Association of increased phosphatidylinositol 3-kinase signaling with increased invasiveness and gelatinase activity in malignant gliomas

TOMAS KUBIATOWSKI, M.D., PH.D., TAICHANG JANG, PH.D., MAHESH B. LACHYANKAR, PH.D., REBECCA SALMONSEN, M.S., ROYA R. NABI, M.S., PETER J. QUSENBERGY, M.D., N. SCOTT LITOFSKY, M.D., ALONZO H. ROSS, PH.D., AND LAWRENCE D. RECHT, M.D.

Departments of Surgery (Neurosurgery), Pharmacology and Molecular Toxicology, Medicine (Cancer Center), and Neurology, University of Massachusetts Medical Center, Worcester, Massachusetts

Object. Glioblastoma multiforme is the most malignant of the primary brain tumors and aggressively infiltrates surrounding brain tissue, resulting in distant foci within the central nervous system, thereby rendering this tumor surgically incurable. The recent findings that both phosphatidylinositol 3-kinase (PI 3-K) and the phosphatase and tensin homolog (PTEN) regulate tumor cell invasiveness have led the authors to surmise that these lipid signaling molecules might play a role in regulating matrix metalloproteinases (MMPs), which are essential for tumor cell invasion.

Methods. Using the C6 glioma cell line, which does not express measurable amounts of PTEN protein and in which in vitro invasiveness is MMP dependent, the authors determined that in vitro glioma cell invasiveness was significantly reduced when cells were preincubated overnight with LY294002 or wortmannin, two specific inhibitors of PI 3-K signaling. Next, using gelatin zymography, it was noted that these compounds significantly inhibited MMP-2 and MMP-9 activities. Moreover, the decrease in MMP activity correlated with the decrease in PI 3-K activity, as assessed by Akt phosphorylation. Finally, using semiquantitative reverse transcriptase–polymerase chain reaction, the authors demonstrated that LY294002 decreased messenger (m)RNA levels for both MMPs. Thus, these in vitro data indicate that PI 3-K signaling modulates gelatinase activity at the level of mRNA.

Using immunostaining of phosphorylated Akt (p-Akt) as a measure of PI 3-K activity, the authors next assessed rat brains implanted with C6 cells. Compared with surrounding brain, there was marked p-Akt staining in C6 glioma cells and in neurons immediately adjacent to the tumor, but not in normal brain. The p-Akt staining in tumors was especially intense in perivascular areas. Using double-labeling techniques, colocalization of p-Akt with MMP-2 and MMP-9 was also noted in perivascular tumor areas.

Conclusions. The increase in p-Akt staining within these PTEN-deficient gliomas is consistent with what would be predicted from unchecked PI 3-K signaling. Furthermore, the immunohistochemically detected colocalization of p-Akt and MMP-2 and MMP-9 supports the authors’ in vitro studies and the proposed linkage between PI 3-K signaling and MMP activity in gliomas.

KEY WORDS • glioblastoma multiforme • tumor invasion • matrix metalloproteinase • lipid signaling pathway • phosphatidylinositol 3-kinase • phosphatase and tensin homolog • gelatinase

Glioblastoma multiforme is the most malignant primary brain tumor and it is characterized pathologically by a marked increase in cell proliferation, neovascularization, and confluent areas of necrosis. Although it is not typically associated with extraneural metastases, it is locally invasive, disseminating along white matter fiber tracts, that is, the corpus callosum, and the basement membrane of blood vessels. This aggressive propensity to infiltrate surrounding brain tissue results in distant foci within the central nervous system that render this tumor surgically incurable. Recently, it has been shown that integrins can enhance motility and invasiveness through activation by PI 3-K of the small G protein, Rac. In subsequent studies the importance of PI 3-K in mediating the invasiveness of bladder, renal, and colon cancer cells has been demonstrated; thus, dysregulation of the PI 3-K pathway may contribute to the invasiveness of gliomas and other tumors.

Although PI represents only a small percentage of total cellular phospholipid, it is the precursor of several second-messenger molecules and is an important cell signal transducer. It is known that PI 3-K phosphorylates the D3 position of the inositol ring and is present in all cell types; it has generated much interest because it is activated by many growth and survival factors. Furthermore, over-
expression of PI 3-K has been implicated in the development of several kinds of nonglial tumors\textsuperscript{3,9,39} and as an oncogene in ovarian cancer.\textsuperscript{50}

The viral oncogene v-Akt's cellular homolog, c-Akt, also known as protein kinase B, is a serine-threonine kinase that is an important intermediary in the downstream signaling of PI 3-K.\textsuperscript{4,11,23} Binding of Akt to PIP initiates Akt phosphorylation and activation; it is widely expressed, with the highest levels being noted in the brain, thymus, heart, and lung.\textsuperscript{11,23} Although it has only weak transforming properties by itself, the Akt gene is overexpressed in cancer and may be associated with tumor aggressiveness.\textsuperscript{3,9,39} Recently, elevated Akt was reported in a cell line derived from a patient with GBM, suggesting that this signal-transducing system is also involved in the generation or maintenance of the glioma phenotype.\textsuperscript{21}

The dual-specificity homolog PTEN is located on chromosome 10 and is deleted or mutated in many GBMs.\textsuperscript{3,9,39} It has been reported recently that PTEN modulates Akt activity by dephosphorylating PIP. Normally, PIP and Akt activity are low in the absence of growth factor stimulation; however, PTEN-deficient tumor cell lines exhibit high basal levels of PIP and Akt phosphorylation.\textsuperscript{18,21} When PTEN is introduced into nonexpressing cancer cells, it results in a dose-dependent and specific inhibition of Akt, and Akt overexpression can rescue cells from PTEN-initiated death.\textsuperscript{12,13,31,51} Furthermore, PTEN can regulate the Akt signaling pathway to modulate cell cycle progression, cell survival, and anoikis,\textsuperscript{13} thus, PTEN and PI 3-K have opposite influences on Akt, and thereby, on tumor cell proliferation and survival.

The invasiveness of gliomas is determined in part by their proteolytic capacity, especially by two MMPs, MMP-2 and MMP-9, that degrade gelatin.\textsuperscript{19} The expression and activity of these MMPs are upregulated in more aggressive tumors, but the mechanisms by which enzyme activity is controlled remain unclear.\textsuperscript{41,44,45,55} We hypothesize that the MMPs represent relevant downstream targets by which PI 3-K signaling affects invasiveness.

To test this hypothesis, we used the C6 glioma model because these cells form intracranial tumors that characteristically invade surrounding brain, both as a mass and as single cells.\textsuperscript{5,20} We demonstrated that C6 cells are PTEN negative, and specific pharmacological inhibition of PI 3-K activity decreased both the invasiveness and gelatinase activity of C6 cells in vitro, the latter due to an effect at the mRNA level. Based on these results, we then assessed the in situ expression of Akt and the gelatinases after intracranial implantation. Our histochemical results demonstrate a distinctive increase of p-Akt, but not Akt, within the tumor mass. Furthermore, this increase paralleled that of the gelatinases. We conclude that the invasiveness of C6 cells is partly caused by unchecked PI 3-K signaling and that MMPs are a downstream target of PI 3-K.

Materials and Methods

Reagents Used in the Study

We used AG3340 that had been commercially synthesized as previously described,\textsuperscript{48} and the reagent was stored dessicated in amber vials at 4°C. The AG3340 was solubilized in H\textsubscript{2}O (pH 2.3) and passed through sterile filters before use. The LY294002 was dissolved in 100% ethanol and stored at 4°C until use.

Invasion of Glioma Cells Through a Matrigel Barrier

Adapting the method of Albinii, et al.,\textsuperscript{1} povidone-free polycarbonate membrane filters (12-μm-diameter pore size) were soaked in ice-cold H\textsubscript{2}O and placed on an ice-cold plate covered with parafilm. Fifty microliters of Matrigel (0.5 mg/ml H\textsubscript{2}O) was added to each filter, after which they were air dried for 1 hour and reconstituted in DMEM plus 1% BSA.

The bottom chamber was loaded with C6-conditioned DMEM at 37°C, and the coated filter (Matrigel side was up) was screwed in place, after which 10\textsuperscript{5} cells removed with 1 mM ethylenediaminetetra-acetic acid from logarithmically growing cultures and resuspended in 800 μl DMEM plus 1% BSA were placed in the top chamber and incubated for 5 hours. The filters were then removed, fixed, and stained. Cells were then counted in 10 randomly chosen high-power fields (x40).

Gelatin Zymography

Adapting previously described methods to measure production of gelatinases,\textsuperscript{46} 2 × 10\textsuperscript{5} glioma cells grown in a 75-cm\textsuperscript{2} tissue culture flask were incubated for 24 hours at 37°C in 10 ml DMEM/F12 medium. Gelatin-agarose beads were washed twice with 10 ml DMEM/F12 at 4°C. To concentrate the gelatinases, the supernatant was cleared of cellular debris at 4°C and mixed with gelatin agarose beads at a ratio of 10 ml supernatant/0.5 ml agarose beads. The mixture was then gently stirred at 4°C for 24 hours. After the incubation, beads were centrifuged for 2 minutes at 4°C at 1000 g and then washed with 0.5 ml of DMEM/F12. Fifty microliters of sample buffer was added to 150 μl of gelatin beads and gently mixed, after which the mixture was incubated at 37°C for 90 minutes, and equal amounts of supernatant protein were loaded onto the gel at 4°C. The separating gel contained 1 mg/ml gelatin. Five units of human MMP-2 and MMP-9 were used as a control. The gel was electrophoresed at 150 V at 4°C, after which gels were washed sequentially three times, first with 2.5% Triton X-100 diluted in distilled H\textsubscript{2}O, then with 2.5% Triton X-100 in 50 mM Tris buffer, and finally with 50 mM Tris buffer, pH 7.4; they were then incubated for 24 hours at 37°C. Finally, gels were placed in 2.3% wt/vol Coomassie Blue in methyl alcohol and glacial acetic acid for 30 minutes and destained for 6 hours to reveal zones of gelatin lysis.

Western Blotting for PTEN, Akt, and p-Akt

Protein was extracted, separated on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted onto nitrocellulose. The nitrocellulose was incubated in blocking buffer (0.01 M TBS containing 5% powdered milk and 0.1% Tween 20) for 3 hours at room temperature, after which the filter was incubated for 1 hour at room temperature with either 1:1000 anti-Akt or p-Akt antibodies in blocking buffer.

Identification of PTEN Protein

Protein was extracted from C6 cells growing exponentially in minimum essential medium supplemented with 10% fetal calf serum and incubated with 1:1000 U4B2 anti-PTEN antisemur.\textsuperscript{55} The PC12 cells grown in DMEM containing 10% horse serum and 5% fetal bovine serum supplemented with 100 ng/ml nerve growth factor to induce differentiation were used as a positive control, based on previous experiments. The filter was then washed in TBS containing 0.1% Tween 20 and incubated with 1:2000 horseradish peroxidase–conjugated anti–rabbit antibodies in TBS with 0.1% Tween 20 for 3 hours at room temperature. The filter was then washed and peroxidase activity was demonstrated with a Western detection kit. Bands were quantified with a scanning densitometer.

Reverse Transcription–Polymerase Chain Reaction

Poly(A)\textsuperscript{+} RNA was prepared using an mRNA purification kit. The RNA was heat denatured at 95°C for 5 minutes and then quickly chilled on ice. The RT buffer, deoxynucleoside triphosphates,
RNase inhibitor, and oligoT (RNA PCR kit) were mixed with 1 μg of poly(A)⁺ RNA. The reaction was performed in a thermal cycler by using the RT program (15 minutes at 42°C followed by 5 minutes at 99°C, and subsequent cooling to 4°C). Oligonucleotide primers, buffer, and Taq polymerase were then added. The primers used were as follows: MMP-2 (predicted length 309 bps) sense: 5'-CTATTTCTGCAGCACTTTGG-3', antisense: 5'-CAGACTTTGGTTCCTCAACTT-3'; MMP-9 (predicted length 309 bps) sense: 5'-AAATGTTGGTTAGACACGGC-3', antisense: 5'-TTACCCCGGTTCCTGAAAATCT-3', and G3PDH (predicted length 983 bps) sense: 5'-TGAAGGTCGGAGCAACGGATTTGGT-3', antisense: 5'-CATGTGGGCCCAGAGGTCCACCAC-3'.

After initial heating of the mixture to 95°C for 2 minutes, PCR amplification was performed for 20, 25, 30, and 35 cycles (MMP-2; 1 minute at 94°C, 1 minute at 57°C, and 90 seconds at 72°C; MMP-9; 45 seconds at 94°C, 45 seconds at 55°C, and 1 minute at 72°C; G3PDH; 45 seconds at 94°C, 45 seconds at 60°C, and 2 minutes at 72°C) with a final extension of 10 minutes at 72°C for all primers. The PCR products were then analyzed on a 2% agarose gel.

Intracranial Inoculations

The methods used in this study were approved by the University of Massachusetts Medical School's Institutional Use and Care of Animals Committee. Nine Wistar-Kyoto adult male rats weighing 150 to 200 g were anesthetized with 50 mg/kg sodium pentobarbital and placed in a stereotactic head holder. Suspensions of 10⁶ exogenous for 3,3′-diaminobenzidine tetrahydrochloride for visualization. Sections incubated without the primary antibody served as negative controls.

Double labeling was performed by simultaneously incubating sections in the combination of primary antibodies, followed by a mixture of non–crossreactive fluorescein isothiocyanate-conjugated and tetramethyl rhodamine isothiocyanate-conjugated secondary antibodies. The MMP-2 and MMP-9 antibodies were used at 20 μg/ml and 20 μg/ml, respectively, in the fluorescence double-labeling experiments.

Photographs were taken of the areas of interest through brightfield and fluorescent microscopes. The negatives were then scanned with a Nikon Coolscan LS 2000 for further analysis.

**Statistical Analysis**

A commercially available software package was used for statistical analysis. One-way ANOVA tests were used to analyze significance between groups. The Dunnett method of multiple compar-
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**Fig. 4.** Representative gelatin zymogram demonstrating decreased inactive and active MMP-9 (92-kD and 86-kD bands, respectively) and MMP-2 (68-kD and 62-kD bands, respectively) secretion by C6 cells after incubation in LY294002. Two times 10⁶ cells were incubated overnight in serum-free defined media, after which the supernatant was collected and analyzed as described in *Gelatin Zymography*; S indicates the gelatinase standard. This experiment was repeated four times with similar results.

**Fig. 5.** Western blot in which specific antibodies were used against Akt and p-Akt, as described in *Histochemical Procedures*. It is apparent that LY294002 inhibits p-Akt; note the selective attenuation of the p-Akt band after incubation in 10 µM LY294002. This experiment was repeated three times with similar results.

**LY 294002 (µM) kDa**

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**LY294002 (µM)**

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**AKT**

| 66 |

**P-AKT**

| 66 |

**Results**

Using a specific polyclonal antibody directed against PTEN, we determined that C6 glioma cells do not express measurable amounts of this protein (Fig. 1), and hence, would be expected to have elevated PI 3-K signaling. In addition, by using a compound that specifically inhibits MMP-2 and MMP-9 as well as MMP-13 and MMP-3 (stromelysin), we noted a significant reduction in C6 invasion through a Matrigel barrier at doses of 25 µM and 100 µM AG3340 (Fig. 2). At the two highest doses used, which had no effect on cell growth, invasiveness was reduced more than 50% (p < 0.05, the Dunnett method of multiple comparisons versus control). Hence, both PI 3-K and MMPs are important for this tumor line; in the remainder of this study we analyze the relationship between these two activities.

We reasoned that if unchecked PI 3-K signaling were enhancing invasiveness, then blocking its activity should inhibit glioma cell invasion. To test this hypothesis, we assessed the effects of PI 3-K inhibitors on the invasiveness of C6 cells through a Matrigel barrier. Preincubation overnight of C6 cells with LY294002 caused a dose-dependent inhibition of invasiveness. Whereas only minimal inhibition was noted at a concentration of 1 µM LY294002, a 73% decrease in the number of penetrating cells was noted at a concentration of 10 µM (p < 0.001, one-way ANOVA; Fig. 3). Similarly, invasiveness was decreased 88% by an overnight preincubation with 5 µM wortmannin (p < 0.001, t-test). Neither inhibitor affected cell viability or proliferation at these concentrations (data not shown), supporting the hypothesis that this effect was due to specific inhibition of PI 3-K.

To address whether this inhibition was associated with decreased MMP activity, we used gelatin zymography to measure the effect of LY294002 on MMP-2 and MMP-9 activities. Preincubation of C6 cells with 10 µM LY294002 for 24 hours markedly decreased the lysis zones corresponding to both the active and inactive forms of MMP-2 and MMP-9. As depicted in Fig. 4, a decrease in the bands corresponding to both the active and inactive forms of MMP-2 and MMP-9 was noted. To support the contention that the effect of LY294002 on MMP activity was specifically directed through the PI 3-K pathway, we used Western blotting to determine Akt phosphorylation. As illustrated in Fig. 5, p-Akt but not Akt expression was decreased at the LY294002 concentration (that is, 10 µM) that also decreased MMP production and tumor invasiveness.

To assess the level at which PI 3-K activity mediates these effects, we used semiquantitative RT-PCR to assay mRNA levels for MMP-2 and MMP-9. For control cells and those treated with 1 µM LY294002, PCR products are clearly apparent for MMP-2 and MMP-9 after 30 cycles. In contrast, even at 35 cycles, only very weak bands are observed.
expression of the two gelatinases MMP-2 and MMP-9. Using antibodies that recognize both the proenzyme and activated forms of these enzymes, we examined rat brains by using double staining for p-Akt and either MMP-2 or MMP-9. Both MMP-2 and MMP-9 were found to coexist with p-Akt–positive cells. This coexistence was more frequently observed in the perivascular areas at the junction of tumor and normal brain tissue (Figs. 9 and 10). Instances of double staining were never seen in the p-Akt–positive neurons noted in peritumoral areas.

Discussion

We hypothesized that unchecked PI 3-K signaling in the absence of PTEN would increase invasiveness of glioma cells, as well as MMP activity. After confirming that C6 glioma cells did not express PTEN, we first analyzed whether modulation of PI 3-K activity altered invasiveness of C6 glioma cells. Using penetration through Matrigel-coated filters as an in vitro measure of C6 glioma cell invasiveness, we noted that invasion was decreased in a dose-dependent fashion when PI 3-K activity was inhibited with either LY294002 or wortmannin, two relatively specific inhibitors of PI 3-K. We next addressed whether inhibition of PI 3-K also decreased gelatinase activity as assessed by gelatin zymography and found that LY294002 substantially reduced both MMP-2 and MMP-9 activity.

To further document that this was a specific effect, we demonstrated that LY294002 decreased p-Akt at the same dosage that inhibited MMP activity. Finally, to assess at what level this effect occurred, we measured mRNA levels by using semiquantitative RT-PCR and found a reduction for both MMPs. There was no change in the level of a housekeeping mRNA, indicating that this effect was selective and specific, rather than a general decrease in transcription resulting from toxicity.

Opposing Influences on Cancer Invasiveness by PTEN and PI 3-K

One of the most common genetic abnormalities in malignant gliomas is a deletion on chromosome 10q23. The product of this gene, the PTEN tumor suppressor protein, was originally proposed to be a dual-specificity phosphatase, but recent studies indicate that the biologically relevant targets of PTEN are inositol phospholipids. Transfection of PTEN in negatively expressing cells inhibits migration and results in anoikis. Further work has established that PTEN inhibits PI 3-K–dependent activation of Akt, thus leading Cantley and Neel to propose a model whereby PTEN acts as a tumor suppressor by inhibiting the downstream effects of PI 3-K. Using this model we would predict increased signaling of PI 3-K in cells lacking PTEN.

The transducer PI 3-K can be activated by growth factors, oncogenes, and integrins and plays a key role in regulating mitogenesis, apoptosis, intracellular vesicle trafficking, motility, and adhesion. It has been shown that PI 3-K is overexpressed in a variety of tumors, and its overexpression can result in cellular transformation. Recently, in two studies it has been shown that integrins can enhance nonglioma tumor cell motility and invasiveness through the activation of PI 3-K. In both of these
studies it was posited that the actions of PI 3-K were mediated via the small G protein, Rac. Subsequent studies have further demonstrated the importance of PI 3-K in mediating invasiveness of bladder, renal, and colon cancer cells. Furthermore, in a recent study by Ling and colleagues, it was noted that inhibition of PI 3-K activity impeded astrocytoma attachment and migration in vitro.

Although Shaw, et al., found no effect on invasiveness by blocking Akt function, specific antibodies that distinguish phosphorylated Akt allow measurement of PI 3-K activity in situ. The present study is the first in which Akt

**Increased p-Akt Staining in Glioma and Surrounding Brain**

![Photomicrographs of tissue sections showing expression of Akt and p-Akt in brains 7 days after inoculation with C6 tumor cells.](image)

A: Low-power magnification of tumor (T) and surrounding neural tissue stained for Akt. Note relative homogeneity of staining. B: Adjacent section stained for p-Akt. Note the small amount of staining in brain compared with tumor. C: Cortical pyramidal cells demonstrating expression of Akt. D: Similar brain area stained for p-Akt demonstrating minimal staining. E: Tumor parenchyma stained for Akt showing homogeneous cellular staining. F: Tumor parenchyma near brain–glioma interface stained for p-Akt, revealing enhanced perivascular staining. F = fimbria; V = blood vessel.
and p-Akt are analyzed in brain tumors in situ. We used an antibody that recognizes Akt molecules phosphorylated at thr308, which is located within the catalytic domain of the molecule.10 We noted an increased expression of p-Akt, but not Akt, within the brain tumor (Fig. 7A and 7B). This differential staining deserves comment for a number of reasons. First, it emphasizes the difference between expression and activity. Such an analysis would not be possible with currently available PI 3-K antibodies. Second, the absence of detectable p-Akt in the surrounding brain indicates that in undisturbed brain, PI 3-K signaling is at a low level. In the growing C6 glioma, however, Akt phosphorylation is much greater. This is consistent with the loss of the modulating influence of PTEN on Akt phosphorylation.

Certain neurons located inferior to the tumor in the region of the thalamus stained strongly for p-Akt. We hypothesize that these cells are under stress because of changes in this environment and have increased Akt phosphorylation to enhance cell survival. The tumor-induced stress could occur by several mechanisms. Glioma cells, for example, secrete cytotoxic concentrations of glutamate;61 it is possible that such cytotoxic molecules secreted by invading tumor cells result in Akt phosphorylation.

**Influence of MMPs on Glioma Invasiveness**

The MMPs represent a large family of Zn\(^{++}\)-dependent proteases that are capable of degrading almost all extracellular matrix macromolecules.36,38,42 MMPs are important in the remodeling of the extracellular matrix during development, growth, and tissue repair.37,62 The invasiveness of glioma cells is, in part, determined by this proteolytic capacity and especially reflects the activity of two MMPs that degrade gelatin: MMP-2 and MMP-9. These enzymes are present in greater quantities in more aggressive gliomas.16,19,41,44–46,57 Thus, modulating the behavior of these MMPs should affect tumor phenotype and favorably influence outcome; in a recent study43 in which a compet-

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**Fig. 8.** Photomicrograph of tissue section showing expression of p-Akt in neurons in the brain region adjacent to the tumor. Neuronal staining.

**Fig. 9.** Photomicrographs showing coexpression of p-Akt and MMP-2 in C6 gliomas. Cells were stained using methods described in *Histochemical Procedures*. Note numerous cells (arrows) that coexpress p-Akt and MMP-2.

**Fig. 10.** Photomicrographs showing coexpression of p-Akt and MMP-9 in C6 gliomas. Cells were stained using methods described in *Histochemical Procedures*. Three cells (arrows) in the perivascular area coexpress both p-Akt and MMP-9.
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itive inhibitor of MMPs was used in an experimental model of glioma, such an effect was indicated, and such a strategy is currently being evaluated clinically.

Increased MMP levels and activity are commonly encountered in GBMs when these tumors are assessed using gel zymography or enzyme-linked immunosorbent type assays. In previous immunohistochemical studies, however, conflicting results were reported in both the presence and absence of MMP-2 and MMP-9 in paraffin-embedded clinical specimens. In our study, wherein freshly prepared frozen sections were examined, we found a heterogeneous expression of both MMP-2 and MMP-9. What the significance of such a pattern is in the determination of glioma aggressiveness remains to be resolved, however.

Influence of PI 3-K Signaling on Expression of MMP

Our in vitro studies indicate a relationship between PI 3-K signaling and gelatinase expression, and, by inference, activity. Moreover, the in situ coexistence of MMP-staining and p-Akt–staining perivascular cells (Figs. 9 and 10) located at the junction of tumor and normal brain where tumor invasion is occurring further indicates that increased PI 3-K signaling might be linked to the GBM’s invasiveness through an effect on MMPs. A number of questions remain to be answered before it can be stated definitively that such a link exists. For instance, these findings still need to be confirmed in clinical specimens obtained in patients with GBM. In this regard, initial studies (T Jang, unpublished observations) demonstrate coexpression of MMP and p-Akt in human GBM, but more work needs to be done to establish a definitive link. Additionally, it would be interesting to know whether a different expression pattern of both p-Akt and MMP would be noted if PTEN expression could be induced in glioma cells. Moreover, would MMP expression in situ be decreased if Akt phosphorylation (or expression) were inhibited? Further studies are also needed to address whether modifications in lipid-signaling pathways could serve as a basis for developing novel therapeutic agents that affect malignant glioma invasiveness.

References

27. Kotelevets L, Noe V, Bruyneel E, et al: Inhibition by platelet-activating factor of Src- and hepatocyte growth factor-


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Address reprint requests to: Lawrence D. Recht, M.D., Department of Neurology, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, Massachusetts 01655. email: RechtL@ummc.org.