Chimeric antisense RNA derived from chromosomal translocation modulates target gene expression

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Online Supplementary Design and Methods

Fluorescent in situ *hybridization (FISH)*

FISH analysis was performed by SRL, Inc. (Tachikawa, Japan) using TEL/ETV6 5′ and 3′ DNA probes (main text, Figure 1A).

DNA and RNA preparation and DNA sequencing

The genomic DNA and the total mRNA was obtained from the patient's primary leukemia cells after obtaining appropriate informed consent. Extraction of genomic DNA and total RNA was performed as described.[1,2] DNA sequencing was performed using ABI PRISM 310 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Reverse transcriptase-polymerase chain reaction (RT-PCR) and three-prime rapid amplification of cDNA ends (3′-RACE)

RT-PCR and 3'-RACE was performed as described.[1,3,4] Briefly, the following primer was used for the RT reaction: QT; 5′-TGA GCA GAG TGA CGA GGA CTC GAG CTC AAG CTT TTT TTT TTT TTT TT-3′. For the first and the second amplification steps, the following primers were used: *5′-TEL41F*; 5′-CTC AGT GTA GCA TTA AGC AGG AAC G-3′ and *3′-Q0*; 5′-CCA GTG AGC AGA GTG ACG-3′, and *5′-TEL338F*; 5′-GAT CTC CTC ATT CAG GTG CTG TG-3′ and *3′-Q1*; 5′-GAT CTC CTC ATT CAG GTG CTG TG-3′.[4] For the RT-PCR of wild-type *TAOK1* mRNA, the following primers were used: TAOK1-5'-U; 5'- CGA GAT GTG CGT ACC AAT GA-3', TAO1-5'-L; 5'- AAG CAG AGC CAA AGT CAG CA-3', TAOK1-3'-U 5'-AAA TGC TCT CCA CAC AAG CC-3', and TAOK1-3'-L; 5'-TTA TGT ATA AGA CAT GTG TGA CC-3'. For *TEL/ETV6* mRNA, the following primers were used: TEL-5'-U; 5'- GAA CTT CCT GAT CTC TCT CG-3', TEL-5'-L; 5'- ATC GAG TCT TCC TCC ATC CT-3', TEL-3'-U 5'- AAG CCC ATC AAC CTC TCT CA-3', and TEL-3'-L; 5'- AGT TTG TAG TAG TGG CGC AG-3'. For confirmation of the *TEL-TAOK1ap* (ap; antiparallel) fusion transcript expression, the following primers were used: TT-U; 5'-AGC CCA GTG CCG AGT TAC G-3' and TT-L; 5'-GTT TGG ATG AAG CAC AGG AAG-3'.

Quantitative RT-PCR using real-time PCR method

Quantitative RT-PCR was carried out using TaqMan PCR (ABI prism 7000, Applied Biosystems) as previously described.[1] The following probe sets were used for the amplification: *5'-TAOK1*; Hs00687856_m1 (Invitrogen, Carlsbad, CA, USA), and *GAPDH*; Human GAPD Endogenous Control (VIC®/TAMRA Probe, Invitrogen). The *3'-TAOK1* probe set covering the translocation break point was designed as followed: forward primer; 5'-GCT TCG CGA GCT TGA ACA G-3', reverse primer; 5'-GCA AAG CCA ACA TCT CTT CTT CAA T-3', and the reporter; 5'-CCG GAG GGC ACT CTT AGA A-3'.

Expression vectors

The coding sequence followed by 3'-untranslated region of *TEL-TAOK1ap* was amplified by PCR using primers TEL-U1; 5′-ATG TCT GAG ACT CCT GCT CA-3′, and TAOKap-L; 5′-GTT TGG ATG AAG CAC AGG AAG-3′. The products were cloned into the pcDNA4-TOPO TA cloning vector. For short-hairpin RNA (shRNA) expression, the short *TAOK1* sequence (GTA AGA GTT TGA AGT CTA AA) was cloned into the pS65R-shH1 vector as previously described.[3]

Transformation of the expression vector

The expression vector was transfected into 293T cells as described[2,3] using the FuGene6 reagent (Roche).

Immunoblotting

Immunoblotting was performed as described[2] using anti-TAOK1 C-terminus (Abcam, Cambridge, UK) and anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies.

References

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