Prognostic Associations of Activated Mitogen-Activated Protein Kinase and Akt Pathways in Glioblastoma

Christopher E. Pelloski,[1] E Lin,[2] Li Zhang,[2] W.K. Alfred Yung,[3] Howard Colman,[3] Juinn-Lin Liu,[3] Shaio Y. Woo,[1] Amy B. Heimberger,[4] Dima Suki,[4] Michael Prados,[6] Susan Chang,[6] Fredrick G. Barker III,[7] Gregory N. Fuller,[5] and Kenneth D. Aldape[5]

Abstract

Purpose: Activation of mitogen-activated protein kinase (MAPK) and members of the Akt pathway have been shown to promote cell proliferation, survival, and resistance to radiation. This study was conducted to determine whether any of these markers are associated with survival time and response to radiation in glioblastoma.

Experimental Design: The expression of phosphorylated (p-)Akt, mammalian target of rapamycin (p-mTOR), p-p70S6K, and p-MAPK were assessed by immunohistochemical staining in 268 cases of newly diagnosed glioblastoma. YKL-40, a prognostic marker previously examined in these tumors, was also included in the analysis. Expression data were tested for correlations with response to radiation therapy in 131 subtotally resected cases and overall survival (in all cases). Results were validated in an analysis of 60 patients enrolled in clinical trials at a second institution.

Results: Elevated p-MAPK expression was most strongly associated with poor response to radiotherapy, a finding corroborated in the validation cohort. For survival, higher expressions of p-mTOR, p-p70S6K, and p-MAPK were associated with worse outcome (all $P < 0.03$). YKL-40 expression was associated with the expressions of p-MAPK, p-mTOR, and p-p70S6K (all $P < 0.02$), with a trend toward association with p-Akt expression ($P = 0.095$). When known clinical variables were added to a multivariate analysis, only age, Karnofsky performance score, and p-MAPK expression emerged as independent prognostic factors.

Conclusions: p-MAPK and activated members of the Akt pathway are markers of outcome in glioblastoma. Elevated expression of p-MAPK is associated with increased radiation resistance and represents an independent prognostic factor in these tumors.

The Ras signaling pathway, consisting of the Raf/mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase/MAP kinase (MAPK, also known as p42/44 or ERK1/ERK2) as well as the phosphoinositide 3-kinase (PI3K)/Akt pathways (Fig. 1), are critical in the malignant phenotype of glioblastoma and have been shown to govern proliferation and survival (1–4), invasiveness (2, 5–7), and radiation resistance (8–11) *in vitro*. The Ras protein seems to be activated by stimulation of surface receptors and other abnormal signaling events because Ras gene mutations have not been found in glioblastoma (12). From *in vitro* experiments, data indicate that addition of YKL-40 protein to connective tissue cells results in increased cell proliferation through activation of Akt and MAPK pathways (13). Therefore, YKL-40 is a possible candidate for regulation of these signaling pathways, as this secreted protein is the product of one of the most expressed genes in glioblastoma (14–18) and is a prognostic marker in these tumors (16, 19).

Attention has recently been turned toward elucidating the effects of PI3K/Akt and Raf/MAP/ERK kinase/MAPK intermediates on clinical outcome in gliomas. One report indicates that the presence of activated [phosphorylated (p-)] PI3K/Akt pathway members is correlated with increased tumor grade, lesser likelihood of apoptosis, and decreased overall survival (20). The presence of activated mammalian target of rapamycin (p-mTOR) was found to be highly correlated with the presence of activated PI3K/Akt pathway intermediates within tissue specimens from glioblastoma patients and p-mTOR is thought to have a stimulatory effect on this pathway (21). It has also been reported that high tumor levels of p-MAPK are associated with shorter survival times and higher proliferation indexes (22). However, no data exist regarding the association of radiation responsiveness, in the clinical setting, to the presence of these markers in human tumors.

Authors' Affiliations: Departments of [1]Radiation Oncology, [2]Biostatistics and Applied Mathematics, [3]Neuro-Oncology, [4]Neurosurgery, and [5]Pathology, University of Texas M.D. Anderson Cancer Center, Houston, Texas; [6]Department of Neurosurgery, University of California San Francisco School of Medicine, San Francisco, California; and [7]Neurosurgical Service, Massachusetts General Hospital, Boston, Massachusetts

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Requests for reprints: Kenneth D. Aldape, Department of Pathology, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 85, Houston TX 77030. Phone: 713-745-3524; Fax: 713-745-1105; E-mail: kaldape@mdanderson.org.

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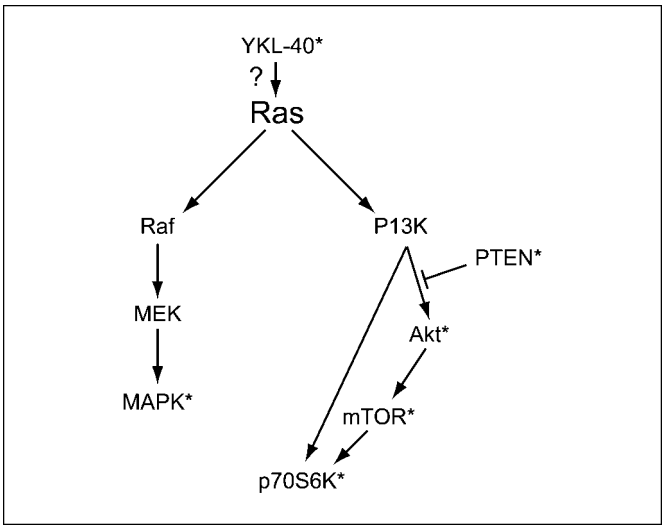

Fig. 1. The signaling pathways involved in this study. *, biomarkers stained for and analyzed.

For these reasons, we investigated the relationship of tumor cell YKL-40 status, survival, and radiation response to the expression of activated (phosphorylated) species of the PI3K/Akt pathway (p-Akt, p-mTOR, and p-70S6K) and the major phosphointermediate of the Raf/MAP/ERK kinase/MAPK pathway, p-MAPK, present in tumors of patients with glioblastoma who were treated at The University of Texas M.D. Anderson Cancer Center from 1994 to 2003. A second population, which consisted of patients with glioblastoma who were enrolled in clinical trials at The University of California-San Francisco during a similar time period, was used as a validation group.

Materials and Methods

Patient selection. Tissues and records of 268 patients who were newly diagnosed with glioblastoma (WHO glioma grade 4) from 1994 to 2003 were retrospectively reviewed. Patient selection was based on the availability of paraffin-embedded tissue and medical records. The extent of surgery comprised two prognostic groups: those who underwent an attempted maximal resection (gross-total or subtotal resection) and those who underwent biopsy alone. The extent of resection was confirmed by postoperative magnetic resonance imaging. The subset of patients who underwent an incomplete resection (i.e., who had measurable disease on imaging after subtotal resection or biopsy) had been previously scored for imaging-assessed radiation response (19). Clinical categories were determined in accordance with the modified Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis classification system for glioblastoma (23–25).

To validate the findings of the initial cohort, tissue samples were obtained from 60 additional patients who were previously enrolled in two clinical trials at the University of California-San Francisco (26, 27). These patients underwent subtotal resection and had clinical follow-up, including magnetic resonance imaging assessment of response after radiotherapy. Survival and radiation response end points were examined.

Tissue processing. The staining for YKL-40 was described previously (19). The tissue for each case underwent immunohistochemical staining for p-Ser^{473} Akt (1:300 dilution), p-Ser^{2448} mTOR (1:100), p-Thr^{389} p70S6K (1:1000), and p-Thr^{202}/Tyr^{204} MAPK (1:100; all from Cell Signaling Technology, Beverly, MA) using previously described techniques (28). A neuropathologist (K.D.A.) identified blocks with sufficient tumor available for analysis of each case. Intratumoral heterogeneity of phosphoprotein expression was noted, some of which may have been due to fixation artifacts. In all cases, scoring was based on the most positive area present in the tumor. Markers were scored separately (by K.D.A.) and the molecular profile was determined while blinded to clinical information. The PI3K negative regulator, protein tyrosine phosphatase (PTEN; 1:200 dilution, Zymed, Carlsbad, CA) was stained in 141 cases, but discontinued because of insignificant findings in an interim analysis. For all markers except p-MAPK, a negative score designated a complete absence of the protein within the tumor cells. As seen in a previous report (29), p-MAPK was always present; therefore, a negative score signifies light (faint or patchy) staining, whereas a positive score designates intense (heavy staining in all tumor cells) for this marker. Scoring for p-MAPK expression in the initial set of 268 cases was independently done by a second neuropathologist (G.N.F.) while blinded to the clinical data as well as the scores determined by the first neuropathologists (K.D.A.).

Immunoblotting. An immunoblot assay was done on cases that were assessed by immunohistochemistry. After confirmation of the presence of tumor by representative H&E slides, specimens frozen in liquid nitrogen were crushed into powder using a pestle and mortar and lysed

Table 1. Clinical characteristics and survival times for glioblastoma patients from M.D. Anderson Cancer Center (n = 268)

Factor	Status	No. cases (%)	Median OS* (wk)	Actuarial 2-y OS (%)	P †
Age (y)	<50	72 (27)	68	38	<0.001
	≥50	196 (73)	47	9	
KPS	90-100	143 (53)	59	22	0.004
	70-80	114 (53)	45	10	
	<70	11 (4)	33	0	
Extent of resection	Gross total	131 (49)	51	19	0.091
	Subtotal	126 (47)	50	15	
	Biopsy alone	11 (4)	39	0	
Radiation response	Regression/stable	57 (44)	83	30	<0.001
	Progression	74 (56)	41	2	

*Overall survival.
† Kaplan-Meier, log-rank.

Table 2. Spearman rank marker-marker correlations in the glioblastoma tumors from M.D. Anderson Cancer patients (n = 268)

		YKL-40	p-Akt	p-mTOR	p-70S6K	p-MAPK
p-MAPK	Corr. coeff.*	0.14	0.12	0.11	0.34	
	P	0.01	0.02	0.03	<0.001	
p-p70S6K	Corr. coeff.	0.18	0.35	0.24		
	P	0.001	<0.001	<0.001		
p-mTOR	Corr. coeff.	0.16	0.32			
	P	0.005	<0.001			
p-Akt	Corr. coeff.	0.09				
	P	0.08				
PTEN† (n = 141)	Corr. coeff.	0.12	0.06	0.23	0.17	0.10
	P	0.175	0.454	0.006	0.041	0.296

*Correlation coefficient.
† Based on an interim analysis.

with radioimmunoprecipitation assay buffer protein extraction reagent [150 mmol/L NaCl, 50 mmol/L Tris-HCl (pH 7.4), 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mmol/L dichlorodiphenyltrichloroethane, 1 mmol/L sodium orthovanadate (all from Sigma)], and 10 μg/mL triple protease inhibitor cocktail as suggested (Roche, Basel, Switzerland) for at least 45 minutes at 4°C. Extracts were cleared by centrifugation at 14,000 rpm using a microfuge for 5 minutes at 4°C. Protein concentrations were determined by using Bradford reagent (Bio-Rad, Hercules, CA). Five hundred sixty micrograms of protein were resolved per lane in 12% gels by SDS-PAGE. The proteins were transferred onto polyvinylidene difluoride filter – type immobilon-P transfer membranes (Millipore, Billerica, MA) at 200 mA and immunoblotted with 1:1,000 dilution of antibodies [p44/42 MAPK and p-p44/42 MAPK (Thr^{202}/Tyr^{204}); both were from Cell Signaling] overnight at 4°C. Respective horseradish peroxidase – conjugated secondary antibodies (Jackson ImmunoResearch, West Grove, PA) were used and the cross-reactivity was visualized by SuperSignal West Dura (Pierce, Rockford, IL) chemiluminescence on Blue Lite Auto Rad films (ISCBioExpress, Kaysville, UT).

Statistical analysis. The primary clinical end points were overall survival time and radiation response. Survival was determined from the date of diagnosis to the date of death or last follow-up. Cases in which patients were alive at last follow-up were censored. The method of imaging-assessed radiation response scoring and its relationship to YKL-40, age, and survival was described previously (19). Response data in relation to survival and age were reported previously in the validation patient cohort (30, 31). Spearman correlation was done to identify molecular and clinical factors that were associated with radiation response. Kaplan-Meier and Cox regression analyses were used to identify relationships between markers and survival. Binary logistic regression was used to determine independent factors related to radiation response. All significant findings relating to survival and radiation response were then tested in the validation group.

Results

Clinical/molecular characteristics and survival. Patient characteristics and their relationships to survival (determined by Kaplan-Meier analysis) are summarized in Table 1. The median age (59 years; range 13-84 years) and median survival (50 weeks) of the 268 cases in the initial group were typical of glioblastoma cohorts (32, 33). Thirty patients (11%) were still alive at last follow-up. The clinical factors significantly associated with shorter overall survival were age, Karnofsky

Table 3. Frequency and associations with survival time in the initial cohort of patients (n = 268)

Marker	Status	No. cases (%)	Median OS* (wk)	Actuarial 2-y OS (%)	*P*†
p-MAPK	Negative	52 (19)	73	31	0.003
	Positive	216 (81)	47	13	
p-mTOR	Negative	66 (25)	66	25	0.021
	Positive	202 (75)	49	13	
p-Akt	Negative	19 (7)	56	28	0.095
	Positive	249 (93)	49	15	
p-p70S6K	Negative	17 (6)	85	41	0.013
	Positive	251 (94)	49	15	
YKL-40	Negative	52 (19)	69	34	0.002
	Positive	216 (81)	47	12	

*Overall survival.
† Kaplan-Meier, log-rank.

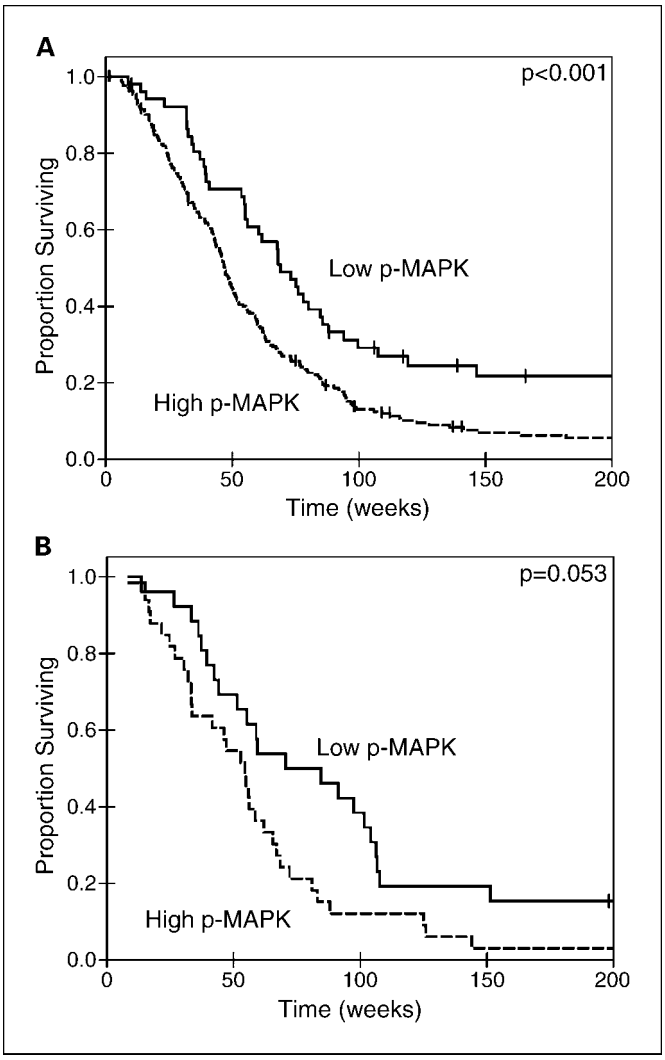

Fig. 2. Kaplan-Meier log-rank survival analysis for patients dichotomized by p-MAPK expression levels. *A,* the initial group (M.D. Anderson patients, $n = 268$). Low levels of p-MAPK confer a survival advantage when compared with patients whose tumors had high expression levels (69 versus 47 weeks, respectively, $P < 0.001$). *B,* the validation group (University of California-San Francisco, $n = 60$). There is a statistical trend toward a survival advantage for those patients with low tumor p-MAPK expression (70 versus 54 weeks, $P = 0.053$).

performance status (KPS) and imaging-assessed response/progression after radiotherapy, as in previous reports (19). All cases were stained/scored for p-Akt, p-mTOR, p-p70S6K, and p-MAPK. YKL-40 staining data was previously done and described (13). PTEN staining was limited to 141 tumor specimens because an interim analysis indicated no relationship with this marker to radiation response, survival, nor an inverse relationship to p-Akt pathway activation. Therefore, PTEN staining was discontinued and excluded from the main analyses. Review of Table 2 shows that the phosphorylated intermediates of the MAP and Akt pathways were significantly associated with each other. The strongest correlations were among the Akt pathway members [p-Akt, p-p70S6K, and p-mTOR (all $P < 0.001$)]. Consistent with prior *in vitro* data (13), YKL-40 positivity was significantly associated with positive staining for p-MAPK, p-mTOR, and p-p70S6K (all $P < 0.02$) and showed a statistical trend with p-Akt staining ($P = 0.095$).

To determine whether marker expression correlated with survival time, univariate (Kaplan-Meier) survival analysis was done and the results are summarized (Table 3). Those factors associated with shorter overall survival were positive staining for p-mTOR, p-p70S6K, p-MAPK, and YKL-40 (all $P < 0.03$). A Kaplan-Meier curve indicating the association of p-MAPK expression with outcome is shown in Fig. 2A. The results of the multivariate survival analysis (which included all significant molecular and clinical markers) are shown in Table 4. Age, KPS, and p-MAPK expression were the only independent prognostic factors in a multivariate Cox analysis. The association between p-MAPK expression and RTOG recursive partitioning analysis class in determining survival time is shown in Fig. 3, which shows a large survival advantage for patients in the most favorable clinical class (class III).

Examples of p-MAPK staining are shown in Fig. 4A and B. Protein from frozen tissues from these cases was extracted, and Western analysis showed the presence of total MAPK in both, but high levels of p-MAPK were observed only in the sample that showed high expression by immunohistochemistry (Fig. 4C). To confirm that p-MAPK expression, as determined by immunohistochemistry, was associated with outcome when interpreted by an independent observer, these 268 cases were reviewed by a second neuropathologist (G.N.F.). Interpretations were concordant in 247 of 268 (92%) of cases. Of the 21 discrepancies, the second neuropathologists gave a higher score in 13 cases and a lower score in eight cases. Further, univariate analysis using p-MAPK scoring data from the second neuropathologist showed a significant correlation with survival, which remained significant in multivariate analysis after adjustment for age, KPS, and extent of resection ($P < 0.01$ for both univariate and multivariate analyses).

Radiation response. Because higher expression of these phosphorylated intermediates was associated with shorter overall survival, we next tested them for associations with radiation response. We previously showed, within this cohort, a significant association between imaging-assessed changes in tumor size after radiotherapy and overall survival (19). Using these data, we found that only p-MAPK was significantly associated with response to radiation therapy (correlation coefficient -0.28, $P < 0.001$, Spearman), whereas the other markers showed statistical trends (correlation coefficients ≈ -0.135, all $P < 0.073$). Binary logistic regression analysis, which included the four molecular markers, YKL-40, and age,

Table 4. Multivariate Cox model analysis of all cases ($n = 328$)

Variable	Initial cohort ($n = 268$)		Validation cohort ($n = 60$)	
	HR* (95% CI†)	*P*	HR (95% CI)	*P*
Age	2.1 (1.5-2.8)	<0.001	2.4 (1.2-4.6)	0.012
KPS	0.7 (0.5-0.9)	0.009	0.4 (0.2-0.8)	0.035
p-MAPK	1.5 (1.1-2.2)	0.009	2.1 (1.2-3.8)	0.012

*Hazard ratio.
† Confidence interval.

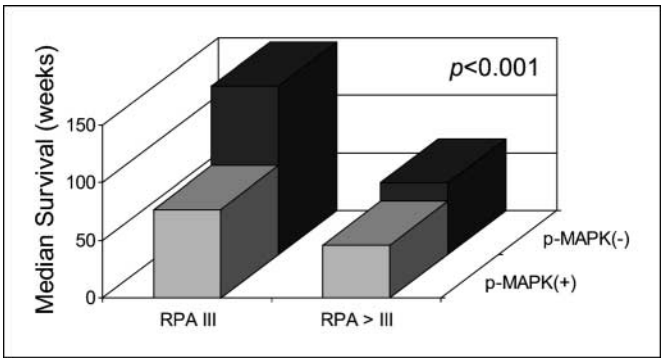

Fig. 3. The effect of p-MAPK expression on survival in patients (from M.D. Anderson, $n = 268$) dichotomized by RTOG recursive partitioning analysis classes. The survival differences were more pronounced among patients in the most favorable RTOG recursive partitioning analysis (*RPA*) class, class III (median overall survival in patients with low p-MAPK was 146 versus 77 weeks in patients with high p-MAPK), but were still significant in patients with >class III (62 weeks for low p-MAPK versus 46 weeks for high p-MAPK, $P < 0.001$).

revealed that only p-MAPK was an independent factor of radiation resistance (hazard ratio, 4.4; 95% confidence interval, 1.7-11.5; $P = 0.002$). Seventy-two percent (18 of 25) of the p-MAPK-negative patients had no tumor progression after radiotherapy. In contrast, only 37% (39 of 106) of patients had no tumor progression after radiotherapy if their tumor had elevated p-MAPK (Fig. 5A).

Validation cohort. The 60 patients in the validation cohort were somewhat younger than those in the initial cohort (49- versus 59-year old, $P < 0.001$). However, the survival times were not significantly different between these two groups (50 versus 56 weeks, $P = 0.341$). As in the initial cohort, the clinical factors associated with survival on univariate analysis were age and KPS. Although these cases were chosen primarily to validate the radiation response findings, univariate analysis revealed a similar trend in survival time ($P = 0.053$) associated with p-MAPK expression (Fig. 2B). Furthermore, the significant results from Cox modeling in the initial cohort were reproduced within the validation cohort (Table 4), thus corroborating the effect of p-MAPK upon survival.

Only p-MAPK expression correlated with radiotherapy response (correlation coefficient: 0.642; $P < 0.001$) in the validation group. All 26 patients (100%) with a negative p-MAPK score had responsive or stable disease, as determined radiographically; in contrast, only 38% (13 of 34) of those patients with a positive p-MAPK score had a radiographically evident response or stabilization after radiotherapy (Fig. 5B). These findings validate the initial observation that p-MAPK expression has a detrimental effect on the response of glioblastoma to radiotherapy.

Discussion

In this study, we showed that activated intermediates of the Akt pathway and MAPK are associated with decreased overall survival in glioblastoma. Furthermore, this is the first report to show that high expression of p-MAPK is associated with increased tumor resistance to radiotherapy in patients with glioblastoma. This association was found to be significant by univariate and multivariate analysis of both a retrospectively selected group of patients treated at a single institution and patients prospectively enrolled in clinical trial at an outside institution. We used immunohistochemistry with phospho-specific antibodies in paraffin-embedded archival specimens. It is acknowledged that artifacts based on differences in formalin fixation may affect expression of phosphospecific markers. The highest staining area in each case was used to minimize this artifact. In addition, we used a large sample size to increase statistical power, as would be needed where random misclassification (due to technical artifact) might occur. The use of an independent validation set that showed similar results also provides additional confidence that immunohistochemistry for phosphospecific markers in archival sections can yield clinically important results. Finally, interobserver differences in interpretation may exist. To address this, a second neuropathologist (G.N.F.) reviewed 268 cases for p-MAPK. Concordance was high (92%) and p-MAPK remained highly correlated with outcome using scores from the second neuropathologist.

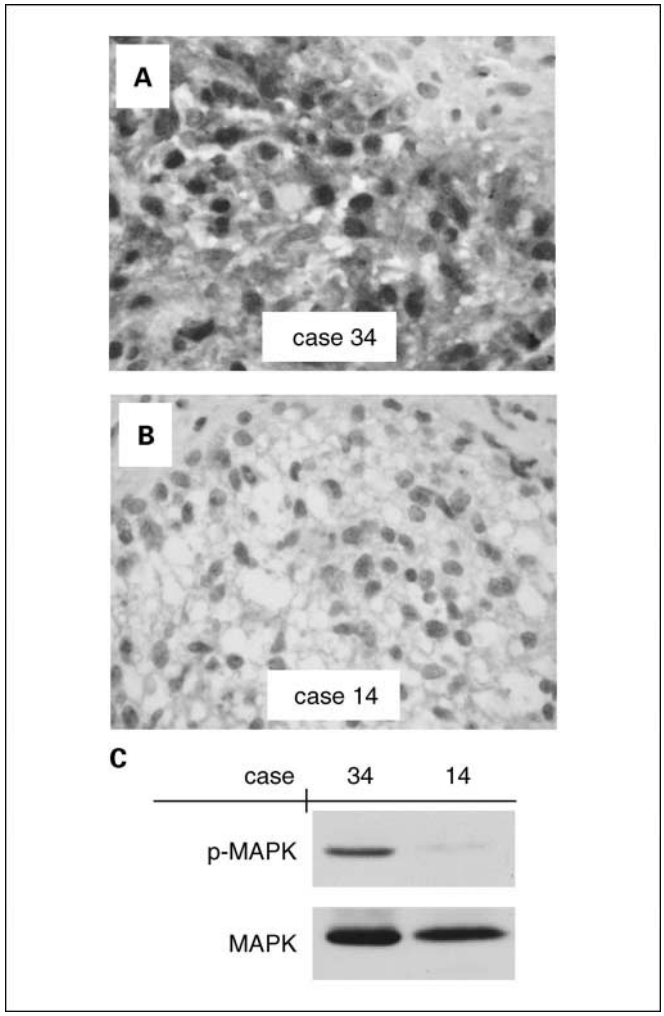

Fig. 4. Immunohistochemical evaluation and immunoblotting for p-MAPK. Representative examples of positive (*A*) and negative (*B*) staining for p-MAPK in paraffin sections of glioblastoma. Case 34 shows strong nuclear/cytoplasmic staining in tumor cells, whereas case 14 shows only weak cytoplasmic staining. *C,* immunoblot with total MAPK and p-MAPK expression of the cases shown in (*A* and *B*), indicting increased p-MAPK detection in the case that was positive by immunohistochemistry.

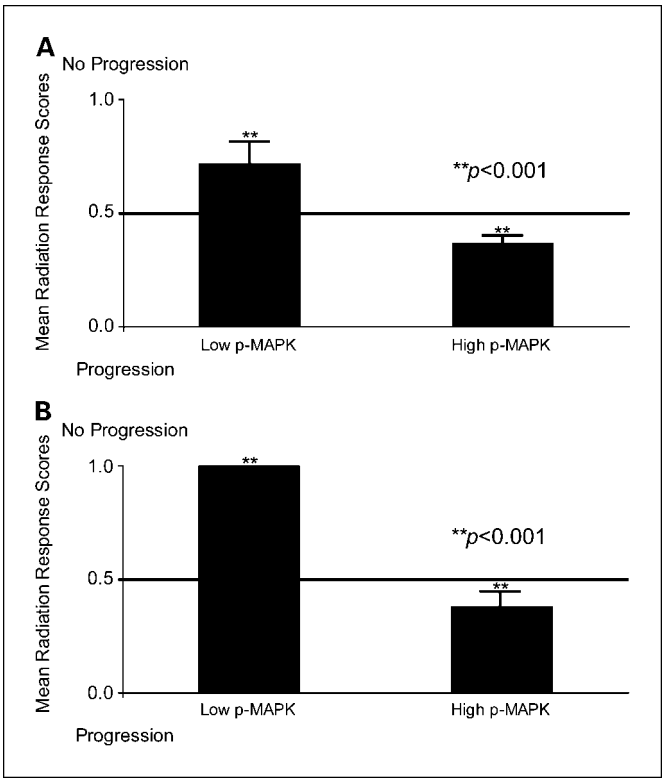

Fig. 5. Mean radiation response scores for patients dichotomized by p-MAPK staining score. Response/stabilization (no tumor progression) as determined by postradiotherapy magnetic resonance imaging was assigned a value of +1, whereas progression was assigned a value of 0. *A,* results from the initial group (M.D. Anderson patients, $n = 268$). *B,* results from validation group (University of California-San Francisco, $n = 60$).

We found that p-p70S6K, but not p-Akt, correlated with decreased overall survival time on univariate, but not multivariate, analysis. A previous report (20) found both markers to be significantly related to survival on univariate analysis but only p-p70S6K to be significant on multivariate analysis. This difference may be explained statistically (the percentages of p-p70S6K- and p-Akt-negative cases in our study, 6% and 7%, respectively, were lower those in the prior study at 44% and 34%, respectively) or because we included, within our study, additional markers that may have more dominant molecular effects within tumor cells (namely YKL-40 and p-MAPK). We found that the absence of p-mTOR was associated with longer survival time on univariate analysis only, similar to a previous analysis of 45 glioblastoma patients (21).

These clinical results support the *in vitro* observations that the MAPK pathway is involved with increased radiation resistance. One study (8) indicated that adding PD98059 (prevents MAPK phosphorylation) to monocytic leukemia cells prolonged the G_2-M phase (the most radiosensitive time of the cell cycle) and increased radiation-induced cell killing. It was also shown in leukemia cells that MAPK inhibits radiation-induced apoptosis by preventing caspase-8/Bid cleavage and the loss of mitochondrial membrane potential, which are important early apoptotic events (34). Within the U87 glioma cell line, MAPK was found to contribute to radiation induction of the early growth response gene (Egr-1) promoter (35). This gene is up-regulated, in a cytoprotective fashion, after radiation exposure and is involved in growth and differentiation. Last, another study (36) showed that inhibition of MAPK, epidermal growth factor receptor, and PI3K (with PD98059, AG1478, and LY294002, respectively) enhanced the radiosensitivity of esophageal cancer cells, demonstrating that this phenomenon occurs in a variety of tumors.

We stained tumor specimens from approximately half of the cases for PTEN ($n = 141$), but could not identify a significant relationship or statistical trend between PTEN expression and radiation therapy response or survival time. Furthermore, we found no correlation between PTEN expression and p-Akt. Some reports have indicated a negative relationship between the immunohistochemical expression of these two markers (21), which would reflect the regulatory function that PTEN has on Akt activation. However, the lack of correlation in this study may be explained by the fact that the functional status of PTEN is not assessed by means of immunohistochemical staining. PTEN is mutated, with subsequent loss of function, in ~30% of all glioblastoma (37). Akt pathway activation may occur by means independent of PTEN regulation, reducing the likelihood of finding a correlation. Surprisingly, among the cases in which both PTEN and phosphorylated components of the Akt pathway were assessed, the expression of PTEN was positively correlated with the expressions of p-mTOR and p-p70S6K (Table 2), as indicated by the significant positive correlation coefficients. This result is consistent with prior findings of an apparent paradoxical relationship between higher PTEN expression and activation of the Akt pathway (38, 39). The conflicting data in the literature indicate that the relationships between PTEN expression and activation of the Akt pathway are likely to be tumor specific and best assessed by methods other than immunohistochemical analysis.

Nonetheless, given the strong associations of activated Akt pathway and MAPK intermediates to shorter survival times and poor radiation response, these downstream Ras-associated pathways seem to play a critical role in the outcome of glioblastoma. These factors should be considered in the risk stratification of patients in future clinical trials and may serve as targets for successful therapy. Support for adopting these strategies resides in two recent clinical trials. In one study (40), 41 glioma patients were treated with erlotinib, which targets epidermal growth factor receptor. None of the 22 tumors (0%) with high levels of p-Akt responded to erlotinib treatment. However, 8 of 18 tumors (44%) with low levels of p-Akt did respond to erlotinib treatment ($P < 0.001$). The higher levels of activated Akt were associated with decreased time to tumor progression as well ($P < 0.001$). In the second study (41), in which 43 patients with recurrent glioblastoma received temsirolimus (an mTOR inhibitor), treatment response was better for those whose tumors had high levels of p-p70S6K, suggesting that the efficacy of temsirolimus is related to activation of p70S6K by mTOR. Our present study showed that the glioblastoma patient group in which p-MAPK had the largest effect was those in the RTOG recursive partitioning analysis class III. These patients represent the most favorable clinical group (with younger age and good performance status) and may benefit from targeted agents designed to inhibit signaling along the MAPK pathway.

Although we previously showed that YKL-40 correlated with shorter overall survival and decreased radiation sensitivity in this cohort of patients, this more recent analysis reveals that p-MAPK may be an equivalent or better molecular marker in predicting clinical outcome. We also found positive associations between YKL-40 and activated Akt pathway and MAPK intermediates, supporting the *in vitro* finding that YKL-40 stimulates these pathways (13). It is tempting to speculate that YKL-40, a secreted protein, may serve as an extracellular signal, inducing the increased downstream activity of Ras. This supposition, however, requires further investigation.

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References

1. Aeder SE, Martin PM, Soh JW, Hussaini IM. PKC-η mediates glioblastoma cell proliferation through the Akt and mTOR signaling pathways. Oncogene 2004; 23:9062–9.
2. Bouterfa HL, Sattelmeyer V, Czub S, et al. Inhibition of Ras farnesylation by lovastatin leads to downregulation of proliferation and migration in primary cultured human glioblastoma cells. Anticancer Res 2000;20: 2761–71.
3. Van Brocklyn J, Letterle C, Snyder P, Prior T. Sphingosine-1-phosphate stimulates human glioma cell proliferation through Gi-coupled receptors: role of ERK MAP kinase and phosphatidylinositol 3-kinase β. Cancer Lett 2002;181:195–204.
4. Suzuki K, Kodama S, Watanabe M. Extremely low-dose ionizing radiation causes activation of mitogen-activated protein kinase pathway and enhances proliferation of normal human diploid cells. Cancer Res 2001;61:5396–401.
5. Lakka SS, Jasti SL, Gondi C, et al. Downregulation of MMP-9 in ERK-mutated stable transfectants inhibits glioma invasion *in vitro*. Oncogene 2002;21:5601–8.
6. Park MJ, Park IC, Hur JH, et al. Modulation of phorbol ester-induced regulation of matrix metalloproteinases and tissue inhibitors of metalloproteinases by SB203580, a specific inhibitor of p38 mitogen-activated protein kinase. J Neurosurg 2002;97:112–8.
7. Grille SJ, Bellacosa A, Upson J, et al. The protein kinase Akt induces epithelial mesenchymal transition and promotes enhanced motility and invasiveness of squamous cell carcinoma lines. Cancer Res 2003;63: 2172–8.
8. Cartee L, Vrana JA, Wang Z, et al. Inhibition of the mitogen activated protein kinase pathway potentiates radiation-induced cell killing via cell cycle arrest at the G_2-M transition and independently of increased signaling by the JNK/c-Jun pathway. Int J Oncol 2000;16:413–22.
9. Gupta AK, Bakanauskas VJ, Cerniglia GJ, et al. The Ras radiation resistance pathway. Cancer Res 2001; 61:4278–82.
10. Eshleman JS, Carlson BL, Mladek AC, et al. Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation therapy. Cancer Res 2002;62:7291–7.
11. Russell JS, Lang FF, Huet T, et al. Radiosensitization of human tumor cell lines induced by the adenovirus-mediated expression of an anti-Ras single-chain antibody fragment. Cancer Res 1999;59:5239–44.
12. Guha A, Feldkamp MM, Lau N, Boss G, Pawson A. Proliferation of human malignant astrocytomas is dependent on Ras activation. Oncogene 1997;15: 2755–65.
13. Recklies AD, White C, Ling H. The chitinase 3-like protein human cartilage glycoprotein 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. Biochem J 2002;365:119–26.
14. Tanwar MK, Gilbert MR, Holland EC. Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma. Cancer Res 2002;62:4364–8.
15. Shostak K, Labunskyy V, Dmitrenko V, et al. HC gp-39 gene is upregulated in glioblastomas. Cancer Lett 2003;198:203–10.
16. Nigro JM, Misra A, Zhang L, et al. Integrated array-comparative genomic hybridization and expression array profiles identify clinically relevant molecular subtypes of glioblastoma. Cancer Res 2005;65: 1678–86.
17. Markert JM, Fuller CM, Gillespie GY, et al. Differential gene expression profiling in human brain tumors. Physiol Genomics 2001;5:21–33.
18. Lal A, Lash AE, Altschul SF, et al. A public database for gene expression in human cancers. Cancer Res 1999;59:5403–7.
19. Pelloski CE, Mahajan A, Maor M, et al. YKL-40 Expression is associated with poorer response to radiation and shorter overall survival in glioblastoma. Clin Cancer Res 2005;11:3326–34.
20. Chakravarti A, Zhai G, Suzuki Y, et al. The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. J Clin Oncol 2004;22: 1926–33.
21. Choe G, Horvath S, Cloughesy TF, et al. Analysis of the phosphatidylinositol 3′-kinase signaling pathway in glioblastoma patients *in vivo*. Cancer Res 2003;63: 2742–6.
22. Mawrin C, Diete S, Treuheit T, et al. Prognostic relevance of MAPK expression in glioblastoma multiforme. Int J Oncol 2003;23:641–8.
23. Shaw EG, Seiferheld W, Scott C, et al. Reexamining the radiation therapy oncology group (RTOG) recursive partitioning analysis (RPA) for glioblastoma multiforme (GBM) patients. Int J Radiat Oncol Biol Phys 2003;57:S135–6.
24. Scott CB, Scarantino C, Urtasun R, et al. Validation and predictive power of Radiation Therapy Oncology Group (RTOG) recursive partitioning analysis classes for malignant glioma patients: a report using RTOG 90–06. Int J Radiat Oncol Biol Phys 1998;40:51–5.
25. Curran WJ, Jr., Scott CB, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst 1993;85:704–10.
26. Prados MD, Wara WM, Sneed PK, et al. Phase III trial of accelerated hyperfractionation with or without difluromethylornithine (DFMO) versus standard fractionated radiotherapy with or without DFMO for newly diagnosed patients with glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2001;49:71–7.
27. Prados MD, Larson DA, Lamborn K, et al. Radiation therapy and hydroxyurea followed by the combination of 6-thioguanine and BCNU for the treatment of primary malignant brain tumors. Int J Radiat Oncol Biol Phys 1998;40:57–63.
28. Simmons ML, Lamborn KR, Takahashi M, et al. Analysis of complex relationships between age, p53, epidermal growth factor receptor, and survival in glioblastoma patients. Cancer Res 2001;61: 1122–8.
29. Rajasekhar VK, Viale A, Socci ND, et al. Oncogenic Ras and Akt signaling contribute to glioblastoma formation by differential recruitment of existing mRNAs to polysomes. Mol Cell 2003;12:889–901.
30. Barker FG II, Prados MD, Chang SM, et al. Radiation response and survival time in patients with glioblastoma multiforme. J Neurosurg 1996;84:442–8.
31. Barker FG II, Simmons ML, Chang SM, et al. EGFR overexpression and radiation response in glioblastoma multiforme. Int J Radiat Oncol Biol Phys 2001;51: 410–8.
32. Scott JN, Rewcastle NB, Brasher PM, et al. Long-term glioblastoma multiforme survivors: a population-based study. Can J Neurol Sci 1998;25:197–201.
33. Sant M, van der Sanden G, Capocaccia R. Survival rates for primary malignant brain tumours in Europe. EUROCARE Working Group. Eur J Cancer 1998;34: 2241–7.
34. Shonai T, Adachi M, Sakata K, et al. MEK/ERK pathway protects ionizing radiation-induced loss of mitochondrial membrane potential and cell death in lymphocytic leukemia cells. Cell Death Differ 2002;9: 963–71.
35. Meyer RG, Kupper JH, Kandolf R, Rodemann HP. Early growth response-1 gene (Egr-1) promoter induction by ionizing radiation in U87 malignant glioma cells *in vitro*. Eur J Biochem 2002;269:337–46.
36. Akimoto T, Nonaka T, Harashima K, et al. Selective inhibition of survival signal transduction pathways enhanced radiosensitivity in human esophageal cancer cell lines *in vitro*. Anticancer Res 2004;24: 811–9.
37. Kleihues PBP, Collins VP, Newcomb EW, Ohgaki H, Cavenee WK. Glioblastoma. In: Kleihues CWP, editor. Pathology and genetics of tumours of the nervous system, 1st ed, vol. 1. Lyon (France): IARC Press; 2000. pp. 29–39.
38. Panigrahi AR, Pinder SE, Chan SY, et al. The role of PTEN and its signalling pathways, including AKT, in breast cancer; an assessment of relationships with other prognostic factors and with outcome. J Pathol 2004;204:93–100.
39. Slipicevic A, Holm R, Nguyen MT, et al. Expression of activated Akt and PTEN in malignant melanomas: relationship with clinical outcome. Am J Clin Pathol 2005;124:1–9.
40. Haas-Kogan DA, Prados MD, Tihan T, et al. Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib. J Natl Cancer Inst 2005; 97:880–7.
41. Galanis E, Buckner JC, Maurer MJ, et al. Phase II trial of temsirolimus (CCI-779) in recurrent glioblastoma multiforme: a North Central Cancer Treatment Group Study. J Clin Oncol 2005;23:5294–304.