A novel TCF3-HLF fusion transcript in acute lymphoblastic leukemia with a t(17;19)(q22;p13)

Ioannis Panagopoulos a,b,*, Francesca Micci a,b, Jim Thorsen a,b, Lisbeth Haugom a,b, Anne Tierens c, Aina Ulvmoen d, Sverre Heim a,b,e

a Section for Cancer Cytogenetics, Institute for Medical Informatics, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; b Centre for Cancer Biomedicine, Faculty of Medicine, University of Oslo, Oslo, Norway; c Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Oslo, Norway; d Women and Children’s Division, Department of Paediatric Medicine, Ulleval Hospital, Oslo University Hospital, Oslo, Norway; e Faculty of Medicine, University of Oslo, Oslo, Norway

A 10-year-old boy was admitted to the hospital because of anemia detected after a two week history of fatigue, dizziness, nausea, headaches, and weight loss. A bone marrow investigation confirmed a diagnosis of acute lymphoblastic leukemia of the B-cell precursor phenotype. Chromosome G-band analysis yielded the karyotype 46,XY,t(17;19)(q22;p13), and fluorescence in situ hybridization (FISH) analysis showed rearrangement of the genes TCF3 (on 19p13; accession number NM_03200 version 3) and HLF (on 17q22; accession number NM_002126 version 4) with the generation of a TCF3-HLF chimera. Polymerase chain reaction and sequencing analyses demonstrated the presence of two in-frame chimeric TCF3-HLF transcripts. In the first one, which corresponds to a type 2 fusion, exon 15 of TCF3 is fused to exon 4 of HLF. In the second, described here for the first time and named type 3, exon 14 of TCF3 is fused to exon 4 of HLF. Whether the type 3 chimeric transcript has the same DNA binding and transcriptional regulatory effect as type 1 and type 2 TCF3-HLF chimeras remains to be seen.

Keywords: Acute lymphoblastic leukemia, cytogenetic, t(17;19)(q22;p13), TCF3, HLF, fusion gene

© 2012 Elsevier Inc. All rights reserved.

The t(17;19)(q22;p13) chromosomal translocation was first described in 1991 by Raimondi et al. (1) in four cases of acute lymphoblastic leukemia (ALL): in two cases as the sole aberration, in one with additional cytogenetic abnormalities, and in the fourth as a der(19)t(17;19)(q22;p13) in a complex karyotype. In the same year, Ohyashiki et al. (2) also reported a t(17;19)(q22;p13) where the breakpoint on chromosome 17 was interpreted as being somewhat more proximal in a patient with ALL, and they established a cell line (HAL-01) from the blood of the patient. In 1992, Hunger et al. (3) focused their work on the HAL-01 cell line and showed that the molecular result of the t(17;19)(q21;p13) is the rearrangement of the genes TCF3 (alias E2A; in 19p13) and HLF (in 17q21, as they saw it) and the generation of a TCF3-HLF chimera. Inaba et al. (4) showed that the t(17;19)(q22;p13) also resulted in the generation of the TCF3-HLF chimera. The molecular data therefore proved that the two aforementioned translocations were in fact one and the same, despite the breakpoint being variably interpreted (17q21 or 17q22). According to the Mitelman database (5), there have now been 10 ALL cases reported with a t(17;19)(q22;p13), nine ALLs described with a t(17;19)(q21;p13), one described with a t(17;19)(q21-22;p13), and six reported with a der(19)t(17;19) with breakpoints attributed at the cytogenetic level to 17q21 or 17q22.

Both the TCF3 and HLF genes encode transcription factors. The TCF3 gene codes for a member of the E protein (class I) family of helix-loop-helix transcription factors (http://www.ncbi.nlm.nih.gov/gene/6929). E proteins activate transcription by binding to regulatory E-box sequences on target genes as heterodimers or homodimers and are inhibited by heterodimerization with the inhibitor of DNA-binding (class IV) helix-loop-helix proteins. The HLF gene codes for a member of the proline- and acidic-rich (PAR) protein family, a subset of the bZIP transcription factors.

* Corresponding author.
E-mail address: ioannis.panagopoulos@rr-research.no

Received August 26, 2012; received in revised form September 26, 2012; accepted October 5, 2012.
was shifted to the ALL Relapse Berlin-Frankfurt-M
and avascular necrosis of the knees. During maintenance
delayed several times due to complications from acute
The MRD on day 79 was negative. His treatment has been
0.14%, and he was allocated to the intermediate risk group.
minimal residual disease (MRD) by flow cytometry was
http://helseforskning.etikkom.no), and written informed
(Regional komité for medisinsk forskningsetikk Sør- Norge,
http://helseforskning.etikkom.no), and written informed
Case history
A 10-year-old boy born in Norway but of Pakistani descent
was admitted to the Children’s Hospital because of anemia
detected after a two week history of fatigue, dizziness,
nausea, headaches, and a 4-kg weight loss. On clinical
examination, he had pale skin and mucous membranes,
multiple hematomas on the legs, and a palpably enlarged
spleen. The peripheral blood values were hemoglobin 45 g/L,
white blood cells 6.0 × 10^9 cells/L, platelets 105 × 10^9 cells/
L, absolute neutrophil count 2.3 × 10^9 cells/L, lactate dehy-
drogenase 1191 U/L, lactate 15.0 mmol/L, pH 7.34, partial
pressure of carbon dioxide 3.5 kPa, bicarbonate 13.8 mmol/
L, base excess -10.7 mmol/L, and anion gap 26 mEq/L.
A bone marrow investigation confirmed a diagnosis of ALL of
L, base excess -10.7 mmol/L, and anion gap 26 mEq/L.
A bone marrow investigation confirmed a diagnosis of ALL of
B-cell precursor ALL, and the prognoses have been dismal.
Relapse follows in cases where complete remission was
achieved, even when stem cell transplantation was tried as consolidation therapy (6).
Materials and methods
Case history
A 10-year-old boy born in Norway but of Pakistani descent
was admitted to the Children’s Hospital because of anemia
detected after a two week history of fatigue, dizziness,
nausea, headaches, and a 4-kg weight loss. On clinical
examination, he had pale skin and mucous membranes,
multiple hematomas on the legs, and a palpably enlarged
spleen. The peripheral blood values were hemoglobin 45 g/L,
white blood cells 6.0 × 10^9 cells/L, platelets 105 × 10^9 cells/
L, absolute neutrophil count 2.3 × 10^9 cells/L, lactate dehy-
drogenase 1191 U/L, lactate 15.0 mmol/L, pH 7.34, partial
pressure of carbon dioxide 3.5 kPa, bicarbonate 13.8 mmol/
L, base excess -10.7 mmol/L, and anion gap 26 mEq/L.
A bone marrow investigation confirmed a diagnosis of ALL of
B-cell precursor phenotype. There was no central nervous
system (CNS) involvement. The boy was treated according to
the Nordic Society of Pediatric Hematology and Oncology
(NOPHO) ALL 2008 non—high risk protocol. On day 29, the
minimal residual disease (MRD) by flow cytometry was
0.14%, and he was allocated to the intermediate risk group.
The MRD on day 79 was negative. His treatment has been
delayed several times due to complications from acute pancreatitis, secondary insulin-dependent diabetes mellitus, and avascular necrosis of the knees. During maintenance
treatment, CNS leukemic relapse was identified, and he
was shifted to the ALL Relapse Berlin-Frankfurt-Münster
(ALL-REZ BFM) 2002 protocol. Currently, the patient is in his
second remission.
The study was approved by the regional ethics committee
(Regional komité for medisinsk forskningsetikk Ser- Norge,
http://helseforskning.etikkom.no), and written informed
consent was obtained from the patient’s parents.
G-banding and karyotyping
Bone marrow cells were cytogenetically investigated by
standard methods. Chromosome preparations were made
from metaphase cells of a 24-hour culture, G-banded using
Leishman stain, and karyotyped according to International
System for Human Cytogenetic Nomenclature (ISCN) 2009
guidelines (7).
Fluorescence in situ hybridization analyses
Bacterial artificial chromosome (BAC) clones were retrieved
from the Human “32K” BAC Re-Array library (BACPAC
Resources, http://bapac.chori.org/home.htm). They were
selected according to physical and genetic mapping data on
chromosomes 17 and 19 (see [section name]) as reported on
the Human Genome Browser at the University of California,
Santa Cruz website (May 2004, http://genome.ucsc.edu/).
Fluorescence in situ hybridization (FISH) mapping of the
clones on normal controls was performed to confirm their
chromosomal location. The clones used were RP11-456C20
(green) and RP11-349F1 (red) for the HLF locus and RP11-
81M8 (yellow) for the TCF3 gene. DNA was extracted and
probes were labeled and hybridized as previously described
(8). Chromosome preparations were counterstained with
0.2 μg/mL 4’,6-diamidino-2-phenylindole(DAPI) and overlaid
with a 24 × 50 mm² coverslip. Fluorescent signals were
captured and analyzed using the CytoVision system (Applied
Imaging, Newcastle, UK).
Polymerase chain reaction analyses
In a 20 μL reaction volume, 1 μg of total RNA was reverse-
transcribed using an iScript Advanced complementary DNA
cDNA) Synthesis Kit for reverse transcription—quantitative
polymerase chain reaction (RT-qPCR) according to the
manufacturer’s instructions (Bio-Rad, Hercules, CA). For the
detection of the TCF3-HLF fusion transcript, the 25 μL PCR
volume contained 12.5 μL of OneTag 2 × Master Mix with
Standard Buffer (New England BioLabs, Ipswich, MA), 1 μL
of diluted cDNA, and 0.2 μM of each of the forward TCF3-1120F
(TCCAGCCCTTTCTACCCCGTGG) and reverse
HLF-1345R (GCATTGGCCAGCTCTTTCTCTCAAA) primers.
In a semi-nested PCR using the forward primer TCF3-1193F
(CCGGTCCTATCGGCCAGTAC) and the reverse
primer HLF-1301R (GGGGCATTGCTCTTTCTCTAGA),
1 μL of the amplified product was used as the template. For
amplification of a TCF3 cDNA fragment, in the first PCR the
TCF3-1120F forward primer was used with the TCF3-1174R
(TCTGTCCTGGGGGAGAAGGTG). In the second (semi-
nested) PCR, the primers TCF3-1193F and TCF3-1174R were used. The PCRs were run on a C-1000 Thermal
cycler (Bio-Rad). The PCR conditions for both the first and
second amplifications were an initial denaturation at 94°C for
30 seconds; 35 cycles of 15 seconds at 94°C, 30 seconds at
58°C, and 1 minute at 68°C; and a final extension for
5 minutes at 68°C. Next, 2 μL of the second PCR amplifi-
cation were run on a 1.5% agarose gel, stained with GelRed
(Biotium, Hayward, CA), and photographed. The amplified
products were excised from the gel, purified using the Qia-
gen gel extraction kit (Qiagen, Hilden, Germany), and cloned
into the pCR4-TOPO vector using TOPO TA Cloning Kits for
Sequencing (Invitrogen, Carlsbad, CA).
Colonies were sequenced at GATC Biotech (Konstanz,
Germany). The Basic Local Alignment Search Tool (BLAST)
software (http://www.ncbi.nlm.nih.gov/BLAST/) was used for
the computer analysis of sequence data.
Results
The G-banding and FISH analyses yielded the karyotype
46,XY,t(17;19)(q22;p13) (Figure 1A). When metaphase
spreads were hybridized with the BAC RP11-81M8, which is a
TCF3-specific probe, a split signal was seen and part of the
probe had moved to the derivative chromosome 17,
Figure 1  Cyogenetic, FISH, and PCR analyses. (A) A partial karyotype showing the two derivative chromosomes, der(17)t(17;19)(q22;p13) and der(19)t(17;19)(q22;p13), from the 17;19-translocation with their corresponding normal homologues; the breakpoint positions are indicated by arrows. (B) FISH using BAC RP11-81M8 (yellow), a TCF3-specific probe, showed that part of the probe had moved to the derivative chromosome 17 indicating that the translocation breakpoint on 19p13 was within the TCF3 locus. FISH with the RP11-456C20 (green) and RP11-349F1 (red) probes for the HLF locus showed that the HLF gene was also rearranged and that probe RP11-349F1 (red) was moved onto the derivative chromosome 19. (C) cDNA fragment amplifications of TCF3-HLF (lane 1) using primers TCF3-1193F and HLF-1301R and normal TCF3 with primers TCF3-1193F and TCF3-1674R (lane 2). M, 1 Kb Plus DNA ladder (GeneRuler, Fermentas). (D) Partial sequence chromatograms of the 170 bp cDNA fragment showing that exon 14 of TCF3 is fused to exon 4 of HLF. (E) Partial sequence chromatograms of the 380 bp cDNA fragment showing that exon 15 of TCF3 is fused to exon 4 of HLF. (F) A diagram showing the three known variants of TCF3-HLF chimeric transcripts.

A novel TCF3-HLF transcript in acute lymphoblastic leukemia
indicating that the translocation breakpoint on 19p13 was within the TCF3 locus (Figure 1B). FISH with RP11-456G20 (green) and RP11-349F1 (red) for the HLF locus showed that the HLF gene was also rearranged and that probe RP11-349F1 (red) was moved to the derivative chromosome 19 (Figure 1B).

PCR amplification generated two fragments, 380 bp and 170 bp in size (Figure 1C). Sequencing of the amplified fragments showed that, in the 380 bp fragment, exon 15 of TCF3 was fused to exon 4 of HLF and, in the 170 bp fragment, exon 14 of TCF3 was fused to exon 4 of HLF (Figures 1D and 1E).

Discussion

Previously, two types of TCF3-HLF chimeric transcripts were detected (9). In the early literature, the TCF3-HLF type 1 transcript was described as having exon 13 of TCF3 fused to exon 4 of HLF. At the junction, there was an insertion of a segment from intron 13 of TCF3 that maintained an open reading frame and led to the encoding of a functional TCF3-HLF chimeric protein. Type 2 was defined as the transcript in which exon 12 of TCF3 was fused to exon 4 of HLF resulting in an in-frame TCF3-HLF transcript. Thus, both type 1 and type 2 TCF3-HLF fusion proteins contain the same portion of HLF but differ in the TCF3 moiety. In vitro experiments have nevertheless shown that the TCF3-HLF type 1 and 2 chimeric proteins have the same behavior in all DNA binding and transcriptional regulatory assays suggesting that the insertion and/or presence of TCF3 exon 13 is of little or no functional consequence (10). Of the 17 patients so far reported with TCF3-HLF chimeric transcripts, six had type 1, nine had type 2, and two had both types (3,4,9,11–15). In early studies, an association between TCF3-HLF type 1 and disseminated intravascular coagulation (DIC) was seen (9). However, DIC was later found in patients with the type 2 transcript (15). Hypercalcemia complications were also found to be frequent in leukemias with the t(17;19) and TCF3-HLF expression, seemingly without any relation to whether the transcript was type 1 or 2 (13).

Only one reported TCF3-HLF chimeric mRNA sequence, M95586, is in the National Center for Biotechnology Information (NCBI) database. The sequence, which was found in the cell line UOC-B1, corresponds to the type 1 TCF3-HLF fusion (4). According to the current reference sequence of TCF3 with accession number NM_003200.3, transcript variant 1, the portion of TCF3 in the M95586 sequence corresponds to exons 1–16 of the TCF3 sequence NM_003200.3. The same applies to the TCF3-HLF fusion protein sequence found in the HAL-01 cell line presented by Hunger et al. (3): the TCF3 part of the chimeric protein is encoded by exons 1–16 of TCF3. Thus, according to the current reference nomenclature, type 1 is the fusion of exon 16 of TCF3 with exon 4 of HLF (accession number NM_002126.4) (Figure 1F). In the same vein, type 2 TCF3-HLF corresponds to the fusion of exon 15 of TCF3 with exon 4 of HLF (Figure 1F). The present case had two TCF3-HLF chimeric transcripts: the type 2, fusion of exon 15 of TCF3 with exon 4 of HLF (accession number NM_002126 version 4), and a novel in-frame type 3 transcript resulting from the fusion of exon 14 of TCF3 with exon 4 of HLF (Figure 1F). Whether the new type 3 chimeric transcript has the same DNA binding and transcriptional regulatory behavior as that displayed by type 1 and type 2 TCF3-HLF chimeras remains to be seen.

Acknowledgments

This work was supported by grants from the Norwegian Cancer Society and Helse Ser-Ost.

References