An Adult Patient with Early Pre-B Acute Lymphoblastic Leukemia with t(12;17)(p13;q21)/ZNF384-TAF15

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Abstract. This is a case report of a 46-year-old man diagnosed with early pre-B acute lymphoblastic leukemia (ALL), bearing the translocation t(12;17)(p13;q21) as the sole chromosomal abnormality. This is a rare chromosomal abnormality that has been reported in approximately 25 cases worldwide. FISH analysis revealed a rearrangement of ZNF384 (12p13) and TAF15 (17q12) genes, which is usually associated with a pre-B ALL phenotype with co-expression of the myeloid markers CD13 and/or CD33. ZNF384 encodes a zinc finger protein, which acts as a transcription factor, regulating the expression of several matrix metalloproteinases and TAF15 belongs to the FET (FUS, EWS, and TAF15) family, consisting of RNA and DNA-binding proteins. Unlike most of the cases where CD10 expression was absent or weak, in our case CD10 was highly expressed. The prognostic significance of ZNF384/TAF15 fusion is not very clear since several reports support a generally good prognosis, while others support a poor clinical outcome. Our patient was treated with the German multicenter ALL (GMAALL) protocol for B-ALL, but experienced a fulminant gram-negative sepsis and eventually died during induction therapy.

Translocation t(12;17)(p13;q21) is very rare and has been reported in 25 cases worldwide (1), either as a sole cytogenetic abnormality or coexisting with other chromosomal abnormalities (2-5), mainly in patients with pre-B acute lymphoblastic leukemia (ALL). Only four cases of acute myelogenous leukemia (AML) with t(12;17)(p13;q21) have been reported, one of which was first diagnosed as pre-B ALL, but on the second relapse it was transformed to AML (6). All but one cases (7) were positive for CD13 and/or CD33.

In 11 cases, molecular testing was performed and a fusion of ZNF384 (mapping on 12p13) and TAF15 (mapping on 17q21) genes was revealed (Table I). ZNF384 encodes for a zinc finger protein, which acts as a transcription factor, regulating the expression of several matrix metalloproteinases (MMP1, MMP3, MMP7 and COL1A1) (12). TAF15 belongs to the FET family (FUS, EWS and TAF15), formerly known as TET family, consisting of RNA and DNA-binding proteins. More specifically, TAF15 is a subunit of transcription factor II D (TFIID), which is part of the polymerase II complex that is formed on the gene promoter site (13). Rearrangements of ZNF384 have been reported with several other partner genes such as TCF3 (on 19p13.3), EP300 (on 22q13.2), EWSRI (on 22q12.2) or ARID1B (on 6q25.3) (14). Again, all gene alterations were reported in patients with pre-B ALL and expressed CD13 and/or CD33.

Case Presentation

A 46-year-old Greek Caucasian man was admitted due to leukocytosis detected during evaluation of a 2-month history of malaise and a 20-day history of fever without an identifiable site of infection. His past medical history was unremarkable.
On clinical examination, he was feverish and had a palpable non-tender spleen about 2 cm below the left costal margin. The initial laboratory examination revealed leukocytosis (14.5×10^9/l), normocytic anemia (Hb, 11.3 g/dl) and thrombocytopenia (115×10^9/l). The biochemistry panel revealed high uric acid and LDH levels, while his blood and urine cultures were negative. Examination of a peripheral blood smear revealed 70% blasts that were identified by flow cytometry as CD19+, CD10+, CD79a+, CD34+, CD123+, HLA-DR+, Tdt+ and CD13+ (dim), a picture compatible with B-acute lymphoblastic leukemia (B-ALL). The patient tested negative for a BCR/ABL rearrangement and the cytogenetic analysis revealed t(12;17)(p13;q11). A cerebrospinal fluid analysis showed absence of blasts and the patient was treated according to the German multicenter ALL (GMALL) protocol for B-ALL.

On day 22, the patient had an episode of neutropenic fever treated with meropenem and vancomycin. Unfortunately, despite timely treatment initiation, he developed severe septic shock, rhabdomyolysis and acute renal injury and eventually died from fulminant gram-negative sepsis.

Materials and Methods

G-Banding preparation. Bone marrow aspirates were obtained at diagnosis and short-term cultures were set up according to standard protocols (15). Cells were cultured in RPMI (Roswell Park Memorial Institute) medium 1640 (1x) complete with Glutamax supplemented with 20% Fetal bovine serum (Gibco, Grand Island, NY, USA) and 2% penicillin/streptomycin. Colcemide (Invitrogen, Carlsbad, CA, USA) was added to the cultures 15 minutes before harvest. Cells were harvested after treatment with 0.075 M KCl hypotonic solution and 362K1 overlapping the TAF15/ZNF384 and ETV6 genes. Both genes were amplified using a random primer and hybridized to Vysis LSI break apart probes for TAFL5, ZNF384 and ETV6, respectively (10).

FISH preparation. Three FISH probes were used: LSI Vysis ETV6 Break Apart FISH Probe Kit (Vysis, Des Plaines, IL, USA) and two different break-apart FISH probes for ZNF384 and TAF15 genes. Both probes were designed from BAC clones RP11-151M4 and RP11-362K1 overlapping the ZNF384 and TAF15 genes, respectively (10).

Slides with fixed cells were co-denatured with ETV6 Break Apart FISH probe on Thermo Brite machine (Abbott, Chicago, IL, USA) for 5 min at 75°C and then incubated in a humidified chamber for 16 h at 37°C. The slides were then washed twice in 50% formamide and 2×SSC (saline sodium citrate) for 10 min each time, to remove random non-specific hybridizations. Finally, the slides were washed with 0.15% NP40 (Vysis) and counterstained with DAPI (4,6-diamidino-2-phenylindole) (Vysis). Slides were analyzed under the Fluorescent Microscope Olympus BX51 using the CytoVision™ v3.6 software system of Applied Imaging. Detailed FISH procedures were previously reported (10).
Results

Cytogenetic analysis. Cytogenetic analysis of G-Banded chromosomes revealed that t(12;17)(p13;q21) translocation was the only identified chromosomal abnormality and was observed in 11 out of 17 metaphases: 46,XY,t(12;17)(p13;q21)(11)/46,XY(6) (Figure 1).

FISH analysis. FISH analysis using ETV6 break-apart probe detected a normal hybridization pattern in interphase nuclei, as well as in metaphase nuclei, excluding the possibility of ETV6 gene rearrangement (Figure 2A). In a metaphase stained with DAPI antifade, it was shown that both fluorescent probes for ETV6 remained on chromosome 12, indicating that the breakpoint position was more distal on 12p (Figure 2B, C).

FISH analysis using break-apart probes for ZNF384 and TAF15 detected rearrangements of both genes, confirming the ZNF384-TAF15 fusion (Figure 2D, E).

Discussion

There is an unequivocal relation between t(12;17)(p13;q21) rearrangement and specific antigen(s) expression. Regardless of the partner gene (TCF3, EP300, EWSR1, ARID1B or TAF15), with which ZNF384 was rearranged, CD13 and/or CD33 antigen expression was consistently observed in all reported cases. In the great majority of cases, CD10 expression was weak or absent, but it was highly expressed in our case (2, 8). CD10 expression has been associated with poor clinical outcome due to decreased tendency of cells to undergo apoptosis (2, 4). Interestingly, from a cohort of 260 pediatric patients with precursor B cell acute lymphoblastic leukemia, 26% of patients that expressed CD13 and/or CD33 but did not express CD10, were positive for ZNF384 rearrangements. Prognosis also seemed to be favorable regarding pediatric patients with several other genetic aberrations and adult patients with the same gene fusion (14).

In reported cases with t(12;17)(p13;q12), several breakpoints had been assigned to derivative chromosome 17, ranging from q11 to q21, but it's highly probable that most, if not all, involved TAF15 gene rearrangements take place on 17q12 (10).

Although several reports support a generally good prognosis for patients with the ZNF384/TAF15 rearrangement (16), other reports support a poor clinical outcome. This is probably because, despite CD13 and/or CD33 expression, these cases show a high degree of heterogeneity concerning different breakpoints within TAF15 ranging from exon 4 to exon 10, broader gene mutation status, additional chromosomal abnormalities, diversity in CD10 expression, age of onset and clinical features (6, 10, 17).

Fusions between genes coding FET proteins and transcription factors have been described in several solid...
tumors of mesenchymal origin and acute leukemia (17). It is thought that transcriptional deregulation is the main oncogenic event. In acute leukemia, the ZNF384 transcription factor is recurrently fused to two FET proteins, EWSR1 and TAF15. On both cases the amino-terminal SYGQ-rich region, is fused to the entire ZNF384 protein sequence (13). The transactivating properties of both fusion proteins were investigated on HEK293T cell extracts. Surprisingly, neither enhanced nor decreased expression of metalloproteinases was observed (17).

Although EWSR1/ZNF384 and TAF15/ZNF384 fusion proteins do not cause tumorigenesis by elevated expression of metalloproteinases, their oncogenic potential in acute leukemias is beyond any doubt. Alternative oncogenic pathways, such as their effect on pre-mRNA splicing, are under investigation.

The incidence of this rare gene rearrangement, followed by a severe infection makes this case interesting. Metalloproteinases, among others, are involved in regulating activation and release of cytokines, chemokines, growth
factors and antibiotic peptides, contributing actively to innate and adaptive immunity (18). Although ZNF384 acts as a transcription factor on several metalloproteinases, its fusion to TAF15 protein does not alter their transcription level in HEK293T cells (17), but this may happen in other cell types. The fact as well, that none of the reported cases, correlated this gene rearrangement with increased infection risk, cannot support a biological connection between the TAF15/ZNF384 fusion and severe sepsis.

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References


