Receptor-type Protein-tyrosine Phosphatase ζ Is a Functional Receptor for Interleukin-34*§

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Background: IL-34 and the known IL-34 receptor, CSF-1R, are differentially expressed in mouse brain; thus, IL-34 may signal via an additional receptor(s).

Results: IL-34 binds to PTP-ζ on U251 human glioblastoma cells to stimulate intracellular signaling and responses.

Conclusion: PTP-ζ is an IL-34 receptor.

Significance: CSF-1R-independent actions of IL-34 via PTP-ζ should be considered in evaluating IL-34 roles in development and disease.

Interleukin-34 (IL-34) is highly expressed in brain. IL-34 signaling via its cognate receptor, colony-stimulating factor-1 receptor (CSF-1R), is required for the development of microglia. However, the differential expression of IL-34 and the CSF-1R in brain suggests that IL-34 may signal via an alternate receptor. By IL-34 affinity chromatography of solubilized mouse brain membrane followed by mass spectrometric analysis, we identified receptor-type protein-tyrosine phosphatase ζ (PTP-ζ), a cell surface chondroitin sulfate (CS) proteoglycan, as a novel IL-34 receptor. PTP-ζ is primarily expressed on neural progenitors and glial cells and is highly expressed in human glioblastomas. IL-34 selectively bound PTP-ζ in CSF-1R-deficient U251 human glioblastoma cell lysates and inhibited the proliferation, clonogenicity, and motility of U251 cells in a PTP-ζ-dependent manner. These effects were correlated with an increase in tyrosine phosphorylation of the previously identified PTP-ζ downstream effectors focal adhesion kinase and paxillin. IL-34 binding to U251 cells was abrogated by chondroitinase ABC treatment, and CS competed with IL-34 for binding to the extracellular domain of PTP-ζ and to the cells, indicating a dependence of binding on PTP-ζ-CS moieties. This study identifies an alternate receptor for IL-34 that may mediate its action on novel cellular targets.

The CSF-1R § kinase (1, 2) plays a critical role in the regulation of macrophage and osteoclast production and function (3–6) as well as the development and regulation of other cell types (7–11). The existence of an additional CSF-1R ligand was proposed based on the greater severity of phenotype of homozygous CSF-1R-null mice compared with the phenotype of homozygous CSF-1-null mutant mice (12). A second ligand for the CSF-1R, interleukin-34 (IL-34) (13), with no apparent sequence similarity to any other growth factor was subsequently identified (13). Although IL-34 and CSF-1 compete for binding to the CSF-1R and have similar CSF-1R-mediated effects, they exhibit significant tissue-specific and developmental differences in their expression patterns (14). In addition, whereas CSF-1-deficient mice exhibit partial loss of microglia, CSF-1R-deficient mice have no microglia (15). This observation together with the high expression of IL-34 in brain suggested an important role of IL-34 in microglial development. In agreement with this, IL-34-deficient (IL-34−/or−) mice were shown to exhibit severe deficits in microglia (16, 17). Despite the similarity of IL-34 and CSF-1 in their CSF-1R-mediated effects (14, 18), IL34 mRNA is expressed at a significantly higher level than either Csf1 or Csf1r mRNA in several regions of the early postnatal and adult brain (14), IL-34 protein is often expressed in regions where there is minimal expression of the CSF-1R or CSF-1-reporter proteins, and IL-34 is significantly more active in suppressing neural progenitor cell proliferation and neuronal differentiation than CSF-1 (9). These observa-

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5 The abbreviations used are: CSF-1R, colony-stimulating factor-1 receptor; CS, chondroitin sulfate; HS, heparan sulfate; GAG, glycosaminoglycan; ECM, extracellular matrix; FAK, focal adhesion kinase; KD, knockdown; PTN, pleiotrophin; PTP, protein-tyrosine phosphatase; TN-R, tenascin-R; CSF, colony-stimulating factor; hPTN, human PTN; mIL-34, mouse IL-34; hIL-34, human IL-34; hCSF-1, human CSF-1; hCSF-1, human recombinant CSF-1; EDC/NHS, 1-ethyl-3(dimethylaminopropyl)-carbodiimide/N-hydroxy succinimide; OG, N-octyl β-glucoside; SPR, surface plasmon resonance; NeuN, neuronal nuclei; GFAP, glial fibrillary acidic protein; t50, time taken for 50% wound closure; GIT1/Cat-1, G protein-coupled receptor kinase inhibitor 1/Cool-associated, tyrosine-phosphorylated 1; OB, olfactory bulb.
tions suggested that IL-34 signals in a CSF-1R-independent manner in brain.

Receptor-type protein-tyroine phosphatase ζ (PTP-ζ) (19, 20), a cell surface receptor and a chondroitin sulfate (CS) proteoglycan, is highly abundant in the brain (21), primarily expressed on neural progenitors and glial cells (22–24), and binds to and signals through the actions of multiple ligands (25), including the growth factor pleiotrophin (PTN) (26, 27), the cell surface protein contactin (28), and the extracellular matrix (ECM) protein tenascin-R (TN-R) (29). The binding of some of these ligands involves the CS glycosaminoglycan (GAG) moiety of PTP-ζ (26, 30). Ligand binding to PTP-ζ leads to increased tyrosine phosphorylation of downstream targets, including β-catenin, β-adducin, Src family kinases (SKF), focal adhesion kinase (FAK), paxillin, and extracellular signal-regulated kinase-1/2 (Erk-1/2) (31–38). PTP-ζ is up-regulated in many human cancers, including glioblastomas, and regulates their proliferation and migration (39–41).

In the present study, an unbiased proteomics approach identified PTP-ζ as an IL-34-interacting membrane protein in mouse brain. Using shRNA-mediated suppression of PTP-ζ expression in a CSF-1R-less U251 human glioblastoma cell line, we demonstrate that IL-34 binds specifically to cell surface PTP-ζ to initiate downstream signaling that leads to the inhibition of cell proliferation, clonogenicity, and motility. We further show that IL-34-binding to PTP-ζ is dependent on the presence of the CS GAG moiety on PTP-ζ. The demonstration of the existence of a novel IL-34 receptor increases the scope of biological effects of IL-34 in development, homeostasis, and disease.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Purified mouse IL-34 (mIL-34), human IL-34 (hIL-34), and purified polyclonal rabbit anti-mIL-34 antibodies were from Five Prime Therapeutics, Inc., and human PTN (hPTN) was from R&D Systems (Minneapolis, MN). Growth factors were suspended in phosphate-buffered saline (PBS) as vehicle. mIL-34 and hIL-34 were biotinylated using a 10 molar excess of EZ-Link Sulfo-NHS-LC-LC-Biotin (sulfosuccinimidyl-6-[biotinamido]-6-hexanamidohexanate; Thermo Scientific) (15 min, 20 °C) following the manufacturer’s instructions. The rabbit anti-C-terminal CSF-1R peptide antibody (C-15) to the mouse CSF-1R and human CSF-1R (hCSF-1R) used for Western blotting and immunoprecipitation has been reported previously (42). Other antibodies used for Western blotting were directed against phosphotyrosine (pY-100) and β-catenin (Cell Signaling Technology); Tyr(P)185paxillin and Tyr(P)395FAK (Invitrogen); hCSF-1R (2-4A5), β-adducin, FAK, and TN-R (Santa Cruz Biotechnology Inc.); paxillin and PTP-ζ (C-209) (BD Biosciences); PTP-ζ (3F8) (Developmental Studies Hybridoma Bank, University of Iowa); PTP-ζ (473-HD) (Santa Cruz Biotechnology Inc.) (43); and EF1α (44). Bovine serum albumin (BSA) was from Gemini. Puromycin dihydrochloride, trypsin blue, crystal violet, DAPI, shark cartilage CS salts, *Proteus vulgaris* chondroitinase ABC, and phalloidin were from Sigma. Polybrene was from Santa Cruz Biotechnology, Inc. Neutravidin Ultralink beads were from Thermo Scientific. Streptavidin-conjugated allophycocyanin-Cy7 was from Bio.legend. LIVE/DEAD® Fixable Dead Cell Stain kits were from Molecular Probes. HTS FluoroBlok™ inserts and 24- and 6-well tissue culture dishes were from BD Biosciences. Accurase was from Stem Cell Technologies (Vancouver, British Columbia, Canada). Human PTP-ζ and CSF-1R extracellular domains (ECD) fused to immunoglobulin Fc domains (hPTP-ζ-ECD-Fc and hCSF-1R-ECD-Fc) were prepared as described previously for the hCSF-1R-ECD-Fc (13). Human recombinant CSF-1 (hCSF-1) was a gift from Chiron Corp. (Emeryville, CA). EDC/NHS, HBS-P, and HBS-P+ buffers were from GE Healthcare.

**Sample Preparation for LC-MS/MS Identification of the Receptor**—Subcellular fractionation was carried out to isolate the membrane fraction from a pool of two postnatal day 7 and two postnatal day 60 mouse brains. Briefly, mouse brain tissue was homogenized in homogenization buffer (65 mM Tris, 150 mM sodium chloride, 1 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM benzamidine, pH 7.4), and the homogenate was centrifuged (1000 × g, 3 min, 4 °C). The supernatant was further centrifuged (100,000 × g, 30 min, 4 °C), and the pellet was dissolved in 2% N-octyl β-D-glucoside (OG) prior to centrifugation (100,000 × g, 30 min, 4 °C). The supernatant containing 26 mg of the OG-solubilized membrane lysate was first preclarified by incubation with 60 μg of anti-CSF-1R peptide antibody (C-15) (4 °C, 16 h) and then incubated with 24 μg of mIL-34 non-covalently bound to 40 μg of immobilized polyclonal rabbit anti-mIL-34 antibody (4 °C, 16 h). mIL-34-antimIL-34 antibody complex was serially washed using 0.1 M glycine HCl, pH 2.2 and 8 M urea and subsequently eluted with 1% SDS. The glycine HCl and urea washes did not result in dissociation of proteins from mIL-34-anti-mIL-34 antibody complex as determined by SDS-PAGE and LC-MS/MS.6 The denatured, reduced, and alkylated SDS eluate was further concentrated by ultra centrifugation using 100-kDa-cutoff filters and subjected to SDS removal, concentration, trypsinization, and detergent extraction with ethyl acetate as described elsewhere (45, 46) followed by LC-MS/MS.

**Nanoelectrospray LC-MS/MS Analyses and Protein Identification**—Tryptic digests were loaded and separated using the UltiMate, FAMOS, Switchos nano-HPLC system (LC Packings, Dionex, Sunnyvale, CA) connected on line to an LTQ linear ion trap mass spectrometer (Thermo Fisher Scientific, Waltham, MA) and equipped with a nanospray source. The mobile phases consisted of 5% acetonitrile, water, and 0.1% formic acid (A) and 80% acetonitrile, water, and 0.1% formic acid (B). After injection (15 μl of sample) and loading onto a C18 trap column (0.3-mm inner diameter × 5 mm), the tryptic peptides were separated on a C18 analytical HPLC column (75-μm inner diameter × 15 cm; PepMap, 3 μm, 100 Å; LC Packings, Dionex). The flow rate for loading and desalting was 15 μl/min for 30 min, whereas the analytical separation was performed at 250 nl/min. The gradient used was as follows: 2–55% B in 65 min, held at 55% B for 10 min, increased to 95% B in 5 min, and then held at 95% B for 5 min. The HPLC eluent was electrosprayed into the LTQ using the nanospray source. After an ini-

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6 S. Nandi, Y. G. Yeung, and E. R. Stanley, unpublished observations.
Novel IL-34 Receptor

Table 1

| PTP-ζ shRNA sequences |
|------------------------|
| Hairpin sequence: 5'- |
| GATCCCCGATTTCTACCAACATCCAAGAGATGGTGAGTAAATCTGTTTTT |
| 3' |
| Corresponding siRNA sequences: |
| • Sense: 5'-CCAGAUUUCACCAACAT-3' |
| • Antisense: 5'-UGUUGUGUGUAACUUCUGIT-3' |

Hairpin sequence: 5'-
GATCCCCGAGATTTCTACCAAGAGATGGTGAGTAAATCTGTTTTT-3'

Corresponding siRNA sequences:
• Sense: 5'-CCAGAGAUUUCACCAACAT-3'
• Antisense: 5'-UCAGAGACUUCUGUGIT-3'

Hairpin sequence: 5'-
GATCCCCGAGATTTCTACCAAGAGATGGTGAGTAAATCTGTTTTT-3'

Corresponding siRNA sequences:
• Sense: 5'-GGGGGAACUGCAACAU-3'
• Antisense: 5'-UAUGUGACAGUUCUUCG-3'

Total mass spectrometry (MS) survey scan (m/z 300–1800), MS/MS scans were obtained from the three most intense ions using a normalized collision energy of 35%. DTA files were generated from the raw data files, merged, and searched against all species of the NCBInr database (July 2, 2010) using Mascot (version 2.3). The search parameters were: fixed modification, carbamidomethyl Cys; variable modifications, Asn/Gln deamidation, oxidized Met, pyro-Glu from Gln, and pyro-Glu from Glu; two missed cleavages; peptide mass tolerance, ±3.5 and ±0.6 Da for the product ions. Scaffold (version 3, Proteome Software Inc., Portland, OR) was used to validate MS/MS-based peptide identifications. Peptide identifications were accepted if they could be established at greater than 95% probability and with unique significant peptide hits (p < 0.05). Utilizing these criteria, nine proteins were identified excluding trypsin and keratin (six of them were membrane proteins listed in supplemental Table 1). The two proteins identified with the highest protein score were TN-R and PTP-ζ. The Mascot protein score for TN-R (three matching protein accession numbers: gi|148707401, gi|226958549, and gi|61216646; 139 kDa) was 932 with 15% protein sequence coverage. The protein score for PTP-ζ (accession number gi|124486807) was 555 with 5% coverage.

Cell Lines, Cell Culture Conditions, and Cell Treatments—The U251, SNB19, and U87MG human glioblastoma cell lines were a gift from Dr. J. Segall, Albert Einstein College of Medicine, New York, NY. NIH-3T3-hCSF-1R cells (47) were a gift from Dr. Martine Roussel, St. Jude Children’s Research Hospital, Memphis, TN. Mouse BAC1.2F5 macrophages (48) were cultured in 36 ng/ml hCSF-1 as described (49). U251 cells were cultured in DMEM-high glucose supplemented with 0.2% BSA for 16 h except where otherwise indicated. Following stimulation, cells were washed in ice-cold PBS and recovered by scraping and centrifugation except where otherwise indicated. For chondroitinase ABC treatment, serum-starved U251 cells were incubated with 4.2 units/ml chondroitinase ABC (37 °C, 1.5 h) and washed extensively before processing for flow cytometry as described below. For treating membrane lysates, 0.3 unit/ml chondroitinase ABC was used.

Generation of U251 PTP-ζ Knockdown (KD) Cells—Lentiviral particles (5 × 10⁴ infectious units) carrying a pool of three different PTP-ζ shRNA or scrambled shRNA plasmids (Santa Cruz Biotechnology; Table 1) were used to infect 3 × 10⁶ U251 cells (50% confluent) in the presence of 5 μg/ml Polybrene in 6-well dishes (37 °C, 16 h). Vector-containing cells were selected using 5 μg/ml puromycin dihydrochloride, and the resistant colonies were further subcloned by a serial dilution method in 96-well plates. The efficiency of knockdown was estimated by Western blotting whole cell lysates from the puromycin-resistant clones with anti-PTP-ζ antibody.

Immunoprecipitation and Western Blot Analysis—Membrane fractions of mouse brain and of BAC1.2F5 macrophages, NIH-3T3-hCSF-1R cells, or U251 cells were solubilized in homogenization buffer (65 mM Tris, 150 mM sodium chloride, 1 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM benzamidine, pH 7.4) containing the appropriate concentration of OG (brain membrane, 2%; cell membrane, 1%) and incubated (4 °C, 16 h) with either immobilized purified polyclonal rabbit anti-mIL-34 antibody beads (preincubated with mIL-34), biotinylated mIL-34, biotinylated hIL-34 (a gift from Five Prime Therapeutics, Inc.), or anti-hCSF-1R antibody. The biotinylated IL-34 complexes were recovered by incubation with neutravidin-agarose, and SDS eluates of IL-34 pulldowns and immunoprecipitates were analyzed by SDS-PAGE and Western blotting. For co-immunoprecipitation experiments, mouse brain membrane lysates were preincubated with mIL-34 (4 °C, 16 h) prior to incubation with anti-PTP-ζ (3F8) antibody. For stimulation and/or immunoprecipitation experiments, serum-starved U251 cells were incubated with hPTN or hIL-34 (120 ng/ml) at 37 °C, and Nonidet P-40 cell lysates (using 1% Nonidet P-40, 10 mM Tris-HCl, 50 mM NaCl, 30 mM Na₂P₂O₇, 50 mM NaF, 100 μM Na₃VO₄, 5 mM ZnCl₂, 1 mM benzamidine, 10 μg/ml leupeptin, and 10 μg/ml aprotinin, pH 7.2) were subjected to immunoprecipitation using antibodies to FAK and paxillin.

Flow Cytometry—For cell surface IL-34 binding, serum-starved U251 cells were gently harvested with 2 mM EDTA in PBS, pH 7.4 and washed, and 1 × 10⁶ cells were preincubated with a 10 molar excess of hIL-34 in Hanks’ balanced salt solution (Invitrogen) in the presence of 1% BSA (4 °C, 1 h). After extensive washing, specific IL-34 binding was detected by incubating the cells with 2 μg/ml biotinylated hIL-34 (4 °C, 1 h) and subsequently with 5 μg/ml streptavidin-conjugated allophycocyanin-Cy7. Flow cytometry was performed using FACS Canto II (BD Biosciences) (gating on viable cells). The FlowJo software (Treestar) was used for data analysis. For detection of hCSF-1R expression, 2 × 10⁶ serum-starved U251 cells were incubated with 5 μg/ml rat anti-hCSF-1 monoclonal antibody (2-4A5) or control rat IgG₁ (e-Biosciences) (4 °C, 45 min) and then subsequently incubated with 5 μg/ml FITC-conjugated F(ab’)₂ anti-rabbit IgG (e-Biosciences) prior to flow cytometric analysis as described above. For the analysis of FLAG-tagged IL-34 and CSF-1 binding to U251 cells by flow cytometry, the expression, purification, and quantitation of the concentrations of IL-34-FLAG and CSF-1-FLAG proteins in the medium of the
transfected 293T cells as well as the detection of cell binding with biotin-labeled anti-FLAG M2 antibody were carried out as described (18).

Cell Proliferation and Clonogenic Assays—U251 cells were seeded at 25% confluence in DMEM-high glucose supplemented with 10% FCS in 24-well tissue culture dishes. 24 h later, cells were washed twice with PBS, and medium was replaced with DMEM-high glucose supplemented with 1% FCS and vehicle (PBS), hIL-34 (20 ng/ml), or hPTN (20 ng/ml). Cell proliferation was assessed by counting viable (trypan blue-excluding) cells harvested at the indicated times. For the clonogenic assays, semiconfluent U251 cells were exposed to a 16-h pulse of hPTN (20 ng/ml), hIL-34 (20 ng/ml), or vehicle (PBS). Cells where then harvested by Accutase digestion, filtered through a 40-μm mesh to ensure single cellularity, and subsequently seeded at 1000 cells/well into 6-well dishes in the presence of 25% conditioned medium (from the 16-h pulse). The number of colonies composed of >50 cells was scored by crystal violet staining 8 days later.

Cell Migration Assays—For wound healing assays, serum-starved (16 h) monolayer cultures of U251 cells were scratched, and the wound was allowed to heal in the continued absence of serum and in the presence of either hPTN or hIL-34 (200 ng/ml). For haptotactic migration assays, 10⁵ serum-starved U251 cells were assayed (37 °C, 4 h) in a 24-well Transwell chamber. Inserts were precoated with BSA (20 μg/ml; no growth factor), hPTN (5 μg/ml), or hIL-34 (10 μg/ml) for 2 h at room temperature prior to the assay. For the random migration studies (37 °C, 4 h), hPTN or hIL-34 (1 μg/ml) was added to both sides of the Transwell chamber. Cells were scraped from the upper side of the chamber, and the lower side was stained with DAPI and phalloidin. Phalloidin-stained cells were counted using a fluorescence microscope.

Surface Plasmon Resonance (SPR) binding analyses were performed at 25 °C on Biacore instruments. For binding of hIL-34 and hCSF-1 to immobilized hPTP-ζ- and hCSF-1R-ECD-Fcs (Biacore T100), all flow cells of a CM4 sensor chip were activated with EDC/NHS (7 min, 10 μl/min), and recombinant Protein A (Pierce, 21184; 50 μg/ml) was added to both sides of the Transwell chamber. Cells were scraped from the upper side of the chamber, and the lower side was stained with DAPI and phalloidin. Phalloidin-stained cells were counted using a fluorescence microscope.

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RESULTS

mIL-34 Associates with Mouse Brain PTP-ζ and TN-R—Compared with the expression patterns of CSF-1 and the CSF-1R, IL-34 expression in postnatal mouse brain is spatially, temporarily, and quantitatively distinct (9, 14). This prompted us to search for an additional IL-34 receptor(s) in detergent-solubilized postnatal mouse brain membranes. To identify novel IL-34-interacting proteins, mouse brain membrane lysate depleted of the known IL-34 receptor, CSF-1R, was subjected to affinity chromatography with mIL-34 non-covalently bound to an immobilized polyclonal rabbit anti-mIL-34 antibody (Fig. 1A, lanes 1 and 2). Bound proteins were eluted with SDS and processed for MS. The two eluted proteins identified with highest certainty were PTP-ζ, a cell surface receptor PTP (Fig. 1B, upper panel), and its ECM ligand, TN-R (Fig. 1B, lower panel). SDS-PAGE and silver staining of the SDS eluates of the mIL-34-anti-mIL-34 antibody affinity purification or of IL-34-associated proteins prepared by the alternative approach of binding to biotinylated mIL-34 and capturing the complexes with immobilized neutravidin (Fig. 1A, lanes 3 and 4) contained a diffuse band of ~400 kDa, another broad band at ~225 kDa, and a ~160/180-kDa species (Fig. 1A, lanes 1–4, upper panel).

Due to alternative splicing, PTP-ζ exists in three isoforms: one soluble and two membrane-spanning molecules (Fig. 1C). The ~400-kDa band was confirmed by Western blotting to cover stainable bands corresponding to both the long (50) and phosphacan isoforms of PTP-ζ (51, 52) as well as multimeric TN-R (53). The ~225-kDa band co-migrated with a band that stained with the 473-HD antibody (43), which sensitively stains the short PTP-ζ isoform and which also stained in the region of the ~400-kDa band corresponding to the long receptor and the phosphacan isoforms. The 160/180-kDa proteins with the apparent molecular mass of the monomeric TN-R isoforms (53) co-migrated with the faster bands Western blotted with the anti-TN-R antibody (Fig. 1A, lanes 1–4, lower panels) that
**Figure 1. Interaction of IL-34 with PTP-ζ.**

A, solubilized membrane fractions of mouse brain. OG-solubilized membrane fractions of mouse brain were incubated (4 °C, 16 h) with either immobilized polyclonal rabbit anti-mIL-34 antibody beads that had been preincubated with mIL-34 (lanes 1 and 2) or with biotinylated mIL-34 (lanes 3 and 4). The SDS eluates of the IL-34 immunoprecipitates or biotinylated IL-34 complexes (recovered with neutravidin beads) were analyzed by SDS-PAGE with silver (Ag) staining. The IL-34-associated proteins identified with 99% certainty by mass spectrometry were PTP-ζ/H9256 and TN-R (supplemental Table 1). Western blots (WB) indicate the PTP-ζ (L, long isoform; P, phosphacan/soluble isoform; S, ~225-kDa short isoform) and TN-R staining bands. Multimeric (S3) (slower migrating bands, ~400 kDa) and alternatively spliced (faster migrating bands, 160/180 kDa) variants of TN-R were co-immunoprecipitated or pulled down. B, LC-MS/MS peptide hits for PTP-ζ/H9256 and TN-R. Identified peptides are highlighted. Upper panel, PTP-ζ protein sequence. The carbonic anhydrase homology domain (CA), the fibronectin type III repeat (F), and the dual phosphatase domains (PTP1 and PTP2) are boxed. The consensus GAG addition sites are underlined, and the transmembrane domain is italicized. Several N-linked glycosylation sites are not indicated. The peptide stretch missing in the short isoform is bold. Lower panel, TN-R protein sequence. C, scheme depicting various PTP-ζ isoforms. Upper panel, the long isoform (active) containing a carbonic anhydrase domain (CA), a fibronectin type III repeat (F), a transmembrane domain (TM), protein-tyrosine phosphatase domains (PTP1 and PTP2), and three GAG addition sites. Middle, phosphacan, the secreted isoform lacking the transmembrane and PTP domains. Lower, the short isoform (active) missing 860 amino acids of the long isoform. 3F8 and C-209 antibodies recognize the extracellular and intracellular regions of PTP-ζ, respectively, and the 473-HD antibody recognizes all three isoforms. 3F8 is directed against rat phosphacan and is not as effective in detecting mouse PTP-ζ. C-209 antibody recognizes the short isoform from mouse brain membrane lysates infrequently. D, co-immunoprecipitation of PTP-ζ with IL-34. The OG-solubilized mouse brain membrane fraction was incubated with mIL-34 (4 °C, 16 h) and immunoprecipitated with immobilized anti-PTP-ζ (3F8) or isotype control (mIgG1) antibody, and the immunoprecipitates were analyzed by gradient SDS-PAGE and Western blotting. L, long isoform; P, phosphacan; S, short isoform. E, SPR analysis showing that hIL-34, but not hCSF-1, binds hPTP-ζ-ECD-Fc. Blank-subtracted sensograms for hIL-34 (left panel) and hCSF-1 (right panel) are shown. RU, resonance units.
correspond to the monomeric TN-R isomers. To confirm the PTP-ζ binding results, we incubated mouse brain membrane lysate with mIL-34 and performed a reciprocal co-immunoprecipitation experiment utilizing 3F8, an antibody that recognizes both the soluble and the long membrane-spanning isoforms of rat PTP-ζ (54) (Fig. 1D), and Western blotting with anti-IL-34 and anti-TN-R antibodies (Fig. 1D). These results show that mIL-34 forms a complex with the larger membrane-spanning isoform of PTP-ζ and with TN-R. However, consistent with earlier reports that the TN-R binding to PTP-ζ is ligand-independent (29, 55), we also observed that TN-R binding to PTP-ζ was IL-34-independent (Fig. 1D).

**hIL-34, but Not hCSF-1, Binds to the Human PTP-ζ ECD in Vitro**—SPR analysis of hIL-34 binding to the full-length hPTP-ζ-ECD-Fc (KD ≈ 10⁻⁷ M) and the hCSF-1R-ECD-Fc (KD ≈ 10⁻¹² M) revealed dose-dependent binding to both (Fig. 1E, left panel), whereas hCSF-1 only bound to the hCSF-1R-ECD-Fc (KD ≈ 10⁻¹¹ M) (Fig. 1E, right panel). Similar analyses of the binding of IL-34 to two other proteoglycans identified by MS, human TN-R (KD ≈ 10⁻⁶ M) and human brevicain (KD = 3 × 10⁻⁸ M), revealed lower affinity binding, whereas the interaction of TN-R with the hPTP-ζ-ECD-Fc was of higher affinity (KD ≈ 10⁻⁸ M) but lower than reported previously (29, 55).

**hIL-34 Binds to Cell Surface PTP-ζ on U251 Glioblastoma Cells**—Because membrane-spanning PTP-ζ, rather than TN-R, is a known signal-transducing receptor for several ligands (25–29), we sought to determine whether PTP-ζ also functions as a receptor for IL-34. As PTP-ζ is up-regulated in human glioblastomas (39–41), we tested glioblastoma cell lines U251, SNB19, and U87MG for PTP-ζ expression. All the tested cell lines expressed high levels of PTP-ζ and we selected U251, which does not express the CSF-1R (Fig. 2A), for hIL-34 binding studies.

Supporting our observations in mouse brain membrane (Fig. 1A), biotinylated hIL-34 primarily formed complexes with the long, membrane-spanning ~400-kDa PTP-ζ (51) and to a lesser extent with the non-glycosaminoglycanated 300-kDa (56) and the short 220-kDa (50) isoforms (Fig. 2B) in U251 membrane lysates. However, we failed to observe TN-R in the biotinylated hIL-34 pulldown fractions of U251 membranes, suggesting that IL-34 binds to PTP-ζ in a TN-R-independent manner. Clones stably expressing PTP-ζ shRNA (KD cells) expressed lower levels of PTP-ζ protein than clones expressing scrambled shRNA (scrambled cells) (Fig. 2C, left panel). Scrambled cells expressed higher levels of total soluble PTP-ζ than uninfected cells (Fig. 2C, right panel), indicating that lentiviral infection per se causes cellular PTP-ζ up-regulation. Consistent with the dependence of IL-34 binding on PTP-ζ expression, flow cytometric studies demonstrated that the ability of biotinylated hIL-34 to bind to the cell surface of intact U251 cells was reduced in PTP-ζ KD cells, particularly in KD2 cells (Fig. 2D). Thus, these results show that hIL-34 binds to PTP-ζ at the surface of intact U251 cells. The specificity and binding of IL-34 to U251 cells was also investigated in binding experiments with FLAG-tagged human IL-34 and FLAG-tagged human CSF-1 (18). At equivalent concentrations, IL-34-FLAG exhibited dose-dependent binding, covering a wide concentration range (0.1 pm−1 nm) (Fig. 2F).

**hIL-34 Inhibits U251 Proliferation, Clonogenicity, and Motility in a PTP-ζ-dependent Manner**—PTP-ζ signaling is involved in neuronal migration (57) and neuritogenesis (58) in mouse and in the in vitro and in vivo growth of human glioblastomas (40, 59, 60). As previous studies have shown that PTN inhibits the growth of glioblastomas, to determine the functional relevance of PTP-ζ receptor engagement by IL-34, we tested the effects of IL-34 and PTN on the U251 glioblastoma cells expressing either PTP-ζ or scrambled (control) shRNA. We found that both IL-34 treatment and PTN treatment slightly, but significantly, reduced the growth of scrambled U251 cells over a 96-h time period (~20% reduction in IL-34 versus vehicle-treated control cells; Fig. 3A, left panel), whereas the growth of the PTP-ζ KD U251 cell lines was not affected (Fig. 3A, right panel). We also examined the effects of IL-34 and PTN on the colony-forming ability of infected U251 cells. After a 16-h pulse with IL-34 or PTN, cells were seeded at clonal density, and the colonies formed at 8 days were stained and counted. IL-34 or PTN treatment strongly decreased the clonogenicity of scrambled U251 cells (reductions of 68% for IL-34 and 53% for PTN versus vehicle control cells) without significantly affecting the clonogenicity of PTP-ζ KD cells (Fig. 3B, left and right panels).

As PTN was also shown previously to affect glioblastoma cell migration (39–41), we compared the wound healing rates of the scrambled and PTP-ζ KD cells (Fig. 4A). In the absence of added ligand(s), KD clones exhibited a slower rate of wound healing, indicating that the constitutively active PTP-ζ receptor facilitates U251 migration (e.g. time taken for 50% wound closure (t₅₀) for KD2 cells was >20 h compared with 5.5 h for scrambled cells; Fig. 4A). Consistent with ligand-induced activation of the receptor (31, 33, 61), PTN (t₅₀ = 10 h) or IL-34 (t₅₀ = 11.5 h) significantly inhibited wound healing in uninfected cells (vehicle t₅₀ = 7.5 h) (Fig. 4B). Furthermore, neither IL-34 nor PTN could suppress PTP-ζ KD cell wound healing (Fig. 4C), thereby indicating that suppression of healing by either ligand is mediated through PTP-ζ. To determine whether IL-34 and PTN suppress directed migration, we utilized a haptotaxis assay in which PTP-ζ ligands were shown to be more effective in regulating migration than in a conventional chemotaxis assay (62, 63). Both IL-34 and PTN when coated on the bottom of the membrane suppressed migration of U251 cells (Fig. 4D). To determine whether IL-34 and PTN also inhibit random migration, we examined migration of the cells through membranes containing these growth factors on both sides. Both IL-34 and PTN inhibited the random migration of the cells (Fig. 4E). Together, these results demonstrate that IL-34 suppresses proliferation, clonogenicity, and motility of U251 cells in vitro in a PTP-ζ-dependent manner.

**hIL-34 Enhances PTP-ζ-mediated Tyrosine Phosphorylation of FAK and Paxillin in U251 Cells**—To function as a receptor for IL-34, IL-34 binding to cell surface PTP-ζ should trigger intracellular signaling. Consistent with the reduction of PTP-ζ phosphatase activity by ligand binding (31, 34, 61), PTP-ζ ligand binding has been shown previously to trigger intracellular protein tyrosine phosphorylation (32, 33, 35, 36). Following

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7 S. Nandi and E. R. Stanley, unpublished observations.
incubation of U251 cells with PTN or IL-34 for various times at 37 °C, we observed a similar ligand-induced tyrosine phosphorylation of proteins, including those with apparent molecular masses of ~190, ~125, ~120, ~70, and ~42 kDa, that peaked within the first 5 min of stimulation (Fig. 5A). PTP-ζ ligands have been shown to increase the tyrosine phosphorylation of FAK in lung and prostate carcinomas and endothelial cells (32, 38, 41) and of paxillin in osteoblastic cells (64), and G protein-coupled receptor kinase interactor 1/Cool-associated, tyrosine-phosphorylated 1 (GIT1/Cat-1) has been shown to be a direct PTP-ζ substrate (65). We showed that either PTN or IL-34 also increased the tyrosine phosphorylation of FAK (~125 kDa), paxillin (~70 kDa) (Fig. 5, A and B), and GIT1/Cat-17 in U251 cells.

In contrast, we failed to detect an increase in the tyrosine phosphorylation of the putative PTP-ζ substrates β-catenin and β-adducin (33, 35) in response to either ligand.7 IL-34-mediated activations of FAK and paxillin were abolished in the PTP-ζ KD2 cell (Fig. 5C), demonstrating that IL-34-induced tyrosine phosphorylation of these proteins is mediated by PTP-ζ.

hIL-34 Binds to PTP-ζ in a Chondroitin Sulfate-dependent Manner—PTP-ζ is a proteoglycan receptor for several ligands (25–29). Furthermore, the PTP-ζ CS chains are known to affect
binding to some of these ligands (26, 30). We therefore tested the requirement of CS for IL-34 binding. Consistent with the previously reported presence of CS on PTP-ζ (20, 66), treatment of solubilized U251 membranes with chondroitinase ABC increased the mobility of a significant fraction of the large PTP-ζ isoform (Fig. 6A). To determine the requirement of CS for cell surface binding, intact U251 cells were incubated with enzyme buffer alone or with chondroitinase ABC to remove cell surface CS. Treatment with chondroitinase ABC reduced binding of biotinylated IL-34 to the level seen in IL-34-competed cells (background levels) (Fig. 6B). Preincubation of U251 cells with a 16 molar excess of IL-34 blocked the subsequent binding of biotinylated IL-34, whereas preincubation with a 16 molar excess of CSF-1 or PTN was without effect (Fig. 6C). Consistent with the removal of binding sites by preincubation with chondroitinase ABC, preincubation of IL-34 with 3 μg/ml shark cartilage CS blocked IL-34 binding (Fig. 6C). Thus, the CS GAG moiety of PTP-ζ is involved in IL-34 binding. SPR analysis further confirmed the inhibition by CS (Fig. 6D).

**Comparative Expression Profiles of PTP-ζ, TN-R, and IL-34 in Adult Brain**—Previous studies have shown that PTP-ζ is primarily expressed in neural progenitors and glial cells (22–24) as well as in a subset of cortical neurons (23, 26). The expression of TN-R overlaps with PTP-ζ expression in rostral brain regions (67–69). IL-34 expression is primarily observed on mature neurons (9, 16, 17), including regions of the brain where PTP-ζ is expressed (24). We have shown previously that IL-34 expression profiles are distinct from those of its cognate receptor, CSF-1R, and also from those of CSF-1 and that it is preferentially increased in specific areas of early postnatal and adult forebrain, thereby suggesting the presence of an alternative signaling receptor (9). As PTP-ζ functions as a cell surface receptor for IL-34 (Figs. 2–5) and also interacts with TN-R (Fig. 1),

**FIGURE 2.** IL-34 binds cell surface PTP-ζ in U251 glioblastoma cells. A, U251 human glioblastoma cells lack the CSF-1R. Left panel, failure of hIL-34 to bind to CSF-1R on U251 cells. OG-solubilized membrane fractions of BAC1.2F5 (lanes 1 and 2) and U251 (lanes 3 and 4) cells were incubated overnight at 4 °C with biotinylated mIL-34 (lanes 1 and 2) or biotinylated hIL-34 (lanes 3 and 4), and the biotinylated IL-34-neutravidin complexes were analyzed by SDS-PAGE and Western blotting (WB) with an antibody that equally recognizes both mouse CSF-1R and hCSF-1R. Middle panel, absence of CSF-1R in U251 cells. OG-solubilized membrane fractions of U251 human glioblastoma (lanes 1) and NIH 3T3-CSF-1R (lanes 2–4) cells were incubated overnight at 4 °C with anti-hCSF-1R antibody (lanes 1 and 2) or biotinylated hIL-34 (lanes 3 and 4), and the biotinylated IL-34-neutravidin complexes and CSF-1R immunoprecipitates were analyzed as above. Right panel, flow cytometric verification. 3T3-CSF-1R or U251 cells (2 × 10⁶) were incubated with 5 μg/ml rat monoclonal anti-hCSF-1R antibody or rat IgG1 (isotype control) for 30 min at 4 °C, washed with PBS, and further incubated with 5 μg/ml anti-rat IgG1-conjugated FITC for 30 min at 4 °C. B, interaction of IL-34 with PTP-ζ in OG-solubilized U251 cell membrane fractions. Membrane lysates were incubated with biotinylated hIL-34 (4 °C, 16 h), and the complexes were captured with neutravidin beads (4 °C, 6 h), eluted with SDS, and analyzed by SDS-PAGE and silver staining or by Western blotting (WB) with antibody to PTP-ζ. Arrowhead, nonspecific band; asterisk, PTP-ζ proteolytic product (52); L, long isoform; NG, non-glycosaminoglycan form; S, short isoform. C, reduced PTP-ζ expression in PTP-ζ KD U251 clones. Left panel, PTP-ζ and control (EF1α) Western blots of OG-solubilized whole cell lysates from cells expressing scrambled or PTP-ζ (KD1 and KD2) shRNAs. Right panel, quantitation of the combined intensities of the three bands (long isoform, non-glycosaminoglycan form, and short isoform) from two independent experiments. Note that the scrambled cell line expressed a higher total PTP-ζ compared with uninfected U251 cells. D, flow cytometric analysis of hIL-34 binding to PTP-ζ KD U251 lines. Serum-starved control (scrambled) and KD (KD1 and KD2) cells were either untreated or incubated with 5 μg/ml biotinylated hIL-34 and then subsequently incubated with streptavidin-conjugated allopuriocyanin (APC)-Cy7 prior to flow cytometric analysis gating on viable cells. Right panel, quantitation. Note that although scrambled cells expressed a higher level of total PTP-ζ, cell surface expression was not significantly different from uninfected cells. Compared with the scrambled line, both KD1 and KD2 lines expressed lower levels of cell surface PTP-ζ, and KD2 cells also had reduced cell surface PTP-ζ compared with uninfected cells. G.M., geometric means of signal intensities of duplicate experiments. E, FLAG-tagged IL-34, but not FLAG-tagged CSF-1, binds U251 cells. F, dose dependence of FLAG-tagged IL-34 binding to U251 cells. Control CM, 293T conditioned medium. In C and D, error bars represent S.D. (n = 3), *, p < 0.05; **, p < 0.01; ***, p < 0.001. PE, phycoerythrin; FSC, forward scatter.
we analyzed the expression profiles of IL-34, PTP-ζ, and TN-R in 8–10-week-old mouse brains (Fig. 7). PTP-ζ and TN-R were co-localized in olfactory bulb (OB), cerebral cortex, rostral migratory stream, and the CA3 region of the hippocampus that have been shown previously to express IL-34 (9). In addition, PTP-ζ expression remained prominent in distinct subcortical structures (thalamic and subthalamic nuclei), midbrain, and brain stem nuclei (inferior colliculus, pontine nuclei, locus coeruleus, and vestibular nuclei) as well as the cerebellum and displayed distinct co-localization patterns with both IL-34 and TN-R (Fig. 7, A–D). Mature postmitotic neurons of the cerebrum were labeled by all three of these markers (Fig. 7, E–H). These observations are consistent with our previous study that localized IL-34 expression preferentially to mature neurons of the adult cerebral cortex extending from layers 2 to 5. In addition, we noted that IL-34 and PTP-ζ co-localization was particularly prominent in layer 5 (Fig. 7B1). Consistent with the existence of the secreted PTP-ζ isoform, PTP-ζ expression was also observed in the ECM of the cerebral cortex in concert with IL-34 (Fig. 7, B1–B3). IL-34 and PTP-ζ appeared to be uniformly distributed in the ECM of layer 5 and at the periphery of mature neuronal cell bodies (Fig. 7B2). In contrast, in cortical layer 6, PTP-ζ expression was reduced in the ECM and neuronal soma, whereas IL-34 expression was virtually absent (Fig. 7B2). Expression of TN-R was evident in cerebral cortical layers 2–5 (Fig. 7C1) and was most prominent in layer 4 where it was co-localized with IL-34 in both the ECM and at the periphery of mature neuronal somas (Fig. 7C2). Its cellular and extracellular expression decreased in cortical layer 5 (Fig. 7C3). Finally, in contrast to the expression profiles of IL-34 and TN-R, PTP-ζ staining was also seen in GFAP+ adult neural stem cells present in the anterior subventricular zone (Fig. 7D1) as well as in those neural species migrating through the rostral migratory stream to the OB (Fig. 7, D2 and D3) (22, 24) but not in GFAP+ astrocytes in the corpus callosum.8

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8 S. Gokhan, unpublished observations.
DISCUSSION

The differential and higher expression of IL-34 compared with CSF-1 and CSF-1R expression in brain coupled with the more pronounced effects of IL-34 compared with CSF-1 on neural progenitor cell self-renewal and differentiation (9, 14) suggested the possibility that IL-34 might signal via an alternate receptor. We have identified PTP-ι/H9256, a cell surface CS proteoglycan, as a second functional receptor for IL-34. IL-34 selectively interacts with PTP-ι/H9256 in membrane lysates from both mouse brain and U251 human glioblastoma cells. It binds to intact U251 cells, stimulates their phosphotyrosine signaling, and suppresses their tumorigenic properties in a PTP-ι/H9256-dependent manner. Furthermore, IL-34, but not CSF-1, binds PTP-ι in vitro ($K_d \sim 10^{-7}$ M) and at the cell surface, and whereas IL-34 and CSF-1 compete for binding to the CSF-1R (14, 70, 71), CSF-1 preincubated at a 16 molar excess failed to compete for IL-34 binding to U251 cells. Thus, PTP-ι fulfills the criteria required for it to function as the postulated IL-34 receptor.

The affinity of PTP-ι for IL-34 is somewhat lower than the affinities reported for other ligands ($K_d$ values of 0.04–1.6 $\times$ 10$^{-9}$ M), including midkine, pleiotrophin, TN-R, and TAG-1 (26, 29, 30, 72). The lower affinity of PTP-ι-ECD-Fc for IL-34 contrasts with the ability of IL-34 to bind to intact cells and to stimulate tyrosine phosphorylation at relatively low concentrations (0.1 nM; Figs. 2F and 5C). This suggests the possible involvement of an auxiliary receptor(s) and/or receptor clustering and cooperative signaling at the cell surface. IL-34 may also elicit downstream signaling at low occupancy.

The IL-34/PTP-ι interaction exhibited a striking dependence on PTP-ι CS GAG chain(s). Like IL-34, PTN also exhibits GAG-dependent binding to PTP-ι/H9256. However, in contrast to IL-34, CS-C, but not shark cartilage CS, inhibits this binding (26). Furthermore, competition experiments showed that PTN fails to compete for the binding of IL-34 (Fig. 6C), suggesting that IL-34 binding could involve a different PTP-ι CS GAG moiety not recognized by PTN. It was also of interest that IL-34 binds to other CS and heparan sulfate proteoglycans with lower affinity. The finding that binding to brevican and glypican was effectively blocked by heparin suggests that the low affinity binding of IL-34 to proteoglycans might involve electrostatic interactions between IL-34 and the proteoglycan GAG chains and that the nature of the GAG chain is the likely determinand of this interaction. Such an electrostatic interaction appears to be an important part but not the sole component of the interaction of IL-34 with PTP-ι and the reason for the CS inhibition we observed.

The PTP-ι receptor phosphatase is constitutively active and thought to be inactivated upon ligand binding (31, 33, 61). Con-
consistent with this notion, IL-34 treatment of U251 cells resulted in increased tyrosine phosphorylation of the downstream signaling molecules FAK, paxillin, and GIT1/Cat-1. Furthermore, compared with the scrambled cells, KD cells exhibited higher baseline levels of FAK and paxillin tyrosine phosphorylation and mimicked IL-34 treatment by possessing reduced wound healing activity (Fig. 4A).

We observed that addition of IL-34 to U251 cells led to an increase in the tyrosine phosphorylation of FAK and paxillin and a suppression of cell motility. Although an increase in tyrosine phosphorylation of FAK is associated with an increase in proliferation and motility in other cells, it has been shown to inhibit these responses in glioblastomas (73). Also, similar to our observations in U251 cells, increased tyrosine phosphorylation of paxillin is correlated with inhibition of motility in macrophages (74) and FAK (Tyr397) dephosphorylation promotes tumor metastasis (73). Thus, signaling via the PTP-ζ receptor can display pronounced context-specific actions possibly dependent on the differential expression of PTP-ζ co-receptors and the activation of specific signaling pathways. For example, in contrast to PTP-ζ signaling in U251 cells, PTP-ζ signaling in hematopoietic stem cells leads to their expansion (75). A positive regulatory role of PTP-ζ in proliferation and migration was also reported in some glioblastomas (32, 41) that could reflect the role of known PTP-ζ co-receptors, such as the integrins β1 (64) and β3 (39), in governing these biological responses.

PTP-ζ is primarily expressed on neural progenitor and glial cells (22–24). Our results indicate that IL-34 signaling via PTP-ζ in U251 glioblastoma cells causes a suppression of clonogenicity, which is similar to the effect of CSF-1 or IL-34 on isolated CSF-1R-expressing neural progenitors (9). This suppression of clonogenicity is correlated with stimulation of cellular tyrosine phosphorylation in either setting. Indeed, IL-34 was shown to be significantly more active in suppressing neural progenitor cell proliferation and enhancing neuronal differentiation than CSF-1 (9), which is consistent with an additional action of IL-34 via the PTP-ζ receptor on neural progenitor cells.
In addition, IL-34 suppressed U251 glioblastoma cell motility. Relevant to this, we observed PTP-ζ expression on immature cells migrating within the rostral migratory stream (Fig. 7, D2 and D3) at the periphery of which IL-34 (9) and TN-R (Fig. 7C) (68) are expressed. It is possible that local regulation of these migrating progenitor cells via IL-34/PTP-ζ/TN-R signaling may have structural and functional consequences related to the integrity of the rostral migratory stream and olfaction.

Both PTP-ζ (23, 26) and IL-34 (9, 16) are expressed on cortical neurons. Our immunofluorescence localization studies revealed that IL-34, PTP-ζ, and TN-R were expressed throughout cortical layers 2–5. IL-34 was often co-localized with PTP-ζ and TN-R on the surface of mature neurons in cortical layers 5 and 4, respectively. Thus, IL-34/TN-R/PTP-ζ signaling in these mature, cortical neurons in an autocrine and/or a paracrine manner may play a role in their maintenance and possibly in the integrity of the rostral migratory stream and olfaction.
mediating higher cognitive functions. Interestingly, we also observed co-localization of IL-34 with secreted PTP-ζ (Fig. 7B2) and with TN-R (Fig. 7C2) in the cerebral cortical ECM consistent with the existence of a stable IL-34-PTP-ζ-TN-R complex (Fig. 1D). Such a stable association may serve to provide high local concentrations of IL-34 to receptor-expressing cells. The identification of PTP-ζ as a novel receptor for IL-34 necessitates a reevaluation of the possible role(s) of IL-34/PTP-ζ signaling in tissues in which both ligand and receptor are expressed. Obviously the CNS is an important organ system to study because of the significant expression of both IL-34 and PTP-ζ in brain and because PTP-ζ has been implicated in several disease settings in the CNS. For example, it is expressed in remyelinating oligodendrocytes, and PTP-ζ-deficient mice display a delayed recovery from demyelinating lesions in a model of experimental autoimmune encephalomyelitis (76). Furthermore, the soluble PTP-ζ isoform has been shown to be necessary for maturation of oligodendrocyte progenitors to differentiated myelin-secreting oligodendrocytes in vitro (77), and PTP-ζ-deficient mice exhibit increased myelin breakdown (78). In addition, the PTPRZ1 gene in humans is a schizophrenia-susceptibility gene (79), and PTP-ζ regulates tyrosine phosphorylation of voltage-gated sodium channels in neurons (80). Given the multiplicity of ligands for PTP-ζ, the role of IL-34 regulation in these settings will need to be further defined. Also, our demonstration that IL-34 modulates tumorigenic properties of the glioblastoma cell line U251 coupled with the fact that PTP-ζ is expressed in neuroblastomas (19) and other tumors (38, 41) strongly suggests that analysis of the CSF-1R-dependent and -independent roles of IL-34 in tumorigenesis is of great translational medicine relevance.

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