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

Harvindar Kaur GILL a PhD, *TEN Sew Keoh b MSc, Jasbir Singh DHALIWAL a PhD, Sarah MOORE c BSc (Hons), Roshida HASSAN c MBCh, MP, Faraiza ABDUL KARIM d MD, MSc (Haematol), Zubaidah ZAKARIA d MBBS, DCP, Shahnaz MURAD e MBCh, MSc, Mahfuzah MOHAMED f MBBS, MMed (Paeds), Hishamshah MOHAMAD IBRAHIM f MD, MMed (Paeds) and Eni Juraida ABDUL RAHMAN f MD, MMed (Paeds)

a Allergy & Immunology Research Centre, Institute for Medical Research, Kuala Lumpur
b Cancer Research Centre, Institute for Medical Research, Kuala Lumpur
c Cytogenetics Department, Institute of Medical & Veterinary Science, Adelaide, South Australia
d Haematology Laboratory, Pathology Department, Kuala Lumpur Hospital
e National Blood Centre, Kuala Lumpur
f Paediatric Institute, Kuala Lumpur Hospital

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 x 10^9/l and a pre-pre-B ALL immunophenotype. Case P120 was a 6-month-old female infant, with a WBC > 615 x 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). These MLL translocations, both individually and collectively, are found in a cross section of leukemias. The most common of these is the reciprocal t(4;11)(q21;q23) translocation 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. 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. 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. 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. 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 lymphocytes while inactivation of MLL results in defective yolk sac haematopoiesis. 11q23 abnormalities feature in 70 to 80 % of infant leukemias, and t(4;11) is the most common translocation in this group. In contrast, t(4;11) is only found in about 5 % of, both, childhood and adult leukemias. 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.
**Malaysian J Pathol December 2004**

**MLL/AF4 positive ALL is characteristically associated with extremely high white cell counts (> 100 x 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.**

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, 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 Superscript™ 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 transcript was carried out using the *MLL*-2 and *FEL-2/AF4*-2 primers. 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 β₂-microglobulin gene with the following primer pair 3'β₂,-5'-GCCCTAGATGCGTCTTACATAGTC-3' and 5'β₂,-5'-ATGTCCTCGCTCGTGCCCTAGCT. 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® PCR Prep 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 x 10^9/l, platelets 10 x 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 protocol 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 x 10^9/l, platelets 27 x 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. 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 transcripts 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. 2: Sequencing of t(4;11) transcripts. In P76, exon 11 of the MLL gene is fused to exon 4 of the AF4 gene, and 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 found in 84% and in 65% of cases, 2) der(11) is also always conserved 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., 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 i.e. t(9;22), t(12;21), t(1;19) and t(4;11).

ACKNOWLEDGEMENTS

The technical assistance of Nadarajah P and Pathianathan M (Haematology Laboratory, National Blood Centre) and Saraswathi R, Fazli MY, Maimunah K and Siti Asanah MS (Pathology Department, Kuala Lumpur Hospital) is gratefully acknowledged. The authors thank the Director, Institute for Medical Research for permission to publish. This study was supported by a grant, SRG-2001-1, from the Ministry of Health, Malaysia.

REFERENCES

3. Secker-Walker LM on behalf of the European
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).