PML-RARα co-operates with Sox4 in acute myeloid leukemia development in mice

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Supplementary Appendix

Design and Methods

Promyelocyte cell staining and sorting strategy

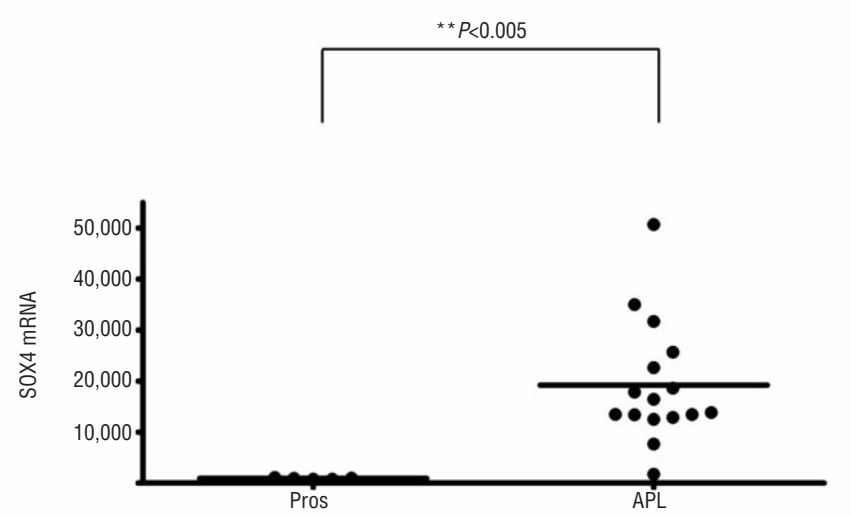
Single-cell suspensions of bone marrow cells were prepared by filtering tissues through 70-μm cell strainers (BD). CD117 enrichment was carried out using MicroBead positive selection according to the manufacturer's instructions (Miltenyi Biotec Ltd., UK). Cells were stained with the following fluorescently labeled monoclonal antibodies (Biolegend, UK): CD117-PE-Cy7, CD16/32-PE, Gr1-Pacific Blue, as well as SA-APC toward the mixture of the following lymphocyte/erythrocyte biotinylated antibodies: CD3, CD4, CD5, CD8, B220, Ter119, CD127, Sca1. Live singlet promyelocyte cell sorting was performed using a FACSAria II flow cytometer (BD) as $7AAD^{neg}$Lymphocyte/erythrocyte$^{neg}CD117^{intermediate}Gr1^{pos}CD16/32^{pos}$.

RNA extraction and quantitative PCR analysis

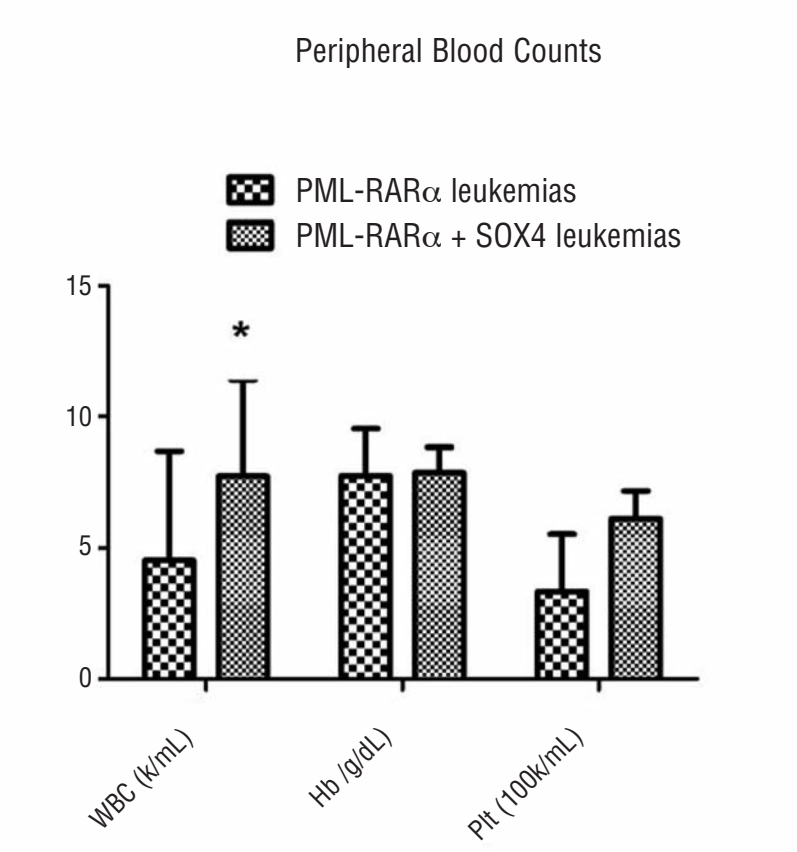
RNA was extracted from unsorted or sorted secondary transplant cells using RNeasy Mini kit (Qiagen, UK). RNA (100 ng) was used in a 20 μL reverse transcription reaction containing 10 x Buffer II, 5 mmol/L $MgCl_2$, 0.5 μmol/L deoxynucleotide triphosphates, 2.5 units reverse transcriptase, 1 unit RNase inhibitor, and 2.5 mol/L random hexamers (Roche Diagnostics, UK). cDNA (2 μL) was used in a Fast SYBR® Green master mix (Invitrogen, UK) on 7500 real-time PCR system (ABI systems, UK) using primer pairs (Sigma-Aldrich, UK) described below according to the manufacturer's instructions. The amount of GapDH mRNA was quantified in all samples as an internal housekeeping control, and quantitative PCR were expressed as normalized target gene values. All experiments were performed in triplicate reactions. Sfpi1: Forward CGA TTC AGA GCT ATA CCA ACG TC; Reverse TGA TCG CTA TGG CTT TCT CCA. Sox4: Forward GAC AGC GAC AAG ATT CCG TTC; Reverse GTT GCC CGA CTT CAC CTT C. GapDH: Forward AGC TTC GGC ACA TAT TTC ATC TG; Reverse CGT TCA CTC CCA TGA CAA ACA.

Online Supplementary Table S1. PML-RARα + Sox4 leukemias are characterized by aneuploidy.

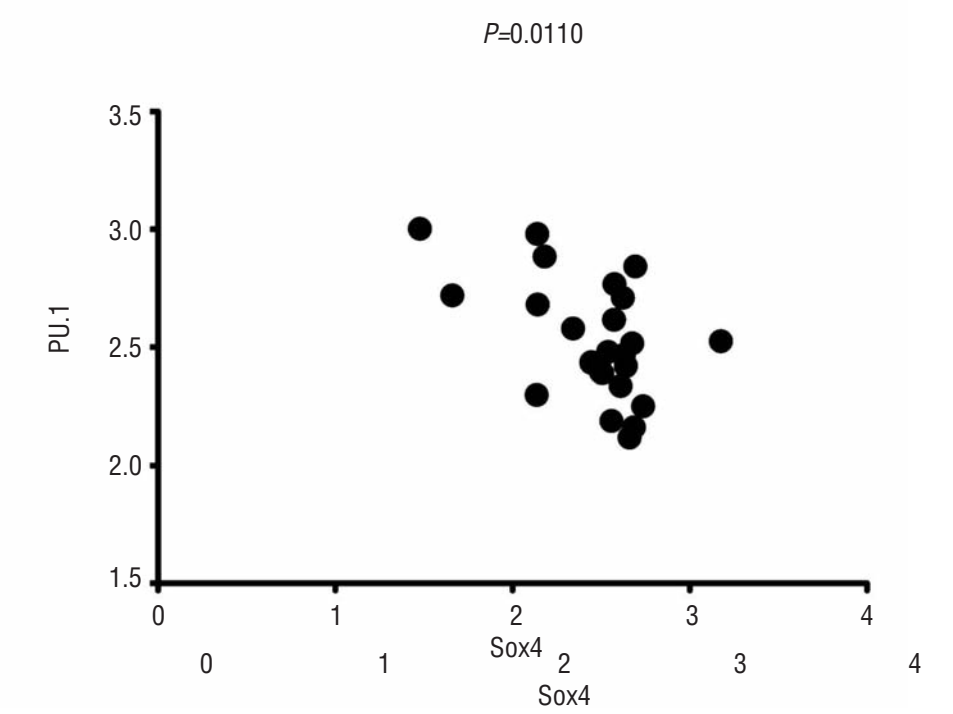
Mouse diagnosis	Karyotype
109 Leukemia	40,X,-X,+8[11]
550 Leukemia	39,X,-X[11]/40,XX[1]
3460 Leukemia	40,XX[7]/40,X,-X,+4,+11,-19[1]/37,XX,-7,-13,der(14;15)(A1;A1),+15,-18[1]/40,XX,-7,+14[1]
3463 Leukemia	40,XX[5]/41,XX,+14[1]/41,X,-X,+8,+15[1]
3877 Leukemia	40,XY[19]/41,XY,+12[1]
5547 Leukemia	39,X,-X[6]/40,idem,+10[1]/40,idem,+15[1]/40,XX[3]
5548 Leukemia	39,X,-X or -Y[1]/40,idem,+8[6]/40,idem,+15[1]/41,idem,+8,+15[1]/80,idemx2[1]



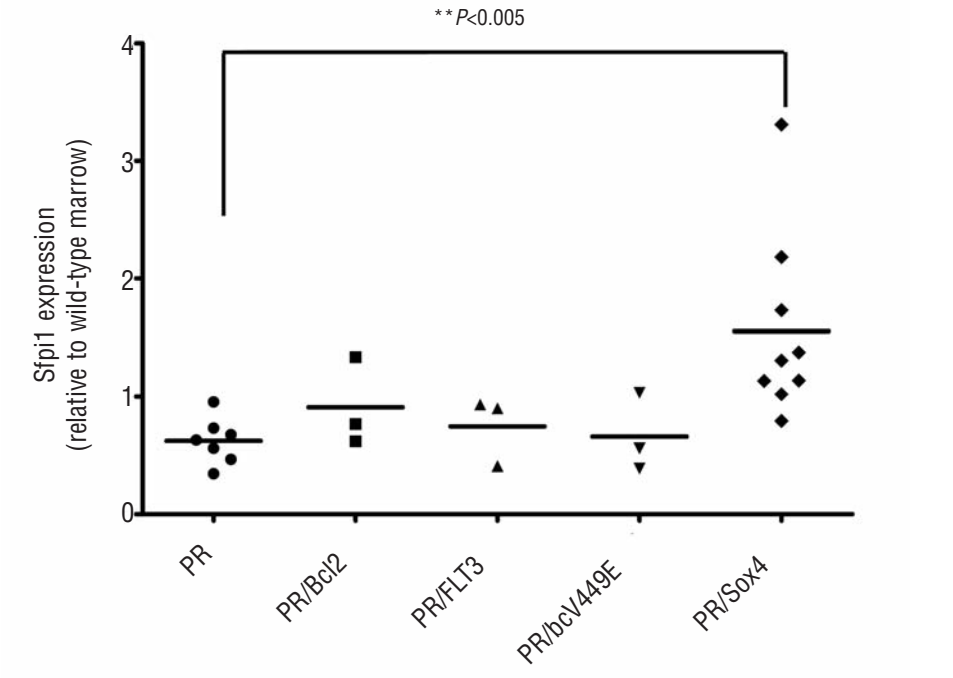
Online Supplementary Figure S1. ***SOX4*** **transcript level is significantly different between normal promyelocytes and myeloid cells from APL patients (*P*<0.005).**[15]



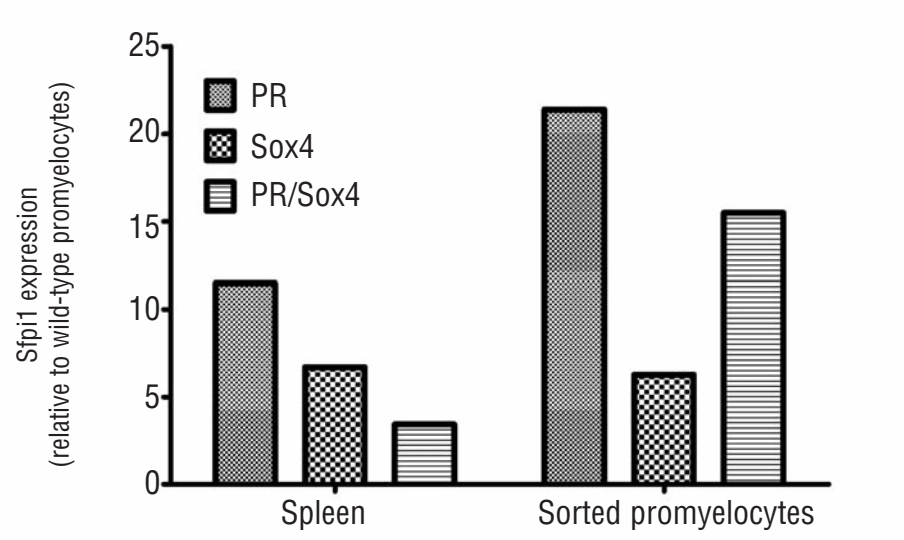
Online Supplementary Figure S2. **Peripheral blood analyses were determined via retro-orbital bleeds of similarly aged animals from cohorts of PML-RARα leukemias (n=10) and PML-RARα + Sox4 leukemias (n=11). Although not statistically significant, surprisingly, peripheral platelet counts were marginally higher in PML-RARα + SOX4 animals as compared to PML-RARα alone animals. A significant difference in WBC was observed between the two groups (**P*<0.05).**



Online Supplementary Figure S3. Expression level plot of one probe out of five (201418_s_at) shows significant inverse correlation between *SOX4* and *PU.1* in 24 human APLs.[15] This probe showed the strongest correlation, but a similar pattern was observed with the four other SOX4 probes (*data not shown*).



Online Supplementary Figure S4. RT-qPCR demonstrates significantly increased *Sfpi1* expression on the background of PML-RARα with overexpression of *SOX4* ($P<0.005$), whereas overexpression of the anti-apoptotic protein Bcl2, of an activating FLT3 mutation or an activating mutation of the common beta subunit of IL3/IL-5/GM-CSF receptor (βcV449E) was not associated with a statistically significant change in *Sfpi1* levels. All values normalized to *Sfpi1* levels in a concurrently run normal bone marrow.



Online Supplementary Figure S5. RT-qPCR demonstrates raised *Sfpi1* expression in spleen cells and sorted promyelocytes in all three leukemias. All values normalized to *Sfpi1* level in concurrently run sorted promyelocytes of normal bone marrow (representative of 2 experiments).