The Sox4/Tcf7l1 axis promotes progression of BCR-ABL-positive acute lymphoblastic leukemia

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SUPPLEMENTARY INFORMATION

Online Supplementary Methods.

Establishment of ALL cell lines.

First, fetal liver cells were isolated or B220⁺ bone marrow B cells were sorted out by magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany) from Sox4^fl/fl^Rosa26-eYFP (experimental) and Sox4^fl/+^Rosa26-eYFP (control) mice. The cells were then cultured with OP-9 bone marrow stromal cells in the presence of IL-7 to obtain pure B220+ pro-B cells. Subsequently, the pro-B cells were transformed with p190 BCR-ABL (mCherry⁺) by retrovirus-mediated transfection. Cherry⁺ cells were then sorted out and transfected again, 5 days post transformation, with self-excising Cre recombinase (SE-Cre) to induce Sox4 inactivation. Finally, about 10 days after SE-Cre transfection, eYFP⁺ Sox4^fl/fl^SE-Cre or Sox4^fl/+^SE-Cre pro-B cells were sorted out for in vitro experiments.

For in vivo experiments, transformed eYPF⁺ Sox4^fl/fl^SE-Cre or Sox4^fl/+^SE-Cre cells (Figure 2a-c) or transformed Sox4^fl/fl^;Cre-ER;eYFP cells (Figure 2d-f) were transfected with luciferase-expressing eCFP retroviral vector. eCFP⁺ cells were then sorted out and expanded for transplantation.

Gene expression microarray.

The experiments were performed with use of an Agilent (Santa Clara, CA, USA) gene expression microarray according to the manufacturer’s instructions.

Virus production and transduction.

Virus particles were prepared using phoenix cells derived from 293T human embryonic kidney cells. Phoenix cells were cultured in DMEM (Gibco) supplemented with 10% heat inactivated FBS (Atlanta biological, Lawrenceville, GA, USA), 100 units/ml penicillin, and 100 µg/ml streptomycin (phoenix medium). For virus production, 1 x 10^6 phoenix cells were seeded in 60-mm dishes and transiently transfected with complexes assembled from 1 µg of viral vector, 1 µg of packaging plasmid (pCL-ECO), and 6 µl of Fugene-6 (Promega, Madison, WI, USA). Complexes were removed from the cells after 18 hours of transfection and fresh medium was replaced. Cell supernatants containing virus particles were harvested after 48 hours of incubation and filtered through 0.45 µM syringe filers (Fisher scientific, Hudson, NH, USA). For virus transduction, pro-B cells were seeded in phoenix medium supplemented with 50 µM β-mercaptoethanol, 1 µg/ml
protamine sulphate (Sigma), and 10 ng/ml IL-7 and filtered virus supernatants were added in 1:1 ratio and centrifuged at 1200 rpm for 45 minutes. Virus-containing medium was replaced with fresh pro-B medium at the end of 15 hours of incubation.

**Vector construction for mutational analysis.**

The 460 bp fragment of Tcf7l1 promoter (nucleotides -460 to -1) was amplified from genomic DNA of a C57BL/6J mouse by PCR with specific primers (forward: 5’ aaaaaagaattcgcagaaggaagaccccaggtagc 3’; reverse: 5’ aaaaaagaattcgcagaaggaagaccccaggtagc 3’). The mutant Tcf7l1 reporter was generated by using the pair of primers with modification in one of them (forward: 5’ aaaaaagaattcgacgagaaggaagaccccaggtagc 3’; reverse: 5’ aaaaaagaattcgacgagaaggaagaccccaggtagc 3’; the mutation site is bolded). The PCR products were then inserted into the EcoRI site of the luciferase-expressing pSIN viral vector.
Online Supplementary Figures.

Figure S1.

**Figure S1.** Effect of Sox4 deletion on the proliferation of ALL cells by MTS proliferation assay. The p190 BCR-ABL (mCherry+) transformed Sox4fl/fl SE-Cre cells exhibited a lower proliferation rate than did the transformed Sox4fl/+ SE-Cre cells. Data are representative of three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 MTS proliferation assay. Briefly, cells were seeded into 96-well plates (10,000 cells per well in 100 μl medium) in 3 replicates. After addition of 20 μl CellTiter 96 Aqueous One Solution (Promega, Madison, WI, USA), the cells were incubated at 37°C for 4 h before the absorbance at 490 nm was measured daily for 4 days.
**Figure S2.** Effect of Sox4 délletion on the apoptosis of ALL cells by Annexin V/7-AAD staining assay. Cells were stained with Annexin V-APC and 7-AAD and analyzed by flow cytometry. Data are representative of three independent experiments.
Figure S3. Effect of Tcf7l1 knockdown on the proliferation of BCR-ABL–transformed wild-type pro-B cells by MTS proliferation assay. Knockdown of Tcf7l1 resulted in a significant reduction in cell proliferation. Data are representative of three independent experiments. Tcf7l1 shRNA: Tcf7l1 specific shRNA; NT shRNA: non-targeting shRNA. *p < 0.05, **p < 0.01, ***p < 0.001
Figure S4.

**Figure S4.** Effect of Tcf7l1 knockdown on the apoptosis of BCR-ABL–transformed wild-type pro-B cells by Annexin V/7-AAD staining assay. Cells were stained with Annexin V-APC and 7-AAD and analyzed by flow cytometry. Data are representative of three independent experiments.