Online methods

Leukemia samples

T-ALL samples (n=196) were collected at various institutions, expression patterns of some of these samples were described previously.[13,25] All samples were obtained according to the guidelines of the local ethical committees. Diagnosis of T-ALL was based on morphology, cytochemistry and immunophenotyping according to the World Health Organization and European Group for the Immunological Characterization of Leukemias criteria.[26,27]

Murine T cells

Primary mouse $CD4^+CD8^+$ leukemic T-cells were isolated from a mouse (Balb/c) suffering from an irradiation induced T-ALL like disease. Cells were expanded in RPMI-1640 medium supplemented with 20% fetal calf serum, IL2 (5 ng/ml) and IL7 (10 ng/ml) (Peprotech). The Ptpn2 locus was not affected in these cells, Notch1 was activated.

Array technology

Array-based comparative genomic hybridization (array CGH) was performed using the Agilent 244K platform (Agilent Technologies) or SNP array 6.0 (Affymetrix).

Cytogenetics and FISH analysis

Cytogenetic and FISH analyses were performed following standard protocols.[7,28] Fosmid probes covering regions within (G248P8043H2) or outside (G248P84981C11) the *PTPN2* gene locus were designed based on UCSC Genome Browser (http://www.genome.ucsc.edu) and purchased from CHORI BACPAC Resources. Whole chromosome painting probes for chromosome 18 (XCP18) were obtained from MetaSystems (MetaSystems, Altlussheim, Germany). LSI BCR-ABL ES (Abbott Molecular International) was used to identify *ABL1* amplification.

Quantitative PCR

Fast SYBR® Green Master Mix (Applied Biosystems) was used for performance of quantitative (real time) PCR with the LightCycler® 480 Real-Time PCR System (Roche Diagnostics). *CDH2* and *RNMT* were used as control. Data were analyzed with the LC480 software (Roche Diagnostics) before applying the comparative ddCT method (Bulletin 2; Applied Biosystems).[11]

Sequence analysis of *PTPN2*

Genomic DNA was isolated from bone marrow or blood samples using the Wizard® Genomic DNA Purification Kit (Promega). PCR reactions were performed with Phusion® Hot Start High-Fidelity DNA Polymerase (Finnzyme). GC-rich exon 1 was amplified using expand long template PCR system (Roche Diagnostics) and reactions were supplemented with betaine (Sigma) for improvement. All sequencing reactions were run on a 3130xl Genetic Analyzer (Applied Biosystems).

mRNA expression analysis

TRIzol LS Reagent (Invitrogen) was used for total RNA extraction. cDNA synthesis and subsequent mRNA expression analysis of PTPN2 by real-time quantitative PCR was performed as described previously.[29] HPRT was used as reference gene for normalization.

Analysis of DNA methylation

Primer pairs were designed (Methyl Primer Express v 1.0 software) to bind sequences lacking any CpG islands. If CpG sites were unavoidable, degenerated bases were used. Genomic DNA was subjected to bisulfate treatment using the EZ DNA Methylation Kit (Zymo) followed by PCR amplification using AmpliTaq Gold 360 Polymerase (Applied Biosystems) or JumpStart REDTaq DNA polymerase (Sigma Aldrich). PCR products were directly sequenced. Nucleotide sequences in electropherograms were analyzed for cytosine or thymidine calls located in CpGs. An unmethylated control sample was included to assure complete bisulfate conversion.

Western blotting and immunoprecipitation

Cells were lysed in lysis buffer (Cell Signaling) with complete protease inhibitor (Roche) and 1 mM $NaVO_4$. For immunoprecipitation of proteins, precleared lysates were incubated overnight with Dynabeads protein G (Invitrogen) coupled to respective antibody.

Antibodies

We used the following antibodies: anti-c-ABL (24-11), anti-ERK (C-16), anti-LCK (3A5), anti-LCK (2102), anti-phospho-JAK1 (Tyr1022/1023) (Santa Cruz), anti-JAK2 (D2E12), anti-JAK3, anti-phospho-SRC (Tyr416), anti-phospho-AKT (Ser473), anti-phospho-ERK1/2 (9101), anti-c-ABL (Cell Signaling), anti-phosphotyrosine (4G10), anti-JAK1 (Upstate), anti-PTPN2 (clone CF4-1D, Calbiochem), anti-Ptpn2 (3E2, MÉDIMABS), anti-phospho-ABL (Tyr245, Sigma). A phospho-STAT antibody sampler kit (Cell Signaling) was used for the detection of STAT proteins. For IP experiments the following antibodies were used: anti-PTPN2 (clone CF4-1D, Calbiochem), anti-LCK (Santa Cruz), anti-JAK1 (Upstate), anti-JAK2 and anti-JAK3 (D2E12) (both Cell Signaling).

Equipment and settings

The LAS-3000 Imaging System (Fujifilm Global) was used for Western blot analysis. Quantification of Western blot data was performed using Image J software.

Vectors and mutagenesis

PTPN2 was amplified from human cells using primers described previously[21] and cloned into the retroviral vector pMSCV-puro (Clontech) or the inducible pRetroX-PTuner system (Clontech). For knockdown of Ptpn2 in mouse cells, shRNA sequences targeting Ptpn2 or Alk (control), were cloned into pMSCV-GFP containing a mir30 flanking cassette (further referred to as GFP-miR30). shRNA sequences: Ptpn2: CACAAAGAAGTTACATCTT; Alk: CGGAAGGAATATTCACTTCTAA. For expression of NUP214-ABL1[22] and simultaneous knockdown of Ptpn2 in Ba/F3 cells, *NUP214-ABL1*

was recloned into a second multiple cloning site of the GFP-CmiR30 vector. PTPN2 trapping D182A mutant of the 45kD form (PTPN2-D182A)[30,31] was constructed. PTPN2-WT, PTPN2-D182A or GFP (negative control) were cloned into pRetroX-PTuner (Clontech). In this vector, the *PTPN2* open reading frame was fused to a 12 kDa destabilizing domain (DD). Addition of a stabilizing ligand (Shield1) resulted in stabilization and accumulation of the DD-tagged protein. Protein expression was induced for 24h (Shield1 concentration: 500nM).

Cell culture, virus production, retroviral transduction

HEK293T and Ba/F3 cells were cultured, transfected, and transduced as described previously.[28] For transformation assays, IL3 was removed and cells were seeded out at $1x10^5$ cells/ml. Cell number and viability was analyzed using Vi-cell^{TM}XR cell viability analyzer (Beckman Coulter). Primary mouse leukemia T-ALL, ALL-SIL and HSB-2 cells were transduced using the spin infection method. Centrifugation of 200 µl of concentrated viral supernatant and 0.5×10^6 cells/ml media containing 8 µg/ml polybrene was carried out for 90 minutes at 2500 rpm. At 48h after transduction, human cell lines were selected with puromycin (1 µg/ml) for a period of 6 days.

Electroporation conditions and siRNA knockdown

$2x10^6$ cells from T-ALL cell lines were washed, resuspended in a total volume of 400 µl SFM and transferred to a 4 mm cuvette (Biorad). Electroporation conditions: HPB-ALL 500µF/350V, ALL-SIL 1000µF/275V, JURKAT 950µF/300V, HSB-2 1000µF/300V. Electroporated cells were transferred to 12-well plates containing 2 ml prewarmed medium supplemented with 40% FBS for recovery. Effective siRNA delivery was assessed 4 hours after electroporation using a fluorescent labeled siRNA (BLOCK-iT™, Invitrogen). siRNA duplexes were used at a final concentration of 200 nM. Cells were electroporated twice within 24 hours (PTPN2 siRNA) using a GenePulser XCell electroporation system (Biorad). Predesigned siRNA oligonucleotides were purchased from the DsRNA sequence library from Integrated DNA Technologies (IDT). Custom designed duplexes targeting PTPN2 were obtained from Qiagen. Scrambled siRNA was used as negative control (IDT). siRNA sequences (sense): PTPN2(1): ACAGUACAUCUACUACAAUUAGAAA; PTPN2(2): CAAAGGAGUUACAUCUUAA; scrambled(1): CUUCCUCUCUUUCUCUCCCUUGUGA; scrambled(2): UCACAAGGGAGAGAAAGAGAGGAAGGA.

Signaling experiments

All signaling experiments were performed at time point of optimal siRNA knockdown. For pathway analysis upon inhibitor exposure, ALL-SIL and HSB-2 cells were incubated with imatinib or PP2 (both 1 µM) for 90 min. In case of cytokine signaling experiments, human T-ALL cells were serum depleted for 4 hours and primary leukemic mouse T- cells were cytokine depleted for 24 hours prior to stimulation with respective cytokines.

Proliferation and drug inhibitor assays

Cells were seeded in triplicate at a cell density of $3x10^5$ cells/ml (24-well plates) 24 hours after second electroporation and either treatment was initiated for inhibitor experiments or cells were left untreated

for proliferation assays. Primary leukemic mouse T- cells were deprived from cytokines for 24 hours before re-stimulation. Proliferation was assayed 24 and 48 hours after addition of either a low concentration of IL7 (0.1 ng/ml) alone or IL7 (0.1 ng/ml) combined with IL2 (5 ng/ml). For dose response curves, ALL-SIL cells were incubated for 48h with increasing concentrations of imatinib. Cell numbers and viability was determined using Vi-cell^{TM}XR cell viability analyzer (Beckman Coulter). IC50 values were calculated using GraphPad Prism. Imatinib (ChemieTek) and PP2 (Calbiochem) were stored in DMSO at a stock concentration of 10 mM and diluted in medium prior to use.

URLs

Locations of genes and probes, and gene structures were determined based on Ensembl data (http://www.ensembl.org). Sequence similarities were analyzed using the BLAST algorithm at http://www.ncbi.nlm.nih.gov/BLAST.

Statistical analysis

The Students t-test was used to compare the mean of two groups. Normality tests were used to test the assumption of a normal distribution. All graphs represent mean values ±s.e.m. *p<0.05 and **p<0.005

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