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

Haiqing Ma,[1,2] Saradhi Mallampati,[1] Yue Lu,[3] Baohua Sun,[1] Enze Wang,[1] Xiaohong Leng,[4] Yun Gong,[5] Haifa Shen,[6] C. Cameron Yin,[7] Dan Jones,[8] Hesham M. Amin,[7] M. James You,[7] Patrick Zweidler-McKay,[9] Yupo Ma,[10] Hagop M. Kantarjian,[11] Ralph B. Arlinghaus,[4] Armand Glassman,[12] and Xiaoping Sun[1]

[1]Department of Laboratory Medicine and the Center for Stem Cell and Developmental Biology, The University of Texas MD Anderson Cancer Center (MDACC), Houston, TX, USA; [2]Department of Oncology, The Fifth Affiliated Hospital of Sun Yat-sen University, Zhuhai, China; [3]Department of Molecular Carcinogenesis, The University of Texas MDACC, Houston, TX, USA; [4]Department of Translational Molecular Pathology, The University of Texas MDACC, Houston, TX, USA; [5]Department of Pathology, The University of Texas MDACC, Houston, TX, USA; [6]Department of Nanomedicine, Houston Methodist Research Institute, TX, and Department of Cell and Developmental Biology, Weill Cornell Medical College, New York, NY, USA; [7]Department of Hematopathology, The University of Texas MDACC, Houston, TX, USA; [8]School of Health Sciences, The University of Texas MDACC, Houston, TX, USA; [9]Department of Pediatrics, The University of Texas MDACC, Houston, TX, USA; [10]Department of Pathology, Stony Brook University Medical Center, Stony Brook, NY, USA; [11]Department of Leukemia, The University of Texas MDACC, Houston, TX, USA; [12]Department of Microbiology and Immunology, The Medical University of South Carolina, Charleston, and Department of Pathology & Laboratory Medicine, The University of Texas Houston Health Science Center, Houston, TX, USA

©2014 Ferrata Storti Foundation. This is an open-access paper. *doi:10.3324/haematol.2014.104695*
Manuscript received on January 23, 2014. Manuscript accepted on July 3, 2014.
Correspondence: xsun@mdanderson.org

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 106 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' aaaaaagaattcgcgagaaaggacttcccagggtagc 3'; reverse: 5' aaaaaagaattccgatcgtttggccgctgcggcgaca 3'). The mutant *Tcf7l1* reporter was generated by using the pair of primers with modification in one of them (forward: 5' aaaaaagaattcgcgagaaaggacttcccagggtagc 3'; reverse: 5' aaaaaagaattccgatcgtttggccgctgcggcg**ctagca**ttttgacagggc 3'; the mutation site is bolded). The PCR products were then inserted into the *E*coRI site of the luciferase-expressing pSIN viral vector.

Online Supplementary Figures.

Figure S1.

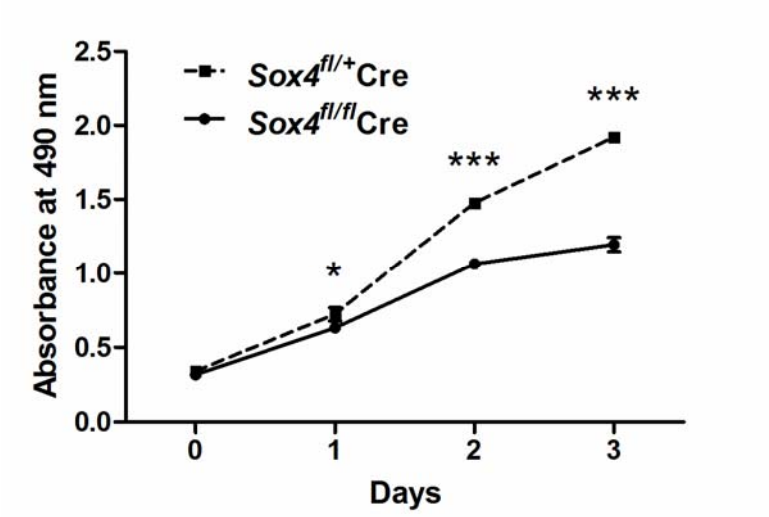

Figure S1. Effect of *Sox4*^{fl} deletion on the proliferation of ALL cells by MTS proliferation assay. The p190 *BCR-ABL* (mCherry+) transformed *Sox4*$^{fl/fl}$SE-Cre cells exhibited a lower proliferation rate than did the transformed *Sox4*$^{fl/+}$SE-Cre cells. Data are representative of three independent 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.

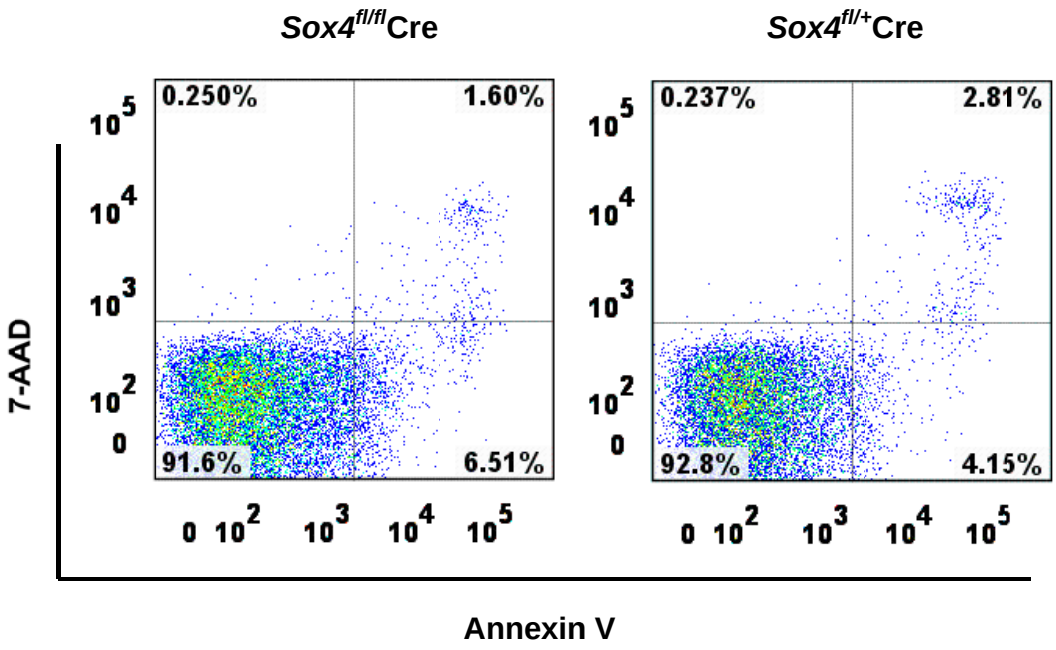

Figure S2. Effect of *Sox4*^{fl} deletion on the apoptosis of ALL cells by Annexin V/7-AAD staining assay. Cells were stained with Annexin V-APC and 7-AAD and analyzed by flow cytometry. Data are representative of three independent experiments.

Figure S3.

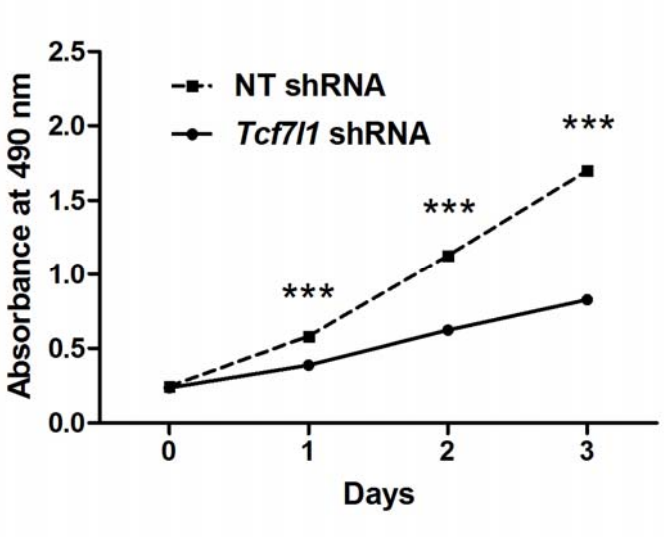

Figure S3. Effect of *Tcf7l1* knockdown on the proliferation of *BCR-ABL*–transformed wild-type pro-B cells by MTS proliferation assay. Knockdown of *Tcf7l1* resulted in a significant reduction in cell proliferation. Data are representative of three independent experiments. *Tcf7l1* shRNA: *Tcf7l1* specific shRNA; NT shRNA: non-targeting shRNA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

Figure S4.

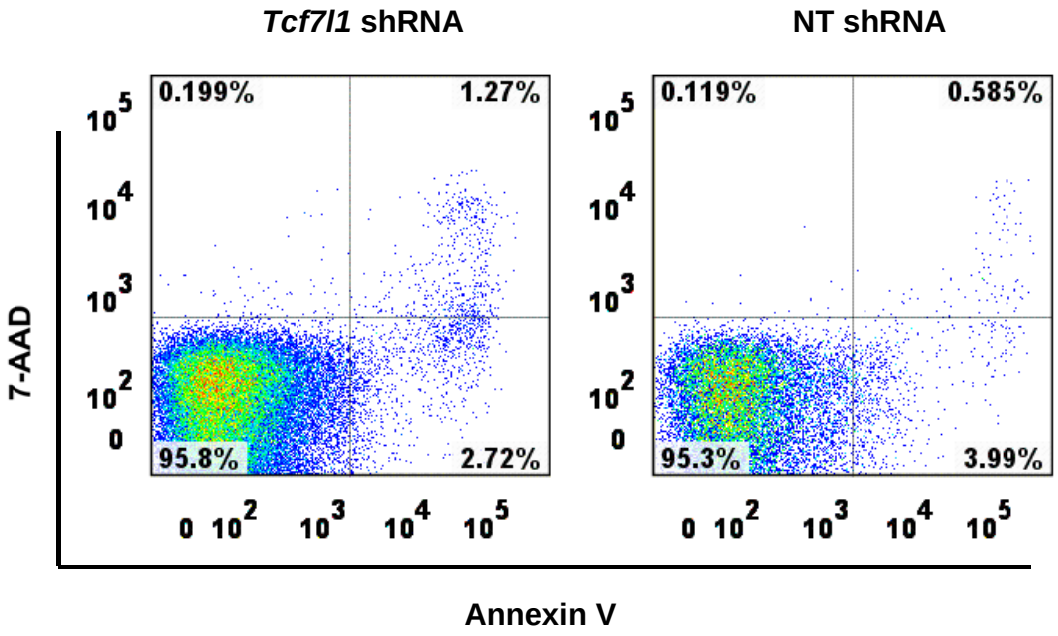

Figure S4. Effect of *Tcf7l1* knockdown on the apoptosis of *BCR-ABL*–transformed wild-type pro-B cells by Annexin V/7-AAD staining assay. Cells were stained with Annexin V-APC and 7-AAD and analyzed by flow cytometry. Data are representative of three independent experiments.