Activation of STAT3, MAPK, and AKT in Malignant Astrocytic Gliomas: Correlation With EGFR Status, Tumor Grade, and Survival

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Abstract
Diffuse astrocytic gliomas are the most common human glial tumors with glioblastoma being the most malignant form. Epidermal growth factor receptor (EGFR) gene amplification is one of the most common genetic changes in glioblastoma and can lead to the activation of various downstream signaling molecules, including STAT3, MAPK, and AKT. In this study, we investigated the activation status of these 3 signaling molecules as well as wild-type (EGFRwt) and mutant (EGFRvIII) EGFR in 82 malignant astrocytic gliomas (55 glioblastomas and 27 anaplastic astrocytomas) using immunohistochemistry. The presence of EGFRwt, but not EGFRvIII, immunopositivity correlated significantly with prevalent EGFR gene amplification in glioblastomas. STAT3 and AKT activation correlated significantly with EGFR status, although the correlation for p-STAT3 was attributed exclusively to EGFRvIII. The distribution of these 3 activated molecules varied significantly with tumor grade; although activation of STAT3 was essentially identical between anaplastic astrocytomas and glioblastomas, an increase in the activation of MAPK and AKT appeared to correlate with the progression of anaplastic astrocytoma to glioblastoma. Finally, activated STAT3 and AKT were marginally predictive of improved and worse prognosis, respectively. Taken together, these findings begin to elucidate the interrelationship between these signaling pathways in astrocytic gliomas in vivo.

Key Words: AKT, EGFR, Glioma, MAPK, Phosphorylation, STAT3.

INTRODUCTION
Diffuse astrocytic gliomas are the most common human glial tumors (1). Based on degree of malignancy, these lesions are graded according to the World Health Organization classification system into grade II (diffuse astrocytoma), grade III (anaplastic astrocytoma), and grade IV (glioblastoma); glioblastoma is the most aggressive malignant glioma with a median survival of approximately 1 year (1). The ability to invade surrounding normal brain tissue accounts for much of the high lethality associated with these diffuse lesions, because it essentially prevents surgical cure. Furthermore, the vast majority of these tumors are resistant to the current standard radiotherapy and chemotherapeutic approaches. In this regard, the recent emphasis on the development of targeted molecular therapies for glial tumors affords the possibility of more effective treatment options in the future (2, 3).

One of the most common molecular genetic alterations in glioblastomas is amplification of the epidermal growth factor receptor (EGFR) gene (4). Furthermore, approximately one third of glioblastomas with EGFR gene amplification display a mutant form of EGFR, the most common being the constitutively active mutant EGFRvIII (5). Aberrant activation of EGFR leads to deregulation of a number of downstream signaling cascades, including the STAT, MAPK, and AKT pathways (2–4, 6). Previous reports have implicated a role for increased activation of each of these molecules in glioblastoma (7–15). However, crosstalk between these pathways results in the formation of complicated signaling networks (14–22). For example, in vitro studies have suggested that AKT and MAPK signaling can inhibit the STAT pathway in melanoma-derived cell lines (21). Furthermore, activation of AKT has been shown to inhibit MAPK signaling, possibly through inhibition of RAF (16, 17, 19). To complicate matters further, crosstalk between these signaling pathways, and the resulting cellular response, may be affected by the type of ligand, concentration, and time course as well as the type and differentiation state of the cells involved (15, 19). Moreover, much remains unclear as to how these pathways are modulated in vivo.

In situ studies examining crosstalk between signaling pathways in gliomas have been limited to date. Better understanding of these complicated signaling networks will lay the foundation for improved development of targeted molecular therapeutic strategies for malignant gliomas. In this study, we used immunohistochemistry to investigate the
status of EGFR, STAT3, MAPK, and AKT in a series of 55 glioblastomas and 27 anaplastic astrocytomas. We examined the correlation among these molecules in situ within astrocytic gliomas and investigated whether the distribution of activated signaling molecules correlated with tumor grade and clinical outcome.

**MATERIALS AND METHODS**

**Tissue Samples**

Samples were collected from Massachusetts General Hospital (Boston, Massachusetts) under approval of the Massachusetts General Hospital Institutional Review Board. A total of 82 malignant gliomas were used in the study: 55 glioblastomas and 27 anaplastic astrocytomas. Of the 55 glioblastomas, 35 were analyzed using tissue microarrays with 3 to 4 representative 1.0-mm cores sampled from each case. Full size sections were used for the remaining 20 glioblastomas and the entire set of 27 anaplastic astrocytomas.

**EGFR Amplification Status**

EGFR amplification status was evaluated for 43 of the 55 glioblastomas by differential polymerase chain reaction (PCR) as described previously (23). For samples demonstrating a discrepancy between EGFR gene amplification status as determined by differential PCR and EGFR immunohistochemistry, dual-color fluorescent in situ hybridization (FISH) was performed as described previously (24). BAC clones CTD-2113A18 and RP11-340A14 were used for the EGFR and 7q control probes, respectively. For direct comparison with areas of EGFR immunopositivity, immunohistochemistry and FISH were performed on serial sections.

**Immunohistochemistry**

All immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue. Antigen retrieval was performed by microwaving 10 to 30 minutes in 10 mM sodium citrate buffer (pH 6.0). After antigen retrieval, the slides were incubated with 10% normal horse serum (Vector Laboratories, Burlingame, CA) for monoclonal antibodies or 10% normal goat serum (Vector Laboratories) for polyclonal antibodies followed by incubation with each primary antibody overnight at 4°C: EGFR.113 monoclonal antibody (1:30; Novocastra Laboratory Ltd., Newcastle upon Tyne, U.K.), anti-EGFRvIII polyclonal antibody (1:25; Zymed Laboratories Inc., San Francisco, CA), phospho-STAT3 (Tyr705) (3E2) monoclonal antibody (1:20; Cell Signaling Technology, Inc., Beverly, MA), phospho-p44/42 MAPK (Thr202/Tyr204) (E10) monoclonal antibody (1:100; Cell Signaling Technology, Inc.), and phospho-AKT (Ser473) polyclonal antibody (1:100; Cell Signaling Technology, Inc.). This was followed by incubation with biotinylated secondary antibodies (Vector Laboratories) for 30 minutes and staining was completed with an avidin–biotin complex (Vectastain Elite ABC kit; Vector Laboratories) in the presence of 3,3′-diaminobenzidine tetrahydrochloride.

As a positive control for wild-type EGFR (EGFRwt), a primary human glioblastoma with documented amplification of the EGFR gene was used; tumor cells demonstrated strong membrane staining. For EGFRvIII, tissue from an EGFRvIII transfected xenograft was used as a positive control and EGFRwt transfected cell lines were used as negative controls. Using our optimized protocol, strong staining was seen in the EGFRvIII transfected xenograft, whereas only scattered EGFRwt transfected cells were immunopositive. Normal brain was negative for both EGFRwt and EGFRvIII. Tissues incubated without primary antibody were also used as negative controls.

As controls for the phospho-specific antibodies, formalin-fixed, paraffin-embedded cell pellets from 2 different prostate cell lines were used: PC3 is a PTEN null cell line that demonstrates a high level of AKT activation (25) and DU145 is a PTEN intact cell line that shows weak AKT activation but strong activation of STAT3 (26, 27). Both cell lines exhibit MAPK activation by Western blot analysis (22). Protocols for all 3 phospho-specific antibodies were optimized to detect the differential staining pattern displayed by these 2 cell lines: PC3 cells showed strong p-AKT positivity, whereas DU145 exhibited only a few scattered positive cells; DU145 demonstrated clear nuclear staining for p-STAT3; and both cell lines showed moderate nuclear and cytoplasmic staining for p-MAPK. Furthermore, normal endothelial cells, which demonstrate activated MAPK (28), were available as an internal positive control. Normal glial cells were negative for all 3 phospho-specific antibodies. Tissues incubated without primary antibody were also used as negative controls.

**Evaluation of Immunohistochemical Staining**

For evaluation of tumor samples, staining was scored semiquantitatively based on both cell number and staining intensity as follows: 0, no staining; 1, less than 20% of cells demonstrating mild to moderate staining intensity; 2, 20% to 50% of cells demonstrating mild to moderate staining intensity in less than 20% of cells; and 3, greater than 50% of cells demonstrating mild to moderate staining intensity or strong staining intensity in greater than 20% of cells. Nuclear and cytoplasmic staining was scored independently. For statistical purposes, only scores of 2 or 3 were considered immunohistochemically positive. Furthermore, only scores of 2 or 3 for nuclear staining were considered positive for phospho-STAT3 (p-STAT3) and phospho-MAPK (p-MAPK), whereas scores of 2 or 3 in either the cytoplasm or nucleus were considered positive for phospho-AKT (p-AKT).

**Statistical Analysis**

Associations among categorical variables were assessed using the Fisher exact test. Associations among activated signaling pathways and patient survival were assessed using Cox proportional hazards regression analyses with adjustment for tumor grade and patient age. Because each of the 3 activated pathways under consideration was of a priori biologic interest, no adjustments were made for multiple testing.
RESULTS

Prevalent EGFR Gene Amplification Correlates With Wild-Type But Not Mutant EGFR Protein Expression

Of 55 glioblastomas, 35 (63.6%) demonstrated positive staining (i.e., immunohistochemical scores of 2 or 3) for EGFR: 14 (25.5%) demonstrated only EGFRwt staining, 10 (18.2%) were positive only for EGFRvIII, and 11 cases (20%) exhibited positive staining for both EGFRwt and EGFRvIII. EGFR gene amplification status was available for 43 of these 55 glioblastomas. Of these 43 cases, 24 glioblastomas demonstrated prevalent EGFR gene amplification as detected by differential PCR. The distribution of EGFRwt immunohistochemical staining correlated with EGFR gene amplification status ($p < 0.0001$): 20 of 24 glioblastomas (83.3%) with EGFR gene amplification demonstrated an EGFRwt immunohistochemical score of either 2 or 3 and 16 of 19 glioblastomas (84.2%) without EGFR gene amplification exhibited an EGFRwt immunohistochemical score of either 0 or 1 (Fig. 1; Table 1). In contrast, the distribution of EGFRvIII immunohistochemical staining did not correlate with EGFR gene amplification status ($p = 1$): 15 of 24 glioblastomas (62.5%) demonstrated an EGFRvIII immunohistochemical score of either 0 or 1 despite having EGFR gene amplification and 8 of 19 (42.1%) exhibited an EGFRvIII immunohistochemical score of either 2 or 3 in the absence of prevalent EGFR amplification (Fig. 1; Table 1).

Differential PCR methods for the detection of EGFR amplification use whole tumor lysates and, as such, the techniques are less likely to detect tumors with isolated EGFR-amplified cells or tumors with low-level amplification or copy number gains. Because there were a number of tumors exhibiting EGFRvIII immunopositivity in the absence of prevalent EGFR amplification, we performed FISH and EGFR immunohistochemistry on serial sections of 5 of these cases. One of these cases did, in fact, demonstrate EGFR amplification in the focal area of EGFRvIII

| Table 1. Correlation of EGFR Protein Expression with Prevalent EGFR Gene Amplification Status in Glioblastomas |
|---------------------------------|-----------------|-----------------|-----------|
| **EGFR Immunohistochemistry**   | **EGFR Amplification** | **EGFR Amplification p Value** |
| Positive (n = 24)               | Negative (n = 19)  |
| Positive EGFRwt                 | 20               | 3                | < 0.0001  |
| Negative EGFRwt                 | 4                | 16               |           |
| Positive EGFRvIII               | 9                | 8                | 1.0       |
| Negative EGFRvIII               | 15               | 11               |           |

**FIGURE 1.** Correlation of EGFR protein expression with prevalent EGFR gene amplification status in glioblastomas. The top 2 panels demonstrate positive immunohistochemical staining for (A) EGFRwt and (B) EGFRvIII in an EGFR-amplified glioblastoma. The bottom 2 panels demonstrate (C) negative immunohistochemical staining for EGFRwt and (D) positive staining for EGFRvIII in a glioblastoma that lacks amplification of the EGFR gene. The (A, B) top and (C, D) bottom panels demonstrate staining of the same region within each glioblastoma; serial sections of the same tissue microarray core were compared.
immunopositivity (Fig. 2). However, the remaining 4 cases demonstrated only isolated amplified cells or low-level amplification/copy number gains with many cells exhibiting a normal copy number of the \( \text{EGFR} \) gene; an example of one of these cases is shown (B). The corresponding immunohistochemistry images are inset. Images demonstrate the same region for each tumor; serial sections of the same tissue block were compared.

**Activation of STAT3 and AKT Correlates With EGFR Status in Glioblastomas**

Within the sample set of 55 glioblastomas, both STAT3 and AKT activation correlated significantly with positive EGFR immunohistochemical staining (\( p = 0.026 \) and 0.001, respectively; Table 2A). Interestingly, when the data were investigated in finer detail to determine associations with either EGFRwt or EGFRvIII specifically, STAT3 activation correlated significantly only with EGFRvIII immunopositivity (\( p = 0.027 \); Table 2C). Although AKT activation also appeared to correlate more strongly with EGFRvIII-positive staining (\( p = 0.002 \); Table 2C), significant correlation was also found in tumors expressing EGFRwt (\( p = 0.046 \); Table 2B). MAPK activation did not correlate significantly with EGFR regardless of mutational status (Table 2).

**Glioblastomas Display Increased Activation of Signaling Molecules as Compared With Anaplastic Astrocytomas**

Prevalence of the 3 activated molecules was significantly different between anaplastic astrocytomas and glioblastomas (\( p < 0.0001 \); Fig. 3). Although activation of STAT3 was essentially identical between anaplastic astrocytomas and glioblastomas (55.6% vs. 56.4%, \( p = 1 \); Table 3; Fig. 4), the difference in AKT activation was highly significant: 43 of 55 glioblastomas (78.2%) showed positive nuclear and/or cytoplasmic staining of p-AKT, whereas only 5 of 27 anaplastic astrocytomas (18.5%) showed p-AKT positivity (\( p < 0.0001 \); Table 3; Fig. 4). The frequency of MAPK activation also appeared higher in glioblastomas (34.5%) as compared with anaplastic astrocytomas (18.5%), but this distinction was not significantly different (\( p = 0.197 \); Table 3; Fig. 4). STAT3 activation was much more frequent in anaplastic astrocytomas than either AKT or MAPK: 55.6% of anaplastic astrocytomas stained positive for p-STAT3, whereas only 18.5% of tumors were immunopositive for each of p-AKT and p-MAPK (Table 3; Fig. 4).

**Correlation Among Activated Signaling Molecules in Malignant Glioma**

Overall correlation of the 3 signaling pathways is summarized for both anaplastic astrocytomas and glioblastomas in Figure 4. When AKT activation was detected in anaplastic astrocytomas, it was significantly correlated with STAT3 activation (\( p = 0.047 \)). Within glioblastomas, the correlation between STAT3 and AKT activation was even more striking, with 30 of 31 (97%) glioblastomas immunopositive for p-STAT3 also staining for activated AKT (\( p = 0.0001 \)). Neither activation of AKT nor STAT3 correlated significantly with MAPK activation in anaplastic astrocytomas (\( p = 0.221 \) and \( p = 0.342 \), respectively) and glioblastomas (\( p = 0.511 \) and \( p = 1 \), respectively).

**Prognostic Significance of Activated Signaling Molecules in Patients With Malignant Astrocytic Gliomas**

It is well established that patients with anaplastic astrocytomas experience longer survival times than those patients with glioblastoma (1). Because there appeared to be a strong trend from predominantly STAT3 activation in anaplastic astrocytomas to AKT activation in glioblastomas,
we investigated whether activation of either of these 2 molecules alone correlated with survival in patients with astrocytic gliomas. After adjusting for tumor grade and patient age, activation of STAT3 alone appeared marginally predictive of improved survival (hazard ratio = 0.63, \( p = 0.083 \)). Furthermore, tumors with activation of AKT in
the absence of p-STAT3 and p-MAPK demonstrated a trend toward a worse prognosis (hazard ratio = 2.125, p = 0.094). EGFR status did not correlate with survival in this study.

**DISCUSSION**

One of the most common molecular genetic alterations in glioblastomas is amplification of the \( EGFR \) gene, which can, in turn, affect signaling through the STAT3, MAPK, and AKT pathways. Although the activation of each of these signaling molecules has been demonstrated in glial tumors (7–15), in situ studies examining crosstalk between signaling pathways in gliomas have been limited to date (11, 13, 14, 29, 30). Because better understanding of the complicated signaling networks at play in malignant gliomas is essential for the development of effective targeted treatment strategies, we examined in situ the correlation among \( EGFR \) and these 3 key signaling molecules within astrocytic gliomas and investigated whether the distribution of these molecules correlated with tumor grade and clinical outcome.

**TABLE 3. Correlation of Activated Signaling Pathways in Anaplastic Astrocytomas and Glioblastomas**

<table>
<thead>
<tr>
<th>Activated Pathway</th>
<th>Glioblastoma (n = 55)</th>
<th>Anaplastic Astrocytoma (n = 27)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>STAT3</td>
<td>31 (56.4%)</td>
<td>15 (55.6%)</td>
<td>1</td>
</tr>
<tr>
<td>MAPK</td>
<td>19 (34.5%)</td>
<td>5 (18.5%)</td>
<td>0.197</td>
</tr>
<tr>
<td>AKT</td>
<td>43 (78.2%)</td>
<td>5 (18.5%)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

**FIGURE 4.** Association of activated STAT3, MAPK, and AKT in 27 anaplastic astrocytomas and 55 glioblastomas. The number of tumors with activation of the given molecules is shown with the percentage in brackets. In general, activation of signaling molecules increased with tumor grade: 41% of anaplastic astrocytomas were negative for all 3 activated molecules, whereas only 15% of glioblastomas were completely negative (depicted in the small ovals under each Venn diagram). Furthermore, with tumor progression, there appeared to be a trend from activation of STAT3 alone in lower-grade tumors to additional activation of MAPK and AKT in glioblastomas.

We began our study by examining the correlation between \( EGFR \) gene amplification and EGFR protein expression in glioblastomas. EGFR protein expression was found in all tumors with \( EGFR \) amplification. Although 4 tumors with \( EGFR \) gene amplification were scored as negative for \( EGFR/WT \) immunopositivity (Table 1), these tumors did demonstrate some degree of staining for \( EGFR/WT \) and/or \( EGFR/vIII \); tumors with less than 20% of cells with mild to moderate staining intensity were scored negative in this study. Not surprisingly, the data demonstrated a strong correlation between amplification of the \( EGFR \) gene and increased expression of wild-type \( EGFR \) protein.

However, \( EGFR/vIII \) expression did not correlate significantly with prevalent \( EGFR \) amplification in this study. Although 9 of 24 (37.5%) glioblastomas with \( EGFR \) amplification stained positive for \( EGFR/vIII \), 8 of 19 (42.1%) glioblastomas stained positively for \( EGFR/vIII \) even in the absence of \( EGFR \) gene amplification. One possible reason for this lack of correlation might be the detection of focal \( EGFR/vIII \)-positive cells by immunohistochemistry; focal \( EGFR/vIII \) staining was considered positive in this study as long as the staining intensity was strong. In contrast, a tumor lysate technique was used for the detection of \( EGFR \) amplification; focal cells with \( EGFR \) amplification might have been below the level of detection for this assay. We have, in fact, demonstrated previously that \( EGFR \) amplification can occur focally (24). Furthermore, Feldkamp et al have demonstrated an effect of assay choice in the detection of \( EGFR/vIII \): 67% of glioblastomas were reported to be positive for \( EGFR/vIII \) when measured by immunohistochemistry, but only 58% and 42% were positive when Western blot and reverse transcriptase-PCR analyses (both are tumor lysate-based approaches) were used respectively (31). As such, to investigate these apparently discrepant cases in more detail, we performed immunohistochemistry and FISH on serial sections for 5 of these cases. Although one of these cases did demonstrate fairly ubiquitous \( EGFR \) amplification in the focal area of \( EGFR/vIII \) immunopositivity, the remaining 4 cases demonstrated only isolated amplified cells or low-level amplification/copy number gains.

It did appear that expression of \( EGFR/vIII \) could be detected in focal regions of tumors in which many cells in the area demonstrated normal copy numbers of the \( EGFR \) gene. An intriguing alternative explanation as to why tumors might genuinely express \( EGFR/vIII \) in the absence of \( EGFR \) amplification is that cells expressing \( EGFR/vIII \) are not under the same degree of selective pressure to maintain high levels of the \( EGFR \) transcript as are tumors expressing only \( EGFR/WT \). Indeed, it has been suggested that \( EGFR/vIII \) confers enhanced tumorigenicity to glioma cells in vivo (32). Furthermore, \( EGFR/vIII \) has been reported to enhance glial tumor invasion (33), which might also provide an explanation for the focal expression seen in some tumors. Under these circumstances, the expression of the mutant form of \( EGFR \) could conceivably effect a tumor growth advantage even in the absence of amplification.

Next, we examined the correlation of activated signaling molecules with EGFR status in glioblastomas. Interestingly, although p-STAT3 correlated significantly with \( EGFR \)
status, this correlation could be attributed exclusively to EGFRvIII, supporting the theory that EGFRvIII might affect biologic signaling in a manner distinct from that of wild-type EGFR. Indeed, it has recently been suggested that STAT3 is a critical mediator of the oncogenic effects of EGFR mutations in nonsmall cell lung cancer cells (34). Similarly, although AKT activation did correlate significantly with EGFRwt, it also appeared to correlate more strongly with EGFRvIII staining. A number of recent studies have indicated a role for EGFRvIII in activation of the PI3K pathway (11, 35, 36) and have suggested the possibility of additional signaling complexity when EGFRvIII is expressed in the presence of PTEN mutations (11, 35).

The prevalence of each of the 3 activated molecules correlated significantly with tumor grade. Although activation of STAT3 was essentially identical between anaplastic astrocytomas and glioblastomas, p-AKT positivity was significantly higher in glioblastomas than in anaplastic astrocytomas. When AKT activation was detected in anaplastic astrocytomas, it was significantly correlated with STAT3 activation. However, the correlation between AKT and STAT3 activation was even more striking in glioblastomas; 97% glioblastomas immunopositive for p-STAT3 also stained for activated AKT. A tendency toward higher activation of MAPK was also seen in glioblastomas as compared with anaplastic astrocytomas and, although activation of AKT did not correlate significantly with MAPK activation in glioblastomas, an increase in the activation of both of these molecules appeared to correlate with the progression of anaplastic astrocytoma to glioblastoma in this study (Fig. 4). These findings are of particular interest in light of recent investigations using mouse models; in one study by Sonoda et al., activation of AKT was associated with malignant transformation from anaplastic astrocytoma to glioblastoma (37), and combined activation of RAS and AKT has been suggested to be necessary for glioblastoma formation (8, 37).

Because there appeared to be a strong trend from predominantly STAT3 activation in anaplastic astrocytomas to AKT activation in glioblastomas (Table 3; Fig. 4), and because patients with anaplastic astrocytomas experience survival times significantly longer than patients with glioblastoma (1), we investigated whether p-STAT3 or p-AKT correlated with survival in patients with astrocytic gliomas. After adjusting for tumor grade and patient age, activation of STAT3 alone appeared marginally predictive of improved survival (hazard ratio = 0.63, p = 0.083). To date, the correlation between STAT3 activation and survival has been somewhat controversial in human tumors. Although several articles have suggested that STAT3 activation was associated with poor prognosis in various cancers (26, 38–41), one study of a large series of breast cancers demonstrated that STAT3 activation correlated with improved overall survival (42). However, the finding that STAT3 expression during cortical development is associated with astrocyte differentiation (43, 44) provides one potential explanation for why activation of STAT3 in astrocytic gliomas might correlate with better prognosis. In contrast, astrocytic tumors with activation of AKT in the absence of p-STAT3 and p-MAPK demonstrated a trend toward a worse prognosis (hazard ratio = 2.125, p = 0.094) after adjusting for tumor grade and patient age. This finding is consistent with a report by Chakravarti et al that demonstrated p-AKT was associated with significantly reduced overall survival times when gliomas of all grades were considered (45).

Recently, treatment options for the most aggressive glial tumors have included the use of targeted molecular therapies. One example, erlotinib, a small molecule inhibitor of EGFR, has demonstrated rather modest efficacy (46–50). Interestingly, in one study, 6 of 10 patients with EGFR amplification did not respond to erlotinib, suggesting that erlotinib may be active only in a subset of gliomas with EGFR expression (46). Furthermore, this study demonstrated that tumors with high levels of EGFR expression and low levels of phosphorylated PKB/AKT responded better to erlotinib than those lesions with low levels of EGFR and high levels of phosphorylated PKB/AKT (46). These clinical results highlight the fact that studies attempting to understand the complicated signaling networks at play in glioblastomas are crucial. Such knowledge could lay the foundation for the development of effective targeted molecular therapeutic strategies for these clinically aggressive tumors and facilitate the discovery of subtypes of glial tumors that might respond differentially to these therapies.

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