LETTER TO THE EDITOR

A novel fusion of SQSTM1 and FGFR1 in a patient with acute myelomonocytic leukemia with t(5;8)(q35;p11) translocation

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Hematological malignancies with FGFR1 abnormality (8p11 myeloproliferative syndrome; EMS) are rare atypical stem cell disorders characterized by eosinophilia, T-cell proliferation and progression to acute myeloid leukemia.1,2 In EMS, fibroblast growth factor receptor 1 (FGFR1) gene at 8p11 is disrupted by chromosomal translocation, resulting in the formation of chimeric products with various partner genes. To date, 13 FGFR1 partners have been identified: ZMYM2 (ZNF198) at 13q12; CNTR at 9q33; FGR1OP at 6q27; BCR at 22q11; HERVK at 19p13.3; FGR1OP2 at 12p11; TRIM24 (TIF1) at 7q34; CPSF6 at 12q15; MYO18A at 17q23; LRRFP1 at 2q13; CXU1 at 7q34; TPR at 1q25; and RANBP2/NUP358 at 2q12.3-5 In all reported cases in which chimeric transcripts were cloned, N-terminal portion of the predicted fusion protein is composed of a partner gene with a dimerization domain, which may induce the constitutive activation of FGR1 tyrosine kinase in the C-terminal portion, leading to the cellular transformation.6

Here, we report the identification of sequestosome 1 (SQSTM1) gene at 5q35 as a novel fusion partner of the FGR1 in a patient with acute myelomonocytic leukemia presenting 8p11 chromosomal abnormality.

A 76-year-old man was referred to our hospital for anemia with hemoglobin level of 5.0 g/dl. The white blood cell count was 4.1 × 10^6 cells/l with 11% blasts, 1% eosinophils, 29% neutrophils and 30% monocytes. Bone marrow (BM) was hypercellular with 43% blasts and 17% monocytes. Dysplastic changes were observed in peripheral neutrophils and BM megakaryocytes. He was diagnosed as acute myelomonocytic leukemia and received blood transfusion therapy for anemia, chemotherapy was not done because of poor performance status. He died of progressive disease 15 months after the diagnosis.

Chromosome analysis of the BM cells showed the karyotype as 46,XY,t(5;8)(q35;p11) (20/20 cells) (Figure 1a). To characterize the 8p11 translocation, Southern blot analysis of DNA from the patient’s peripheral leukemic cells was performed using a genomic probe containing FGR1 exon 9 and 10, as chromosomal breaks occur within intron 8 in most cases with EMS. Abnormal bands were observed in sample with BamHI or HindIII digestion (Figure 1b), confirming that FGR1 was rearranged by the translocation.

To determine the FGR1 partner gene, we searched for a FGR1 chimeric transcript by 5'-rapid amplification of cDNA end (RACE) method using 5'-Full RACE core set (Takara Bio, Otsu, Japan). Total RNA was extracted from the leukemic cells and the first cDNA strand was synthesized with phosphorylated primer in FGR1 exon 12 (5'-GCCAGATACTCCATGGCTC-3') by reverse transcription. After ligation, cDNA was amplified by PCR with FGR1-10R1 (5'-ACACACACCTGGCAAAGCAGC-3') and FGR1-10F1 (5'-AGGCT ATCGGCGCTGGAAGG-3') primers in exon 10. The PCR product was further amplified with FGR1-10R2 (5'-CAGGGGTTTTGGCCCTAA GACGAG-3') and FGR1-10F2 (5'-CTGTGAGGCAAAGTGCCCTTG-3') primers in exon 10. An abnormal band was amplified and sequencing of the PCR product revealed that FGR1 was fused to a 5' foreign sequence that was identified as exon 9 of SQSTM1 at chromosome 5q35, showing that SQSTM1 was juxtaposed to FGR1 as the result of the chromosomal translocation.

To ascertain the formation of chimeric transcript, we performed reverse transcription PCR (RT-PCR) analysis, using SQSTM1-8F1 (5'-CTGGAAGACGTGGAGAGG-3') for exon 8 and FGR1-10R1 primers. An amplified product was obtained from the patient’s leukemic cells (Figure 1c). Sequencing analysis of the PCR product revealed that the SQSTM1 exon 9 was fused to FGR1 exon 9 (Figures 1c and d).

A reciprocal FGR1-SQSTM1 fusion transcript was also detected in using forward FGR1-7F1 (5'-GTAATCCTATCGGACTCCTCCC-3') for exon 7 and reverse SQSTM1-11R1 (5'-CCGGGGATGTGGTTAAGA-3') for exon 11 primers (Figures 1c and d).

To identify the chromosomal breakpoints, genomic DNA was amplified by the long-and-accurate PCR method using LA Taq polymerase (Takara Bio). The following primers were used: FGR1-7F1 and FGR1-7F2 (5'-TCTGCAGTTGGAGCTGCTG-3') for exon 7 outer and inner; FGR1-10R1 and FGR1-10R2 for exon 10 outer and inner; SQSTM1-9F1 (5'-GATATCAGGTGAGATCGGG-3') and SQSTM1-9F2 (5'-TCCAGAGAGTCCAGACAGAGG-3') for exon 9 outer and inner; and SQSTM1-10R1 (5'-TGGTTTACAGGCTTCCC-3'), SQSTM1-10R2 (5'-TTGAGCTTCGTTTCACTGG-3') for exon 10 outer and inner, respectively. Genomic fragments corresponding to the der(5) and der(8) chromosomes were amplified by primers around the predicted breakpoints on both chromosomes (Figures 1e and f). The sequence analysis of the PCR products identified the chromosome 5 and 8 breakpoints within SQSTM1 intron 9 and FGR1 intron 9, respectively (data not shown).

As sequence databases contain alternative transcripts for SQSTM1 coding N-terminal truncated protein (NCBI Reference Sequence: NM_001142298.1 and NM_00114299.1), we performed RT-PCR using primers located in SQSTM1 alternative exons 1, 2 or 4 and FGR1 exon 9 and detected only a chimeric transcript from SQSTM1 exon 4 coding the full N-terminal end of SQSTM1 (data not shown).

SQSTM1 at chromosome 5q35 encodes a multifunctional protein that binds ubiquitin and regulates activation of the NF-κB signaling pathway, associating with oxidative stress and autophagy.6-9 Mutations in this gene were reported to cause Paget disease of bone.10 The SQSTM1-FGFR1 transcript encodes a predicted chimeric protein of 718 amino acids, containing the N-terminal protein-protein interaction domain, P1, of SQSTM1 (ref. 11) and the tyrosine kinase domain of FGFR1 (Figure 2). SQSTM1-FGFR1 may induce the constitutive activation of tyrosine kinase by P1-mediated dimerization, thus leading to the cellular transformation.

Recently, the generation of SQSTM1-NUP214 and SQSTM1-ALK fusion genes were reported in one case with adult T-cell acute lymphoblastic leukemia presenting t(5;9)(q35;q34) translocation and two cases with anaplastic lymphoma kinase (ALK)-positive large B-cell lymphoma, respectively.12-15 SQSTM1-ALK was shown to possess transforming activity in 3T3 fibroblasts, probably by the constitutive activation of ALK tyrosine kinase by P1-mediated
To our knowledge, our case is the third instance of hematologic malignancy, in which SQSTM1 was involved in chromosomal translocation. SQSTM1 may be a recurrent target of chromosomal aberration.

Our patient, diagnosed as acute myelomonocytic leukemia, lacked typical EMS features, such as myeloproliferation, eosinophilia or lymphadenopathy involved by T-cell proliferation. Multilineage dysplasia indicated the leukemic transformation at early myeloid precursor level, but involvement of the T-cell lineage was not determined. SQSTM1-FGFR1 and reciprocal FGFR1-SQSTM1 fusion gene may exert to promote proliferation of immature myelomonocytic cells preferentially. Further investigation is needed to clarify this point.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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