Molecular characterization of identical, novel *MLL-EPS15* translocation and individual genomic copy number alterations in monozygotic infant twins with acute lymphoblastic leukemia

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Citation: Kotecha RS, Ford J, Beesley AH, Anderson D, Cole CH, Kees UR. Molecular characterization of identical, novel MLL-EPS15 translocation and individual genomic copy number alterations in monozygotic infant twins with acute lymphoblastic leukemia. Haematologica 2012;97(9):1447-1450. doi:10.3324/haematol.2012.065730

Online Supplementary Design and Methods

Samples

To search for novel and rare mixed lineage leukemia (*MLL*) translocation partners, we performed banded chromosomal analysis and fluorescence *in situ* hybridization on diagnostic bone marrow samples of all infants with acute lymphoblastic leukemia (ALL) presenting to our institution, Princess Margaret Hospital for Children, between January 1983 and December 2011. Of 16 patients, 2 were unique, being monozygotic twins bearing a t(1;11) translocation. We undertook further molecular analysis of the leukemic cells of these twins.

Peripheral blood and bone marrow samples were collected at diagnosis and on remission from both twins according to cell collection protocols approved by the institutional review board of Princess Margaret Hospital for Children after informed consent was obtained in accordance with the Declaration of Helsinki. Total cellular DNA and RNA were extracted from the primary samples of both twins at diagnosis and remission using the AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. All samples and intermediate products from the 2 twins were stored in separate locations and were manipulated at different times and in different areas of the laboratory to avoid cross-contamination

Polymerase chain reaction and direct sequencing

Total cellular RNA was reverse transcribed using the Omniscript RT Kit (Qiagen), according to the manufacturer's instructions, to generate cDNA. The cDNA was amplified by polymerase chain reaction (PCR) using the following primers:

- 5'-GAAACCTACCCCATCAGCAA and 5'-AACGTGAGGAGGATCAATGC were used to amplify the *MLL-EPS15* transcript;
- 5'-GATTTGGGATTTAGCCGACA and 5'-TTTCGGCACTTATTACACTC were used to amplify the *EPS15-MLL* transcript.

Total cellular DNA was amplified by PCR with the following primers:

- 5'-TGCAGTGAGCTGTGACTGTG and 5'-TTAGCTTTTTCTGCAGGGGA were used to amplify the *MLL-EPS15* genomic fusion;
- 5'-CAGCCTGCCTCTTGTCAGAT and 5'-CCAGTTGGTGCTGATTTCCT were used to amplify the *EPS15-MLL* genomic fusion.

Each PCR was performed using an optimized GoTaq Flexi DNA polymerase protocol (Promega, Madison, WI, USA) with cDNA/DNA template at a concentration of between 100-200 ng. The PCR conditions used to amplify the samples consisted of initial denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 4 min. A final extension at 72°C was performed for 10 min following the 35 cycles. The positive control consisted of amplification with beta actin primers.

Direct sequencing was performed according to the manufacturer's instructions using the BigDye Terminator v3.1 Cycle Sequencing Kit and an automated sequencer (ABI 3130x1 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The same internal forward and reverse primers as specified in the PCR amplification reactions were used at a concentration of 1mM.

Whole-genome cytogenetic arrays

The Affymetrix Cytogenetics Whole-Genome 2.7M Array (Affymetrix, Santa Clara, CA, USA) technology was applied to detect submicroscopic aberrations and copy-number neutral loss of heterozygosity in each twin at diagnosis and remission. The arrays provide unbiased, whole-genome coverage with 2.7 million markers, including 400,000 single nucleotide polymorphisms and 2.3 million non-polymorphic copy-number markers. For each sample, 100 ng of genomic DNA were amplified by whole-genome amplification reaction, purified by magnetic beads, and fragmented to generate small (<300bp) products. These were subsequently labeled and loaded onto a single array. After hybridization, the chip was washed, stained and scanned. Raw data were analyzed with Affymetrix Chromosome Analysis Suite 1.01 software (Affymetrix) and compared to the manufacturer's recommended normalized reference. Filters that were applied included 45kbp for gains, 25kbp for losses and 1500kbp for copy-number neutral loss of heterozygosity. A 20-marker filter and 85% confidence was applied throughout. The filters selected were based on the manufacturer's recommendations and previous analysis of t(4;11) infant ALL using the same platform.[1] All reported lesions were visually examined, and for each identified region of interest a further in-depth analysis was undertaken in the other twin below the filter thresholds to ensure that no relevant results were missed. A tumor-associated copy-number alteration was defined as a copy-number alteration present in the tumor but not the remission DNA of each patient and for which there was no overlap with the Database of Genomic Variants.[2]

References

1. Bardini M, Galbiati M, Lettieri A, Bungaro S, Gorletta TA, Biondi A, et al. Implementation of array based whole-genome high-resolution technologies confirms the absence of secondary copy-number alterations in MLL-AF4-positive infant ALL patients. Leukemia. 2011;25(1):175-8.
2. http://projects.tcag.ca/variation/