

## Soluble protein tyrosine phosphatase receptor type Z (PTPRZ) in cerebrospinal fluid is a potential diagnostic marker for glioma

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### Abstract

**Background.** High-grade glioma is the most pervasive and lethal of all brain malignancies. Despite advances in imaging technologies, discriminating between gliomas and other brain diseases such as multiple sclerosis (MS) often requires brain biopsy. Several reports show that protein tyrosine phosphatase receptor Z (PTPRZ) is highly expressed in glioblastoma, and we found that a soluble cleaved form of PTPRZ (sPTPRZ) was present in the cerebrospinal fluid (CSF). The aim of this study was to determine whether the sPTPRZ level in CSF has utility as a diagnostic marker for glioma.

**Methods.** Microarray datasets from normal brain tissue and brain tumors were obtained from the Gene Expression Omnibus. PTPRZ protein expression in clinical specimens was evaluated by immunohistochemistry. Semiquantitative western blotting was used to measure sPTPRZ levels in CSF samples from patients with glioma, schwannoma, MS, or nontumor disorders.

**Results.** Expression of PTPRZ mRNA and protein was markedly increased in glioblastoma, astrocytoma, oligodendroglioma, and schwannoma tissues compared with control brain tissue. sPTPRZ was present at significantly elevated levels in the CSF of patients with glioma (grades 1–4), but not in patients with schwannoma or MS, compared with the control samples. Receiver operating characteristic curve analysis showed that sPTPRZ in CSF could discriminate between glioma and MS patients (area under the curve 0.9676;  $P < .0001$ ).

**Conclusions.** sPTPRZ in CSF is a promising diagnostic biomarker for glioma and could reduce the need for a surgical biopsy.

### Key Points

- PTPRZ mRNA and protein were elevated in glioma tissue compared with a normal brain.
- sPTPRZ in CSF from glioma patients was elevated 10-fold compared with control CSF.
- sPTPRZ in CSF is a potential diagnostic marker for glioma.

## Importance of the Study

Despite advances in brain imaging technology, it can be difficult to differentiate between glioma and other diseases, and a definitive diagnosis often requires a brain biopsy. Here, we examined the expression of cellular protein tyrosine phosphatase Z (PTPRZ) and its cleaved soluble form (sPTPRZ) as a possible diagnostic marker for glioma. Analysis of normal brain and brain tumor RNA microarray datasets (Gene Expression Omnibus) revealed

markedly higher *PTPRZ* mRNA expression in glioma tissues compared with control tissue, and this was confirmed at the protein level by immunohistochemical analysis. Finally, we found that sPTPRZ was present at 10-fold higher levels in the CSF of glioma patients compared with control CSF. Taken together, our data suggest that sPTPRZ in CSF may be a useful biomarker for discriminating between glioma and other diseases.

Glioma is a brain parenchymal tumor that accounts for about 25% of intracranial tumors and about 40% of gliomas are malignant tumors such as glioblastoma.<sup>1,2</sup> Most brain tumors are diagnosed by imaging methods such as CT, MRI, and PET, which provide information on tumor location and, in some cases, identity. However, the diagnostic ability of imaging is limited, and a biopsy is usually necessary to obtain a pathological diagnosis. For example, discriminating between malignant glioma and inflammatory diseases such as multiple sclerosis (MS) can sometimes be difficult.<sup>3</sup>

Diagnostic biomarkers for brain tumors would ideally be present in serum or CSF to avoid the need for tumor biopsy. Such biomarkers, however, have been established for only a limited number of tumors, such as  $\beta$ -human chorionic gonadotropin, alfa-fetoprotein, carcinoembryonic antigen, and placental alkaline phosphatase for intracranial germ cell tumors<sup>4</sup> and soluble interleukin 2 receptor for malignant lymphoma.<sup>5</sup>

Protein tyrosine phosphatase receptor type zeta (PTPRZ) is a membrane-bound protein predominantly expressed in the CNS,<sup>6</sup> and recent transcriptomic analyses have shown particularly high expression in astrocytes and oligodendrocyte precursor cells.<sup>7</sup> PTPRZ is a heavily glycosylated protein modified with chondroitin sulfate,<sup>8</sup> keratan sulfate,<sup>9,10</sup> N-linked glycans, and O-mannose (O-Man) glycans.<sup>11</sup> The glycosylated extracellular region is cleaved and shed, and the soluble form is known as sPTPRZ or phosphacan.<sup>12</sup> We recently found that sPTPRZ is detectable in CSF, raising the possibility that sPTPRZ in CSF could be a useful diagnostic marker for some disorders. Although several groups have reported significantly higher PTPRZ expression in glioma tissue compared with normal brain,<sup>13–15</sup> several other microarray analyses have not identified *PTPRZ* as one of the genes upregulated in gliomas.<sup>16,17</sup>

In this study, we investigated whether PTPRZ and/or sPTPRZ have value as a diagnostic biomarker for glioma. We analyzed *PTPRZ* mRNA datasets and PTPRZ protein expression in brain tissues and performed western blot analysis of PTPRZ in microsome fractions derived from glioma tissues and sPTPRZ in CSF. We show that PTPRZ expression is elevated in glioma compared with control tissues and that sPTPRZ levels are higher in CSF from glioma patients than that from schwannoma and MS patients. Our results indicate that sPTPRZ in CSF may be a promising

biomarker and alternative to brain biopsy for the diagnosis of glioma.

## Materials and Methods

### Subjects

This study was approved by the ethics committee of Fukushima Medical University (approval numbers 2466 and 2478), which is guided by local policy, national laws, and the World Medical Association Declaration of Helsinki. CSF samples were collected from 75 patients at Fukushima Medical University and 11 patients at Takasaki General Medical Center between September 2004 and May 2019. They consisted of 24 patients with glioma, 14 with schwannoma, 27 with MS, and 21 with nontumor disorders (idiopathic normal pressure hydrocephalus [iNPH], unruptured cerebral aneurysms, facial spasm, or trigeminal neuralgia), which were the control subjects. Tumors were diagnosed and graded according to the current WHO Classification.<sup>2,18</sup> MS diagnosis was based on the McDonald criteria<sup>19,20</sup>. iNPH was diagnosed according to the iNPH guidelines<sup>21</sup> and ventriculomegaly with an Evans' index of more than 0.3. The clinical profiles of the patients are summarized in Table 1.

**Table 1.** Clinical Characteristics of Subjects for CSF Analysis

Tumor/disease type	No. of patients	Male/female	Age range (years)
Glioma <sup>a</sup>	24	14/10	3–78
Schwannoma	14	2/12	18–80
Multiple sclerosis	27	11/16	18–69
Control <sup>b</sup>	21	8/13	32–88

<sup>a</sup>Subtypes of gliomas (number of patients) were as follows: pilocytic astrocytoma (3), diffuse astrocytoma (1), anaplastic oligodendroglioma (4), anaplastic astrocytoma (8), glioblastoma (6), gliosarcoma (1), and unclassified glioma (1).

<sup>b</sup>Control samples were obtained from patients with iNPH, unruptured cerebral aneurysm, facial spasm, and trigeminal neuralgia.

## CSF Sampling

All attempts were made to obtain CSF samples as noninvasively as possible depending on the disease, ensuring that they would fulfill scientific quality standards as well as ethical considerations. For gliomas, schwannomas, facial spasm, trigeminal neuralgia, and unruptured cerebral aneurysms, CSF samples were only collected during surgery in a noninvasive manner, avoiding lumbar puncture. For MS, they were collected upon lumbar puncture for diagnostic purposes. The sites and timings of CSF sampling were as follows: gliomas, deep sulci, or fissures outside of the tumor (cerebral cisterns) were sampled right after the dural opening before the tumor removal procedure; schwannomas, facial spasm, and trigeminal neuralgia, the cerebellomedullary cistern were sampled before initiating manipulation of the tumors or nerves; unruptured cerebral aneurysm, Sylvian fissure, or interhemispheric fissure (cerebral cisterns) were sampled when exposing the above; iNPH was sampled from the lateral ventricle during ventriculoperitoneal shunting, from the lumbar sac upon lumbar puncture; and MS was sampled from the lumbar sac upon lumbar puncture. CSF samples were centrifuged at 1500 rpm for 5 min to remove cells and debris and were then stored at  $-80^{\circ}\text{C}$  until biochemical analysis.

## Reagents

Most chemicals were purchased from Sigma or Wako Chemicals. The following antibodies were purchased: mouse IgM anti-PTPR $\zeta$  (sc-33664; Santa Cruz Biotechnology), hereinafter referred to as “anti-PTPRZ (Santa Cruz);” mouse IgM anti-chondroitin sulfate proteoglycan (MAB1581; Merck Millipore), referred to by its clone name, as “Cat-315;” mouse monoclonal anti-aggrecan (6-B-4; Abcam), rabbit polyclonal anti-PTPRZ1 (HPA015103; Sigma), hereinafter referred to as “anti-PTPRZ1 (Sigma);” anti-pan cadherin (ab16505; Abcam), polyclonal sheep anti-transthyretin (ab9015; Abcam), referred to as “TTR;” horseradish peroxidase (HRP)-conjugated goat anti-mouse IgM (SAB-110; Stressgen), goat anti-rabbit IgG (NA934; GE Healthcare), and donkey anti-sheep IgG-HRP (A16041; Thermo Fisher Scientific).

## Microarray Expression Analysis

Three gene expression microarray datasets (GSE15824,<sup>22</sup> GSE4290,<sup>23</sup> and GSE108524<sup>24</sup>) were downloaded from the National Center of Biotechnology Information Gene Expression Omnibus (GEO). GSE15824 and GSE4290 datasets were obtained on the GPL570 platform of [HG-U133\_Plus\_2] Affymetrix Human Genome U133 Plus 2.0 Array (Affymetrix), and the GSE108524 dataset was obtained on the GPL17586 platform of [HTA-2\_0] Affymetrix Human Transcriptome Array 2.0 (Affymetrix). We analyzed normalized mRNA expression values for *PTPRZ* and the  $\gamma$  isoform *PTPRG* from each dataset. Because *PTPRG* gene expression was represented by multiple probes (1569323\_at, 204944\_at, 227126\_at, and 244574\_at for the GPL570 platform; TC03000384.hg.1, TC03002363.hg.1, TC03002364.hg.1, and TC03002365.hg.1 for the GPL17586 platform), the expression values of each were summed.

## Western Blot Analysis

### Sample preparation and experimental protocols.

—Glioma tissues were homogenized with 7 volumes of 50 mM Tris-HCl buffer (pH 7.4)–0.15 M NaCl containing a protease inhibitor cocktail (Roche) using Dounce homogenizer. Homogenates were ultracentrifuged at  $100\,000 \times g$  for 30 min at  $4^{\circ}\text{C}$ , and the resultant pellet was lysed with T-PER Tissue Extraction Reagent (Thermo) containing a protease inhibitor cocktail and used as microsome fraction. Microsome fractions (6  $\mu\text{g}$  protein) and CSF samples (10  $\mu\text{L}$ ) were digested with 0.2 mU chondroitinase ABC (Sigma) in Tris-acetate buffer (pH 7.4) containing a protease inhibitor cocktail (Nacalai) for 1 h at  $37^{\circ}\text{C}$ . Some samples were additionally digested with 3  $\mu\text{L}$  endo- $\beta$ -galactosidase (R&D Systems) in sodium citrate buffer (pH 6.0) for 16 h at  $37^{\circ}\text{C}$ .

Proteins in the digested samples were resolved by SDS-PAGE (3–10% gradient gels, Atto) and transferred to nitrocellulose membranes. After blocking with 1% bovine serum albumin in phosphate-buffered saline (pH 7.4) overnight at  $4^{\circ}\text{C}$ , the membranes were probed with anti-PTPRZ (Santa Cruz, 1:200), Cat-315 (1:5000), anti-PTPRZ1 (Sigma, 1:750), anti-aggrecan (1:500), anti-cadherin (1:500), or anti-TTR (1:2000) antibodies for 2 h at  $25^{\circ}\text{C}$  and then with the appropriate HRP-conjugated secondary antibodies (1:10 000 dilution) for 2 h at  $25^{\circ}\text{C}$ . The blots were developed with SuperSignal West Femto maximum sensitivity substrate (Thermo Fisher Scientific) for PTPRZ and Cat-315 detection, and SuperSignal West Dura (Thermo Fisher Scientific) for aggrecan, cadherin, and TTR detection. The signals were detected with EZ-Capture MG (Atto) and quantified using a CS Analyzer ver3.0 (Atto).

### Exploratory experiments.

—We recently found that sPTPRZ is detectable in CSF. Given our finding that PTPRZ expression is elevated in glioma, we anticipated that sPTPRZ levels in the CSF of glioma patients might be higher than in the control subjects and/or MS patients. To investigate this, we first conducted exploratory experiments using CSF and/or microsome samples from small numbers of patients and different antibodies for characterization of PTPRZ protein expression and shedding to CSF. For comparison of sPTPRZ levels at different sites of CSF sampling, the CSF samples of 2 sites, the lateral ventricle and the lumbar sac, from a single iNPH patient were investigated. To evaluate the influence of timings of CSF sampling during surgery on the result, CSF samples were analyzed in 1 glioblastoma patient for whom CSF sampling was undertaken twice during surgery: just after dural opening (before the tumor removal procedure) and after the tumor removal procedure.

### Semi-quantitative evaluation of sPTPRZ in the CSF among glioma, schwannoma, MS, and control samples.

—For quantification of cleaved PTPRZ, semiquantitative western blot analysis with anti-PTPRZ (Santa Cruz) was performed using CSF samples from glioma ( $n = 24$ ), MS ( $n = 27$ ), schwannoma ( $n = 14$ ), and control ( $n = 21$ ) patients. TTR levels in CSF samples were determined by ELISA for the internal standard. The ratio of signal intensities of

sPTPRZ-Long and sPTPRZ-Short compared with the levels of the internal standard protein TTR is calculated. The receiver operating characteristic (ROC) curve analysis of the value of the sPTPRZ-Long to TTR signal intensity ratio in CSF was performed between glioma and MS patients, as well.

### TTR Sandwich ELISA

A PreAlbumin (Transthyretin) ELISA kit (ab108895; Abcam) was used to measure TTR in CSF (1:4000 dilution) according to the recommended protocol.

### Immunohistochemistry and Histology

Tissues were formalin-fixed, paraffin-embedded, sliced into 4- $\mu$ m-thick sections, and either stained with hematoxylin and eosin (H&E) or processed for immunohistochemical staining as follows. Endogenous peroxidase was quenched by incubation with 0.3% hydrogen peroxide solution, and the sections were then incubated with anti-PTPRZ1 (Sigma, 1:400) or Cat-315 (1:2500) antibodies overnight at 4°C. After washing, the sections were incubated with goat anti-mouse IgM-HRP (1:200), and bound antibodies were visualized with a Histofine SAB-PO (M) kit (Nichirei Biosciences). Immunohistochemical staining was evaluated by an independent observer who had no knowledge of the patients' clinical information.

### Statistical Analysis

Data are presented as the mean  $\pm$  SEM. Comparisons between 2 group means were performed with a Mann-Whitney *U* test. Multiple comparisons were performed by one-way analysis of variance with Dunnett's test for data with normal distribution and by Kruskal-Wallis test with Dunn's test for all other data. All analyses were performed using GraphPad Prism 8.21 (Statcon).

## Results

### PTPRZ mRNA Is Upregulated in Several Brain Tumors

We first compared the expression of the  $\zeta$  and  $\gamma$  isoforms of PTPR (*PTPRZ* and *PTPRG*<sup>25</sup>) both of which are expressed in the brain,<sup>26</sup> by analysis of RNA microarray datasets from

GEO. The 3 datasets GSE15824, GSE4290, and GSE108524 consisted of specimens from 89 glioblastoma, 34 astrocytoma, 57 oligodendroglioma, and 27 schwannoma patients and 32 control patients (Table 2). Figure 1A–C shows that *PTPRZ* mRNA levels were significantly higher in all brain tumors tested compared with the controls. *PTPRZ* expression was particularly high in glioma; specifically, 4-fold higher in GSE15824 and 2-fold higher in GSE4290 compared with the controls. In contrast, *PTPRG* expression was slightly elevated in oligodendroglioma but not in other glioma as compared with control specimens (Figure 1D and E). Interestingly, *PTPRG* expression was significantly lower in schwannoma samples than in control samples (Figure 1F).

### PTPRZ Protein Is Expressed in Brain Tumors

To determine whether the elevated *PTPRZ* expression in glioma is derived from glioma cells or other tumor-associated cells, we assessed *PTPRZ* protein expression by immunohistochemical staining of tissue sections. For this, we employed 2 antibodies: anti-PTPRZ1 (Sigma), which is raised against the PTPRZ peptide, and Cat-315, which binds to the abnormal HNK-1-capped *O*-Man glycan plus PTPRZ peptide region,<sup>27,28</sup> as previously described in reactive astrocytes in demyelinated brain lesions<sup>11,28,29</sup> (Figure 2A). H&E staining of tissue sections (glioblastoma, IDH-wildtype) revealed typical histopathological features of glioblastoma, including microvascular proliferation and tumor cells with nuclear atypia<sup>30</sup> (Figure 2B). Immunohistochemical staining showed that some tumor cells were PTPRZ-positive, whereas endothelial cells were PTPRZ-negative (Figure 2C). Tumor cells also showed positive staining with the Cat-315 antibody (Figure 2D). Oligodendroglioma, *IDH*-mutant, and 1p/19q-codeleted samples showed moderately cellular tumors composed of glial tumor cells with uniformly rounded nuclei and relatively rich cytoplasm, proliferating on a fibrillary background (Figure 2E). Although the histological findings may not be typical of oligodendroglioma, the diagnosis was confirmed with genetic/chromosomal profiles (positive *IDH1* mutation and 1p/19q-codeletion). Some tumor cells showed positive staining with anti-PTPRZ1 antibody (Sigma) and weakly positive staining with the Cat-315 antibody (Figure 2F and G). In the schwannoma samples, spindle cells were arranged in wavy or interlacing bundles, and most of the tumor cells were stained positively with anti-PTPRZ1 (Sigma, Figure 2I) but negatively with Cat-315

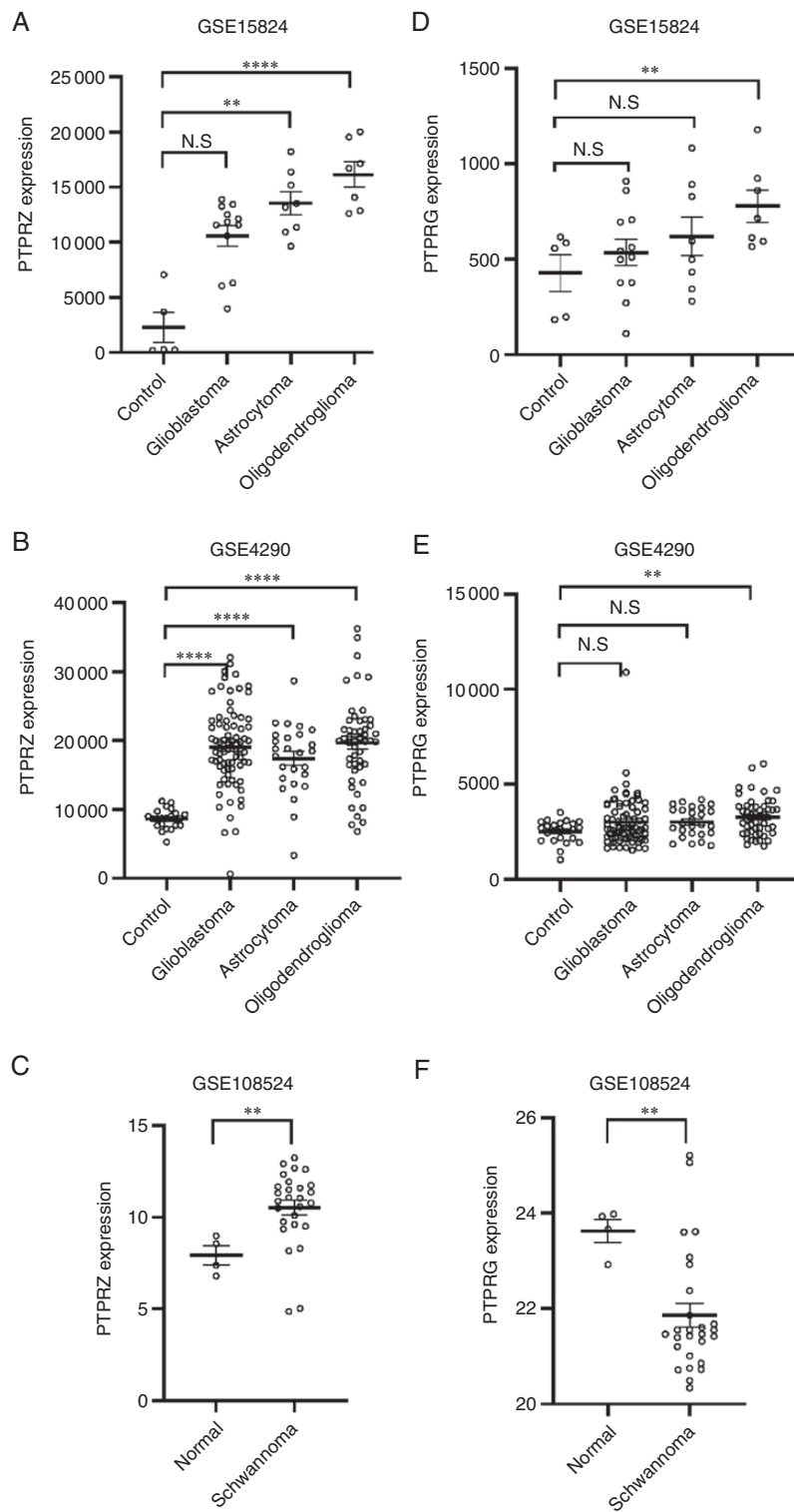
**Table 2.** List of NCBI-GEO Datasets Used in This Study

GEO ID	Control	Glioblastoma	Astrocytoma	Oligodendroglioma	Schwannoma	References
GSE15824	5 <sup>a</sup>	12	8	7	—	28
GSE4290	23 <sup>b</sup>	77	26	50	—	29
GSE108524	4 <sup>c</sup>	—	—	—	27	30

<sup>a</sup>Normal brain tissues and astrocytes.

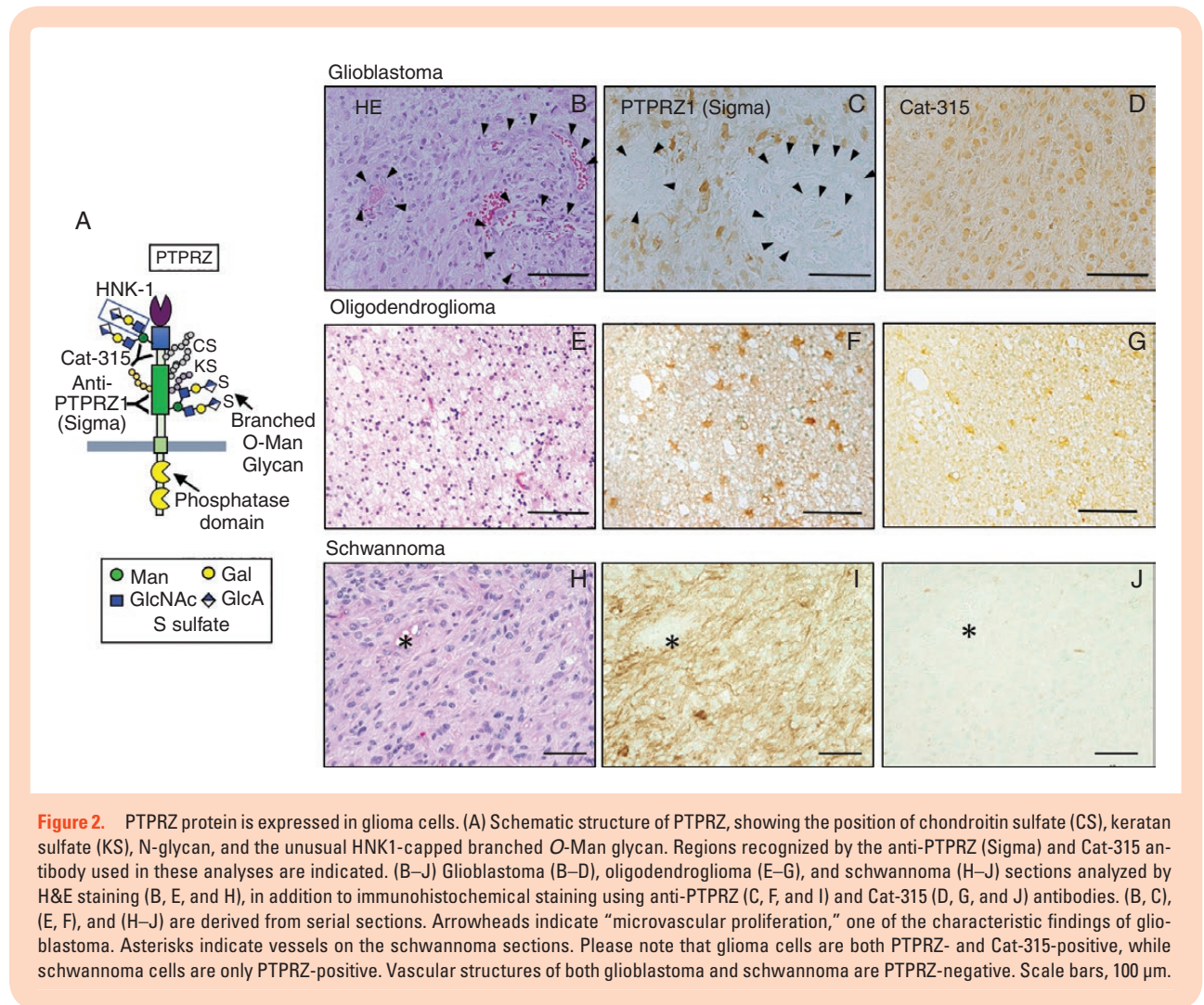
<sup>b</sup>Diagnosed as epilepsy.

<sup>c</sup>Normal vestibular nerve.



**Figure 1.** *PTPRZ* mRNA is highly expressed in brain tumors. (A–F) *PTPRZ* (A–C) and *PTPRG* (D–F) mRNA levels in the GSE15824 (A and D), GSE4290 (B and E), and GSE108524 (C and F) RNA microarray datasets. All data, corresponding with the gene expression levels, are normalized signals and are presented as the mean  $\pm$  SEM, with individual data points represented by circles. \*\* $P < .01$ , \*\*\*\* $P < .0001$  by Mann–Whitney *U* test (C and F), one-way ANOVA with Dunnett’s test (B), and Kruskal–Wallis test with Dunn’s test (A, D, and E).





**Figure 2.** PTPRZ protein is expressed in glioma cells. (A) Schematic structure of PTPRZ, showing the position of chondroitin sulfate (CS), keratan sulfate (KS), N-glycan, and the unusual HNK1-capped branched *O*-Man glycan. Regions recognized by the anti-PTPRZ (Sigma) and Cat-315 antibody used in these analyses are indicated. (B–J) Glioblastoma (B–D), oligodendroglioma (E–G), and schwannoma (H–J) sections analyzed by H&E staining (B, E, and H), in addition to immunohistochemical staining using anti-PTPRZ (C, F, and I) and Cat-315 (D, G, and J) antibodies. (B, C), (E, F), and (H–J) are derived from serial sections. Arrowheads indicate “microvascular proliferation,” one of the characteristic findings of glioblastoma. Asterisks indicate vessels on the schwannoma sections. Please note that glioma cells are both PTPRZ- and Cat-315-positive, while schwannoma cells are only PTPRZ-positive. Vascular structures of both glioblastoma and schwannoma are PTPRZ-negative. Scale bars, 100  $\mu$ m.

(Figure 2J). Taken together, these results are in good agreement with the gene expression microarray data and show increased PTPRZ protein in glioma cells, particularly the unusually glycosylated form detected with the Cat-315 antibody. This differential expression between brain tumors probably reflects cell type-specific expression of glycosylation enzymes.

### sPTPRZ Levels Are Elevated in the CSF of Glioma Patients

#### Exploratory experiments

##### Different sPTPRZ isoforms in the CSF of glioma patients.

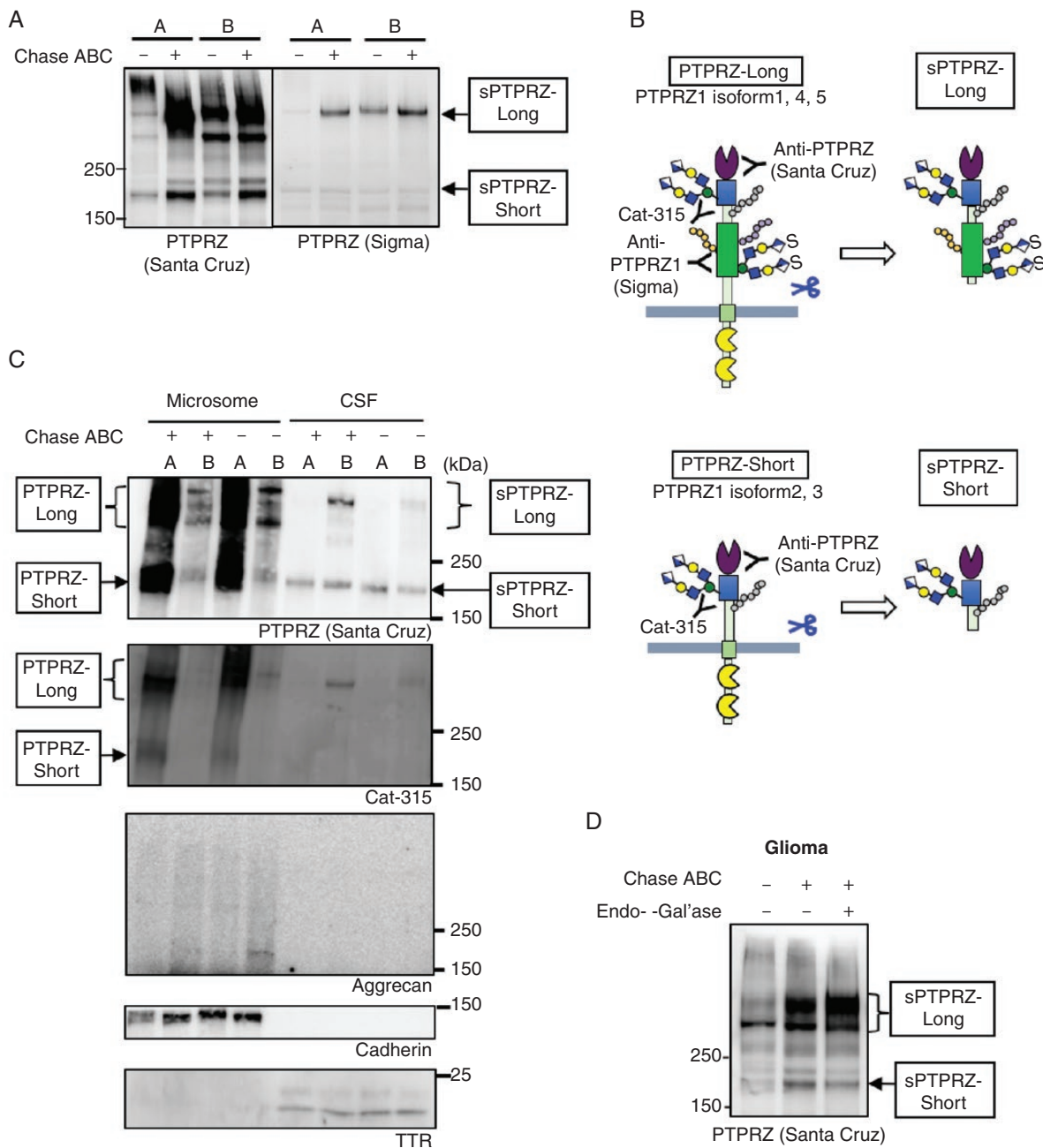
Chondroitinase treatment of CSF samples from glioma patients resulted in the formation of 2 sPTPRZ bands: one of approximately 400 kDa (sPTPRZ-Long) and one of approximately 190 kDa (sPTPRZ-Short), detected with anti-PTPRZ (Santa Cruz) (Figure 3A and Supplementary Figure 1A). Human PTPRZ has 5 isoforms.<sup>31</sup> Isoform 1 consists of 2315 amino acids (aa), isoform 4 of 2308 aa, and isoform 5 of 2301 aa (Figure 3B). Both PTPRZ isoforms 2 (1455 aa) and 3 (1448 aa) lack the heavily glycosylated 755–1614 residues.

When we stained the CSF samples with anti-PTPRZ antibody (Sigma), which is raised against the 755–1613 peptide sequence of human PTPRZ, only the upper band was reactive (Figure 3A).

##### Membrane-bound PTPRZ in the glioma tissues.

We then performed western blot analysis using a microsomal fraction from glioma tissues and CSF samples from glioma patients to detect full-length PTPRZ and sPTPRZ, respectively. Cadherin and TTR were detected as internal standards of microsomal and CSF samples (Figure 3C). Analysis of the microsomal fraction by anti-PTPRZ (Santa Cruz) showed an upper band corresponding with 300–500 kDa (sPTPRZ-Long) and a lower band corresponding with 200 kDa (sPTPRZ-Short). Despite the presence of heterogeneous bands, the upper band corresponding with PTPRZ-Long and the PTPRZ-Short bands had 70–100 kDa higher molecular weights than their cleaved forms in CSF samples, possibly because the latter lacks a membrane-bound region. The Cat-315 antibody also detects 2 bands. *Cat-315 and anti-PTPRZ antibodies detect CSF sPTPRZ.*

We then performed cross-immunoprecipitation western blot studies. Cat-315 immunoprecipitates were stained with anti-PTPRZ (Santa Cruz), and vice versa, revealing



**Figure 3.** Western blot analysis of PTPRZ and sPTPRZ in microsomal fractions and CSF from glioma patients reveals 2 forms. (A) CSF samples from glioma patients A and B were treated with or without chondroitinase ABC (Chase ABC) and probed with Santa Cruz or Sigma anti-PTPRZ antibodies. (B) Schematic of the structures of PTPRZ-Long, PTPRZ-Short, and their proteolytic cleaved forms, sPTPRZ-Long and sPTPRZ-Short, respectively. Regions recognized by the anti-PTPRZ1 (Sigma), anti-PTPRZ (Santa Cruz), and Cat-315 antibodies used are indicated. (C) Microsomal fractions prepared from glioma tissues and CSF samples were treated with or without chondroitinase ABC (Chase ABC) and used for western blot analysis with anti-PTPRZ (Santa Cruz), Cat-315, and anti-Aggregan antibodies. Cadherin and TTR were probed as loading controls for microsomal fractions and CSF, respectively. A and B are from different patients. (D) Western blot analysis of CSF samples from a glioma patient treated with chondroitinase ABC (Chase ABC) or endo- $\beta$ -galactosidase and probed with anti-PTPRZ (Santa Cruz).

that Cat-315 detected the sPTPRZ protein (Supplementary Figure 1B). Even though the Cat-315 antibody is known to detect aggrecan,<sup>32</sup> the Cat-315-positive signal differs from aggrecan signals, and aggrecan is not observed in the CSF

samples (Figure 3C). The reason why the Cat-315 antibody preferentially detects the upper band could be because HNK-1-capped *O*-mannosylated glycans are enriched in the 755–1614 residues. In addition to chondroitinase digestion,

treatment with endo- $\beta$ -galactosidase reduced the molecular weight of sPTRZ (Figure 3D), indicating that it also contains keratan sulfate.<sup>10</sup>

*Pilot experiment to detect CSF sPTRZ from iNPH, schwannoma, and MS patients.*

We next performed western blot analysis of CSF samples from glioma, iNPH, schwannoma, and MS patients (1 sample each). We again observed strong sPTRZ-Long and sPTRZ-Short signals in the CSF from glioma patients (Figure 4A). In contrast, CSF from iNPH, MS, and schwannoma patients exhibited a similarly strong sPTRZ-Short signal but a weak signal representing sPTRZ-Long. Similar results were obtained using the Cat-315 antibody.

*Influence of CSF sampling site and timing on the sPTRZ level.*

In order to evaluate the potential influence of the sampling site and timing on the above result, we conducted additional experiments. Samples of the ventricle and lumbar CSF from 1 iNPH patient showed that the levels of sPTRZ in the 2 were quite similar (Supplementary Figure 1C). sPTRZ levels (sPTRZ/TTR) in the CSF sample of 1 glioma patient demonstrated a significant difference between the 2 timings of sampling during surgery. The sample obtained before tumor manipulation was approximately 7 times higher than in that from the controls (Figure 4B). The sample taken after tumor removal, which had apparent blood contamination, showed levels that were 6 times higher than the first one. Even though the same volume of CSF samples was used for western blot analysis, the second CSF sample showed markedly higher levels of TTR, most likely caused by contaminating blood in the CSF samples.

*Semiquantitative evaluation of sPTRZ in the CSF among glioma, schwannoma, MS, and control samples*

Semiquantitative western blot analysis using 86 CSF samples collected from glioma, MS, schwannoma, and control patients demonstrated that the level of sPTRZ-Long was more than 10 times higher in CSF from glioma patients compared with that from patients with MS or schwannoma (Figure 5A), whereas the level of sPTRZ-Short was similar in all subjects (Figure 5B). Interestingly, sPTRZ levels in CSF from schwannoma patients and control subjects were similar, even though both microarray and immunohistochemical analyses revealed higher PTPRZ expression in schwannoma tissue. We found no significant difference in sPTRZ-Long levels in the CSF of patients with grade 1, 2, 3, or 4 disease (Figure 5C). The area under the ROC curve between glioma and MS patients was 0.9676 ( $P < .0001$ ), indicating that sPTRZ had good discriminative power (Figure 5D).

## Discussion

### Clinical Significance of a Glioma Biomarker

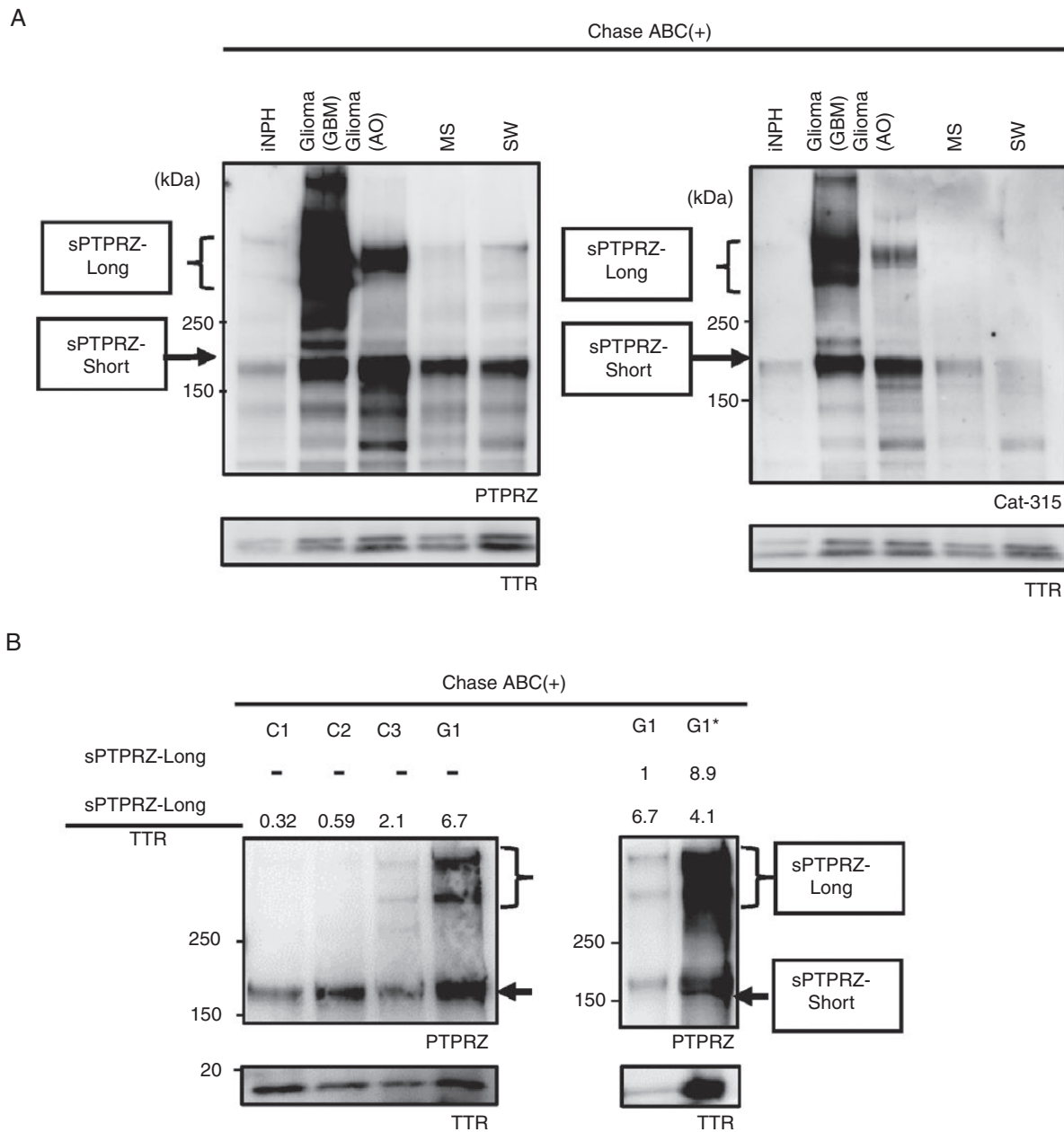
Preoperative diagnosis of brain disorders is crucial in designing appropriate treatment protocols, particularly because of the marked differences in the treatment of brain

tumors compared with infectious or inflammatory diseases such as MS. Similarly, surgical strategies differ for gliomas, where radical resection is the usual choice, and other tumors such as CNS lymphomas, for which biopsy is the surgical option. Nevertheless, there are no established preoperative biomarkers other than imaging to discriminate between the various brain tumors, and it is quite common to encounter abnormal findings on imaging studies suggestive of glioma or other diseases. In these cases, a biopsy is unavoidable.<sup>33</sup> Several methods of brain biopsy exist, such as stereotactic needle biopsy, endoscopic biopsy, and craniotomy, but all are invasive and carry potential complications such as intracerebral hemorrhage. The time between biopsy and a definitive diagnosis is another drawback and can be days or weeks. Thus, preoperative biomarkers that can distinguish between glioma and other brain disorders are urgently needed. In this preliminary study, we demonstrated for the first time that sPTRZ in CSF is a promising candidate diagnostic biomarker for gliomas. The levels of sPTRZ in gliomas were strikingly high, at more than 10 times higher than those in the control samples. Our findings must be confirmed in a larger clinical study exploring the profiles of CSF sPTRZ levels in various brain tumors including CNS lymphomas, metastatic brain tumors, and other intra-axial brain tumors. We predict that the biomarker will support the diagnostic process of not only gliomas but also various CNS diseases for which differentiation from glioma is necessary. Moreover, it could eventually be a useful diagnostic marker for evaluating the efficacy of treatments and detecting tumor recurrence by comparing data from different time points. Furthermore, accumulating data indicate that the PTPRZ-pleiotrophin signaling axis<sup>34</sup> enhances tumor growth and invasion.<sup>35,36</sup> Therefore, it is possible that sPTRZ in CSF may also have utility as a prognostic marker in gliomas.

### Balancing Scientific Quality and Ethical Considerations

We designed this study protocol carefully to ensure that it met scientific quality standards as well as all ethical considerations. It was, at the start of this study, considered to be unethical to perform a lumbar puncture on glioma patients (or glioma-suspected patients) or for other diseases to obtain CSF for study purposes alone because of the invasive nature of the procedure itself. Hence, we attempted to take CSF samples from the patients as noninvasively as possible, depending on the disease. For gliomas, schwannomas, facial spasm, trigeminal neuralgia, and unruptured cerebral aneurysms, CSF samples were obtained from cerebral cisterns during surgery before the manipulation of tumors, nerves, and cerebral arteries. However, for all MS and some iNPH patients, CSF was evacuated from the lumbar sac during lumbar puncture as clinical practice. It is therefore possible that our findings were influenced by the site and timing of CSF sampling during surgery. However, we believe that the elevation in CSF sPTRZ level in gliomas is still significant for the reasons described below. First, CSF sPTRZ levels in gliomas are much higher than in schwannomas

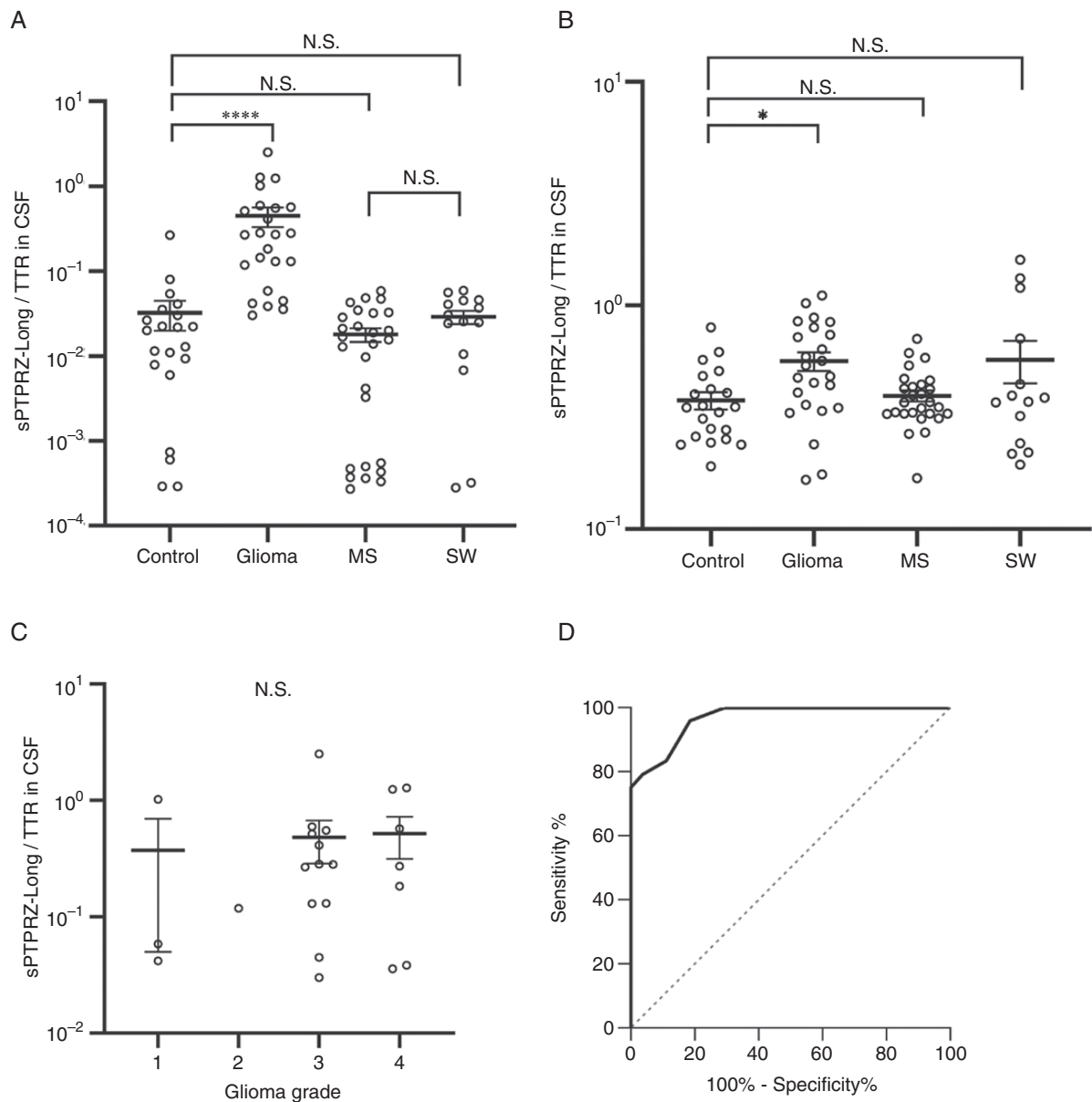




**Figure 4.** Western blot analysis shows elevated levels of sPTPRZ in the CSF of glioma patients. (A) Western blot analysis of CSF samples from patients with iNPH, glioma (glioblastoma [GBM] and anaplastic oligodendroglioma [AO]), multiple sclerosis (MS), or schwannoma (SW). Blots were probed with anti-PTPRZ (Santa Cruz; upper) or with Cat-315 antibody (lower). Transthyretin (TTR) was probed as a loading control. (B) Western blot analysis of CSF samples from control subjects (C1, facial spasm; C2, unruptured cerebral aneurysm; and C3, iNPH) and from glioma patients collected from cerebral cisterns before surgery (G1) and from ventricles after surgery (G1\*) for sPTPRZ and TTR. Relative signal intensities of sPTPRZ and sPTPRZ/TTR are shown.

and other control samples, even when comparing CSF samples only from “cerebral cisterns,” suggesting that our findings are true, at least, in CSF from cerebral cisterns. Second, CSF protein levels are known to be higher in the lumbar sac than in the ventricles. Cerebral cisterns are located between the two.<sup>37</sup> Our finding that, contrary to the above, sPTPRZ levels in CSF samples from cerebral

cisterns (gliomas) were much higher than even those from the lumbar sac (MS), indicates that the site of CSF sampling cannot account for these observations. In fact, analysis of CSF from the ventricle and lumbar sac in 1 iNPH patient showed that the sPTPRZ levels of the 2 were quite similar (Supplementary Figure 1C), suggesting that sPTPRZ levels are not remarkably different between the 2



**Figure 5.** Quantitative analysis shows elevated sPTPRZ in the CSF of glioma patients. (A and B) For quantification of cleaved PTPRZ, semiquantitative western blot analysis was performed using CSF samples from glioma ( $n = 24$ ), MS ( $n = 27$ ), schwannoma (SW,  $n = 14$ ), and control ( $n = 21$ ) patients. TTR levels in CSF samples were determined by ELISA. Data show the ratio of signal intensities of sPTPRZ-Long (A) and sPTPRZ-Short (B) compared with the levels of the internal standard protein TTR. Data are presented as the mean  $\pm$  SEM, with individual data points represented by circles. N.S., not significant,  $*P < .05$ ,  $****P < .0001$  by Kruskal–Wallis test with Dunn’s test. (C) As described for sPTPRZ-Long in (A) except the CSF samples were from patients with grade 1 ( $n = 3$ ), 2 ( $n = 2$ ), 3 ( $n = 12$ ), and 4 ( $n = 7$ ) glioma. N.S., Not significant by Kruskal–Wallis test with Dunn’s test. (D) Receiver operating characteristic curve analysis of the value of the sPTPRZ-Long to TTR signal intensity ratio in CSF for discriminating between glioma and MS patients. The area under the curve was 0.9676 ( $P < .0001$ ).

sites. Taken together, it is reasonable to assume that our findings would be essentially true regardless of the CSF sampling site. However, one must be cautious about the timing of CSF sampling during glioma surgery. We experienced a patient for whom CSF sampling was performed twice during surgery, just after dural opening (before the tumor removal procedure) and after the tumor removal

procedure. sPTPRZ levels in the first sample were approximately 7 times higher than in those of the controls, but the second samples were 6 times higher than the first (approximately 40 times higher than the controls). The tumor removal procedure itself seemed to enhance PTPRZ elevation in CSF during surgery. To avoid this contamination, CSF samples should be obtained in gliomas before surgical

manipulation of tumors when they are taken during surgery. To exclude the possibility of CSF contamination with cellular constituents from brain tumors, all CSF samples should be centrifuged to remove cells and debris.

### PTPRZ Isoforms and its Glycosylation

Using western blot analysis, we detected the long and short forms of sPTPRZ (~400 kDa and ~190 kDa, respectively). The 2 forms of sPTPRZ are most likely derived from alternatively spliced mRNA isoforms (Figure 3B). Also, considering the number of amino acid residues in each PTPRZ isoform, our result suggests that sPTPRZ-Long comprises isoforms 1, 4, and 5, and sPTPRZ comprises isoforms 2 and 3. It seems likely that several anti-PTPRZ (Sigma) reactive bands observed at 150–200 kDa are the degradation products of sPTPRZ-Long.

Interestingly, only sPTPRZ-Long was elevated in the CSF of glioma patients compared with MS, schwannoma, and control patients. We are currently developing a more rapid and quantitative method for detecting sPTPRZ-Long in CSF. As compared with full-length PTPRZ, we found that sPTPRZ is highly modified with chondroitin sulfate. To develop a high-affinity antibody against sPTPRZ, we might have to consider this glycosylation event. Although we found that PTPRZ is highly expressed in several brain tumors, immunohistochemical analysis using the Cat-315 antibody shows that the presence of an unusual glycosylated form was limited to gliomas. In schwannoma, PTPRZ expression was high but it lacked this unusual glycosylation and there was no evidence of an increase in its cleavage. The specific elevation of sPTPRZ observed here in glioma might suggest that PTPRZ cleavage is most prominent in this tumor type. Limited information is available about the protease responsible for PTPRZ cleavage and release,<sup>12</sup> and thus further investigation is necessary. Immunohistochemical analyses of brain tissue from MS patients and from mice with cuprizone-induced demyelination<sup>29</sup> have shown that reactive astrocytes express an abnormal HNK-1-capped branched O-Man glycan on PTPRZ. Notably, genetic deletion of the glycosylation enzyme *N*-acetylglucosaminyltransferase IX (*MGAT5B*), which is responsible for the branching of O-Man glycan, enhances PTPRZ instability and decreases PTPRZ expression in astrocytes, thereby suppressing astrogliosis.<sup>29</sup> In this regard, unusual PTPRZ glycosylation may be a therapeutic target for the inhibition of astrocyte activation in pathological states.

### Supplementary Data

Supplementary data are available at *Neuro-Oncology Advances* online.

### Keywords

cerebrospinal fluid | diagnostic marker | glioma | glycosylation | PTPRZ

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**Authorship Statement.** M.F., Y.H., K.S., and S.K. designed the project; Y.Y., Y.M., K.N., and K.H. prepared the human samples and performed the experiments; T.H. interpreted the immunohistochemical data; and M.F. and S.K. wrote the manuscript.

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