**Human Cancer Biology**

**PID1 (NYGGF4), a New Growth-Inhibitory Gene in Embryonal Brain Tumors and Gliomas**

Anat Erdreich-Epstein,1,2 Nathan Robison,1 Xiuhui Ren,1 Hong Zhou,1 Jingying Xu,1 Tom B. Davidson,1 Mathew Schur,1 Floyd H. Gilles,2 Lingyun Ji,5 Jemily Malvar,1 Gregory M. Shackleford,1,3,6 Ashley S. Margol,1,2 Marcel Kool,8 Marcel Kool,8 and Shahab Asgharazadeh1,2

**Abstract**

**Purpose:** We present here the first report of PID1 (Phosphotyrosine Interaction Domain containing 1; NYGGF4) in cancer. PID1 was identified in 2006 as a gene that modulates insulin signaling and mitochondrial function in adipocytes and muscle cells.

**Experimental Design and Results:** Using four independent medulloblastoma datasets, we show that mean PID1 mRNA levels were lower in unfavorable medulloblastomas (groups 3 and 4, and anaplastic histology) compared with favorable medulloblastomas (SHH and WNT groups, and desmoplastic/nodular histology) and with fetal cerebellum. In two large independent glioma datasets, PID1 mRNA was lower in glioblastomas (GBM), the most malignant gliomas, compared with other astrocytomas, oligodendrogliomas and nontumor brains. Neural and proneural GBM subtypes had higher PID1 mRNA compared with classical and mesenchymal GBM. Importantly, overall survival and radiation-free progression-free survival were longer in medulloblastoma patients whose tumors had higher PID1 mRNA (univariate and multivariate analyses). Higher PID1 mRNA also correlated with longer overall survival in patients with glioma and GBM. In cell culture, overexpression of PID1 inhibited colony formation in medulloblastoma, atypical teratoid rhabdoid tumor (ATRT), and GBM cell lines. Increasing PID1 also increased cell death and apoptosis, inhibited proliferation, induced mitochondrial depolarization, and decreased serum-mediated phosphorylation of AKT and ERK in medulloblastoma, ATRT, and/or GBM cell lines, whereas siRNA to PID1 diminished mitochondrial depolarization.

**Conclusions:** These data are the first to link PID1 to cancer and suggest that PID1 may have a tumor inhibitory function in these pediatric and adult brain tumors. Clin Cancer Res; 20(4); 827–36. © 2013 AACR.

**Introduction**

Here, we report on PID1 (Phosphotyrosine Interaction Domain containing 1) in 2 important groups of brain tumors: embryonal brain tumors [medulloblastomas and atypical teratoid rhabdoid tumors (ATRT)] and gliomas. Medulloblastomas are the most common malignant brain tumors in children, where in high-risk disease prognosis remains poor. Moreover, in younger children, therapies that include whole brain irradiation are associated with serious long-term sequelae that hamper quality of life of survivors (1, 2). ATRT is another poor prognosis, highly malignant embryonal brain cancer of young children. Most ATRTs have lost expression and/or function of the INI1 (SMARCB1) tumor suppressor gene, which is part of the SWI/SNF chromatin-remodeling complex (3–5). Malignant gliomas, including glioblastoma multiforme (GBM), comprise the most common primary malignant brain tumors in adults and also carry poor prognosis (6, 7). Here, we provide novel clinical correlations of PID1 in medulloblastomas and gliomas, and demonstrate tumor-inhibitory effects of PID1 in cell lines of these 3 brain tumors.

The PID1 gene [also called NYGGF4 and phosphotyrosine binding (PTB)-containing, cubulin, and LRPI-interacting protein; PCL1; rhymes with “BID-1”] was identified in 2006 based on its differential expression in adipose tissue of obese compared with nonobese subjects (8). To date, PID1 has not been reported in the context of cancer. Although PID1 is known to contain a PTB domain/phosphotyrosine interaction domain (PID; ref. 8), the molecular mechanism...
Translational Relevance

We present here the first report of PID1 (Phosphotyrosine Interaction Domain containing 1; NYGGF4) in cancer. PID1 was identified in 2006, and its role and molecular mechanism are still poorly understood. Using 6 independent datasets in 2 common brain tumors, medulloblastomas and gliomas, we show that: (i) PID1 mRNA is lowest in least favorable subgroups of medulloblastomas and gliomas; (ii) higher PID1 mRNA is directly correlated with longer patient survival; and (iii) PID1 causes increased cell death and decreased proliferation in medulloblastoma, glioma, and atypical rhabdoid tumor cell lines. These data in 2 brain tumor types suggest that it may be possible to incorporate PID1 into personalized molecular prognostic signatures that predict patient response and outcome. Ongoing work on the molecular function of PID1 intends to utilize this knowledge in the design of improved approaches to therapy.

Materials and Methods

Details for additional methods can be found in Supplementary Materials.

Patients, samples, and mRNA expression data

Medulloblastoma specimens and clinical records from 81 children diagnosed at Children’s Hospital Los Angeles (CHLA) between 1989 and 2008 were obtained according to a protocol approved by the local Institutional Review Board. Patient and sample characteristics are described in Supplementary Table S1. The Heidelberg microarray dataset includes 446 medulloblastomas, profiled on Affymetrix U133 plus2.0 arrays, and is a combination of published data on 230 patients obtained from the Gene Expression Omnibus (GSE10327, GSE12992, and GSE37418; refs. 11–13) and unpublished data on 216 additional patients from Heidelberg (M. Kool and S. Pfister; unpublished data). The published Toronto microarray dataset includes 103 medulloblastomas profiled on Affymetrix Human Exon Array data (GSE21140), and the published Boston microarray dataset includes 194 medulloblastomas profiled on Affymetrix U133A (14, 15). Molecular subgroups of medulloblastoma were identified either by using available published data, or by cluster analyses to assign molecular subgroup for the unpublished datasets (11, 12, 14), or for the CHLA analysis, by quantitative reverse transcription PCR (qRT-PCR) and a medulloblastoma gene signature derived from prior microarray studies (15, 16) and a CHLA study (manuscript in preparation). Glioma PID1 mRNA microarray data in REMBRANDT (REpository for Molecular Brain Neoplasia Data) were from Affymetrix U133 plus2.0 arrays (17). Glioblastoma PID1 mRNA data in TCGA (The Cancer Genome Atlas; The Cancer Genome Atlas Research Network, National Cancer Institute and National Human Genome Research Institute, Bethesda, MD) were from Agilent G4502A_07 microarrays (18). For GBM clinical and PID1 correlations, publicly available data for 196 patients was obtained from TCGA and Verhaak and colleagues (19), and was accessed at https://tcga-data.nci.nih.gov/docs/publications/gbm_exp/ (files: unifiedScaled.txt and TCGA_unified_CORE_ClaNC840.txt) and https://tcga-data.nci.nih.gov/tcga/dataAccessMatrix.htm?mode=ApplyFilter&diseaseType=GBM (files: clinical_patient_gbm.txt and clinical_follow_up_v1.0_gbm.txt) on February 18, 2013.

RNA from CHLA medulloblastoma samples was extracted and processed using a previously published method (20). PID1 mRNA levels were obtained by qRT-PCR (PID1: forward primer: 5'-GATTGCTGGCAACCACCTGATG-3', reverse primer: 5'-AAATGAAAGTGGCAGGACCAC-3', probe: 5'-TCCAGAGCATGTTCCTCCAGAGG-3') and after normalization to 3 housekeeping genes (GAPDH/SDHA/HPRT1).

Annexin V, cell proliferation, and mitochondrial depolarization

Assays were performed on green fluorescent protein (GFP)-positive cells 24 hours after transfections. Annexin V staining was done by flow cytometry using the APC Annexin V Kit (Cat. No. 550474; BD Pharmingen) according to manufacturer’s instructions. Cell proliferation and viability were assessed by flow cytometry using the APC BrdUrd Flow Kit (Cat. No. 552598; BD Pharmingen). Mitochondrial depolarization was measured by flow cytometry using the MitoProbe DiIC1; (5) Assay Kit for flow cytometry (Cat. No. M34151; Invitrogen).
Cell culture
The cell lines used were: GBM (U87, U251, LN18, LN229, CHLA-07-BSGBM), medulloblastoma (D283MED, D425MED, UW-228-2, CHLA-259, CHLA-01-MED, CHLA-01R-MED), and ATRT (BT-12, CHLA-05-ATRT, CHLA-06-ATRT). Details for their culture are in Supplementary Materials.

Plasmids
PID1 variant 1 (NM_017933.4; PID1-1) and variant 2 (NM_001100818.1; PID1-2) human cDNA ORFs with a turbo-GFP (tGFP) C-terminal tag in pCMV6-AC-tGFP (pCMV6-AC-PID1-tGFP) were from OriGene (Cat. No. PS100010, RG212451, RG212505). PID1 in the pCL5-2A-tGFP plasmid (ref. 21; from Dr. M.A. Lawlor, University of Michigan, Ann Arbor, MI) was expressed 5' to the foot and mouth disease virus–derived 2A self-cleaving peptide sequence. In pCIENS (a pcDNA3.1-based CMV promoter-driven expression vector that also expresses eGFP via an EMCV IRES), PID1 (variant 1) was expressed from the CMV promoter.

Statistical analysis
Details of the statistical analysis of patient-related information can be found in the Supplementary Materials.

In vitro experiments were analyzed using GraphPad Prism version 5.0 for MAC (GraphPad Software; www.graphpad.com). Results are depicted as mean ± SEM from at least 3 independent experiments unless stated otherwise. P values represent unpaired 2-sided Student t-test unless stated differently.

Results
PID1 mRNA is higher in favorable medulloblastomas and correlates with longer rf-PFS and OS in patients with medulloblastoma
qRTPCR of 81 pediatric medulloblastoma tumors showed that mean PID1 mRNA levels were significantly higher in medulloblastomas with desmoplastic/nodular histology compared with those with anaplastic histology (P < 0.001; Fig. 1A; patient characteristics in Supplementary Table S1). Supporting this, analysis of PID1 in the 4 medulloblastoma core molecular subgroups (11, 22, 23) showed that SHH group medulloblastomas had higher mean PID1 mRNA compared with the less favorable subgroup, groups 3 and 4 (Fig. 1B). Microarray data from 3 other independent medulloblastoma datasets similarly revealed higher mean PID1 mRNA in the more favorable subgroups (SHH and WNT) compared with medulloblastomas in groups 3 and 4 (Fig. 1C and Supplementary Fig. S1A; refs. 11–13). Mean expression of PID1 mRNA was higher in fetal cerebellum compared with groups 3 and 4 medulloblastomas, and was similar to PID1 mRNA in the WNT and SHH groups (Fig. 1C).

In univariate analysis of the CHLA medulloblastoma patients (n = 81; Supplementary Table S1), rf-PFS was significantly longer in children whose tumor PID1 mRNA was higher than the median of the cohort, compared with those with tumor PID1 mRNA lower than median (rf-PFS 73% ± 11% vs. 11% ± 10%, respectively; P < 0.001; Fig. 1D). Difference in rf-PFS also remained significant when dividing the group into equal tertiles according to PID1 mRNA (Supplementary Fig. S1B). In the non-CHLA medulloblastoma patients from Fig. 1C for whom survival data were available, overall survival (OS) was significantly longer in patients with higher tumor PID1 mRNA compared with those with lower PID1 mRNA (Fig. 1E), supporting the findings in the CHLA cohort (Fig. 1D). In multivariate analysis of the CHLA cohort, risk of radiation-free disease progression was significantly higher in patients with lower-than-median PID1 mRNA compared with those with higher-than-median PID1 mRNA (Table 1).

These analyses demonstrate that the clinically favorable medulloblastomas (desmoplastic/nodular histology, or WNT and SHH molecular subgroups) had higher mean PID1 mRNA compared with clinically unfavorable medulloblastomas (anaplastic histology or groups 3 and 4), and that higher medulloblastoma PID1 mRNA correlated with longer rf-PFS and OS.

GBM have lower PID1 mRNA compared with other gliomas and nontumor brains; higher PID1 mRNA correlates with longer overall survival in patients with glioma
Analysis of PID1 mRNA data using the REMBRANDT glioma clinical genomics database (17) revealed that PID1 mRNA levels were lower in the highest grade gliomas, GBMs, compared with nontumor brains, astrocytomas, and oligodendrogliomas (Fig. 2A). The TCGA glioma dataset (18) similarly showed that mean PID1 mRNA in GBM was significantly lower than its level in nontumor brains [95% confidence interval (CI), 0.34–0.38; P < 0.0001; Fig. 2B]. For patients with GBM for whom tumor molecular subtype (classical, mesenchymal, neural, proneural) and clinical data were available and unified PID1 mRNA expression values from 3 platforms in TCGA were analyzed (see Materials and Methods), there were significant differences in mean PID1 mRNA among the molecular subtypes (P < 0.0001; Supplementary Fig. S2A and Table S2). Neural and proneural GBM subtypes showed higher PID1 mRNA compared with classical and mesenchymal subtypes. All paired comparisons between GBM with known subtypes were also significantly different (after Bonferroni adjustment for multiple comparisons) with the exception of the neural versus proneural pairing (P = 0.88). Differences in PID1 mRNA between GBM subtypes remained significant even after adjusting for age at diagnosis (P < 0.0001).

Survival analysis using the REMBRANDT dataset revealed significant differences in OS among patients who were stratified according to PID1 mRNA. PID1 mRNA was directly correlated to OS in patients with any diagnosis of glioma, or within the subset of astrocytomas that does not include GBMs (Fig. 2C and D, Supplementary Fig. 2B–E; using 2 available PID1 probesets). For GBMs in the TCGA dataset, PID1 mRNA was univariately significantly associated with survival (Cox regression analysis, P = 0.031; Fig. 2E), but the REMBRANDT dataset did not reveal such correlation (not shown). Although the PID1 mRNA association with OS in GBM (TCGA) was no
longer significant after adjusting for molecular subtype \( (P = 0.15) \), the relative failure rate decrease per unit increase in \( \text{PID1} \) mRNA was only slightly attenuated in our analysis \( (0.83 \pm 0.071 \text{ univariate}, 0.85 \pm 0.094 \text{ multivariate}) \). In Cox multivariate analysis, with age at diagnosis, and \( \text{PID1} \) expression and subtype as variables (TCGA), \( \text{PID1} \) was marginally significant \( (P = 0.056) \). The relative failure rate decrease per unit increase in \( \text{PID1} \) mRNA was \( 0.81 \pm 0.091 \).

Thus, similar to our findings in medulloblastomas, the most malignant gliomas (i.e., GBMs) had lower \( \text{PID1} \) mRNA compared with other gliomas or with nontumor brains. In addition, higher tumor \( \text{PID1} \) mRNA correlated with longer OS in patients with glioma.

**\text{PID1 confers growth disadvantage in brain tumor cell lines}**

We next asked if the clinical correlations we found for \( \text{PID1} \) mRNA pointed to a possible biological role for \( \text{PID1} \) in brain tumors. To evaluate a role in growth, we assessed the effect of \( \text{PID1} \) on colony formation in U251 GBM.
LN229 GBM, D283MED medulloblastoma, and CHLA-06-ATRT brain tumor cell lines as follows: cells were transfected with PID1-tGFP or tGFP control for 24 hours, tGFP-expressing cells were sorted by flow cytometry and plated at equal numbers, and colonies were allowed to form over 2 weeks without further selection. Each of the cell lines formed significantly fewer colonies when transfected with PID1-tGFP than did cells transfected with the control tGFP vector, despite an equal number of sorted tGFP-positive cells plated for the tGFP control and PID1-tGFP transfected cells (Fig. 3A and B). Both variants of PID1 (variant 1: NM_017933, variant 2: NM_001100818) showed similar effects in this assay. Similar inhibition of colony formation was also observed using the self-cleaving bicistronic pCLS-PID1-2A-eGFP compared with its control vector, pCLS-2A-eGFP (21) as well as with the bicistronic pCIENS-PID1 compared with pCIENS control vector (Supplementary Fig. S3A and S3B). Expression of PID1 at the time of flow sorting and plating of the tGFP-expressing cells was verified by Western blotting (24 hours after transfection; Supplementary Fig. S3C). These data indicate that PID1 confers growth and/or survival disadvantage upon brain tumor cell lines ectopically expressing PID1.

### PID1 inhibits proliferation and promotes cell death of cultured brain tumor cell lines

To characterize the PID1-induced growth disadvantage, we analyzed proliferation and cell death, measuring BrdUrd/7AAD uptake in tGFP-positive cells 24 hours after transient transfection with pCMV6-PID1-tGFP or pCMV6-tGFP control. GBM (U251) and medulloblastoma (UW-228-2) cell lines as well as primary medulloblastoma cells (CHLA-259) expressing PID1-tGFP had significantly more cells in the sub-G0–G1 phase compared with cells expressing tGFP control vector (Fig. 4A and B and Supplementary Fig. S4), indicating that PID1 increased cell death. PID1-tGFP also decreased proliferation, as indicated by the decreased proportion of cells in S-phase (Fig. 4A and B). Apoptosis accounted for at least some of the cell death, as annexin V binding was higher in PID1-tGFP–expressing cells compared with tGFP controls (Fig. 4C). There were very few 7AAD-positive/annexin V-negative cells, indicating that necrosis was only minimal. As mitochondria are involved in metabolism and several forms of cell death and PID1 has been reported to induce mitochondrial dysfunction in adipocytes and myocytes (24–28), we examined the effect of PID1 on mitochondrial depolarization. U251 GBM, D283MED medulloblastoma, and CHLA-06-ATRT cell lines transfected with PID1-tGFP showed increased depolarization of mitochondrial membrane potential compared with tGFP-transfected cells 24 hours after transfection (Fig. 4D). Conversely, siRNA knockdown of PID1 in U87 GBM (U87 express PID1 protein; Supplementary Fig. S5) diminished baseline depolarization of the mitochondrial membrane potential compared with nonsilencing siRNA control (Fig. 4E). siPID1 knockdown similarly diminished baseline depolarization.

### Table 1. Cox proportional hazards model for progression-free survival (patients who received irradiation as part of the primary treatment were censored at time of irradiation)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate analyses</th>
<th>Multivariate analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR $^a$</td>
<td>95% CI $^a$</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relative risk increase per 1 year</td>
<td>1.2</td>
<td>(1.05, 1.4)</td>
</tr>
<tr>
<td>≥3 years vs. &lt;3 years</td>
<td>3.5</td>
<td>(1.3, 9.1)</td>
</tr>
<tr>
<td>Stage $^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>M $^+$</td>
<td>4.7</td>
<td>(1.4, 15)</td>
</tr>
<tr>
<td>Histology $^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classic</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Desmoplastic/nodular</td>
<td>0.42</td>
<td>(0.09, 2.0)</td>
</tr>
<tr>
<td>Anaplastic/large cell</td>
<td>2.0</td>
<td>(0.54, 7.6)</td>
</tr>
<tr>
<td>Molecular subgroup $^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHH/WNT</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Group 3</td>
<td>6.2</td>
<td>(1.5, 26)</td>
</tr>
<tr>
<td>Group 4</td>
<td>3.2</td>
<td>(0.58, 18)</td>
</tr>
<tr>
<td>PID1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Low</td>
<td>6.8</td>
<td>(2.2, 21)</td>
</tr>
</tbody>
</table>

$^a$HR and CI from univariate Cox models.
$^b$HR, CI, and P-values from the Cox model that included age, stage, histology, molecular subgroup, and PID1 expression.
$^c$HR, CI, and P-values from the Cox model that included stage and PID1 expression.
$^d$Missing data for these variables were included in the models as a “missing” category.
mitochondrial depolarization in D283MED medulloblastoma cells (Fig. 4E). These data demonstrate that the inhibitory effect of PID1 on brain tumor cell line growth is the result of both inhibition of proliferation and increased cell death, and is associated with depolarization of mitochondrial membrane potential.

Finally, to begin investigating the molecular mechanism of PID1, we examined its effect on serum-mediated phosphorylation of AKT and ERK, 2 effectors that are central to a number of proliferation and survival signaling pathways. LN229 GBM cells transiently transfected with PID1, serum starved overnight and acutely stimulated with 20% FBS showed decrease in the serum-induced phosphorylation of AKT compared with empty vector-transfected cells (Fig. 4F). In addition, transient transfection of PID1 into D283MED medulloblastoma cells grown in FBS-containing medium caused decrease in phosphorylation of both AKT and ERK (Fig. 4G and Supplementary Fig. S6). This indicates that...
PID1 inhibits serum-mediated signaling pathways, which involve AKT and ERK.

In summary, using 6 independent datasets, we have shown that PID1 mRNA is lower in the more aggressive medulloblastomas and gliomas compared with their relatively more favorable counterparts, and that higher PID1 mRNA correlates with longer rf-PFS and OS in patients with medulloblastoma and longer OS in patients with glioma and GBM. We also showed that PID1 conferred a growth-

inhibitory effect on cell lines of 3 types of brain tumors (medulloblastoma, GBM, and ATRT), which manifested as decreased proliferation and/or increased cell death. Finally, PID1 induced mitochondrial depolarization and inhibition of serum-mediated phosphorylation of AKT and ERK. Taken together, these findings suggest that PID1 is a novel growth inhibitor in gliomas and embryonal brain tumors.

Discussion

The small number of articles reporting on PID1 to date have mostly been in the context of obesity and diabetes in adipocytes and muscle cells (8, 28–30) and in Alzheimer’s brains (9). Our work here is the first to report on PID1 in cancer.

Our clinical findings for PID1 using 6 independent datasets comprising 2 different types of brain cancers emphasize the validity of the association between lower PID1 mRNA and less favorable tumor subgroups. The in vitro experiments suggest that PID1 confers growth suppression because of combined inhibition of proliferation and increase in cell death. Regulation of PID1 expression in embryonal brain tumors and gliomas is currently unknown.

The molecular mechanism by which PID1 affects growth of brain tumor cells in culture is also unknown at this time. Our finding on depolarization of mitochondrial membrane potential by PID1 in brain tumor cell lines is consistent with reports that PID1 disrupts mitochondrial function in adipocytes and muscle cells (24–28). In adipocytes and muscle cells, PID1 also inhibits insulin-mediated phosphorylation of IRS-1 and AKT, and insulin-mediated translocation of the GLUT-4 glucose transporter to the membrane, resulting in decreased glucose uptake (28–30). Our experiments showing that PID1 inhibits phosphorylation of AKT and ERK suggest that PID1 may modulate signaling pathways involved in cell proliferation and survival. Although insulin signaling is less likely to be a target of PID1 modulation in brain tumor cell lines because of their low and/or infrequent expression of the insulin receptor, it will be interesting to examine if PID1 modulates signaling through the insulin-like growth factor receptor (IGF1R), which is important in many brain tumors (31–40). The inhibition of insulin receptor signaling in adipocytes was hypothesized to be mediated via interaction of the PID1 PTB domain with the NPXY motif in the cytoplasmic tail of the insulin receptor (29); however, co-immunoprecipitation of the 2 proteins has not been reported yet. Using yeast 2 hybrid screens and pull-down experiments, several groups reported direct interaction between the PTB domain of overexpressed PID1 and the NPXY motif of low-density lipoprotein receptor-like-1 (LRP1; refs. 9 and 10). However, it is unlikely that LRP1 is a major player in the PID1 (aa84-230)-induced cell-cycle arrest in NIH-3T3, because the arrest could not be overcome by overexpression of LRP1 (10).

Cellular effects of PID1 may differ between cell types. In 3T3-L1 preadipocytes, PID1 increased proliferation (8), but in NIH3T3 cells a PID1 fragment that includes the PTB domain (aa84-230) caused cell-cycle arrest (10). Our findings that PID1 has a growth-suppressive effect in multiple brain tumor cell lines are consistent with the latter.
Examination of the Catalogue of Somatic Mutations in Cancer (COSMIC; ref. 41) revealed that among 7,055 tumors reported to date (July 18, 2013), there were only 27 mutations, of which 15 were nonsynonymous. No mutations or copy number variations of PID1 were found in the 510 brain tumors reported. The lack of PID1 copy number variations and PID1 mutations in brain tumors suggests that PID1 does not function as a classical tumor suppressor gene. The direct correlation of PID1 mRNA and survival in medulloblastoma and gliomas suggests that PID1 may render these brain tumors more susceptible to therapy, accounting for the better outcome in patients whose tumors expressed higher PID1 mRNA. This possibility is currently under investigation in our laboratory.

In summary, this is the first report to link PID1 to cancer and to brain tumors, to demonstrate correlation between PID1 mRNA level and survival in medulloblastomas and gliomas, and to show growth-inhibitory effect of PID1 in cultured medulloblastoma, GBM, and ATRT cell lines. Our data therefore suggest that PID1 may have a growth-modulating function in brain tumors. It will now be important to further investigate the molecular mechanism(s) of PID1’s effects in pediatric and adult malignant brain tumors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: A. Erdreich-Epstein, J. Xu, G.M. Shackleford
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Erdreich-Epstein, X. Ren, H. Zhou,
Acknowledgments

The authors thank J. Scott, M. Kang, and Dr. M. Sheard for their expert technical assistance and helpful discussions. The authors thank Dr. M. Torres for critically reviewing this manuscript and Dr. S. Lee for assistance in the identification of PIDI1 by proteomic analysis.

Grant Support

This work was supported by grant 1R21NS077007-01 from the National Institute of Neurological Disorders and Stroke, a Hyundai Hope on Wheels research grant, a ThinkCure research grant, and support from the Concern Foundation and M. Feldman to A. Erdreich-Epstein. The work was also supported by grants from the American Cancer Society, St. Baldwin’s Foundation, and Alex’s Lemonade Stand Foundation to S. Asgharzadeh. M. Schur was supported by a Rose Hills Foundation Science and Engineering Undergraduate Research Fellowship and a Provost’s Research Fellowship from the University of Southern California. N. Robison was supported by a Fellowship award from the Hope Fund Foundation. This work was also supported in part by generous funding from The Nautica Malibu Triathlon Fund, Grayson’s Gift, The Rachel Ann Hage Neuro-Oncology Fund, The Brad Kaminsky Foundation and the Heroes of Hope Race Teams and The T.J. Martell Foundation for Leukemia, Cancer, and AIDS Research.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby markedadvertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 25, 2013; revised October 17, 2013; accepted November 4, 2013; published OnlineFirst December 3, 2013.

References


Correction: PID1 (NYGGF4), a New Growth-Inhibitory Gene in Embryonal Brain Tumors and Gliomas

In this article (Clin Cancer Res 2014;20:827–36), which was published in the February 15, 2014, issue of Clinical Cancer Research (1), an author’s name was misspelled. The corrected name should read as follows: “Shahab Asgharzadeh.” The authors regret this error.

Reference

Published online September 15, 2015.
©2015 American Association for Cancer Research.
**PID1 (NYGGF4), a New Growth-Inhibitory Gene in Embryonal Brain Tumors and Gliomas**

Anat Erdreich-Epstein, Nathan Robison, Xiuhai Ren, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-13-2053

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2013/12/03/1078-0432.CCR-13-2053.DC1

Cited articles
This article cites 41 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/20/4/827.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/20/4/827.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.