The t(4;22)(q12;q11) in atypical chronic myeloid leukaemia fuses BCR to PDGFRA

E. Joanna Baxter1,2, Andreas Hochhaus3, Pascual Bolufer4, Andreas Reiter3, José M. Fernandez5, Leonor Senent5, José Cervera5, Federico Moscardo5, Miguel A. Sanz5 and Nicholas C.P. Cross1,2,*

1Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury SP2 8BJ, UK, 2Human Genetics Division, University of Southampton School of Medicine, Southampton SO16 6YD, UK, 3III Medizinische Universitätsklinik, Klinikum Mannheim, 68305 Mannheim, Germany and 4Servicio de Hematología and 5Department of Medical Biopathology, Hospital Universitario La Fe, 46009 Valencia, Spain

Received January 17, 2002; Revised and Accepted March 25, 2002

Chronic myeloid leukaemia (CML) is characterized by the presence of the BCR–ABL fusion gene, usually in association with the t(9;22)(q34;q11) translocation. We report here the identification and cloning of a rare variant translocation, t(4;22)(q12;q11), in two patients with a CML-like myeloproliferative disease (MPD). RT–PCR indicated that both patients were negative for BCR–ABL, but FISH analysis suggested that the BCR gene was rearranged. Since other translocations in MPDs frequently involve tyrosine kinases, we designed a multiplex PCR to search for mRNA fusions between BCR and three potential partner genes at 4q12: KIT, KDR and PDGFRA. An unusual inframe BCR–PDGFRA fusion mRNA was identified in both patients, with either BCR exon 7 or exon 12 fused to short BCR intron-derived sequences, which were in turn fused to part of PDGFRA exon 12. Sequencing of the genomic breakpoint junctions showed that the chromosome 22 breakpoints fell in BCR introns whereas the chromosome 4 breakpoints were within PDGFRA exon 12. This is the first report of a fusion gene that involves PDGFRA. Our findings indicate that apparently simple cytogenetic variants of t(9;22) do not always mask a cryptic BCR–ABL fusion, even when found in association with clinical and haematological indications of CML.

INTRODUCTION

Chronic myeloid leukaemia (CML) is a chronic myeloproliferative disease (CMPD) that is characterized by the presence of the BCR–ABL fusion gene in all myeloid lineage cell types, as well as in some lymphoid cells (1). The BCR–ABL chimeric protein is a deregulated, constitutively active tyrosine kinase that is believed to be the primary, and possibly the only, driving force behind the disease (2). Bone marrow-derived metaphases from approximately 90% of CML cases harbour the t(9;22)(q34;q11) translocation, the smaller derivative of which is known as the Philadelphia (Ph) chromosome. The remaining patients either have a cryptic translocation between BCR and ABL that cannot be detected by routine cytogenetic analysis, or have a complex translocation that involves a third or more chromosomes in addition to chromosomes 9 and 22 (3). Depending on the precise positions of the breakpoints, some of these abnormalities may appear cytogenetically as a simple translocation between either 22q11 or, less commonly, 9q34 and a third chromosomal region. These translocations are usually referred to as simple variants of the Ph chromosome (3).

A minority of patients present with clinical and haematological features that are suggestive of CML, but are found to lack BCR–ABL. These individuals are considered to suffer from a related CMPD or myelodysplastic/myeloproliferative disorder (MDS/MPD), a heterogeneous spectrum of conditions for which the molecular pathogenesis is not well understood (1). Most of these cases have a normal karyotype, but a minority have a reciprocal translocation that disrupts specific tyrosine kinase genes, most commonly platelet-derived growth factor receptor β (PDGFRB) or fibroblast growth factor receptor 1 (FGFR1), resulting in the synthesis of constitutively active chimeric proteins that function in a manner analogous to BCR–ABL (4,5). With the advent of targeted signal transduction therapy, an accurate clinical and molecular diagnosis of CML and related diseases has become increasingly important. For example, the small-molecule ATP binding-site-directed compound imatinib mesylate (STI571) is effective in blocking the activity of ABL or PDGFRB fusion proteins, but is inactive against those that involve FGFR1 (5–7). Most patients with a CML-like disease in association with an apparently simple variant of the Ph chromosome are found to...
be BCR-ABL-positive on molecular analysis. Occasional patients, however, are BCR-ABL-negative and instead have distinct gene fusions; for example, the t(9;12)(q34;p13) fuses ETV6 (TEL) to ABL (8), the t(9;22)(p24;q11) fuses BCR to JAK2 (9) and the t(8;22)(p11;q11) fuses BCR to FGFR1 (10,11). Here we report two patients with a CML-like MPD in association with an acquired t(4;22)(q12;q11), which we demonstrate fuses BCR to the platelet-derived growth factor receptor α (PDGFRA). Unusually, one of the breakpoints in both cases falls within a coding exon and both have inframe mRNA fusions that include a BCR intron-derived sequence. Although overexpression or amplification of PDGFRA has been described in some malignancies (12–14), the t(4;22) is the first known translocation to involve this gene.

RESULTS

The t(4;22) disrupts BCR but not ABL

Cytogenetic analysis revealed the presence of a t(4;22) (q12;q11) in the bone marrow-derived metaphases from two patients with a presumptive diagnosis of CML based on clinical and haematological criteria (see below). A previously described multiplex RT-PCR (15) was negative, indicating the absence of either common or rare variant BCR-ABL mRNA fusions. To determine if the t(4;22) disrupted the BCR gene, we performed fluorescence in situ hybridization (FISH) using BAC bK143F12 as a probe against metaphases from patient 2, the only individual for whom sufficient material was available. As shown in Figure 1A, this clone hybridized to both the der(4) and the der(22) in addition to the normal chromosome 22, indicating a rearrangement within, or close to, BCR.

The chromosome 4 breakpoint disrupts PDGFRA

Most translocations that have been cloned from patients with chronic MPDs result in the fusion of a widely expressed partner gene to the entire catalytic domain of a tyrosine kinase that may or may not be normally expressed in haemopoietic cells. Chromosome band 4q12 contains three known tyrosine kinase genes, PDGFRA, KIT and KDR, all of which might be considered as candidates for involvement in the t(4;22). To test this possibility, we employed a multiplex RT-PCR assay that included four forward BCR primers (E1N+, exon 1; E5/6+, exon 5/6 junction; NB1+, exon 12; and C3+, exon 19) plus three reverse primers (PDGFRA-R, KIT-R and KDR-R) designed to regions encoding the beginning of the PDGFRA, KIT and KDR tyrosine kinase domains. Amplification products were obtained from cDNA of both patients, but not from controls (not shown). Using individual primer pairs, we were able to amplify products of approximately 300 bp and 970 bp for patient 1 and 2, respectively, using primers E5/6+ and PDGFRA-R (Fig. 2). To confirm disruption of PDGFRA, we performed FISH analysis with BAC RP11-231C18 (PDGFRA). Each clone hybridizes to both the der(4) and der(22), indicating that they span the chromosome 22 and chromosome 4 breakpoints, respectively.

![Figure 1. FISH analysis of patient 2 with (A) BAC bK143F12 (BCR) and (B) BAC RP11-231C18 (PDGFRA). Each clone hybridizes to both the der(4) and der(22), indicating that they span the chromosome 22 and chromosome 4 breakpoints, respectively.](image)

![Figure 2. RT-PCR analysis of t(4;22) and control patients using primers e5/6+ and PDGFRA.](image)
Unusual structure of the BCR-PDGFRA fusion mRNAs

Sequencing of the amplified products revealed the presence of unexpected additional nucleotides inserted between BCR and PDGFRA coding sequences. The chimeric mRNA junction from patient 1 consisted of BCR exon 7 followed by 24 bp of the beginning of BCR intron 7, followed by PDGFRA sequence that started within exon 12. For patient 2, BCR exon 12 was followed by a 12 bp insert followed by PDGFRA sequence, again starting within exon 12 but at a different position from that seen in patient 1 (Fig. 3A). The origin of the 12 bp insert in patient 2 was unclear: it did not

---

**Figure 3.** (A) Structure of the fusion mRNAs. Patient 1: BCR exon 7 (upper case) followed by 24 nucleotides of BCR intron 7 (lower case) followed by PDGFRA sequence within exon 12 (position 1874, accession no. M21574). Patient 2: BCR exon 12 (upper case) followed by a 12 bp insert (lower case) followed by PDGFRA sequence within exon 12 (position 1830). (B) Domain structure of BCR, PDGFRA and BCR-PDGFRA. The breakpoints in patients 1 and 2 are indicated. Abbreviations: TM, transmembrane domain; TK, tyrosine kinase; KI, kinase insert; OD, oligomerization domain; S/TK, serine/threonine kinase; Rho-GEF, Rho guanidine exchange factor homology domain; RacGAP, Rac GTPase domain.
correspond to the start of BCR intron 12 and it matched perfectly to multiple sites in the genome. In both cases, the reading frame between BCR and PDGFRA was maintained and the fusion mRNAs are predicted to be translated into chimeric proteins that contain the N-terminal coiled-coil domain of BCR and the entire tyrosine kinase domain of PDGFRA (Fig. 3B). The breakpoints in both patients fell within the region encoding the juxtamembrane domain of PDGFRA, which lies between the transmembrane and tyrosine kinase domains. Within BCR, the breakpoint for patient 1 fell within the region encoding the Rho-GEF (guanidine exchange factor) homology domain, whereas in patient 2 this domain if fully retained in BCR–PDGFRA. The fusion gene in patient 1 is predicted to encode a 1177-amino-acid protein of 131 kDa, whereas the fusion in patient 2 is predicted to encode a 1397-amino-acid protein of 157 kDa.

Primers were designed to amplify the reciprocal fusion mRNA (patient 1, BCRe8R and PDAe11F; patient 2, BCRe13– and PDAe11F) but PDGFRA–BCR transcripts were not detected in either patient (not shown).

Definition of the genomic breakpoints

For patient 1, inspection of the genomic sequences surrounding the point of fusion between BCR intron 7 and PDGFRA exon 12 did not reveal the expected GT and AG motifs that would be expected had these two regions been spliced together. An alternative possibility was that the translocation itself had directly fused BCR intron 7 to the middle of PDGFRA exon 12. To test this hypothesis, primers designed to BCR exon 7 (BCRe7+) and PDGFRA exon 12 (PDAe12–) were used to amplify genomic DNA from patient 1 and control individuals. These primers amplified the expected 110 bp product from patient 1 cDNA, and, in addition, amplified an identical size band from patient 1 genomic DNA from but not from DNA of controls (Fig. 4A). The sequence of the genomic product was identical to that obtained from cDNA, confirming that the translocation breakpoints corresponded to the points at which BCR and PDGFRA were fused in the mRNA (Fig. 5).

To determine if a similar event had taken place in patient 2, genomic DNA was amplified with primers NB1+ and PDAe12–. In this case, a genomic band that was specific to patient 2 was amplified that was considerably larger than the product obtained from cDNA (Fig. 4B). The sequence of this band showed that the chromosome 4 breakpoint lay within PDGFRA exon 12 at the same point as that seen in the mRNA fusion and that the chromosome 22 breakpoint was within BCR intron 12, immediately following sequence corresponding to the 12 bp mRNA insert. The 12 bp insert was immediately preceded by an AG dinucleotide and a polypyrimidine tract, which must have served as a cryptic splice to generate the mature composite mRNA (Fig. 5).

DISCUSSION

Several distinct, rare chromosomal translocations that disrupt chromosome bands 4q11 or 4q12 have been described in patients with leukaemia. Probably the most common is the t(4;12)(q11–12;p13) in acute myeloid leukaemia, which fuses the CHIC2/BTL gene at 4q12 to ETV6 at 12p13 (16). Here we describe two patients with a CM L-like MPD in association with a t(4;22)(q12;q11) that results in a fusion of BCR to PDGFRA.
Thus at least two distinct 4q12 genes are involved in haematological malignancies. As far as we are aware, only a single patient with a t(4;22)(q12;q11) has been described previously, in a patient described as having CML that was negative for BCR–ABL by FISH (17). A number of other patients, however, have been described with a CML-like CMPD and a translocation that involves 4q11–12 and chromosomes 3, 7 or 16 (18–21). In view of the similar patient phenotypes, is possible that PDGFRA is also disrupted in these cases.

Translocation-associated tyrosine kinase fusions proteins are constitutively active as a consequence of partner gene-dependent dimerization or multimerization, which mimics the normal activation process that is induced by binding of cognate ligands. For example, BCR contributes a coiled-coil oligomerization domain, encoded by exon 1 of the gene, that is essential for BCR–ABL-mediated transformation (22). PDGFRA encodes a receptor tyrosine kinase that is very similar in structure and sequence to PDGFRB, for which several fusion partners have been identified as a result of translocations in myeloid malignancies (reviewed in 23). Activated forms of both PDGFRA and PDGFRB are known to transform the murine myeloid cell line Ba/F3 to interleukin-3 independence (4,24), and it is highly likely therefore that BCR–PDGFRA has transforming activity in primary haematopoietic cells.

The anatomy of the t(4;22) was unusual in that the chromosome 4 breakpoints in both patients fell within PDGFRA exon 12 and short stretches of BCR intron were incorporated into the mature mRNA that maintained the reading frame. This is surprising, since the great majority of translocation breakpoints fall within introns, and the associated fusion mRNAs result from splicing together of whole exons. Occasional BCR–ABL fusions have been reported to result from translocation breakpoints within exons, but these are extremely uncommon (2). The genomic structure of the two genes cannot explain this finding, since no less than 12 BCR exons (numbers 2, 3, 4, 5, 7, 8, 10, 11, 15, 16, 17 and 22) are inframe with PDGFRA exon 12. One possible explanation is that PDGFRA exon 12 is particularly prone to double-strand breaks; however, this seems unlikely in view of the extreme rarity of PDGFRA fusions. Alternatively, inclusion of the first 12 amino acids encoded by the beginning of PDGFRA exon 12 might interfere with the catalytic activity of the fusion protein.

Our findings, and the recent identification of BCR–JAK2 and BCR–FGFR1 fusions, demonstrate that apparently simple variants of the t(9;22) in association with a clinical and haematological picture of CML do not necessarily indicate a masked BCR–ABL fusion. Currently there are reports of simple variant translocations involving 22q11 and various loci on nearly every other chromosome (http://cgap.nci.nih.gov/Chromosomes/Mitelman). The most common partners are 1p36, 7p22, 11p15, 12p13, 17q25, 19q13 and 22q13. Nevertheless, most patients with these translocations do in fact turn out to be BCR–ABL-positive, and so fusions of BCR to genes other than ABL are clearly very rare. However, with the advent of targeted therapeutic drugs such as imatinib mesylate, it is...
clearly imperative to define any disease at the molecular level. Imatinib mesylate is active against PDGFRs (7,25), and recent data have indicated that patients with PDGFRB fusions respond very well to treatment with this compound (26). Patients with a t(4;22) and BCR-PDGFRB fusion are therefore very good candidates for imatinib mesylate treatment.

MATERIALS AND METHODS

Case reports

Patient 1. A 37-year-old male with an unremarkable history was found to have a leukocytosis of 57.10^9/l during a routine health check. The differential showed 2% promyelocytes, 16% myelocytes, 6% metamyelocytes, 9% bands, 47% neutrophils, 9% lymphocytes, 4% monocytes, 2% basophils and 5% eosinophils. The haemoglobin level was 119 g/l and platelet count 96.10^11/l. Leukocyte alkaline score was 36 and serum biochemistry revealed an increased lactate dehydrogenase (LDH) of 421 U/l. The spleen size was enlarged. Morphology of the marrow showed a massive myeloid hyperplasia with pronounced promyelocytes and some blasts. There was a marked increase of eosinophils and also of normal and atypical megakaryocytes. Cytogenetics of bone marrow showed the presence of a reciprocal translocation t(4;22)(q12;q11); Southern blot analysis for BCR rearrangement and RT-PCR for BCR-ABL were negative. A diagnosis of atypical CML was made. The patient received an allograft from his HLA-identical sister 9 months after diagnosis. Currently the patient is alive and in good physical condition 85 months post transplant.

Patient 2. A 3-year-old boy with a history of repeated tonsillitis and otitis episodes was admitted to hospital with fever and right cervical lymphadenopathy. Physical examination revealed enlarged tonsils, small supravacular and inguinal lymphadenopathy, as well as liver and spleen enlargement. The white blood cell count was highly elevated at 101.10^9/l, with 37% neutrophils, 3% lymphocytes, 22% eosinophils, 1% basophils, 6% promyelocytes, 21% myelocytes, 10% metamyelocytes and 10% isolated blast cells. The haemoglobin level was 93 g/l and the platelet count was 143.10^9/l. Leukocyte alkaline phosphatase score was 20, and an increase in LDH levels (973 U/l) was the only remarkable finding in the serum biochemistry. The bone marrow aspirate showed myeloid hyperplasia (M:E ratio 10:1), with 69% of myeloid cells, 7% erythroid cells, 12% lymphocytes and 10% eosinophils without excess of blasts or myelodysplastic changes. Histological studies of the lymph node tissue revealed diffuse proliferation of small to medium-sized cells with scant basophilic cytoplasm, non-convoluted nuclei and inconspicuous nucleoli. There were isolated eosinophils and mature myeloid cells. Immunohistopathology of lymph node cells indicated that they were T-lineage (100% positivity for surface markers CD2, CD5, CD1a and CD7, 73% CD4, 24% CD8, 18% CD4/CD8 and 38% TdT). Reactivity was negative for myeloperoxidase and CD22, but positive for CD3 (98%) and CD79a (27%). Cytogenetics of directly cultured bone marrow and lymph node cells showed the presence of t(4;22)(q12;q11) in all metaphases (20/20). Phosphoaminoglutanin-stimulated peripheral lymphocytes showed a normal male karyotype, which ruled out a constitutional abnormality. FISH and RT-PCR for BCR-ABL were negative. PCR analysis for rearrangements of the immunoglobulin heavy chain (FR2) and T-cell receptor 3’/5’ showed germline configuration of both. Based on these findings, the patient was diagnosed with a CML-like myeloproliferative disorder with extramedullary T-lymphoid blast crisis, and was intensively treated with vincristine, cyclophosphamide, dexamethasone, asparaginase, high-dose methotrexate and cytarabine, followed by autologous peripheral blood stem cell transplantation. Central nervous system prophylaxis with intrathecal chemotherapy was also administered. The lymph nodes rapidly returned to normal size and the patient returned to chronic phase, with persistent myeloid hyperplasia and eosinophilia. The t(4;22) persisted, with a progressive increase in abnormal metaphases after transplant. After another course of intensive reinduction chemotherapy, he received an allograft from an unrelated donor. Unfortunately, the patient died on day 50.

FISH

BAC bK143F12 contains most of the BCR gene (10) and was provided by Dr M Rocchi, University of Bari, Italy. BAC RP11-231C18 contains the entire PDGFRB locus (Ensembl Genome Server, http://www.ensembl.org/) and was obtained from the Sanger Institute, Hinxton, UK. BAC DNA was isolated from 1.5 ml cultures using a standard miniprep protocol, and FISH was performed as described previously (10).

RT-PCR and PCR

RNA was extracted, reverse-transcribed with random hexamer primers and amplified as described previously (15). Control RNA was obtained from patients with Ph-chromosome-positive CML. Amplification reactions were performed for 30–32 cycles at an annealing temperature of 60°C. Primer sequences were:

E1N+ 5'-AGATCTGGCCCAACAGTGACCA-3'
E5/6+ 5'-GAAATCCCGGACAGACCTGAGA-3'
NB1+ 5'-GAGCCGTGACAGTGAGGGAGACA-3'
C3+ 5'-AGGTCTAAGTGGCCCTACAAT-3'
PDGFRB-R 5'-GGTTTTTAGCATCTTCACTGC-3'
KIT-R 5'-TTTCTCCCCAATCTAGCCCTGT-3'
KDR-R 5'-GCAAGTTGCTGTTCTTGTCAA-3'
PDAe11F 5'-GTCCTGTTGTCATTGTTGAA-3'
BCRE8R 5'-CTCTTCTACCCGTCATGACT-3'
BCRE13- 5'-ATGTCAGGGAGAGAGTTCTG-3'
BCRE7+ 5'-TACAAGCCTGTCACCCTGT-3'
REFERENCES


