clinically and morphologically distinct chronic and acute leukaemias (Chase & Cross, 2006). By far the most frequent and best-studied ABL1 fusion gene is BCR-ABL, which results from an acquired reciprocal t(9;22)(q34;q11) (Shtivelman et al, 1985; Ben-Neriah et al, 1986). The t(9;22)(q34;q11) is found in virtually all patients with chronic myeloid leukaemia (CML), in approximately 20% of adults with B-cell acute lymphoblastic leukaemia (ALL), and in rare cases of acute myeloid leukaemia (Fröhling & Döhner 2008).

The second most frequent and biologically distinct ABL1 fusion gene is NUP214-ABL1 in T-ALL (Graux et al, 2004). Remarkably, this fusion is generated by circularisation of the 500 kb genomic region from ABL1 to NUP214 and subsequent extrachromosomal (episomal) amplification. Other ABL1 fusion genes have been described but are uncommon. The ETV6-ABL1 fusion gene is the product of a t(9;12)(q34;p13) and is found in occasional patients with acute leukaemias or atypical CML (Papadopoulos et al, 1995; Golub et al, 1996; Andreasson et al, 1997). EML1-ABL1 was found in a single female patient with T-ALL and a cryptic t(9;14)(q34;q32) (De Keersmaecker et al, 2005). Recently, RCSD1, ZMIZ1 and SFPQ were identified as novel ABL1 fusion partners in single cases of B-ALL (De Braekeleer et al, 2007; Soler et al, 2008; Hidalgo-Curtis et al, 2008).

Imatinib is a specific inhibitor of several tyrosine kinases including ABL1 and induces long-term complete haematologic and cytogenetic remissions in most patients with chronic phase CML (Druker et al, 2001; Deininger et al, 2009). However, a substantial proportion of patients with advanced phase CML or BCR-ABL1 positive ALL are initially refractory to
imatinib treatment or lose imatinib sensitivity over time and relapse (Apperley, 2007; Pui et al., 2008). More potent second-generation ABL1 kinase inhibitors (e.g. dasatinib, nilotinib, bosutinib) have been developed for the treatment of imatinib-resistant patients and emerging clinical data indicate that these drugs have promising efficacy (Weisberg et al., 2007, Gruber et al., 2009). Both, first- and second-generation ABL1 kinase inhibitors are expected to achieve similar remission rates in patients with ABL1 fusion genes other than BCR-ABL1. However, because the majority of these cases have only been reported in individual patients, insufficient data on the in-vivo efficacy of tyrosine kinase inhibitors are currently available. 

NUP214-ABL and EML1-ABL have been shown to be imatinib sensitive in-vitro and two single case reports documented dasatinib sensitivity for NUP214-ABL and RCSD1-ABL in-vivo (Deenik et al., 2009; Mustjoki et al., 2009).

In this study, we have investigated two B-ALL patients with acquired chromosomal rearrangements each involving the chromosomal band 9q34 and identified two novel ABL1 fusion genes potentially sensitive to imatinib treatment. Although these fusion genes occur rarely, their identification is essential in order to detect patients in whom targeted treatment with tyrosine kinase inhibitors is likely to be successful. Furthermore, molecular definition of novel fusion partners for known kinases remains important both for rapid molecular diagnosis of new cases and for monitoring an individual patient’s response to treatment.
Patients and methods

Patients

Case 1: *FOXP1-ABL1* male/female B-ALL with t(3;9)(p12;q34).

A 16-year old caucasian woman was diagnosed with pre B cell acute lymphoblastic leukaemia in October 2001, with an initial white blood cell count of >50,000/nL. Her past medical history was significant only for recurrent deep venous thrombosis secondary to protein S deficiency. Bone marrow cytogenetics was 46XX,t(3;9)(p12;q34)[20]. FISH revealed loss of one copy of the p16 locus. Based on her age and an initial white blood count she was considered high risk. Treatment was initiated according to the standard arm for high risk patients of the COALL-97 protocol of the German Cooperative Study Group for Childhood Acute Lymphoblastic Leukaemia (COALL). Therapy was complicated by recurrent DVT, catheter-associated septicaemia and steroid-induced diabetes mellitus. She achieved a complete response and in February 2002 was consolidated with a haplo-identical transplant from her father. Complications included grade III GvHD of the skin and fungal pneumonia, both of which resolved on therapy. On last follow-up in spring of 2010 she continued in CR, with excellent functionality (attending medical school).

Case 2: *SNX2-ABL1* male B-ALL with t(5;9)(q23;q34).

The patient was diagnosed in April 2004 aged 29 when he presented with a WBC of 161 x10^9/l. The cell showed a classical B-cell immunophenotype. Bone marrow cytogenetics showed a t(5;9)(q23;q34) and a 20q-. FISH showed evidence of the ABL gene on both derivative chromosomes. BCR was apparently not involved. He responded well to initial
chemotherapy but relapsed early. He then received hyper-C-VAD and again responded. He relapsed again at the end of 2004 and was started on imatinib, it was transient and despite other supportive measures including hydroxyurea he died in Spring 2005.

Fluorescence In Situ Hybridization (FISH)

To our knowledge, no other cases have been reported with a t(3;9)(p14;q34) or t(5;9)(q23;q34). For the t(5;9) FISH was performed using the BCR-ABL probe from Abbott.

5’ Rapid Amplification of cDNA Ends (5’ RACE) PCR

Partner genes were identified using the Gene-Racer™ Kit (Invitrogen, Paisley, UK). Briefly, ~ 5 µg of total RNA extracted using the Qiagen RNeasy kit (Qiagen, Boundary Court, UK) was dephosphorylated, decapped, and ligated to the GeneRacer™ RNA oligo according to the manufacturer’s instructions. The ligated RNA was reverse transcribed using Superscript II™ reverse transcriptase (Invitrogen, Paisley, UK) and random primers (100 ng). Single step 5’ RACE PCR was performed using the 5’ GeneRacer™ primer from the kit in combination with a reverse primer from ABL1 exon 4 (1R: 5’-cca ccg ttg aat ga t gat gaa cc-3’). The PCR cycles were designed to amplify fragments up to 3 kb, with an annealing temperature of 66°C using the High Fidelity PCR Master kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions. Products were cloned with the TOPO TA Cloning Kit for Sequencing (Invitrogen, Paisley, UK) and sequenced.

RT-PCR Methods
Case 1: The presence of FOXP1-ABL1 mRNA was confirmed on random hexamer reverse transcribed cDNA using primers to FOXP1 exon 18 (5’-gca gta tgg aca gtg gat gaa gta-3’) with the reverse ABL1 exon 4 (see above). These primers were also used to amplify the genomic breakpoint.

Case 2: The presence of SNX2-ABL1 mRNA was confirmed on random hexamer reverse transcribed cDNA using primers to SNX2 1F new (5’-aag agt atg tct gct ccc gtg atc tt-3’) with the reverse ABL1 exon 4. These primers were also used to amplify the genomic breakpoint.

Results

Characterization of the t(3;9)(p14;q34)

FISH using the probes that immediately flank the ABL1 gene hybridized to the normal copy of chromosome 9, also the der(9) and the der(3), indicating that the translocation targeted ABL1; however, no BCR-ABL1 fusion was seen by FISH or RT-PCR. Most ABL1 fusions reported to date result in the partner gene fusing to ABL1 exons 2 or 3. To identify the t(3;9) partner, we, therefore, performed 5’-RACE PCR from both these exons. These initial attempts failed despite the fact that normal 5’ ABL1 sequence was readily obtained (data not shown). 5’ RACE primers were subsequently designed in ABL1 exon 4 (ENST00000318560). Sequencing of the products revealed several clones in which FOXP1 (ENST00000318789) exon 19 was fused in frame to ABL1 exon 4.

Confirmation of the FOXP1-ABL1 fusion

The presence of the FOXP1-ABL1 fusion was confirmed initially by RT-PCR. The reciprocal ABL1-FOXP1 product was detectable by single step PCR. Cloning and sequencing revealed
that the reciprocal product fuses ABL1 exon 3 to an alternative RNA isoform (NM_032682) of FOXP1. As shown in Figure 1a and 1b, the FOXP1-ABL1 fusion was specifically amplified from patient cells by single step PCR, but was not detectable in normal controls. To further confirm the presence of the fusion the genomic breakpoint was amplified by long PCR, cloned, and sequenced.

**Characterization of the t(5;9)(q23;q34)**

FISH using the Abbott BCR-ABL1 probes that immediately flank the ABL1 gene hybridized to the normal copy of chromosome 9, also the der(9) and the der(5), indicating that the translocation targeted ABL1; however, no BCR-ABL1 fusion was seen by FISH or RT-PCR. 5’ RACE PCR form ABL1 exons 2 and 3 failed, after the previous cloning of the t(3;9) translocation, 5’-RACE PCR primers were designed in ABL1 exon 4 (ENST00000318560). Sequencing of the products revealed several clones in which SNX2 (ENST00000379516) exon 3 was fused in frame to ABL1 exon 4.

**Confirmation of the SNX2-ABL1 fusion**

The presence of the SNX2-ABL1 fusion was confirmed initially by RT-PCR. The reciprocal ABL1-SNX2 product was not detectable by single step PCR. As shown in Figure 2a and 2b, the SNX2-ABL1 fusion was specifically amplified from patient cells by single step PCR, but was not detectable in normal controls. To further confirm the presence of the fusion the genomic breakpoint was amplified by long PCR, cloned, and sequenced (data not shown).
Discussion

This study has characterised the genomic breakpoints in two B-ALL cases with chromosome 9q34 rearrangements and identified FOXP1 at 3p14 and SNX2 at 5q23 as novel ABL1 fusion partners. This brings the number of known ABL1 partner genes to nine. In the chimeric protein, N-terminal sequences of ABL1 are replaced by amino-acids derived from the partner protein that provides dimerisation motifs essential for the transforming activity of ABL1. Fusion genes involving ABL1 are excellent drug targets as exemplified by the activity of imatinib in BCR-ABL1 positive diseases, particularly CML. The clinical course of patients with ABL1 fusion genes other than BCR-ABL1 may be different and highly dependent on the underlying disease. Response rates and risk of relapse may be similar to CML in patients with chronic phase myeloproliferative neoplasms or similar to BCR-ABL1 positive ALL in patients with acute leukaemias, respectively. As the fusions described here were referred specifically for investigation of 9q rearrangements and were not part of a series that underwent systemic cytogenetic investigation, it is not possible to accurately determine the frequency of these abnormalities. However, we estimate that they probably account for <1% of ALL cases.

The ABL1 gene is located on chromosome band 9q34 and encodes a ubiquitously cytoplasmic and nuclear protein tyrosine kinase that has been implicated in processes of cell differentiation, cell division, cell adhesion, and stress response. Simplified, the structure of ABL1 consists of N-terminal SH3 and SH2 domains (which mediate binding to proline-rich and phosphotyrosine proteine ligands, respectively), a catalytic tyrosine kinase domain that contains phosphotransferase activity and a DNA-binding domain with yet largely unknown effects and a C-terminal actin-binding domain. Most previously described fusion genes involving ABL1 fuse with ABL1 exon 2. However, similar to the recently described fusion
partners SFPQ and RCSD1 (Hildalgo-Curtis et al, 2008; Mustjoki et al, 2009) both partner genes described here fuse to ABL1 exon 4. These are structurally unusual ABL1 fusion proteins as the SH3 and SH2 domains are not included. Mouse models with BCR-ABL1 constructs in which the SH2 domain was inactivated have shown that the fusion protein retained the ability to cause leukaemia in mice (Roumiantsev et al, 2001).

The FOXP1 gene is located at 3p14 and encodes a member of the forkhead box (FOX) family of transcription factors with diverse functions in development, metabolism, organogenesis and cancer (Hannenhalli & Kaestner, 2009). FOXP1 was first cloned from the mouse B-cell leukaemia cell line BCL1 and later described to be an essential transcriptional regulator of B lymphopoiesis via direct regulation of the B-cell specific Erag enhancer (Li & Tucker, 1993; Hu et al, 2006). FOXP1 has been described as a recurrent translocation partner of the IGH gene in a subset of MALT lymphomas and diffuse large B-cell lymphomas with t(3;14)(p14;q32) (Streubel et al, 2005; Wlodarska et al, 2005). Furthermore, FOXP1 was identified as a translocation partner of PAX5 in a single case of paediatric B-ALL (Mullighan et al, 2007). In contrast to previously published studies, we found FOXP1 as the 5’ partner of the fusion gene. Nearly the entire FOXP1 gene including the forkhead domain was fused in 5’ position to ABL1.

The SNX2 gene is located at 5q23 and belongs to the sortin nexins (SNX) family that function within the endocytic network, including endocytosis, endosomal sorting and endosomal signalling (Cullen, 2008). SNX2 is expressed at high levels in CD34 positive bone marrow cells and thought to heterodimerise with SNX1 in a retromer protein complex. The retromer is a vesicle coat complex that helps in protein sorting between the Golgi and endosomes, playing a key role in protecting some receptors from lysosomal destruction (Seaman, 2005). The hallmark of SNX family is the presence of a PX domain - a sequence of approximately 100-
130 amino acids that has been shown to bind various phosphatidylinositol phosphates, thereby potentially targeting these proteins to specific cellular membranes that are enriched in these phospholipids (Worby & Dixon, 2002). As described above, constitutive activation of ABL fusion genes is mediated by an oligomerisation domain in the partner protein. The \( \text{SNX2-ABL1} \) fusion gene is unusual as it lacks a clearly recognisable dimerisation motif. The N-terminal domain of SNX2 - without the PX domain - is fused to ABL1 exon 4.

Although rare the potential sensitivity to imatinib implies that these fusions should be searched for in acute leukaemia. The screen for such uncommon, but clinically significant fusion transcripts can easily be included in multiplex PCR panels used in routine diagnostic work-ups. Targeted therapy has revolutionised the treatment of CML and there is considerable optimism that targeting \( \text{ABL1} \) fusion genes other than \( \text{BCR-ABL1} \) will bring at least short term benefits for those patients. On the other hand, targeted therapy is very expensive and it will be important to develop markers to identify those patients who are most likely to benefit from treatment.

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References


t(3;9)  N1  N2  N3  B1  B2

1 kb →
500 bp →
mRNA breakpoint

FOXp1 exon 19  \rightarrow  ABL exon 4
t(5;9) N1 N2 N3 B1

1 kb

500 bp
mRNA breakpoint

SNX2 exon 3  ABL exon 4