Chimeric antisense RNA derived from chromosomal translocation modulates target gene expression

Chromosomal translocations in hematologic malignancies[1] are closely related to the molecular pathogenesis. Usually, the directions of the two genes involved in the chromosomal translocations are the same, resulting in chimeric proteins that retain their functional domains. Here we report a chromosomal translocation from a myelodysplastic syndrome (MDS) patient resulting in a fusion gene consisting of the sense strand of the *TEL/ETV6* gene on 12p13 fused with the antisense strand of the Thousand-and-one amino acid protein kinase 1 (*TAOK1*) gene on 17q11. We suggest the possibility that the chimeric transcript may act as an antisense RNA on wild-type *TAOK1* mRNA, resulting in downregulation of *TAOK1* protein expression.

A 73-year old man was admitted to our hospital because of severe anemia (hemoglobin 5.5 g/dL) and thrombocytopenia (platelets 72×10⁹/L). Bone marrow aspiration indicated dysplasia in three lineages with 11% blasts. The patient was diagnosed with MDS-RAEB-II. Chromosomal analysis of bone marrow cells revealed t(12;17)(p13;q11). Four months after diagnosis, disease progression to acute myelogenous leukemia was confirmed without additional chromosomal abnormalities. Conventional chemotherapy was performed, but he died of leukemia progression nine months after diagnosis.

Using leukemia cells at diagnosis, fluorescent *in situ* hybridization (FISH) analysis showed a split signal of *TEL/ETV6* gene (Figure 1A and B). The *TEL/ETV6* fusion transcript was amplified by 3′-RACE[2] and analyzed with DNA sequencing (*Online Supplementary Appendix*). The 3′ end of the exon 2 of *TEL/ETV6* was fused with antisense sequences of intron 19 of *TAOK1*, which was followed by antisense sequences of exon 19 and intron 18 of *TAOK1* (Figure 1C) (GenBank #JN603181). Amino acid sequences of the carboxyl (C)-terminus of exon 2 of TEL/ETV6 were followed by nonsense sequences derived from the antisense *TAOK1* sequences of intron 19 (Figure 1C and D).

Expression of the fusion transcript (*TEL-TAOK1ap*) was checked in primary bone marrow cells from MDS patients and cell lines by RT-PCR (TT-U and TT-L primers in Figure 1D), and showed that only the patient's sample that held t(12;17) expressed the *TEL-TAOK1ap* transcript (Figure 2A, lane 1). Expression of the wild-type (WT) TAOK1 protein was confirmed with immunoblotting using whole-cell lysates from cell lines and primary bone marrow cells from MDS patients (Figure 2B), indicating that the level of WT-TAOK1 protein expression was much lower in the patient's cells that held t(12;17) (lane 8) than in normal bone marrow cells (lane 1) and several leukemia cell lines (lanes 2-7). Samples from other MDS patients (lanes 9-13) were analyzed; some patients also showed lower expression of WT-TAOK1 protein (lanes 9 and 10). Expression levels of *TAOK1* mRNAs were confirmed with quantitative (real-time) RT-PCR using the 3' and 5' region-specific probe sets for *WT-TAOK1* transcripts (Figure 2C). Expression of 5'- and 3′-*TAOK1* was lower in the t(12;17) patient's cells than in leukemia cell lines (Figure 2D). The expression levels of 5' and the 3' *TEL/ETV6* mRNAs were mostly similar in the patient's cells in semi-quantitative RT-PCR analysis (*data not shown*). These data suggest that WT-TAOK1 protein expression is down-regulated in some MDS cases by unknown molecular mechanisms.

Next, we hypothesized that the antiparallel portion of exon 19 of *TAOK1* in *TEL-TAOK1ap* transcript may act as an antisense RNA to knock-down *WT-TAOK1* mRNA expression. To test this hypothesis, the *TEL-TAOK1ap* transcript was over-expressed in 293T cells. As a control, shRNA for *TAOK1* mRNA was transfected into 293T cells, resulting in a significant reduction in endogenous TAOK1 protein (Figure 2E). When using the *TEL-TAOK1ap* expression vector in 293T cells, endogenous TAOK1 protein expression was reduced in a dose-depen-

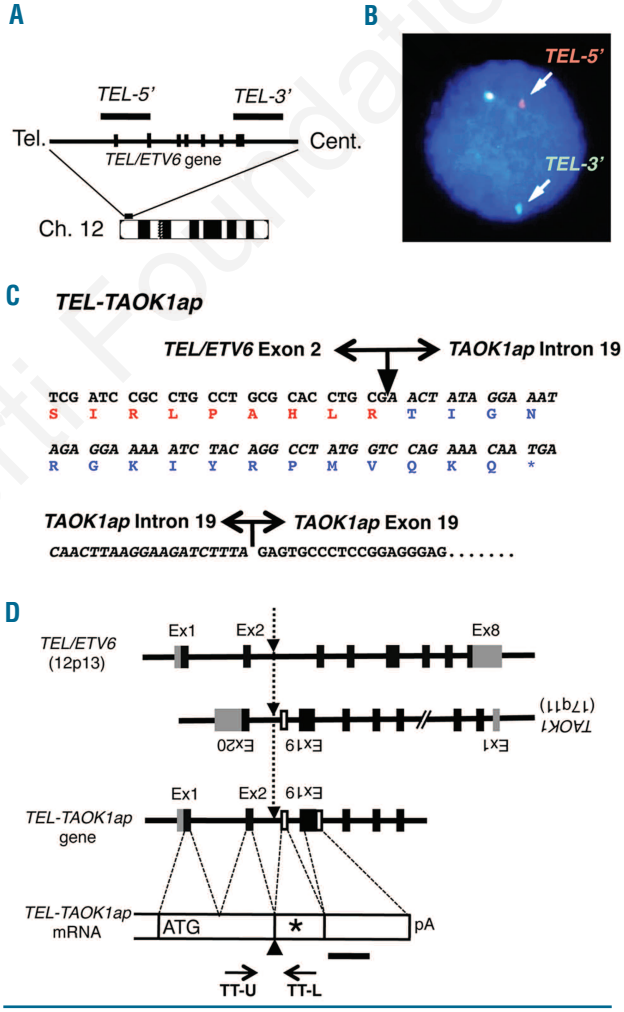

Figure 1. Aberrant fusion transcript derived from chromosomal translocation t(12;17)(p13;q11). (A) FISH probes for *TEL/ETV6*. (B) A split signal was observed in the patient's leukemia cells. Red signal is from the TEL-5' probe, and green signal is from the *TEL*-3' probe. Non-split signal from wild-type *TEL/ETV6* is observed as a yellow dot. (C) DNA sequence of the chimeric transcript (TEL-TAOK1ap) is shown in black letters. Amino acid sequence from exon 2 of TEL/ETV6 is indicated in red letters. Amino acid sequence from the antiparallel sequence of intron 19 of *TAOK1* is indicated in blue letters. Note that amino acid sequence in blue letters is completely different from the sequence of WT-TAOK1 protein. (D) Schematic representation of genomic structure around the breakpoint. Tel: telomere; cent: centromere; ap: antiparallel sequence; Ex; exon, *: stop codon; pA: poly adenine tail, black boxes; exons, shaded boxes; untranslated exon, white boxes; intron sequences that are utilized as exons in *TEL-TAOK1ap*.

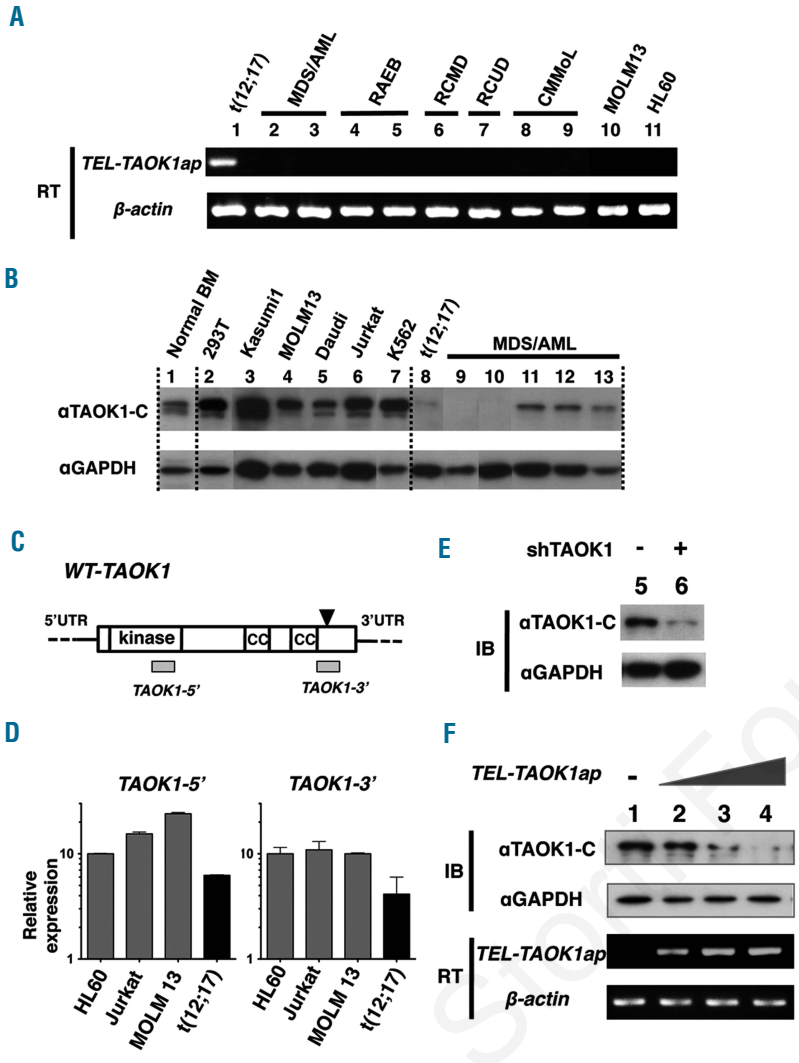

Figure 2. Expression of *TEL-TAOK1ap* chimeric transcript and its RNA interfering effect in regulating endogenous TAOK1 expression. (A) *TEL-TAOK1ap* transcript and (B) WT-TAOK1 protein expression in the patient's primary cells and human leukemia cell lines. RT-PCR (RT) (A) and immunoblotting (IB) using an anti-TAOK1-C-terminus antibody (B) were performed. The patient's samples holding t(12;17)(p13;q11) are indicated as t(12;17). Primary patient's bone marrow cells were used in lanes 1 to 9 in (A) and lanes 8 to 13 in (B). Beta-actin and GAPDH are the positive controls for RT-PCR and immunoblotting, respectively. (C) Schematic representation of *WT-TAOK1* mRNA. Shaded boxes indicate probes for quantitative RT-PCR. Black triangles indicate break point in the *TEL-TAOK1ap* fusion. (D) Quantitative RT-PCR using TAOK1-specific probes as indicated in (C). (E) Expression vector for shRNA against *TAOK1* mRNA or control mock vector (3 mg each) was transfected into 293T cells (5×10^5 cells). Immunoblotting for endogenous TAOK1 was performed using anti-TAOK1 C-terminus antibody. (F) Expression vector for *TEL-TAOK1ap* (0, 0.5, 1, and 3 μg in each sample) or the control mock vector (3, 2.5, 2, and 0 μg in each sample) was transfected into 293T cells (5×10^5 cells). Endogenous TAOK1 protein expression (upper panel) and over-expressed TEL-TAOK1ap transcript (third panel) were confirmed by IB and RT. Note that the endogenous TAO1 protein expression level was decreased in a dose-dependent manner. MDS/AML; acute myelogenous leukemia followed by MDS; RAEB: refractory anemia with excess blasts; RCMD: refractory cytopenia with multilineage dysplasia; RCUD: refractory cytopenia with unilineage dysplasia; CMMoL: chronic myelomonocytic leukemia; UTR: untranslated region; CC: coiled-coil domain.

dent manner (Figure 2F). These findings support our hypothesis that *TEL-TAOK1ap* has an RNA-interfering effect on *WT-TAOK1* mRNA.

A previous report showed that translocation of t(12;17)(p13;p12-p13) in secondary AML results in fusion of *TEL/ETV6* and the antisense strand of *PER1*.[3] Expression of the chimeric transcript containing antisense sequences to *PER1* was confirmed in this case. Recently, RNA interfering activity by small non-coding RNAs, such as small interfering RNA, micro-RNA, and PIWI-RNA,[4] has been reported. Furthermore, several reports have indicated that long non-coding RNAs[5] and natural antisense transcripts[6] play crucial roles in regulating mRNA expression of target genes. Our findings suggest a mechanism in which a chimeric transcript regulates target gene expression via an RNA interfering effect.

The *TEL-TAOK1ap* chimeric transcript may have dual functions, including an antisense effect to interfere with *WT-TAOK1* mRNA and production of C-terminally-truncated TEL/ETV6. A previous report indicated that TAOK1 is a serine/threonine kinase that plays an important role in the p38 MAPK signaling pathway.[7] Knockdown of TAOK1 in HeLa cells disrupts normal cell division due to a disorder in the spindle checkpoint function.[8] In our experiment (Figure 2B), some MDS patients showed relatively lower expression of WT-TAOK1 protein compared with acute leukemia cell lines, suggesting that lower expression might be related to the pathogenesis of MDS, such as aberrant cell division and/or dysplasia. Furthermore, there are many reports of TEL/ETV6 fusion proteins that contain the N-terminus of TEL/ETV6 in leukemia patients,[9] suggesting that aberrant substitution or truncation of the C-terminus of TEL/ETV6 may contribute to leukemia biology. The biological significances of *TEL-TAOK1ap* chimeric transcripts and their relationship with MDS/leukemia genesis require further study.

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