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

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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.
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-Ser473 Akt (1:300 dilution), p-Ser2448 mTOR (1:100), p-Thr389 p70S6K (1:1000), and p-Thr202/Tyr204 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.

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![Table 1](https://www.aacrjournals.org/journals/clin-cancer-research/article-pdf/12/13/3936/3559893/clin-cancer-research-3936.pdf)

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

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

*Overall survival.
† Kaplan-Meier, log-rank.
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 (Thr202/Tyr204); 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).

**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 post. 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.

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

<table>
<thead>
<tr>
<th></th>
<th>YKL-40</th>
<th>p-Akt</th>
<th>p-mTOR</th>
<th>p-70S6K</th>
<th>p-MAPK</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-MAPK</td>
<td>Corr. coeff. *</td>
<td>0.14</td>
<td>0.12</td>
<td>0.11</td>
<td>0.34</td>
</tr>
<tr>
<td>p-p70S6K</td>
<td>Corr. coeff.</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-mTOR</td>
<td>Corr. coeff.</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>p-Akt</td>
<td>Corr. coeff.</td>
<td>0.006</td>
<td>0.006</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PTEN†</td>
<td>Corr. coeff.</td>
<td>0.12</td>
<td>0.06</td>
<td>0.23</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.175</td>
<td>0.454</td>
<td>0.006</td>
<td>0.041</td>
</tr>
</tbody>
</table>

*Correlation coefficient.
†Based on an interim analysis.

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

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

*Overall survival.
†Kaplan-Meier, log-rank.
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>
<thead>
<tr>
<th>Variable</th>
<th>Initial cohort (( n = 268 ))</th>
<th>Validation cohort (( n = 60 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR* (95% CI)</td>
<td>( P )</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>2.1 (1.5-2.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>KPS</td>
<td>0.7 (0.5-0.9)</td>
<td>0.009</td>
</tr>
<tr>
<td>p-MAPK</td>
<td>1.5 (1.1-2.2)</td>
<td>0.009</td>
</tr>
</tbody>
</table>

\*Hazard ratio.  
\( {\text{CI}} \) Confidence interval.
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.
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 G2-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.

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).
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 associations between YKL-40 and activated Akt 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.

Acknowledgments

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References

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