

Heterogeneous t(4;11) fusion transcripts in two infants with acute lymphoblastic leukemia

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Abstract

An RT-PCR assay detected the t(4;11) translocation in two infants with acute lymphoblastic leukemia (ALL). Case P76 was a 10-month-old, female infant, who presented with a WBC of $137.4 \times 10^9/l$ and a pre-pre-B ALL immunophenotype. Case P120 was a 6-month-old female infant, with a WBC $> 615 \times 10^9/l$ and a pre-pre-B ALL immunophenotype. RT-PCR of cDNA from both these cases generated a 656 bp and a 542 bp respectively, which sequencing confirmed as t(4;11) fusion transcripts. The primers and conditions selected for this assay are compatible with a one-step multiplex PCR for the main translocations in childhood ALL.

Key words: RT-PCR assay, acute lymphoblastic leukemia

INTRODUCTION

The *MLL* gene, on chromosome band 11q23, is a partner in more than 30 translocations, including t(4;11), t(6;11), t(9;11), t(11;19).¹ These *MLL* translocations, both individually and collectively, are found in a cross section of leukemias.² The most common of these is the reciprocal t(4;11)(q21;q23) translocation³ which is an in-frame fusion of the *MLL* gene, also known as *HRX*, *HTRX* and *ALL-1*, to the *AF4* or *FEL* gene on chromosome 4.

The *MLL* gene codes for a protein that is found in the nuclei of most cell types including haematopoietic cells.⁴ The genomic structure of the 37-exon *MLL* gene, viewed from the 5' to the 3' end, features an A-T hook domain, a methyltransferase domain, a transrepression domain, two zinc-finger motifs which span the central break point cluster (bcr) region, a transactivation domain, and a 3' region which is homologous to the 3' end of the *Drosophila Trithorax* gene and includes the conserved SET (Suvar3-9, Enhancer - of zeste, Trithorax) domain.^{5,6} This homology to the *Drosophila* gene, *Trithorax*, is also seen in the two central zinc-

finger regions. The genomic structure and the homology to the *Drosophila Trithorax* gene suggest that *MLL* is a transcription factor. The fusion partner gene, *AF4*, codes for a protein that is also found in the nucleus of most cells.⁷ The gene has 21 exons and features nuclear localization (NLS) and guanosine triphosphate (GTP)-binding sequences downstream of its bcr which spans exon 3 to exon 7.^{8,9} It is a member of the *AF4*, *LAF-4*, and *FMR-2* gene family coding for serine/proline rich proteins, which appear to be transcription factors. Inactivation of *AF4* impairs the development of T and B lymphocytes¹⁰ while inactivation of *MLL* results in defective yolk sac haematopoiesis.¹¹

11q23 abnormalities feature in 70 to 80 % of infant leukemias, and t(4;11) is the most common translocation in this group.¹² In contrast, t(4;11) is only found in about 5 % of, both, childhood and adult leukemias.¹³ In all age groups, this translocation generally associates with acute lymphoblastic leukemia (ALL). However, it does occur in a small percentage of acute myeloid leukemias (3 %) and, interestingly, in a small percentage of treatment-related leukemias.¹⁴

MLL/AF4 positive ALL is characteristically associated with extremely high white cell counts ($> 100 \times 10^9/l$), CD10-negative pre-pre-B blast cells which often also express myeloid antigens, organomegaly, a noticeable female bias and a poor prognosis.¹⁴

The poor prognosis conferred by *MLL/AF4* makes early detection of the fusion transcript vital, especially for treatment decisions. While the fusion transcript is detectable by cytogenetic analysis, the possibility of failure does justify a back-up test, especially one that can later serve to monitor residual disease. The main purpose of this study was to establish an RT-PCR assay to detect t(4;11). The conditions of this assay were selected so as to ensure its compatibility with a multiplex PCR, which detects the other clinically important childhood translocations.

MATERIALS AND METHODS

Patients

Bone marrow/peripheral blood or stored cDNA, when available, from cases admitted to the Paediatric Institute, Kuala Lumpur Hospital, and included in the *TEL-AML1* study,¹⁵ were used in this study.

RT-PCR assay for the detection of t(4;11)

RNA was extracted from bone marrow/blood using the RNeasy Mini Blood Kit (Qiagen, Germany) as recommended by the manufacturer. First strand cDNA was synthesized with 1-3 μg of total RNA using SuperscriptTM II RNase H⁻ Reverse Transcriptase (Invitrogen, San Diego, CA) and random hexamers (Promega, USA) incubated at 42°C for 60 mins.

PCR amplification to detect the t(4;11) fusion transcript¹⁶ was carried out using the *MLL-2* and *FEL-2/AF4-2* primers.¹⁷ Essentially, cDNA corresponding to 80 - 200 ng of RNA was amplified in a 50 μl reaction containing 200 μM of each dNTP, 1 x PCR buffer, 1 mM MgCl₂, 2.5 U of AmpliTaq Polymerase (Applied Biosystems, USA), and 15 pmol of each primer. The cycling conditions were as follows: 94°C for 2 mins, followed by 40 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, with a 10 min final extension at 72°C. RNA integrity was ascertained by amplification of the β_2 -microglobulin gene with the following primer pair 3' β_2 , 5'-CCTCCATGATGCTGCTTACATGTC-3' and 5' β_2 , 5'-ATGTCTCGCTCCGTGGCCTTAGCT. Both negative and positive controls were used in all assays. cDNA from the MV4-11 cell line, kindly provided by

S. Moore, IMVS, Adelaide, which carries the *MLL/AF4* fusion transcript was used as the positive control. Rigorous precautions were used to prevent cross contamination.

7.2 μl of the PCR products were resolved on a gel of 2.5 % Agarose (Promega, USA), which was stained with ethidium bromide and then photographed under ultraviolet light.

Sequencing of the PCR product

The PCR products obtained were purified by Wizard^R PCR Preps DNA Purification System (Promega, USA) and submitted to Research Biolabs (M) Pte. Ltd. for sequencing. The purified DNA was directly sequenced using the Big Dye sequencing kit (Applied Biosystems, UK) in an ABI PRISM 377 DNA sequencer (Applied Biosystems).

RESULTS

Case P76

Case 76 was a 10-month-old, female, Malay infant, who had suffered intermittent fever for a month. She was pale, breathless and had a petechial rash on her lower limbs at presentation. She had a hepatomegaly of 4 cm, a splenomegaly of 9 cm and palpable cervical and inguinal lymph nodes. Her Hb was 5.4 gm %, WBC $137.4 \times 10^9/l$, platelets $10 \times 10^9/l$ and 89 % blasts in the peripheral blood. CSF was negative for blast cells. The bone marrow showed a hypercellularity with >80 % blasts. The case was classified as acute lymphoblastic leukemia with L1 morphology. The immunophenotype was CD10-, CD19+, CD22+, HLA-DR+, CD34+ *ie.* pre-pre-B ALL. The karyotype could not be determined at presentation. The child was treated according to the UKALL infant protocol¹⁸ and then a bone marrow transplant (BMT) was carried out. She is currently in remission with chronic graft-versus-host disease (GVHD).

Case P120

Case 120 was a 6-month-old, female, Chinese infant, who had suffered intermittent fever, pallor and a swelling in the neck for about three months. She had a hepatomegaly of 8 cm and a splenomegaly of 6 cm and palpable cervical and inguinal lymph nodes. The Hb was 9.6 gm %, WBC $> 615 \times 10^9/l$, platelets $27 \times 10^9/l$ and 92 % blasts in peripheral blood. CSF was negative for blast cells. The case was classified as ALL. Immunophenotyping showed the blasts to be pre-pre-B ALL in that they were CD10-, CD19+, CD22+, HLA-DR+, CD34+ and TdT+.

The karyotype could not be determined due to short spreads. The child was treated according to the infant UKALL protocol.¹⁸ She was treated with a BMT and is currently in remission.

RT-PCR detection and sequencing of t(4;11)

Amplification of cDNA generated a 656 bp product from P76, a 542 bp product from P120, and a 390 bp product from the MV4;11 cell line (Fig. 1). Sequencing (Fig. 2 and Fig 3) followed by a blast search and comparison with published sequences of the *MLL* and *AF4* genes revealed that while the fusion transcript from P76 comprised exon 11 of the *MLL* gene joined in frame to exon 4 of the *AF4* gene, that from P120 comprised exon 10 of the *MLL* gene joined in frame to exon 4 of the *AF4* gene. The 390 bp product of the MV4;11 cell line consisted of exon 9 of the *MLL* gene joined to exon 5 of the *AF4* gene.

DISCUSSION

The two female infants displayed characteristic t(4;11) presenting features in that they both had high white cell counts, extramedullary disease and leukemic blast cells which were CD10-negative, pre-pre-B in immunophenotype.

Although cDNA amplification from both patients generated two different bands, sequencing

confirmed that the bands were *MLL/AF4* fusion transcripts. As the break-point cluster region in *MLL* spans exon 8 to 12, and that in *AF4* spans exon 3 to 7, the different transcripts¹³ reflect the various possible combinations. Thus the 656 bp fusion transcript from P76 is an example of an e¹¹ - e⁴ transcript, in which exon 11 of the *MLL* gene is joined to exon 4 of the *AF4* gene. The 542 bp fusion transcript from patient P120 is of the e¹⁰ - e⁴ type, which is a fusion of *MLL* exon 10 of *MLL* to *AF4* exon 4. The relative frequencies of the various types of transcripts have been studied and the e¹¹ - e⁴ is the most common in infant leukemia, accounting for 55 % of the transcripts in this group. The P120 e¹⁰ - e⁴ fusion transcript is found in 18 % of infant leukemias. The fusion transcript in the control MV4;11 cell line is the e⁹ - e⁵ type which yields a relatively short transcript¹³ of 390 bp.

The amplified products described above are all examples of der(11) fusion transcripts. In these transcripts about a third of the 5' end of the *MLL* gene is joined to approximately three-quarters of the 3' end of the *AF4* gene. However, in a reciprocal translocation, such as t(4;11), both der(11) and der(4) fusion transcripts will be generated and this raises the issue of which transcript mediates leukemogenesis. In this regard, it has been shown that 1) while der(11) is consistently associated with t(4;11) cases, der(4)

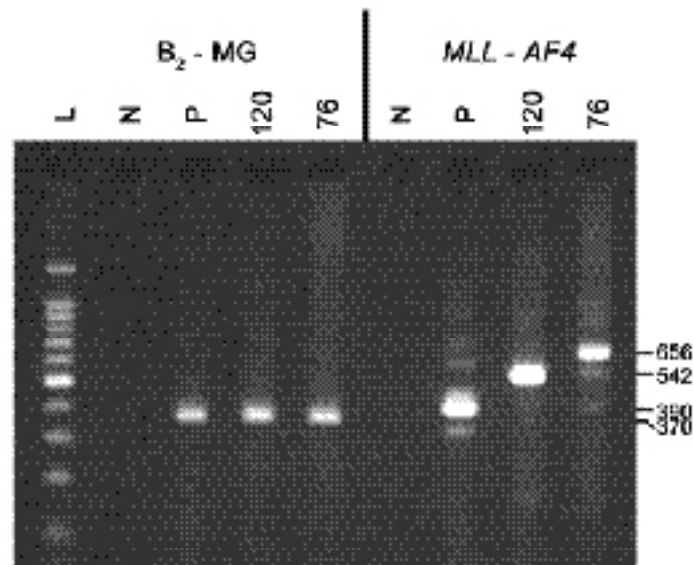


Fig. 1: Detection of t(4;11) transcripts by RT-PCR. Amplification generated a 656 bp transcript in P76, a 542 bp transcript in P120 & a 390 bp transcript in the MV4-11 cell line (P = MV4-11 cell line, N = Negative Control). The β_2 -MG gene was amplified to check the integrity of the RNA.

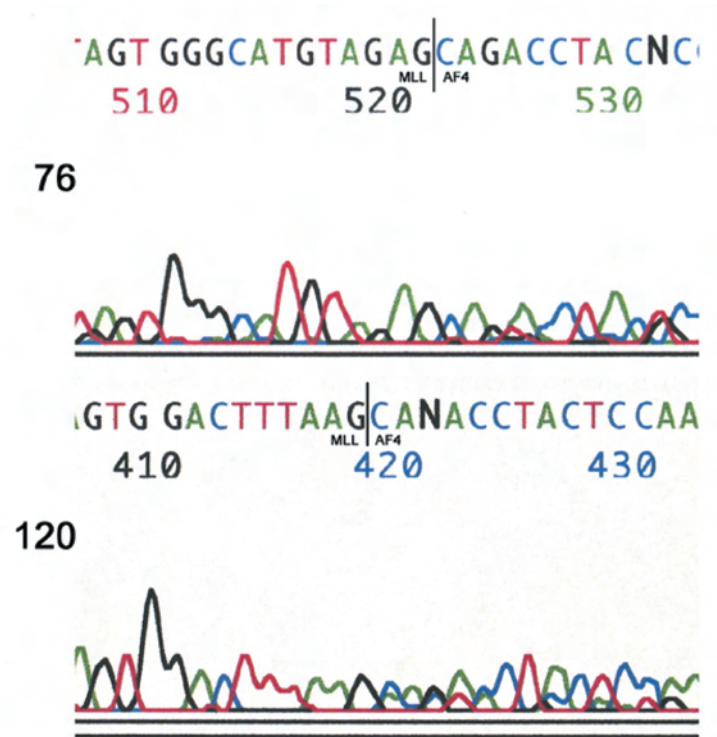


Fig. 2: Sequencing of t(4;11) transcripts. In P76, exon 11 of the *MLL* gene is fused to exon 4 of the *AF4* gene & in P120, exon 10 of the *MLL* gene is fused to exon 4 of the *AF4* gene. In both cases, the *MLL* gene lies to the left and the *AF4* gene to the right of the fusion junction, indicated by the vertical line.

was in found in 84 %¹⁹ and in 65 %²⁰ of cases, 2) der(11) is also always conserved²¹ in complex 11q23 translocations involving either 4q21, 6q21, 9p22 or 19p13 and a third gene, which appear to indicate that leukemogenesis is mediated by der(11). Marschalek *et al.*²², however, pointed out that a substantial portion of the literature on t(4;11) is focused on the der(11) fusion transcript. They postulate that the presence of der(4) transcript is more usual than unusual, and that its structural organization may enable it to arrest cell differentiation while that of der(11) may promote proliferation, which provides a role for both transcripts in leukemogenesis. If found to be true, this would further distinguish the *MLL* abnormalities, which are already quite distinct in that they feature a variety of partner genes, a short latency period in infants and treatment-related leukemias. Regardless of the outcome, it would appear that the *MLL* abnormalities provide a unique leukemogenesis pathway for study.

RT-PCR offers a rapid assay for the detection of t(4;11). It also offers a means of monitoring residual disease. Furthermore, we developed this particular assay as a first step towards a multiplex PCR, which detects the prognostically

significant childhood translocations *ie.* t(9;22), t(12;21), t(1;19) and t(4;11).

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P76

ttcnngnncga aaggccgntg cgtagnnctg ggcngancaa aaaggccacc actccagcct
 tccaggaagt caagcaagca ggtctccag ccagcactgg tcatcccgcc tcagccacct
 actacaggac cgccaagaaa agaagttccc aaaaccactc ctagttagcc caagaaaaag
 cagcctccac caccagaatc aggtccagag^{Exon 9} cagagcaaac agaaaaaagt ggctccccgc
 ccaagtatcc ctgtaaaaca aaaaccaaaa gaaaaggaaa^{Exon 10} aaccacctcc ggtcaataag
 caggagaatg caggcacttt gaacatcctc agcactctct ccaatggcaa tagttctaag
 caaaaaattc cagcagatgg agtccacagg atcagagtgg actttaagga^{Exon 11} ggattgtgaa
 gcagaaaatg tgtgggagat gggaggctta ggaatcttga cttctgttcc tataacacc
 aggggtggtt gcttctctg tgccagtagt gggcatgtag agcagaccta^{MLL ← → AF4 - Exon 4} cnccaatgaa
 gtccattgtg ttgaagagat tctgaaggaa atgaccatt catggccgcc tcctttgaca
 gcaatncata cgcctagtac agnnngacca

P120

ttcccnagng aagtanggat cgtatagtcg nctggagtgc tggnttacia nanagncana
 caggccgggc ncgtaggaan tcgnncaagc aggtctcca tananatnng ntcatcccgn
 ctcnncnacc tannacagga ccgccaagaa aagaagttcc caaaaccact cctagttagc
 ccaagaaaaa gcagcctcca ccaccagaat caggtccaga^{Exon 9} gcagagcaaa cagaaaaaag
 tggctccccg ccaagtatc cctgtaaaac aaaaccaaaa agaaaaggaa^{Exon 10} aaaccacctc
 cggtaataa gcaggagaat gcaggcactt tgaacatcct cagcactctc tccaatggca
 atagttctaa gcaaaaaatt ccagcagatg gagtccacag gatcagagtg gactttaagc^{MLL ←}
^{→ AF4 - Exon 4} anacctactc caatgaagtc cntngtgtg aanagatnct gaagganatg accattnat
 ggccgcctcc nttgacagna atncatcgc ctagtacang ntgaacca

Fig. 3: A partial nucleotide sequence of the der(11) fusion transcripts from Case P76 and Case P120. The fusion junction and the exons are indicated (*MLL* Accession no. NM_005933; *AF4* Accession no L13773).

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