**NFKBIA Deletion in Glioblastomas**


**ABSTRACT**

**BACKGROUND**
Amplification and activating mutations of the epidermal growth factor receptor (EGFR) oncogene are molecular hallmarks of glioblastomas. We hypothesized that deletion of NFKBIA (encoding nuclear factor of κ-light polypeptide gene enhancer in B-cells inhibitor-α), an inhibitor of the EGFR-signaling pathway, promotes tumorigenesis in glioblastomas that do not have alterations of EGFR.

**METHODS**
We analyzed 790 human glioblastomas for deletions, mutations, or expression of NFKBIA and EGFR. We studied the tumor-suppressor activity of NFKBIA in tumor-cell culture. We compared the molecular results with the outcome of glioblastoma in 570 affected persons.

**RESULTS**
NFKBIA is often deleted but not mutated in glioblastomas; most deletions occur in nonclassical subtypes of the disease. Deletion of NFKBIA and amplification of EGFR show a pattern of mutual exclusivity. Restoration of the expression of NFKBIA attenuated the malignant phenotype and increased the vulnerability to chemotherapy of cells cultured from tumors with NFKBIA deletion; it also reduced the viability of cells with EGFR amplification but not of cells with normal gene dosages of both NFKBIA and EGFR. Deletion and low expression of NFKBIA were associated with unfavorable outcomes. Patients who had tumors with NFKBIA deletion had outcomes that were similar to those in patients with tumors harboring EGFR amplification. These outcomes were poor as compared with the outcomes in patients with tumors that had normal gene dosages of NFKBIA and EGFR. A two-gene model that was based on expression of NFKBIA and O⁶-methylguanine DNA methyltransferase was strongly associated with the clinical course of the disease.

**CONCLUSIONS**
Deletion of NFKBIA has an effect that is similar to the effect of EGFR amplification in the pathogenesis of glioblastoma and is associated with comparatively short survival.
Glioblastoma multiforme is the most common and most deadly primary brain tumor. It is a complex disease, in which many signaling pathways are disrupted. Almost all glioblastomas have excessive activation of the epidermal growth factor receptor (EGFR) pathway, often brought about by amplification (see the Glossary for this and other key terms) or activating mutations of the EGFR oncogene. Alternative mechanisms of the activation of the EGFR pathway may exist in tumors that do not have alterations of EGFR.

Nuclear factor of κ-light polypeptide gene enhancer in B-cells (NF-κB) is a transcription factor activated by the EGFR pathway. Aberrant constitutive activation of NF-κB has been observed in glioblastomas. NF-κB inhibitor-α (NFKBIA) represses NF-κB and, hence, signaling in the NF-κB and EGFR pathways. The discovery of mutations of NFKBIA, as well as research showing that there is an enrichment of specific single-nucleotide polymorphisms and haplotypes of NFKBIA in Hodgkin’s lymphoma, colorectal cancer, melanoma, hepatocellular carcinoma, breast cancer, and multiple myeloma, suggests that NFKBIA is a tumor suppressor. This possibility, together with evidence of the activation of NF-κB by EGFR activity in glioblastomas and our previous studies showing an association between the down-regulation of NFKBIA in glioblastoma cells and a lack of response to therapy, prompted our investigation of deletions, mutations, and expression of NFKBIA in glioblastomas, their associations with EGFR amplification and mutation, and the association between these molecular features and the clinical outcome.

Methods

Tumor Samples and Patients
We used 10 study sets of patients with glioblastoma who were treated between July 26, 1989, and August 12, 2009, and studied the patients and their tumors. The demographic characteristics of the patients, the characteristics of the disease, and the types of data that were used are shown in Table 1.

Cell Lines and Preparation of Genomic DNA
We obtained glioblastoma cell lines LN229, U87, and U118 from the American Type Culture Collection. PT67 retroviral packaging cells were grown according to the instructions of the manufacturer (Clontech). Primary tumor-cell cultures were generated from malignant glioma specimens from patients enrolled in a study that was conducted at Northwestern University with approval from the institutional review board. Primary cancer stem-like cell cultures were generated from nine glioblastomas in Study Set 4. Genomic DNA from tumor samples and cell lines was isolated with the use of DNeasy kits (Qiagen) and was quantified with the use of spectrophotometry. Detailed descriptions of cell biologic and molecular biologic analyses and experimental design are provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Copy-Number Variation and Mutational Analyses
Details of the tissue collection, methods of generation and preprocessing of multidimensional genomic data, analysis of copy-number variation, and sequence analysis are provided in the Supplementary Appendix. We sequenced the NFKBIA coding region in 32 glioblastomas in study set 5 and, along with the promoter region, in 15 cell lines in study set 6. We analyzed activating EGFR mutations in 91 patients with glioblastoma in study set 1 and DNA samples from non-neoplastic tissue from those patients and tested for an association between the presence of activating EGFR mutations and the presence of a deletion affecting NFKBIA.

Statistical Analysis
Survival curves were estimated with the use of the Kaplan–Meier product-limit method, and survival distributions were compared across groups with the use of the log-rank test. We performed univariate and multivariate Cox proportional-hazards regression analyses, with overall survival as the dependent variable and NFKBIA and EGFR dosage or NFKBIA and O6-methylguanine DNA methyltransferase (MGMT) expression as the primary predictor. In interpreting hazard ratios, we dichotomized NFKBIA expression (in all models) at the median, and in the NFKBIA–MGMT combined risk-group model, we dichotomized MGMT expression at the 60th percentile (i.e., 60% of tumors with comparatively high MGMT expression vs. 40% of tumors with comparatively low MGMT expression). The 60th percentile of MGMT expression was prespecified to define MGMT “high-risk” tumors (i.e., the 60% of tumors that showed the highest expression of MGMT) on the
Deletions of NFKBIA

We observed a common heterozygous deletion encompassing NFKBIA in 53 of the 219 glioblastomas (24.2%) in study set 1 (Fig. 1A). An analysis of NFKBIA copy number in the glioblastomas in study set 2 revealed fewer than 1.5 copies of NFKBIA in 37 of the 182 tumors (20.3%). There were heterozygous deletions of NFKBIA in 13 of the 46 glioblastomas (28.3%) in study set 3 and in 6 of 27 glioblastomas (22.2%) and 2 of 9 glioblastoma-derived cancer stemlike cell populations (22.2%) in study set 4.

In the 175 tumors in study set 1 with data on NFKBIA dosage and expression, we found significantly lower NFKBIA mRNA expression in tumors in which NFKBIA was deleted than in those with two intact copies of NFKBIA (P = 8×10^-9 by the Wilcoxon rank-sum test) (Fig. 1B).

We sequenced the coding region of NFKBIA in the 32 glioblastomas in study set 5 and both promoter and coding regions of NFKBIA in the 15 cell lines in study set 6. We found no mutations in either coding or promoter sequences, suggesting that inactivation of NFKBIA in glioblastoma cells occurs primarily through the loss of gene copy number (i.e., a reduction of gene dosage).
Recent studies have distinguished between classical and nonclassical (i.e., mesenchymal, neural, and proneural) subtypes of glioblastoma. EGFR amplifications are common (80.0%) in the classical subtype (Fig. 1C). Among the 188 glioblastomas in study set 1 with data on gene dosage and subtype, we found that **NFKBIA** deletions are rare (5.9%) in classical glioblastomas and more common (32.1%) in nonclassical glioblastomas (P = 5×10\(^{-4}\) by Pearson's chi-square test; odds ratio for deletions in classical glioblastomas, 0.13; 95% confidence interval [CI], 0.04 to 0.42) (Fig. 1C). Irrespective of subtype, we observed a pattern suggesting a degree of mutual exclusivity between **NFKBIA** deletion and **EGFR** amplification (P = 2×10\(^{-3}\) by Pearson's chi-square test; odds ratio for concomitant deletion and amplification, 0.33; 95% CI, 0.16 to 0.69) (Fig. 2A). We observed a similar pattern in 46 glioblastomas in study set 3 (P = 0.01 by Fisher's exact test; odds ratio, 0.00; 95% CI, 0.00 to 0.56): no tumor harbored both alterations (Fig. 2B).

In the 83 tumors in study set 1 for which data on gene dosage and somatic mutation for **EGFR** were available, **NFKBIA** deletions and **EGFR** alteration (amplification, activating mutation, or both) were unlikely to occur in the same tumor, although the relative mutual exclusivity of these events reached only marginal significance (P = 0.05 by Fisher's exact test; odds ratio, 0.35; 95% CI, 0.13 to 1.00). The pattern of relative mutual exclusivity between alterations of **NFKBIA** and **EGFR** extended to gene expression; tumors with diminished **NFKBIA** expression from gene deletion had comparatively low **EGFR** expression, and

**Table 1. Patient Characteristics and Types of Data Used in 10 Study Sets.**

<table>
<thead>
<tr>
<th>Study Set</th>
<th>No. of Patients</th>
<th>No. with Matched DNA from Non-neoplastic Tissue</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Vital Status</th>
<th>Median Weeks of Follow-up (Range)</th>
<th>Data Type†</th>
<th>Source</th>
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<tr>
<td>1</td>
<td>219‡</td>
<td>91</td>
<td>Female: 77 Male: 130</td>
<td>55.8±15.1</td>
<td>Dead: 192 Alive: 15</td>
<td>50.6 (1.1–503.4)</td>
<td>G, E, C</td>
<td>Cancer Genome Atlas Project</td>
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<tr>
<td>2</td>
<td>182</td>
<td>0</td>
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<td>NA NA</td>
<td>NA</td>
<td>NA G</td>
<td>REMBRANDT release 1.5.4.1</td>
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<tr>
<td>3</td>
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<td>0</td>
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<td>NA NA</td>
<td>NA</td>
<td>NA G</td>
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<td>NA NA</td>
<td>NA</td>
<td>NA G</td>
<td>Northwestern University</td>
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<td>5</td>
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<td>0</td>
<td>NA NA NA</td>
<td>NA NA</td>
<td>NA</td>
<td>NA S</td>
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<tr>
<td>6</td>
<td>15</td>
<td>0</td>
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<td>Barrow Neurological Institute</td>
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<tr>
<td>7</td>
<td>49§</td>
<td>0</td>
<td>Female: 15 Male: 34</td>
<td>49.9±12.1</td>
<td>Dead: 46 Alive: 3</td>
<td>70.0 (3.0–313.0)</td>
<td>E, C</td>
<td>M.D. Anderson Cancer Center (GEO accession no., GSE4271)</td>
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<tr>
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<td>47</td>
<td>0</td>
<td>Female: 25 Male: 22</td>
<td>50.5±15.6</td>
<td>Dead: 34 Alive: 13</td>
<td>42.0 (1.0–178.0)</td>
<td>E, C</td>
<td>University of California, Los Angeles (GEO accession no., GSE4412)</td>
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<tr>
<td>9</td>
<td>191</td>
<td>0</td>
<td>Female: 74 Male: 117</td>
<td>53.8±13.6</td>
<td>Dead: 176 Alive: 15</td>
<td>55.6 (1.0–479.0)</td>
<td>E, C</td>
<td>Multi-institutional (GEO accession no., GSE13041)</td>
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<td>10</td>
<td>76</td>
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<td>Female: 21 Male: 55</td>
<td>51.1±9.1</td>
<td>Dead: 63 Alive: 13</td>
<td>66.9 (6.1–311.9)</td>
<td>E, M, C</td>
<td>Phase 2 trial, EORTC-NCIC phase 3 trial (GEO accession no., GSE7696)¶</td>
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</table>

* Plus–minus values are means ±SD. GEO denotes Gene Expression Omnibus, and NA not available.
† The types of data include clinical-outcome data (C), gene-expression data (E), gene copy number data (G), methylation status of the **MGMT** promoter (M), and sequencing data (S).
‡ There were 219 patients in study set 1; clinical data were available for 207 of those patients.
§ Twenty-two patients had matched tumor pairs from the initial diagnosis and recurrent disease; therefore, 71 tumors were assessed in study set 7.
¶ Study set 10 included 76 patients with glioblastoma who were treated as part of a phase 2 trial or a European Organization for Research and Treatment of Cancer (EORTC)/National Cancer Institute of Canada (NCIC) randomized phase 3 trial (22981-26981/CE.38), both of which evaluated the addition of concomitant and adjuvant temozolomide to radiotherapy.31,32
NFKBIA Deletions in Glioblastomas

The analysis of copy-number variation for chromosome 14 (Panel A) was based on circular binary segmentation in 219 glioblastomas in study set 1. Gene dosages are mapped according to gene order on chromosome 14. NFKBIA is deleted (del) in 24.2% of tumors (yellow line, NFKBIA locus on 14q13). The bar diagram at the bottom of the panel shows the gene-dosage profiles for NFKBIA. Gene-dosage values indicate the log₂ ratio of red (R, Cy5) to green (G, Cy3) intensity of the fluorescence dye (or log₂ R/G), as estimated with the use of the circular binary segmentation algorithm. The deletion of NFKBIA is associated with significant loss of NFKBIA expression in the 175 glioblastomas in study set 1 that had combined gene and transcript data (Panel B). Values for gene dosage and gene expression are presented as log₂ R/G ratios, as estimated by the circular binary segmentation and robust multigene average preprocessing algorithms, respectively. The box plots show the smallest and largest observations (upper and lower whiskers, respectively), the interquartile range (box), and the median (red line). Data points that are more than 1.5 times the interquartile range lower than the first quartile or 1.5 times the interquartile range higher than the third quartile were considered to be outliers. The P value was calculated with the use of the Wilcoxon rank-sum test. Gene-dosage profiles for NFKBIA and EGFR across 188 glioblastomas in study set 1 are shown (Panel C), along with their relationship to four molecular subtypes of glioblastoma (classical, mesenchymal, neural, and proneural). A corresponding two-way contingency-table analysis reveals a significant association of NFKBIA deletion with the nonclassical subtypes. CI denotes confidence interval.

Figure 1. NFKBIA Deletions in Glioblastomas.

TUMOR SUPPRESSION IN CELL CULTURE

Retrovirally mediated reexpression of NFKBIA in established glioblastoma cell lines with heterozygous NFKBIA deletions (Fig. 2 in the Supplemen-
In primary tumor cultures from three human glioblastomas with different \( \text{NFKBIA} \) and \( \text{EGFR} \) status — deleted \( \text{NFKBIA} \) and wild-type (i.e., normal-gene-dosage) \( \text{EGFR} \), wild-type \( \text{NFKBIA} \) and amplified \( \text{EGFR} \), and wild-type \( \text{NFKBIA} \) and \( \text{EGFR} \) (i.e., both genes present in two copies) — retroviral expression of \( \text{NFKBIA} \) substantially reduced cell viability in the \( \text{NFKBIA} \)-deleted tumor and in the \( \text{EGFR} \)-amplified tumor (\( P=2\times10^{-4} \) and \( P=0.02 \), respectively, by unpaired \( t \)-test) but not in the tumor with normal dosages of each gene (\( P=0.21 \)) (Fig. 3). These data support our conclusion that \( \text{NFKBIA} \) suppresses the growth of glioblastomas in which \( \text{EGFR} \) signaling pathway dependence is brought about by deletion of \( \text{NFKBIA} \) or amplification of \( \text{EGFR} \).

**NFKBIA and Outcomes in Patients with Glioblastoma**

Cox proportional-hazards regression analysis of the 188 glioblastomas in study set 1 for which data on gene copy number and survival were available showed that patients with two copies of \( \text{NFKBIA} \) survived significantly longer than did patients with tumors harboring a deletion of \( \text{NFKBIA} \) (hazard ratio for death with two copies vs. deletion of \( \text{NFKBIA} \), 0.45; 95% CI, 0.23 to 0.89; \( P=0.02 \)). A multivariate Cox model suggested that this association is independent of the prognostic covariate, the age of the patient (hazard ratio, 0.40; 95% CI, 0.21 to 0.79; \( P=8\times10^{-3} \)). Similarly, models incorporating the patient’s age and either \( \text{EGFR} \) dosage or clinically relevant molecular subtypes of glioblastoma\(^6\) confirmed an independent association between survival and normal dosage of...
NFKBIA (hazard ratio for death, 0.40; 95% CI, 0.20 to 0.78; P = 7 \times 10^{-3}; and hazard ratio, 0.39; 95% CI, 0.20 to 0.77; P = 7 \times 10^{-3}, respectively).

Among the 171 patients in study set 1 with newly diagnosed glioblastoma for whom data on both gene dosage and survival were available, we found no difference in time to death between the patients with an isolated NFKBIA deletion (i.e., NFKBIA deletion without EGFR amplification) and those with isolated EGFR amplification (i.e., EGFR amplification without NFKBIA deletion) (hazard ratio for death with isolated NFKBIA deletion vs. isolated EGFR amplification, 1.13; 95% CI, 0.72 to 1.79; P = 0.57 by the Cox model) (Fig. 4A). In contrast, patients with tumors that had either an NFKBIA deletion or EGFR amplification had shorter survival, as measured from the time of diagnosis, than did those with normal dosages of both NFKBIA and EGFR (hazard ratio for death with NFKBIA deletion, 1.69; 95% CI, 1.09 to 2.63; P = 0.02 by the Cox model; and hazard ratio for death with EGFR amplification, 1.48; 95% CI, 1.02 to 2.13; P = 0.04 by the Cox model) (Fig. 4A). The estimated median survival times were 46 weeks for patients whose tumors harbored an isolated NFKBIA deletion, 53 weeks for those whose tumors had isolated EGFR amplification, and 67 weeks for those whose tumors had normal dosages of both NFKBIA and EGFR.

A correlation between NFKBIA expression, as assessed by microarray analysis, and survival was established in three different groups. In a Cox proportional-hazards regression analysis of the 49 glioblastomas in study set 7, greater NFKBIA expression was associated with longer survival (hazard ratio for death, 0.51; 95% CI, 0.35 to 0.75; P = 6 \times 10^{-4}). A multivariate Cox model incorporating the patient’s age, molecular subtype,5,37 and MGMT expression — currently the most potent predictor of response to temozolomide therapy33 — yielded an independent association of NFKBIA with survival (hazard ratio, 0.44; 95% CI, 0.29 to 0.66; P = 7 \times 10^{-4}). A two-class model in which patients were stratified according to median NFKBIA expression also showed an association between NFKBIA expression and longer survival (hazard ratio with high vs. low expression of NFKBIA, calculated with the use of a Cox model, 0.31; 95% CI, 0.16 to 0.58; P = 2 \times 10^{-5} by the log-rank test); the estimated median survival for patients with tumors that had high NFKBIA expression was 131 weeks, as compared with 57 weeks for patients with low NFKBIA expression (Fig. 4B). This relationship was also present in the 47 glioblastomas in study set 8 and the 191 glioblastomas in study set 9, both in multivariate models (hazard ratio, 0.57; 95% CI, 0.33 to 0.98; P = 0.04; and hazard ratio, 0.73; 95% CI, 0.60 to 0.90; P = 3 \times 10^{-3}, respectively) and in two-class models (hazard ratio for high vs. low NFKBIA expression, calculated with the use of a Cox model, 0.43; 95% CI, 0.21 to 0.86; P = 0.02 by the log-rank test; and hazard
Figure 4. NFKBIA and Survival in Patients with Glioblastomas.

Kaplan–Meier estimates of overall survival are shown for 171 patients in study set 1 with newly diagnosed glioblastomas (Panel A), with patients stratified into three subgroups according to the presence of tumors with NFKBIA and EGFR wild-type (wt) status, NFKBIA deletion (del) without EGFR amplification (amp), or EGFR amplification without NFKBIA deletion (9 patients with tumors that had alteration of both NFKBIA and EGFR were omitted owing to the small sample size). Kaplan–Meier estimates of survival are shown for the 49 patients in study set 7 (Panel B), with patients stratified according to median NFKBIA expression. The combined NFKBIA and O6-methylguanine DNA methyltransferase (MGMT) risk-group models are shown for 191 patients with glioblastomas in study set 9 (Panel C) and for 42 patients with newly diagnosed glioblastomas in study set 10 who were treated with radiotherapy plus concomitant and adjuvant temozolomide (Panel D). Assignment of patients to low-, intermediate-, or high-risk groups was based on NFKBIA expression (dichotomized at the median) and MGMT status (MGMT expression dichotomized at the 60th percentile or based on MGMT promoter methylation status). In Panel C, NFKBIA expression higher than the median combined with MGMT expression lower than the 40th percentile denotes a low-risk group, and NFKBIA expression lower than the median combined with MGMT expression higher than the 60th percentile denotes a high-risk group. In Panel D, NFKBIA expression higher than the median combined with methylated MGMT promoter status denotes a low-risk group, and NFKBIA expression lower than the median combined with unmethylated MGMT promoter status denotes a high-risk group; all other cases were assigned to an intermediate-risk group. Small vertical lines indicate patients who were alive at the last follow-up assessment. P values were calculated with the use of the log-rank test.
NFKBIA deletion in glioblastomas

Deletion in Glioblastomas

The association between NFKBIA expression and longer survival was also present in the case of tumors with high-risk MGMT status. One third to 45% of glioblastomas have a comparatively long survival (hazard ratio for death with high vs. low NFKBIA expression, calculated with the use of a Cox model, 0.32; 95% CI, 0.12 to 0.88; P = 0.02 by the log-rank test) (Fig. 6 in the Supplementary Appendix).

To determine the usefulness of a two-gene outcome-predictor model that is based on the status of both NFKBIA and MGMT, we divided the 191 glioblastomas in study set 9 into groups that were defined according to the prespecified cutoff points for expression of NFKBIA (median) and MGMT (60% of tumors with comparatively high MGMT expression vs. 40% of tumors with comparatively low MGMT expression): one high-risk group with low NFKBIA and high MGMT expression, one low-risk group with high NFKBIA and low MGMT expression, and one intermediate-risk group with either low NFKBIA and low MGMT expression or high NFKBIA and high MGMT expression. This model yielded a strong association between risk status and survival (P = 2 × 10\(^{-6}\)) by the log-rank test) (Fig. 4C). The estimated median survival in the low-risk, intermediate-risk, and high-risk groups was 92 weeks, 59 weeks, and 44 weeks, respectively. A model with groups defined according to the median expression of both NFKBIA and MGMT produced similar results; the estimated median survival was 89 weeks in the low-risk group, 57 weeks in the intermediate-risk group, and 45 weeks in the high-risk group (P = 6 × 10\(^{-4}\)) by the log-rank test) (Fig. 8A in the Supplementary Appendix).

When the 74 patients in study set 10 with known MGMT promoter status (methylated vs. unmethylated) were similarly stratified into three risk groups, we found significant differences in estimated median survival: 91 weeks in the low-risk group, 63 weeks in the intermediate-risk group, and 45 weeks in the high-risk group (P = 7 × 10\(^{-4}\)) by the log-rank test) (Fig. 8B in the Supplementary Appendix). The association between risk and survival was even more pronounced in the case of patients with newly diagnosed tumors who were treated with radiotherapy and temozolomide (P = 3 × 10\(^{-6}\)) by the log-rank test) (Fig. 4D); the estimated median survival in the low-risk, intermediate-risk, and high-risk groups was 122 weeks, 71 weeks, and 35 weeks, respectively.
DISCUSSION

Our data support a role for NFKBIA in the suppression of glioblastoma tumors. The presence of NFKBIA deletions in some glioblastoma cancer stem cells suggests that such deletions can emerge early in the pathogenesis of glioblastoma. Our data show that loss of NFKBIA can also be associated with disease progression and tumor recurrence.

The general, albeit not absolute, mutual exclusivity of NFKBIA deletion and EGFR amplification has been reported in the case of other gene pairs in signaling pathways pertinent to the biologic nature of glioblastomas. For example, a decrease in retinoblastoma pathway signaling is achieved through a mutually exclusive mutation of the tumor-suppressor gene RB1 or deletion of the tumor-suppressor genes CDKN2A and CDKN2B. Similarly, mutations in the tumor-suppressor gene TP53 and deletions affecting CDKN2A, both of which reduce TP53 pathway signaling, appear to be mutually exclusive in glioblastomas.

The fact that tumors with deletion of NFKBIA and those with EGFR amplification have similarly poor outcomes suggests that NFKBIA deletion can substitute for EGFR amplification in the pathogenesis of glioblastoma. This finding is consistent with our observation that deletion of NFKBIA occurs more commonly in nonclassical glioblastomas than in classical glioblastomas, which have EGFR amplification more often than do nonclassical glioblastomas. Which aberration occurs may depend on the tumor’s cell of origin and its pattern of accumulation of the other genetic lesions that define glioblastoma subtypes.

We have observed, in a previous study, that glioblastoma cells that do not respond to temozolomide chemotherapy have comparatively low expression of NFKBIA and, in this study, that increasing NFKBIA expression in these cells sensitizes them to temozolomide. Our findings, together with research showing the role of NFKBIA as a gatekeeper for EGFR signaling and the involvement of EGFR activation in the lack of response of glioblastoma cells to chemotherapy and radiotherapy, collectively suggest that NFKBIA-mediated sensitization of glioblastoma cells to temozolomide reflects NFKBIA abrogation of EGFR signaling.

Our observation that NFKBIA status is independently associated with survival in several patient groups supports the importance of NFKBIA as a determinant of glioblastoma behavior, including the response to temozolomide, and suggests that it would be useful to include the gene dosage or expression of NFKBIA in models predicting survival. Our data show that a risk model combining NFKBIA status and MGMT status (currently the best single predictor of response to temozolomide therapy for glioblastomas) was strongly associated with the clinical course of the disease. This makes sense mechanistically: concomitant down-regulation of NFKBIA (enhancing the pro-survival effect of NF-kB) and up-regulation of MGMT (enhancing the repair of DNA damage) could have a synergistic, positive effect on resistance to therapeutic response and cell death.

Our finding that increased expression of NFKBIA inhibited the malignant behavior of tumors that had amplified EGFR and normal dosage of NFKBIA (in addition to tumors with deletions of NFKBIA) suggests that NFKBIA-stabilizing therapies may be effective against glioblastomas that have alterations of EGFR. The limited efficacy of molecular therapies targeting EGFR in glioblastomas suggests that the therapeutic effect of EGFR inhibition can be circumvented through cross-coupled signaling from other growth factor receptors that are mutated, amplified, or overexpressed in these tumors, such as PDGFR, ERBB2, or MET. Because NFKBIA is a major node downstream of such cross-coupled signaling, therapies that stabilize NFKBIA might more effectively restrain oncogenic signaling.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

REFERENCES


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