PME-1 Protects Extracellular Signal-Regulated Kinase Pathway Activity from Protein Phosphatase 2A–Mediated Inactivation in Human Malignant Glioma

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Abstract
Extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase pathway activity is regulated by the antagonist function of activating kinases and inactivating protein phosphatases. Sustained ERK pathway activity is commonly observed in human malignancies; however, the mechanisms by which the pathway is protected from phosphatase-mediated inactivation in the tumor tissue remain obscure. Here, we show that methylesterase PME-1–mediated inhibition of the protein phosphatase 2A promotes basal ERK pathway activity and is required for efficient growth factor response. Mechanistically, PME-1 is shown to support ERK pathway signaling upstream of Raf, but downstream of growth factor receptors and protein kinase C. In malignant gliomas, PME-1 expression levels correlate with both ERK activity and cell proliferation in vivo. Moreover, PME-1 expression significantly correlates with disease progression in human astrocytic gliomas (n = 222). Together, these observations identify PME-1 expression as one mechanism by which ERK pathway activity is maintained in cancer cells and suggest an important functional role for PME-1 in the disease progression of human astrocytic gliomas. [Cancer Res 2009;69(7):2870–7]

Introduction
Protein phosphatase 2A (PP2A) is a human tumor suppressor that inhibits cellular transformation by regulating activity of several signaling proteins critical for malignant cell behavior (reviewed in refs. 1, 2). Recent studies have indicated that phosphorylation and methylation of the COOH terminal tail of the catalytic PP2A subunit (PP2Ac) play an important role in the regulation of both catalytic activity of PP2Ac and recruitment of different substrate specific B subunits to the PP2A complex (reviewed in ref. 3). The nature of PP2Ac COOH terminal kinase(s) remains elusive, but the methylation status of PP2Ac Leu190 is regulated by a methylating enzyme, LCMT, and methylesterase PME-1 (3–6). Recent structural analysis of PME-1–PP2A complex revealed that, in addition to the previously identified role of PME-1 as PP2Ac Leu190 methylesterase (4, 5), PME-1 inhibits PP2A activity by directly binding to the active site of PP2Ac (6). However, PP2A target proteins regulated by PME-1–mediated PP2A inhibition have not been elucidated.

Ras proteins (H-Ras, K-Ras, and N-Ras) are small GTPases that mediate signals from activated receptor tyrosine kinases (RTK) to intracellular signaling pathways. In addition, activating Ras mutations and/or increased Ras activity is detected in the majority of human malignant tumors (7). Although Ras activates several cellular signaling pathways that promote malignant cell behavior, recent studies have shown that Ras-mediated activation of two of its effector cascades, extracellular signal-regulated kinase (ERK) and RalA, is required for the transformation of some types of human cells (8, 9), whereas simultaneous activation of the ERK and phosphatidylinositol 3-kinase (PI3K)–Akt pathway is sufficient to replace activated H-Ras in other cell types (10). Interestingly, all Ras effector pathways described above (ERK, RalA, and PI3K–Akt) are targeted by PP2A tumor suppressor activity (2, 11). Although a few cancer-relevant mechanisms of inhibiting PP2A tumor suppressor activity have been reported (2, 11–14), cancer-associated mechanisms preventing PP2A-mediated inactivation of the ERK pathway remain elusive.

The ERK pathway is a classic three-tiered mitogen-activated protein kinase (MAPK) cascade consisting of Raf, MAPK/ERK kinase (MEK) and ERK proteins. This pathway mediates the effects of activated Ras in the regulation of transcription, cell survival, proliferation, and differentiation via ERK-mediated target protein phosphorylation (reviewed in refs. 15, 16). As elucidated above, PP2A has been shown to be a key negative regulator of ERK pathway activity (17, 18). Importantly, PP2A inhibition results in sustained ERK pathway activation and prolongs transient 12-O-tetradecanoylphorbol-13-acetate (TPA)–elicited ERK phosphorylation (19, 20). Moreover, increased ERK pathway activity in brain tissue was observed in transgenic mice overexpressing a dominant-negative form of the PP2A catalytic subunit C (21). Altogether, these results suggest that PP2A inhibition is required for sustained ERK pathway activity, a phenomenon observed in several human malignancies in vivo (7, 22).

Here, we show that PME-1 promotes both basal and growth factor induced ERK pathway activity in malignant cells. Moreover, in human glioblastoma tissues PME-1 expression correlates with both ERK pathway activity and proliferation. In addition, PME-1 expression correlates with progression of low-grade astrocytic gliomas to malignant glioblastomas. Thus, results of this study provide evidence that PME-1, through inactivation of PP2A, promotes in vivo ERK pathway activity and tumor progression in human glioblastoma.
Small interfering RNA (siRNA) transfections were performed by transfecting B-RafE600 or MEKDD together with myr-Akt) have been described in ref. 10.

A

supplemented with 10% heat-inactivated FCS and penicillin (100 units/mL)–streptomycin (100 μg/mL). HeLa, HT-1080, NIH-3T3, and T98G cells were cultured in Eagle’s MEM (BioWhittaker) containing five different shPME-1 were provided by the RNAi Consortium expressing lentivirus. The pLKO.1-Scr-Puro and pLKO.1-puro vectors generated by infecting cells with shRNAs specific for PME-1 (shPME-1)–

Materials and Methods

Cell culture and small interfering RNA transfections. HeLa, HT-1080, U118-MG, NIH-3T3, and HEK293 (Phoenix) cells were cultured in DMEM (Sigma-Aldrich Co.) and T98G glioma cells in Eagle’s MEM (BioWhittaker) supplemented with 10% heat-inactivated FCS and penicillin (100 units/mL)–streptomycin (100 μg/mL). HeLa, HT-1080, NIH-3T3, and T98G cells were a kind gift from Dr. N. Nupponen (University of Helsinki). HEK-TER cells (overexpressing RasV12) and HEK-TemA cells (overexpressing either B-RafV600 or MEK1/2 together with myr-Akt) have been described in ref. 10. Small interfering RNA (siRNA) transfections were performed by transfecting scrambled (5′-GUACAAUUGAGACCGG-3′) or PME-1 (5′-GGUAGACCUAGAGGAC-3′) specific double-stranded siRNA with Oligofectamine or Lipofectamine. Proteins exclusively identified in PR65TAP eluates were excised from silver-stained gel and identified by mass spectrometric peptide sequencing. B-D, representative data from two to three independent experiments with similar results.

Tandem affinity purification and Strep purification experiments. To identify PR65-interacting proteins from human cancer cells, HT-1080 cells were stably transfected with tandem affinity purification (TAP)–tagged PR65 protein, and TAP purification of protein complexes was performed as described previously (13). HT-1080 cells stably expressing StrepIII-tagged PME-1 and mock lines were harvested, resuspended in membrane lysis buffer [50 mmol/L Tris (pH 7.4), 7.5% glycerol, 1 mmol/L EDTA, 150 mmol/L NaCl with freshly added complete protease inhibitors (Roche), 1 mmol/L Na3VO4, and 0.5 mmol/L DTT] and homogenized with a Dounce homogenizer. Lysates were centrifuged for 10 min at 750 × g; supernatants were collected and centrifuged for 1 h at 100,000 × g in +4°C. The Strept purification from subsequent supernatants was performed using 0.2 mL Strep-Tactin Superflow columns (IBA) following the manufacturer’s instructions. MEK immunoprecipitation and PP2A activity assay. HeLa cells were treated with siRNAs for 48 h and lysed in 1× cell lysis buffer [20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 1% Triton X-100, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerophosphate, 1 mmol/L Na3VO4, 1 μg/mL leupeptin]. To immunoprecipitate
MEK complex, 200 μL of cell lysate were incubated with protein A coupled with MEK1 antibody (0.1 μg; Santa Cruz) for 5 h at +4°C. Immunoprecipitates were briefly washed 3× with cell lysis buffer and once with 1× PP2A reaction buffer (serine/threonine phosphatase 2× reaction buffer; 100 mmol/L Tris-HCl at pH 7.0 containing 0.2 mmol/L CaCl2, 250 μg/mL bovine serum albumin, and 0.1% Tween 20). PP2A activity of the MEK complex was measured with 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; RediPlate 96 EnzChek serine/threonine phosphatase assay kit, R-33700; Molecular Probes) as substrate using 60 min of reaction time, and fluorescence was measured in a fluorescence microplate reader using excitation at 355 nm and fluorescence detection at 460 nm.

**Proliferation assays.** For soft agar assays, HeLa cells were seeded on 3-cm plates 72 h after siRNA transfection. Agar assays were performed in medium containing 10% fetal bovine serum, as described in ref. 13, and colonies were counted after 14 d. Anchorage-independent colonies were classified according to a number between 200 and 10,000 pixels. For foci formation assays, HeLa cells were treated as above and seeded on six-well plate and methanol/crystal violet–stained colonies were counted after 8 d. The number and size of colonies were analyzed from microscopy images (magnification, 10×) using ImageJ 1.33u software. For proliferation assays, U118-MG, HeLa, or HT-1080 cells were plated in duplicates or triplicates a day before transfection and transfected with scrambled or PME-1–specific siRNAs for 48 or 72 h. Transfected cells were left untreated or treated with 10 μmol/L of UO126 for 48 or 72 h. 1×10⁴ HEK TER cells overexpressing H-RasV12 and HEK-TE cells overexpressing B-RafG60D or MEKG10D were plated in triplicates for 6 d. The number of viable cells was determined using a Z2 particle count and size analyzer (Beckman-Coulter).

**Immunohistochemistry.** The expression of PME-1, phosphorylated MEK, and phosphorylated Elk-1 proteins were studied immunohistochemically from 222 grades 2 to 4 astrocytic gliomas. Sections from (thickness, 5 μm) routinely processed tumor microarray paraffin blocks were cut and mounted on SuperFrost Plus slides and dried overnight at 37°C. The sections were then dehydrated and rehydrated. High-temperature antigen retrieval was carried out in 10 mmol/L Tris-HCl/1 mmol/L EDTA buffer (pH 9.0). Immunostainings were done with the TechMate staining automate using the EnVision detection system (Dako Ltd.). The sections were incubated with antibodies against PME-1, phosphorylated MEK1/2, and phosphorylated Elk-1 using dilutions 1:100, 1:50, and 1:50, respectively, for 30 min at room temperature. Immunostaining present in cytoplasm or nuclei were scored negative (−), positive (+), or strongly positive (++) based on the highest intensity observed in the sample. Scores were given independently by two different observers.

**Results**

PME-1 regulates PP2Ac Leu309 methylation in human cancer cells. To identify mechanism(s) regulating PP2A activity in cancer, proteins that interact with PR65α, the scaffolding subunit of PP2A,
were affinity-purified from HT-1080 fibrosarcoma cells using the TAP strategy (13). TAP from cytoplasmic extracts of either mock-transfected or PR65TAP-expressing HT-1080 cells revealed several proteins that copurified only with the PR65TAP (Fig. 1A; Supplementary Table S1). Importantly, B35α, elf4A, PME-1, and PP2Ac were recently identified as PR65-associating proteins also by using Strep tag purification strategy (24). One of the PR65 interacting proteins was PME-1, which has originally been identified to preferentially interact with inactive PP2Ac (5). The interaction between PME-1 and PP2Ac complex was further confirmed here by coprecipitation of endogenous PP2Ac and PR65 with overexpressed PME-1–Strep protein in Streptactin pull-down experiment (Fig. 1B).

PME-1 yeast homologue PPE1 has been shown to inhibit catalytic activity of PP2Ac in yeast cells (25). However, the cellular target proteins for PME-1–regulated PP2A activity have not been identified yet. To characterize the cellular function of PME-1 in human cancer cells, we first verified that PME-1 regulates methylation of PP2Ac Leu^{309} in cancer cells. For this purpose, we used an antibody that specifically recognizes the demethylated form of PP2Ac Leu^{309} (5). We found that PME-1 depletion resulted in an increase of PP2Ac Leu^{309} methylation in HeLa, U-118MG, and HT-1080 cancer cell lines (Fig. 1C and data not shown). PP2Ac demethylation was not altered between untransfected and scrambled siRNA-transfected cells, showing that PP2Ac Leu^{309} methylation is not subjected to regulation by siRNA response (Supplementary Fig. S1). In addition, retroviral-mediated overexpression of PME-1 in NIH-3T3 fibroblasts caused a decrease in PP2Ac Leu^{309} methylation (Fig. 1D). Together, these results verify the role of PME-1 as the PP2A COOH terminal methyltransferase in cultured cells.

**PME-1 supports both basal and growth factor–stimulated MEK-ERK activity.** To determine whether PME-1 regulates the phosphorylation/activity of the PP2A target proteins, we analyzed the phosphorylation status of different established PP2A target proteins in HeLa cells transfected with either scrambled or PME-1–specific siRNA. Among the MAPK proteins, PME-1 depletion was found to inhibit ERK phosphorylation without any notable effects on p38 or c-Jun NH2-terminal kinase MAPK phosphorylation (Fig. 2A and data not shown). In addition to HeLa cells, PME-1 depletion inhibited ERK phosphorylation in HT-1080 and U118MG cells (Fig. 2B).

These observations show that PME-1 depletion inhibits basal ERK pathway activity. Phorbol ester TPA treatment activates the ERK pathway via protein kinase C (PKC) activation in Ras-independent but Raf-dependent manner. To study whether PME-1 supports ERK signaling downstream of PKC, TPA-elicited activation of MEK and ERK was compared in cells transfected with either scrambled or PME-1–specific siRNA. We found that TPA treatment resulted in robust MEK and ERK activation after 10 minutes in scrambled siRNA-transfected cells, whereas Akt Thr^{308} phosphorylation was not altered (Fig. 2C). However, compared with scrambled siRNA-transfected cells, PME-1 depleted cells displayed clearly diminished MEK and ERK activation in response to TPA (Fig. 2C). In addition, PME-1 depletion inhibited serum-induced ERK phosphorylation (Fig. 2D). Taken together, these results show that PME-1 supports basal and growth factor–stimulated ERK activity downstream of growth factor receptors and PKC, but upstream of MEK.

**Inhibition of the ERK pathway activity by PME-1 depletion is mediated by PP2A activity.** Viral oncoprotein SV40 small t inhibits PP2A activity and stimulates ERK signaling at the level upstream of MEK activation (18, 26). To question whether PME-1 supports ERK signaling via its effects on PP2A activity, MEK phosphorylation was analyzed in PME-1–depleted human embryonic kidney epithelial cells (HEK-TER) expressing SV40 large T antigen, hTERT, and HRAS (10) and either a SV40 small t or a control vector. As shown in Fig. 3A, PME-1 depletion by various shRNAs correlated with MEK inactivation in control cells, whereas Akt Thr^{308} phosphorylation was not altered (Fig. 3B). However, compared with scrambled siRNA-transfected cells, PME-1 depleted cells displayed clearly diminished MEK and ERK phosphorylation (Fig. 3C). In addition, PME-1 depletion inhibited serum-induced ERK phosphorylation (Fig. 3D). These observations indicate that PME-1 supports basal and growth factor–stimulated ERK activity downstream of growth factor receptors and PKC, but upstream of MEK.

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critical for signal propagation (15, 16). To further confirm that PME-1 regulates PP2A activity directed toward the Ras/Raf/MEK signaling complex, we analyzed MEK complex–associated PP2A activity in PME-1 or scrambled siRNA-transfected cells. MEK antibody was used for coimmunoprecipitation to avoid the risk of immunoprecipitating Ras or Raf isoforms that are not involved in MEK activation in HeLa cells. As shown in Fig. 3B, an increase in coimmunoprecipitation of the PP2Ac subunit with MEK immunocomplex was observed in PME-1–depleted cells. Accordingly, PME-1 siRNA-transfected cells showed an increase in MEK complex–associated PP2A activity in an in vitro PP2A assay using DiFMUP as the substrate (Fig. 3C).

Our data, thus far, suggest that PME-1 depletion inhibits ERK pathway activity at the level downstream of Ras, but upstream of MEK. To further pinpoint the level on the ERK pathway wherein PME-1 functions, the effect of PME-1 depletion on ERK phosphorylation was studied in HEK-TEmA clones, in which the pathway activity was stimulated, rather than with active H-Ras mutant (Fig. 3A), by constitutively active alleles of either B-Raf (B-RafE600) or MEK (MEKDD; ref. 10). In addition, these cells express activated Akt (10). We found that, in contrast to H-RasV12 expressing cells (Fig. 3A), PME-1 depletion did not inhibit ERK phosphorylation in either B-RafE600 or MEKDD expressing cells (Fig. 3D). Taken together, these results show that PME-1 protects the ERK pathway from PP2A-mediated inactivation. Moreover, these findings indicate that PME-1 functions upstream of Raf to promote ERK pathway activity.

**PME-1 supports malignant cell growth.** MEK-ERK signaling promotes cancer cell proliferation and survival (7, 15). To ascertain the functional role of PME-1, HeLa cells depleted of PME-1 were assessed for their ability to form dense foci on a monolayer. We found that PME-1 depletion resulted in a significant inhibition of colony growth (Fig. 4A). To study whether PME-1 contributes to malignant cell growth, PME-1 depleted HeLa cells were analyzed for their capacity to grow in an anchorage-independent manner. As shown in Fig. 4B, PME-1 depletion significantly inhibited anchorage-independent proliferation of HeLa cells. In addition to HeLa cells, PME-1 depletion inhibited proliferation also in HT-1080 cells (data not shown). Importantly, no signs of cleavage of the caspase substrate protein poly(ADP-ribose) polymerase were

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### Figure 4

**PME-1 supports malignant cell growth.**

**A,** dense foci formation of HeLa cells transfected with scrambled or PME-1 siRNAs. **B,** anchorage-independent growth of HeLa cells transfected with scrambled or PME-1 siRNA. **C,** proliferation of HeLa cells transfected with scrambled or PME-1 siRNA or treated with UO126 MEK inhibitor for 48 to 72 h. **D,** effect of PME-1 depletion on proliferation rates of indicated HEK-TE cells. Data are presented as percentage change of number of colonies compared with scrambled siRNA transfected cells, which were set in each experiment as 100%. Columns, mean of four to five experiments; bars, SD. *, P < 0.05; **, P < 0.01, Student’s t test.
observed in PME-1–depleted cells 72 hours after siRNA transfection (Supplementary Fig. S2), suggesting that inhibition of malignant cell growth by PME-1 depletion is not caused by induction of a programmed cell death.

To examine whether PME-1 affects cell growth through regulation of the ERK pathway, we analyzed the correlation of proliferation rates between cells treated either with a specific MEK inhibitor, UO126, or with PME-1 siRNA. We found that inhibition of MEK activity with UO126 resulted in a 40% reduction in cell number after 48 hours and 60% inhibition after 72 hours (Fig. 4C). Instead, a 50% reduction in cell number was observed in PME-1–depleted cells 72 hours after siRNA transfection (Fig. 4C). These results show that maximal proliferation of HeLa cells is dependent on both PME-1 expression and ERK pathway activity. To determine if inhibition of cell proliferation induced by PME-1 depletion correlates with ERK pathway inactivation, we analyzed the effect of PME-1 shRNA on proliferation of cell clones, in which ERK pathway activity was activated either by H-RasV12, B-RafV600E, or MEKDD overexpression (10). We found that, whereas PME-1 shRNAs inhibited both MEK phosphorylation and proliferation of H-RasV12–infected cells (Figs. 3A and 4D), PME-1 depletion failed to inhibit either of these properties in cells infected with B-RafV600E or MEKDD (Figs. 3D and 4D). These observations show that PME-1 promotes cell proliferation, whereas inhibition of PME-1 partially suppresses the tumorigenic phenotype of cancer cells. Our results also indicate that PME-1 enhances cell proliferation, at least in part, through its ability to maintain ERK pathway activity.

Figure 5. PME-1 promotes ERK activity and proliferation in human malignant glioblastoma. A, Western blot analysis of ERK pathway activity in U118-MG and T98G glioma cells transfected with scrambled or PME-1 siRNA for 72 h. B, expression of PME-1 and Ki-67 proliferation index was studied immunohistochemically from 222 grades 2 to 4 astrocytic glioma samples. Representative Ki-67 staining of samples negative or positive for PME-1 expression. C, correlation of PME-1 expression and phosphorylation of MEK and Elk-1 was studied immunohistochemically from PME-1–positive and PME-1–negative glioblastoma samples (each n = 20). D, Western blot analysis of serum-induced ERK activation in U118-MG glioma cells infected with indicated PME-1 or scrambled shRNAs. Representative of three independent experiments using two different clones (shScr and shPME-1).
Identification of PME-1 as a protein that supports proliferation of human glioblastoma cells prompted us to study the clinical significance of PME-1 in human astrocytic gliomas. The specificity of the PME-1 antibody used for immunohistochemical stainings of glioma samples was verified by siRNA approach (Supplementary Fig. S4). Altogether 98 samples of 222 grades 2 to 4 astrocytic gliomas were found positive for PME-1 protein expression. The representative samples of PME-1–positive tumors are shown in Fig. 5B and Supplementary Fig. S5. Importantly, increased PME-1 expression was found to correlate with malignant progression of astrocytic gliomas: PME-1 immunopositivity correlated with increasing malignancy grade ($P = 0.021$; Supplementary Table S2). Moreover, PME-1 immunopositivity very significantly correlated with increasing Ki-67 proliferation index in the total tumor material ($P < 0.001$; Supplementary Table S3; Fig. 5B). The difference in cell proliferation was significant even when the comparison between PME-1–positive and PME-1–negative tumors was made separately within grade 2 gliomas ($n = 62$) and glioblastomas of grade 4 ($n = 160$; Supplementary Table S3). Importantly, neither epidermal growth factor receptor (EGFR) amplification nor p53 expression status of the tumors corresponded with the very tight correlation of PME-1 expression with glioblastoma proliferation (Supplementary Table S3). EGFR and p53 status of tumors was also unrelated to PME-1 expression, as glioblastoma proliferation (Supplementary Table S3). EGFR and p53 status of tumors was also unrelated to PME-1 expression, as glioblastoma proliferation (Supplementary Table S3). EGFR and p53 status of tumors was also unrelated to PME-1 expression, as glioblastoma proliferation (Supplementary Table S3). EGFR and p53 status of tumors was also unrelated to PME-1 expression, as glioblastoma proliferation (Supplementary Table S3). 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Discussion

The ERK pathway is an important mediator of the oncogenic activity of RTKs and Ras GTPases (16), as well as oncogenic alleles of Ras (8). However, the mechanisms that sustain RTK and Ras-elicited ERK pathway activity in malignant cells have remained elusive. In this work, we show that PME-1 protects ERK pathway activity from PP2A–mediated inactivation in human malignant glioma. Our data show that PME-1 depletion results in increased methylation of PP2Ac Leu<sup>99</sup> and dephosphorylation of MEK, ERK, and Elk-1 proteins. PME-1 depletion inhibited ERK pathway activity induced by RasV12, whereas cells expressing constitutively active alleles of either B-Raf or MEK were found resistant to PME-1 shRNA (Fig. 3A and D). Together, these observations suggest that PME-1 supports ERK pathway activity at the upstream level of Raf. As PP2A–mediated dephosphorylation of Raf has been shown to stimulate rather than inhibit Raf kinase activity (17, 30), the target for PME-1–regulated PP2A activity could be a protein involved in Raf activation. Candidates for such proteins could be scaffolds involved in linking the RTK proteins to Raf/MEK/ERK complex (15, 16). However, it is clear that future studies are needed to identify the molecular target for PME-1–regulated PP2A activity. Also, based on the data presented here, we cannot exclude the possibility that, in addition to its role in supporting ERK activity, PME-1 would in part support malignant cell growth by regulating other PP2A target pathways.

Although the role of RTK activation in response to growth factors is well studied, the role of phosphatases in this process remains incompletely understood. Here, we show that PME-1 enhances the cellular growth factor response (Figs. 2D and 5D). Based on these results, it is tempting to speculate that, in conditions of limited growth factor supply in tumor tissues, cancer cells with high PME-1 expression levels may retain ERK pathway activity and thereby maintain their proliferative capacity. In addition to results of our cell culture experiments, this notion is clearly supported by in vivo correlation of PME-1 expression, proliferation, and ERK pathway activity in human malignant glioblastoma.

Human glioblastoma patients display extremely poor survival, and there are currently no effective treatment regimens for this disease (27). We have found that PME-1 expression correlates with ERK pathway activity in human malignant glioblastoma (Fig. 5C).

### Table 1. In vivo correlation of PME-1 status to MEK and Elk-1 phosphorylation, EGFR amplification status, and p53 immunopositivity in human glioblastomas

<table>
<thead>
<tr>
<th>PME status</th>
<th>Phosphorylated MEK</th>
<th>Phosphorylated Elk-1</th>
<th>EGFR</th>
<th>p53</th>
</tr>
</thead>
<tbody>
<tr>
<td>PME-1 negative</td>
<td>6 of 20</td>
<td>4 of 20</td>
<td>89 of 159</td>
<td>71 of 109</td>
</tr>
<tr>
<td>PME-1 positive</td>
<td>18 of 20</td>
<td>12 of 20</td>
<td>70 of 159</td>
<td>38 of 109</td>
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$\chi^2$ test: $P < 0.001$; $P = 0.022$; n.s.

Abbreviation: n.s., not significant.
and supports ERK activity in glioblastoma cells (Fig. 5A). These results indicate that PME-1 could represent a potential diagnostic marker to identify subgroups of patients that would benefit from treatment with small molecule Raf/MAPK pathway inhibitors, currently in clinical trials for glioblastoma (27). In addition, as expression of phosphorylated ERK was recently shown to be associated with increased radiation resistance of malignant glioblastoma (29), inhibition of PME-1 could sensitize glioblastoma cells to radiotherapy. In this regard, identification of small molecule inhibitors of PME-1 methyltransferase activity or PME-1–PP2Ac interaction (6, 31) would be important to probe potential suitability of PME-1 as a drug target in malignant glioblastoma.

These observations identify PME-1 expression as a hitherto unrecognized mechanism supporting ERK pathway activity in cancer cells. Importantly, our data suggest that, in addition to growth factors and their receptors, PME-1–regulated PP2A functions as an important modulator of the cellular growth factor response. Considering the established role for growth factor–elicited ERK signaling in several human malignancies (7, 22), we postulate that the importance of these results reaches beyond the shown role of PME-1 in human glioblastomas. Moreover, this first identification of a cellular role for PME-1 in the regulation of cellular signaling further highlights the novelty of the presented results. Together with other recently published data (12–14), these findings further emphasizes the relevance of identifying mechanisms that regulate the tumor suppressor function of PP2A in human malignancies. Finally, the results of this work may open novel opportunities for the treatment and diagnosis of human cancers addicted to oncogenic ERK pathway activity (32).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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