Supporting Information

Dagnell et al. 10.1073/pnas.1302891110

SI Materials and Methods

Cell Culture and Reagents. The mouse embryonic fibroblasts used in this study have been described before in the context of TrxR1 (1) and protein tyrosine phosphatase (PTP)1B (2) and were cultured in DMEM with 10% (vol/vol) FBS, 2 mM L-glutamine, and 20 nM sodium selenite with penicillin and streptomycin. The following antibodies were used with the indicated concentrations: PDGF-β receptor (PDGFβR) (1:1,000; Cell Signaling), pY579-581 [1:1,000 (3)], PTP1B (1 μg/mL; R&D Systems), SHP2 (1:1,000; Santa Cruz Biotechnology), Trx1 (1:1,000; Santa Cruz Biotechnology, sc-58439), and secondary HRP-coupled α-mouse, α-rabbit, and α-goat antibodies (1:15.000; Pierce). Auranofin was purchased from Sigma Aldrich, and PDGF-BB ligand was purchased from Peprotech. Human PTP1B for in vitro studies came from ATGen (catalog no. PTB2001). Recombinant TrxR1 was expressed and purified as previously described (4), and human WT Trx1 was a kind gift from Arne Holmgren, Karolinska Institutet, or bought from IMCO Corporation Ltd. His-tagged variants of human Trx1 used for PTP1B trapping experiments are described below.

Cysteinyl-Labeling Assay of PTP Oxidation. The assay was performed as previously described (5). In brief, cells were starved for 24 h in phenol-red free DMEM with 1% FBS. For PDGF-BB stimulations, cells were stimulated with 50 ng/mL PDGF-BB for 5 min and lysed in degassed lysis buffer [50 mM sodium scetate (pH 5.5), 150 mM NaCl, 1% Nonidet P-40, and 10% (vol/vol) glycerol] supplemented with 25 μg/mL aprotinin, 25 μg/mL leupeptin, 10 mM iodoacetic acid (IAA), 250 U/mL catalase, and 125 U/mL superoxide dismutase, after which alkylation was continued for 1 h at 20 °C. Lysates were cleared by centrifugation at 21,000 × *g* for 10 min, and buffer exchanged with 1 mM tris(2-carboxyethyl)phosphine (TCEP)-containing lysis buffer. Lysates were reduced for 30 min and supplemented with 5 mM biotin-labeled iodoacetic acid probe (Pierce). Labeled PTPs were pulled down with agarose-streptavidin, boiled for 5 min in sample buffer, and used for immunoblotting.

Analyses of PDGFβR Phosphorylation. Cells were grown to 70% confluence and then starved for 24 h in medium containing 1% FCS. Where indicated, starvation was followed by a 1 h treatment with 1 μM auranofin. Cells were subsequently stimulated with 50 ng/mL PDGF-BB for 5 min, washed twice with ice-cold 50 mM PBS (pH 7.4), and lysed in lysis buffer [0.5% Triton X-100, 0.5% sodium deoxycholate salt/deoxycholic acid, 150 mM NaCl, 20 mM Tris (pH 7.5), 10 mM EDTA, and 30 mM sodium pyrophosphate (pH 7.5), supplemented with 200 μM sodium orthovanadate and a protease inhibitor mixture]. Lysates were cleared by centrifugation, incubated with wheat germ agglutinin (WGA)-agarose for 1 h, washed three times with lysis buffer, and processed for immunoblotting.

PTP Activity Assay. PTP activity of cell lysates and recombinant protein was determined using a radioactive labeled phosphotyrosine containing peptide as a substrate (AEEEIpYGEFEAKKK) as previously described (6). Cultured WT and *Txnrd1*$^{-/-}$ mouse embryonic fibroblasts, starved for 24 h in phenol-red free DMEM with 1% FCS, were washed with degassed 20 mM ice-cold Hepes buffer (pH 7.4) and lysed with freshly prepared degassed lysis buffer [25 mM sodium acetate (pH 7.4), 1% Nonidet P-40, 150 mM NaCl, and 2 μg/mL aprotinin]. Lysates were cleared by centrifugation at 21,000 × *g* at 4 °C for 10 min. SHP2 and PTP1B were immunoprecipitated from cell lysates and immobilized with magnetic Dynabeads Protein G (Life Technologies). Beads were washed with degassed lysis buffer and 25 mM Imidazole (pH 7.4) before mixing with the substrate. Dephosphorylation of substrate was allowed for 7 min before termination of reaction by addition of a charcoal mixture [0.9 M HCl, 90 mM sodium pyrophosphate, 2 mM NaH_2PO_4, and 4% (vol/vol) NoritA] and centrifugation. Supernatant containing released phosphate was transferred to scintillation vials containing 5 mL of Ecoscint A (National Diagnostics), and measurement of radioactivity was performed in a Wallac Winspectral 1414 Liquid Scintillation Counter.

Treatment of Cell Lysates with Thioredoxin System Components. Cultured NIH 3T3 cells were treated with 400 μM H_2O_2 for 5 min and washed two times with degassed 20 mM Hepes buffer (pH 7.4), followed by addition of lysis buffer (1% Nonidet P-40, 150 mM NaCl, 20 mM Tris, 2 mM EDTA, and 2 μg/mL aprotinin). Cleared cell lysates were treated with a combination of thioredoxin (Trx) system components (10 μM Trx1, 0.5 μM TrxR1, and 1 mM NADPH) or 50 mM DTT for 30 min. Activity of PTP1B and SHP2 was performed as described above.

In Vitro Treatments of Recombinant PTP1B and SHP2 with Trx System Components. Recombinant PTP1B (0.67 nM) and SHP2 (1.05 nM) were incubated with Trx system components [Trx1 (IMCO-Trx-04) or TRP14 10 μM, TrxR1 0.5 μM (IMCO-TR-03B), and NADPH 1 mM] in 50 mM Tris and 2 mM EDTA for 30 min before analysis with PTP activity assay. PTP activity was subsequently measured at 5, 10, and 20 min after addition of the substrate, and turnover values were calculated using the slope that was generated from linearly fitted curves over the time course data points.

Substrate Trapping in Vitro of PTP1B by Trx1. WT and mutant (C32S, C35S, and active site double mutant C32SC35S) forms of N-terminally His-tagged recombinant human Trx1 (14.6 kDa) were used for the substrate trapping experiments. Prereduction of Trx1 was performed using 20× molar excess of DTT for 20 min, whereupon the protein was desalted. Reduced Trx1 variants (500 nM) were subsequently incubated with equal amounts of PTP1B for 20 min at 20 °C and subsequently analyzed by SDS/PAGE in the presence or absence of DTT, followed by detection of Trx1 using Western blot. Control experiments without PTP1B using subsequent nonreducing SDS/PAGE were included for the validation of a predominantly monomeric nature of the prereduced Trx1 species, illustrating that the desalting of the protein did not lead to artificial intermolecular complex formation.

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