Mutation analysis of the tyrosine phosphatase PTPN2 in Hodgkin's lymphoma and T-cell non-Hodgkin's lymphoma

Maria Kleppe,[1,2] Thomas Tousseyn,[3] Eva Geissinger,[4] Zeynep Kalender Atak,[2] Stein Aerts,[2] Andreas Rosenwald,[4] Iwona Wlodarska,[2] and Jan Cools[1,2]

[1]Department of Molecular and Developmental Genetics, VIB, Leuven, Belgium; [2]Center for Human Genetics, K.U.Leuven, Leuven, Belgium; [3]Department of Pathology, University Hospitals Leuven, and Laboratory for Morphology and Molecular Pathology, K.U.Leuven Leuven, Belgium; [4]Institute of Pathology, University of Würzburg, Germany

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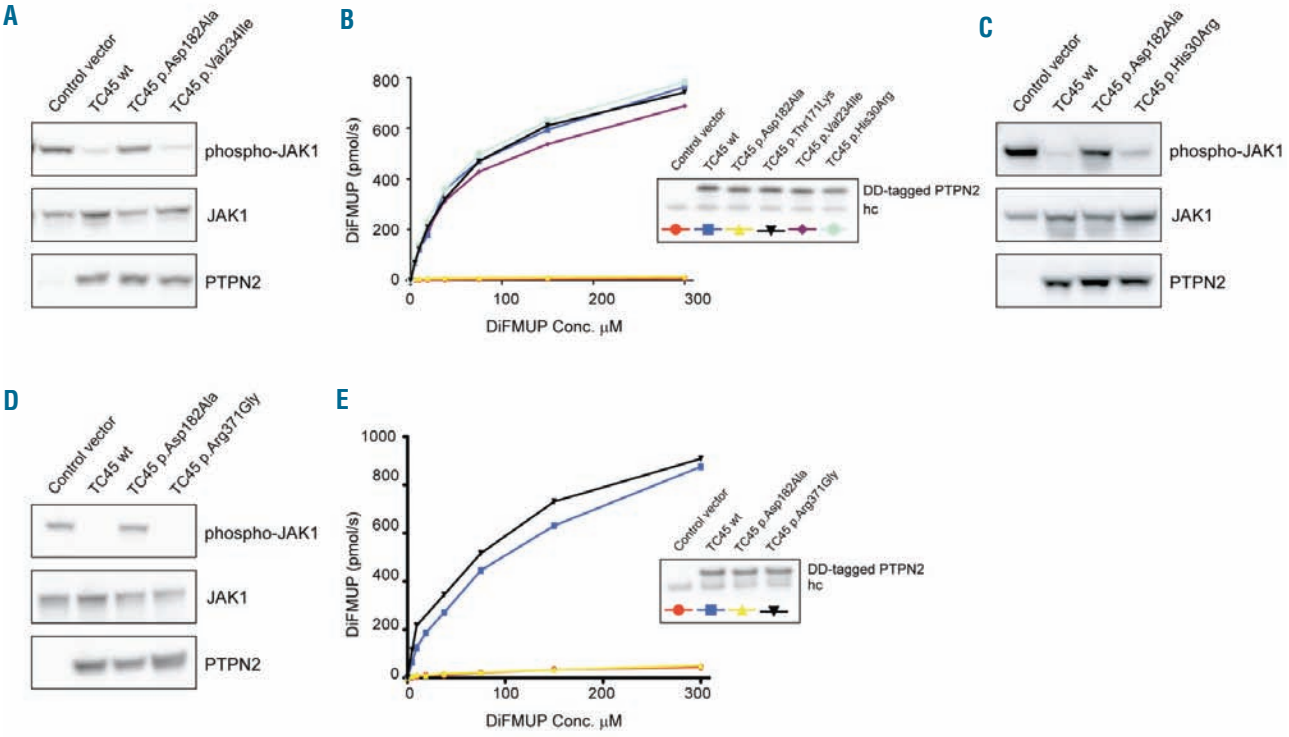
Online Supplementary Design and Methods

Immunecomplex phosphatase activity assay

Stabilization of respective DD-tagged PTPN2 forms expressed from pRetroX-PTuner constructs were induced by adding Shield1 compound (24 h, 500nM) to stably transduced Ba/F3 cell lines. Cells were washed once in ice cold TBS and lysed on ice for 30 min (50 mM Tris HCl (tris(hydroxymethyl)aminomethane), 150 mM NaCl, 1mM EDTA (ethylenediaminetetraacetic acid), 1% Triton X-100 containing complete protease inhibitors (Roche)). Monoclonal PTPN2 antibody (CF4-1D) was coupled to Dynabeads protein G (Invitrogen) for 45 min at room temperature. Lysates were cleared by centrifugation and 1 mg was incubated with PTPN2-coupled dynabeads overnight at 4°C. IgG coupled-beads were included as control to test for unspecific binding. Beads were washed two times in lysis buffer and two times in assay buffer (50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 0.1 mg/ml BSA, 3 mM DTT, 25 mM NaCl and 1mM EDTA) before resuspension and dilution in assay buffer. A fraction of PTPN2 immunoprecipitate was used for Western blot analysis to assess protein expression levels in IP samples. All assays were performed using 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, Molecular Probes) as a substrate. A rate analysis was performed to determine the optimal amount of enzyme quantity for kinetic analysis. For kinetic assays 50 µl of diluted bead solution was added to black 96-well plates and the reaction initiated by addition of 50 µl of increasing concentrations of DiFMUP (final dilution 0-300 mM). Emitted fluorescence was measured every 30 s for 15 min using the Victor X4 Multilabel Plate Reader (Perkin Elmer; excitation 355 nm and emission 460 nm). Initial rates were calculated from the slope of the linear curve of fluorescence *versus* time for each substrate concentration. All measurements were corrected for fluorescence background of substrate alone and three independent experiments were performed.



Online Supplementary Figure S1. Identified variants do not effect PTPN2 phosphatase activity. (A) 293T cells were co-transfected with JAK1 mutant p.Ala634Asp (pMSCV-puro, Clontech) together with empty vector, PTPN2 wt, PTPN2 p.Asp182Ala mutant or PTPN2 variant p.Val234Ile. Whole cell lysates were collected 48 h after transfection and analyzed for phosphorylation levels of JAK1. Consistent transfection efficiency was assured by detection of total JAK1 protein levels. (B) Protein tyrosine phosphatase activity assay of anti-PTPN2 immune complexes. Mutated forms of PTPN2 possessed similar enzymatic capacity as assessed by hydrolysis rates of DiFMUP substrate. Reaction rates are plotted against substrate concentration (0-300 μM). Recovered samples were analyzed by Western blot to ensure equal PTPN2 protein levels in immunoprecipitates. DD-tagged PTPN2, inducible variant of PTPN2 (57 kD); hc: heavy chain of Ig. A representative experiment is shown; similar data were obtained in three separate experiments. PTPN2 variants p.Thr171Lys and p.Val234Ile have been identified as single nucleotide polymorphisms by the 1000 Genomes Project (rs78174797 and rs77573141). (C) Co-expression of PTPN2 variant p.His30Arg strongly affected activation status of JAK1 p.Ala634Asp. Protein analysis of whole cell lysates from transfected 293T cells clearly showed dephosphorylation of JAK1 similar to the magnitude of PTPN2 wild type (TC45 wt). Consistent transfection efficiency was assured by detection of total JAK1 protein levels. (D) Co-expression of PTPN2 variant p.Arg371Gly diminished the phosphorylation level of JAK1 mutant p.Ala634Asp. No obvious difference between the catalytic activity of PTPN2 wild type and the PTPN2 variant p.Arg371Gly was detected. Protein analysis of whole cell lysates from transfected 293T cells is shown. Consistent transfection efficiency was assured by detection of total JAK1 protein levels. (E) Protein tyrosine phosphatase activity assay of anti-PTPN2 immune complexes. PTPN2 variant p.Arg371Gly possessed similar enzymatic capacity as assessed by hydrolysis rates of DiFMUP substrate. Reaction rates are plotted against substrate concentration (0-300 μM). Recovered samples were analyzed by Western blot to ensure equal PTPN2 protein levels in immunoprecipitates. DD-tagged PTPN2: inducible variant of PTPN2 (57 kD); H chain: heavy chain of Ig. A representative experiment is shown; similar data were obtained in three separate experiments.

Online Supplementary Table S1. 454 data sequencing coverage[a].

Samples (n=50)	N. of readings	N. of mapped readings	N. of uniquely mapped readings	N. of readings mapped to the target region	N. of readings uniquely mapped to the target region	Mean depth in the target region
1 (n=7)	11816	11528	6012	11400	5986	1183
2 (n=7)	19252	19005	9182	18913	9132	1929
3 (n=7)	24927	24575	12369	24451	12287	2639
4 (n=5)	14673	14440	7120	14280	7061	1646
5 (n=8)	20479	20087	8462	20017	8412	1994
6 (n=8)	25874	25503	12453	25451	12418	2559
7 (n=8)	25356	24987	12369	24844	12287	2639

[a]*Mean depth in the target region was defined as the sum of coverage per base, averaged over all targeted bases.*

Online Supplementary Table S2. Characteristics of T-NHL patients (n=69).

Patient ID	Subtype	Gender, age (y)	% tumor cells	*PTPN2* status
TNHL1	NOS	F, 59	75-100	p.Arg45* *PTPN2*$^{+/-}$[a]
TNHL2	NOS	M, 84	90	p.Glu41fs and p.Arg45fs
TNHL3	AITL	Unknown, 31	40	p.Arg371Gly, heterozygous
TNHL4	ALCL, ALK-	M, 42	10-50	wt
TNHL5	ALCL, ALK+	F, 77	<10	wt
TNHL6	ALCL, ALK-	M, 45	>50	wt
TNHL7	ALCL, ALK-	Unknown,16	10-50	wt
TNHL8	ALCL, ALK+	M, 55	10-50	wt
TNHL9	ALCL, ALK-	M, 61	>50	wt
TNHL10	ALCL, ALK+	M, 17	10-50	wt
TNHL11	ALCL, ALK+	F, 41	10-50	wt
TNHL12	ALCL, ALK+	M, 6	10-50	wt
TNHL13	ALCL, ALK+	M, 23	10-50	wt
TNHL14	AITL	F, 54	50	wt
TNHL15	AITL	M, 68	35	wt
TNHL16	AITL	M, 63	<10	wt
TNHL17	AITL	F, 78	25	wt
TNHL18	AITL	M, 65	35	wt
TNHL19	AITL	F, 69	<10	wt
TNHL20	AITL	M, 73	20	wt
TNHL21	AITL	M, 63	<10	wt
TNHL22	AITL	F, 55	<10	wt
TNHL23	AITL	M, 79	<20	wt
TNHL24	AITL	F, 80	40	wt
TNHL25	AITL	F, 71	35	wt
TNHL26	AITL	F, 82	40	wt
TNHL27	AITL	M, 77	40	wt
TNHL28	NOS	F, 53	20	wt
TNHL29	NOS	F, 83	40	wt
TNHL30	NOS	M, 54	10	wt
TNHL31	NOS	M, 78	70	wt
TNHL32	NOS	M, 76	60	wt
TNHL33	NOS	M, 46	50	wt
TNHL34	NOS	M, 68	70	wt
TNHL35	NOS	F, 67	90	wt
TNHL36	NOS	F, 82	Unknown	wt
TNHL37	NOS	F, 73	25	wt
TNHL38	NOS	M, 59	>95	wt
TNHL39	NOS	F, 84	>95	wt

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TNHL40	NOS	F, 77	50-75	wt
TNHL41	NOS	F, 72	50-75	wt
TNHL42	NOS	M, 62	Unknown	wt
TNHL43	NOS	F, 55	50-75	wt
TNHL44	NOS	F, 68	>95	wt
TNHL45	NOS	F, 71	5% total sample/ 75-100% lymphoid infiltrate	wt
TNHL46	NOS	M, 74	25-50	wt
TNHL47	NOS	M, 65	75-100	wt
TNHL48	NOS	F, 94	50-75	wt
TNHL49	NOS	M, 62	>95	wt
TNHL50	NOS	F, 32	>95	wt
TNHL51	NOS	M, 80	50-75	wt
TNHL52	NOS	M, 70	50-75	wt
TNHL53	NOS	M, 88	>95	wt
TNHL54	NOS	F, 75	>95	wt
TNHL55	NOS	M, 60	50-75	wt
TNHL56	NOS	M, 48	75-100	wt
TNHL57	NOS	F, 72	50-75	wt
TNHL58	NOS	F, 58	75-100	wt
TNHL59	NOS	M, 82	75-100	wt
TNHL60	NOS	M, 55	>95	wt
TNHL61	NOS	M, 51	>95	wt
TNHL62	AITL	M, 57	>95	wt
TNHL63	NOS	F, 75	>95	wt
TNHL64	NOS	F, 59	50-75	wt
TNHL65	NOS	M, 80	25-50	wt
TNHL66	NOS	F, 61	25-50	wt
TNHL67	AITL	M, 58	50-75	wt
TNHL68	ALCL, ALK-	M, 50	25-50	wt
TNHL69	NOS	M, 70	25-50	wt

[a]Determined by FISH analysis using PTPN2 specific probes; n: number of individuals; y: years ; wt: no PTPN2 alteration detected by Sanger sequencing; M: male; F: female; ALCL: anaplastic large-cell lymphoma; ALK+: ALK-positive; ALK-: ALK-negative ; PTCL: peripheral T-cell lymphoma; NOS: not otherwise specified; AITL: angioblastic T-cell lymphoma.