Prognostic Effect of Epidermal Growth Factor Receptor and EGFRvIII in Glioblastoma Multiforme Patients

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ABSTRACT

Purpose: **The epidermal growth factor receptor (EGFR) is overexpressed in ~50% to 60% of glioblastoma multiforme tumors, and the most common EGFR mutant, EGFRvIII, is expressed in 24% to 67% of cases. We sought to determine whether glioblastoma multiforme expression of either overexpressed wild-type EGFR or the mutant EGFRvIII is an independent predictor of overall patient survival.**

Experimental Design: **Glioblastoma multiforme patients (n = 196) underwent a ≥95% volumetric tumor resection followed by conformal radiation. Their EGFR and EGFRvIII status was determined by immunohistochemistry and survival analyses were done.**

Results: **In our study of glioblastoma multiforme patients, 46% (n = 91) failed to express EGFR, 54% (n = 105) had overexpression of the wild-type EGFR, and 31% (n = 61) also expressed the EGFRvIII. Patients within groups expressing the EGFR, EGFRvIII, or lacking EGFR expression did not differ in age, sex, Karnofsky performance scale score, extent of tumor resection, or radiation. The median overall survival times for patients with tumors having EGFR expression absent, overexpressed only, or mutant (EGFRvIII) were 0.96, 0.98, and 1.07 years, respectively. However, for patients surviving ≥1 year, these values were 2.03, 2.02, and 1.21 years (P < 0.0001; log-rank test comparing EGFRvIII with all others). This effect remained significant in the multivariate analysis after adjustment for all other cofactors including age and Karnofsky performance scale score (rate ratio 4.34; 95% confidence interval, 2.21-8.51).**

Conclusions: **Neither the overexpressed wild-type EGFR nor EGFRvIII was an independent predictor of median overall survival in this selected cohort of patients who underwent extensive tumor resection. However, in patients surviving ≥1 year, the expression of EGFRvIII was an independent negative prognostic indicator.**

INTRODUCTION

Glioblastoma multiforme is the most common primary malignant neoplasm of the central nervous system in adults. Despite multimodal therapies, the median survival time of patients with glioblastoma multiforme is ~1 year; however, there is considerable variability among these patients. Prognostic indicators have included age (1, 2), Karnofsky performance scale (KPS) score (3), and extent of surgical resection (4, 5). The most frequent genetic alteration associated with glioblastoma multiforme is amplification of the epidermal growth factor receptor (*EGFR*) gene, which results in overexpression of the EGFR, a transmembrane tyrosine kinase receptor (6). The majority of glioblastoma multiformes with EGFR amplification also contain the mutant EGFR gene, *EGFRvIII* (7), which is characterized by the deletion of exons 2 to 7, resulting in a sense mutation that has a truncated extracellular domain with ligand-independent constitutive activity (8). Previous work has shown that EGFR amplification is evident in all glioblastoma multiformes expressing EGFRvIII, and glioblastoma multiformes lacking the amplified EGFR are not positive for EGFRvIII protein. In addition, we have previously shown that the positive staining with the 528 antibody, which recognizes an unspecified extracellular epitope of the EGFR, is highly correlated with EGFR amplification (9).

The role of the overexpressed EGFR (wild type) and the variant (vIII) receptor in malignant progression of glial tumors and their respective effects on progression-free survival and overall survival has been debated in the literature. The overexpressed wild-type EGFR was not found to be an independent prognostic indicator of survival in several studies (10–12), and one study was inconclusive (13). One study did identify the EGFR as a negative prognostic indicator in younger patients (14), and several others found the EGFR to be an independent unfavorable predictor of survival (15–17). In some of these studies, analysis was limited by small sample size, uncharacterized extent of surgical resection, and variable postoperative treatment. The prognostic effect of EGFRvIII has not been as extensively studied, but in studies that addressed this variable, the presence of EGFRvIII was found to be an independent and significant unfavorable prognosticator of survival (18).

The first purpose of our study was to establish in a large number of similarly treated glioblastoma multiforme patients whether the presence of the overexpressed EGFR or the EGFRvIII was a negative predictor of survival. Furthermore, we wished to ascertain the effect of these markers on the natural history of this disease. The final issue was to determine whether

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such glioblastoma multiforme patients were more likely to have systemic complications (i.e., pulmonary embolus or deep venous thrombosis).

To determine whether overexpressed EGFR and EGFRvIII were independent prognostic indicators, we retrospectively evaluated 196 newly diagnosed glioblastoma multiforme patients who had undergone ≥95% surgical volumetric tumor resection and subsequently received standard-of-care conformal irradiation. In contrast to the previously published reports, the rate ratios for EGFR and EGFRvIII in glioblastoma multiforme patients, after correcting for confounding factors, indicated the absence of a prognostic effect on median overall survival. However, in patients surviving for ≥1 years, EGFRvIII was an independent negative prognostic indicator.

MATERIALS AND METHODS

Study Population. The study was conducted according to an Institutional Review Board–approved protocol (LAB03-0228). Patients (n = 196) with glioblastoma multiforme (WHO grade 4) who had undergone resection of at least 95% of the tumor volume (defined as the contrast-enhancing component on the preoperative T1-weighed MR image as analyzed by Vitrea 3 software) and who subsequently received conformal irradiation were retrospectively reviewed to determine whether tumor expression of EGFR or EGFRvIII conferred a poor prognosis. Clinical and survival information was obtained from the Department of Neurosurgery Clinical and Imaging Database and the University of Texas M.D. Anderson Cancer Center Tumor Registry. All tissue specimens were acquired at initial diagnosis and resection and were classified morphologically and graded according to WHO criteria.

Immunohistochemical Detection of Overexpressed Epidermal Growth Factor Receptor and EGFRvIII. Immunostaining was done as previously described (14). Briefly, 5-μm tumor tissue sections were mounted on positively charged slides, deparaffinized, and rehydrated in PBS. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS/0.05% Tween 20 for 20 minutes. Sections were washed in PBS and blocked for 20 minutes in the appropriate serum (from the same species as the secondary antibody) diluted to 10% in PBS. The primary antibody for EGFR detection was the monoclonal mouse anti-human pan-EGFR clone 528 (Oncogene Research, Product, San Diego, CA; 1:50 dilution; ref. 19) and for EGFRvIII detection was a rabbit anti-human polyclonal antibody (Zymed, San Francisco, CA; 1:1,200 dilution). For EGFRvIII staining, microwave antigen retrieval was done by placing the slides in 50 mmol/L citrate buffer (pH 6.0) and microwaving for 12 minutes at full power and 10 minutes at 20% power followed by cooling for 15 minutes and two to three 5-minute washes in PBS. For EGFR staining, pretreatment consisted of placing 0.025% trypsin on the tissue and incubating for 30 minutes at room temperature. Primary antibodies, diluted in PBS/10% serum, were applied to the sections in a humid chamber overnight at 4°C. Sections were washed two to three times in PBS, and secondary antibodies were applied using the Dako Envision kit (Carpinteria, CA), according to the manufacturer's instructions. Detection of bound secondary antibody was done with diaminobenzadine for 5 minutes. Sections were then counterstained with hematoxylin and mounted.

Statistical Analysis. The frequencies and descriptive statistics of demographic and clinical variables were recorded for the patients in this study. The χ^2 or exact test (StatXact 3 for Windows) was used for categorical variables as appropriate. The ANOVA was used for continuous variables. Cumulative survival times from the time of surgery at our institution were computed using the Kaplan-Meier method (20). Survival curves for the various subgroups were compared using the log-rank test. The Cox proportional hazards model was used to obtain crude rate ratios, adjusted rate ratios, and their 95% confidence intervals (95% CI) for the various EGFR categories (21). Adjustments were done for age, sex, KPS score, tumor location, radiographic enhancement, radiographic necrosis, extent of edema, midline shift, presence of leptomeningeal disease, multifocal disease, or gliomatosis cerebri and ependymal involvement. Rescaled Schoenfeld residuals were obtained.

RESULTS

Demographic Characteristics. In our study of 196 glioblastoma multiforme patients, 46% (n = 91) failed to express EGFR, 54% (n = 105) had overexpression of the wild-type EGFR, and 31% (n = 61) also expressed the EGFRvIII. Within the overexpressed wild-type EGFR group, 42% (n = 44) failed to express EGFRvIII. There was no significant difference in sex, age, KPS score, or radiation therapy, among the patients whose tumors failed to express EGFR, overexpressed the wild-type EGFR, or expressed EGFRvIII (Table 1). Furthermore, the incidence of known complications, such as deep venous thrombosis or pulmonary embolus, was not increased in patients during their life span with tumors expressing the overexpressed wild-type EGFR or the EGFRvIII mutation compared with patients whose tumors did not express EGFR.

Radiographic Characteristics. There was no significant difference in the location, extent of necrosis, amount of MR image contrast enhancement, extent of edema, or amount of brain midline shift among the three EGFR expression categories of glioblastoma multiformes (Table 2).

Natural Disease History Based on Epidermal Growth Factor Receptor and EGFRvIII Expression in Glioblastoma Multiformes. There were no significant differences in the

Table 1 Demographic characteristics of patients with glioblastoma multiforme categorized according to EGFR expression of the tumor

Variable	EGFR negative	EGFRwt positive only	EGFRvIII positive
Total of 196, *n* (%)	91 (46)	44 (23)	61 (31)
Sex, *n* (%)*			
Male	60 (66)	25 (57)	32 (52)
Female	31 (34)	19 (43)	29 (48)
Age, y, median (range)*	58 (17-82)	57 (21-76)	59 (31-82)
KPS score, median (range)*	90 (50-100)	90 (70-100)	90 (60-100)
MIB-1, median (range)*	8.5 (5.8-15.5)	8.9 (4.4-30.6)	11.0 (5.6-12.0)

Abbreviations: wt, wild type; vIII, vIII mutant; MIB-1, MIB-1 antigen.

*There was no statistically significant difference among the three EGFR expression categories.

Table 2 Radiographic characteristics of glioblastomas according to epidermal growth factor receptor expression category

Variable*	EGFR negative	EGFRwt positive	EGFRvIII positive
Total of 196,	91 (46)	44 (23)	61 (31)
Location,			
CW	60 (66)	24 (54)	32 (52)
E	31 (34)	20 (46)	29 (48)
Eloquency relationship,			
Noneloquent brain	8 (9)	5 (11)	5 (8)
Near-eloquent brain	48 (53)	19 (43)	29 (48)
Eloquent brain	35 (38)	20 (46)	27 (44)
Leptomeningeal disease,			
Yes	6 (7)	2 (4)	4 (7)
No	85 (93)	42 (96)	57 (93)
Multifocal disease,			
Yes	19 (21)	11 (25)	15 (25)
No	72 (79)	33 (75)	46 (75)
Gliomatosis cerebri,			
Yes	3 (3)	2 (5)	2 (3)
No	88 (97)	42 (95)	59 (97)
Necrosis,			
Yes	66 (72)	34 (77)	51 (84)
No	25 (28)	10 (23)	10 (16)

Abbreviations: wt, wild type; vIII, vIII mutant; CW, cortical and/or white matter involvement; E, ependymal involvement.

*All values expressed as *n*(%).

percentages of patients who had leptomeningeal disease, multifocal disease, or gliomatosis cerebri, irrespective of EGFR or EGFRvIII expression status (Table 2), and these variables affected overall survival similarly within EGFR expression categories.

Effect of Epidermal Growth Factor Receptor or EGFRvIII on Survival. In contrast to previously published reports, overexpressed wild-type EGFR or EGFRvIII was not an independent predictor of overall survival and did not confer a worse prognosis (Fig. 1). Both the crude and adjusted rate ratios for the overexpressed EGFR and the EGFRvIII were not significant at $P > 0.5$ compared with the negative EGFR group. The adjusted rate ratio for the overexpressed only EGFR was 1.06 (95% CI, 0.68-1.63) and for EGFRvIII was 1.07 (95% CI, 0.72-1.60). This adjustment was done in a multivariate Cox model analysis as noted under the statistical section. However, in patients surviving for ≥1 year, the expression of EGFRvIII was a negative prognostic indicator (Fig. 2) with $P < 0.0001$ by the log-rank test comparing EGFRvIII with all others. The rate ratio for EGFRvIII after adjustment for all the other cofactors including age and KPS in a multivariate analysis was 4.34 (95% CI, 2.21-8.51). The 1-year cut off was chosen based on the survival curve, as well as the rescaled Schoenfeld residuals, which showed that the hazard ratio didn't significantly differ from one until 1 year after the surgery.

Established Prognostic Factors. Our results were consistent with prior findings that showed that advanced age has a negative prognostic effect on survival (Table 3). Furthermore, there was a trend towards a negative effect on survival in patients ages under 40 years among EGFR expression groups (the median overall survival for nonexpressing, overexpressed only, and EGFRvIII patients was 3.48, 2.22, and 1.82 years, respectively, but this trend was not statistically significant). This effect was not apparent in the ≥40 years group. The age-EGFR interaction was not significant in the Cox hazard model. KPS score was found to be an independent prognostic indicator by both univariate and multivariate analysis in our study, consistent with previous studies that have validated KPS as a prognostic indicator in glioblastoma multiforme patients. Radiographically visualized necrosis was not statistically more common in EGFRvIII-expressing tumors ($P = 0.14$). The percentages of patients that are nonexpressing, overexpressed wild-type EGFR, or EGFRvIII who also had tumor necrosis on the presenting MR image were 72%, 77%, and 84%, respectively. Such necrosis was found to be a negative prognosticator by univariate and multivariate analysis adjusting for all other cofactors ($P = 0.002$ and $P = 0.04$, respectively across groups).

DISCUSSION

To our knowledge, this study reports the largest series of similarly treated glioblastoma multiforme patients analyzed for the prognostic value of the overexpressed EGFR and the mutant EGFRvIII, employing both univariate and multivariate analysis to account for *confounding* variables. In contrast to earlier reports, overexpressed wild-type EGFR or EGFRvIII did not effect median overall survival. In a study by Shinojima et al. (18), the authors concluded that EGFR amplification in glioblastoma multiformes was associated with shorter patient survival. However, the study population was a heterogeneous group of patients who underwent a wide variety of treatments including gross-total resection, partial resection, and biopsy. We have previously shown that the extent of surgical resection is an independent predictor of survival in glioblastoma multiforme patients (4). Our study evaluated the effect on survival of EGFR and EGFRvIII expression by glioblastoma multiformes in a similarly treated patient population (i.e., with respect to gross-total resection). We cannot exclude the possibility that tumor

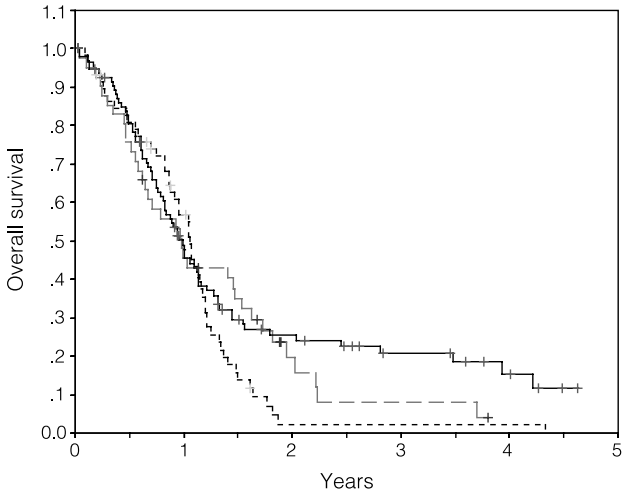

Fig. 1 Kaplan-Meier estimates of overall survival in glioblastoma multiforme patients who underwent gross-total resection followed by standard-of-care radiation therapy. Patients with tumors not expressing the EGFR ($n = 91$; *solid black line*), expressing amplified EGFR ($n = 44$; *dashed grey line*), and expressing EGFRvIII ($n = 61$; *dotted black line*) had median overall survival times of 0.96 year (95% CI, 0.81-1.11), 0.98 year (95% CI, 0.68-1.28), and 1.07 years (95% CI, 0.98-1.16), respectively, which were not statistically significantly different.

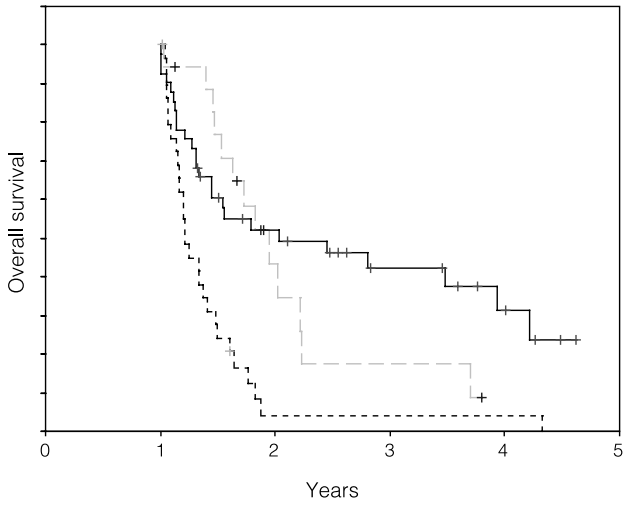

Fig. 2 Kaplan-Meier estimates of overall survival in patients who underwent gross-total resection and radiation therapy and have survived for >1 year. Patients with tumors not expressing the EGFR (n = 38; *solid black line*), expressing amplified EGFR (n = 19; *dashed grey line*), and expressing EGFRvIII (n = 32; *dotted black line*) had median overall survival times of 2.03 (95% CI, 0.50-3.56), 2.02 (95% CI, 1.58-2.46), and 1.21 (95% CI, 1.06-1.36), respectively.

expression of EGFR or EGFRvIII may affect overall survival in patients with unresected or partially resected glioblastoma multiformes. The observed lack of prognostic effect may indicate a bias toward patients who can undergo surgery. Patients who are deemed ''unresectable'' because of extensive tumor invasion and multifocality of disease may be more likely to have the overexpressed EGFR or the EGFRvIII. The studies that have previously shown a prognostic effect of EGFR overexpression by glioblastoma multiformes have included patients who have undergone subtotal resections and biopsy. Laboratory investigation has shown that the EGFRvIII has transforming capabilities and increases glioma invasiveness (22, 23). This disparity between laboratory findings of increased virulence in EGFRvIII-positive gliomas and the lack of effect of EGFR or EGFRvIII expression by glioblastoma multiformes on overall survival seen in our current study can be resolved based on our selection of a subcategory of patients (i.e., those that can undergo gross-total resection). Alternatively, it is possible that EGFR and EGFRvIII are important cofactors and that an additional factor or factors must be present for the negative effect of EGFR expression to manifest itself.

Our study showed that for patients who survive for ≥1 year, the presence of EGFRvIII is an independent negative predictor of survival. This indicates that although EGFRvIII does not affect median survival time, it may have an effect on the overall survival of the subpopulation of glioblastoma multiforme patients who survive for >1 year. This is a small number of patients (n = 19 for those with overexpressed only EGFR, and n = 32 for EGFRvIII-positive patients), and the effect on this subpopulation was not reflected in the median overall survival. Given the relatively small number of glioblastoma multiforme patients who are reported to survive beyond 1 year, this finding may not be discernable or reproducible in other studies.

Simmons et al. (14) showed that EGFR overexpression was an unfavorable prognostic factor in patients who were <55 years old and whose TP53 status was normal. In our study, the median overall survival times for patients not >40 years old whose tumors expressed either no EGFR, overexpressed EGFR, or EGFRvIII were 3.48, 2.22, and 1.82 years, respectively, although this trend was not statistically significant. Thus, our data corroborate that in younger patients, overexpressed EGFR and EGFRvIII may have greater prognostic effect. Furthermore, in the study by Shinojima et al. (18), age bias was likely because 97% of their patients were <70 years old. This age bias may have been sufficient to influence their conclusion that EGFR overexpression affects survival. The differential effect of EGFR within different age groups needs further investigation.

Finally, this article addresses the natural history of glioblastoma multiforme based on tumor EGFR and EGFRvIII expression in patients capable of undergoing gross-total resection. EGFR and EGFRvIII expression have been shown to increase the infiltrative and invasive properties of glioma cells (22, 23). Therefore, one could hypothesize that patients expressing these markers may be more likely to present with leptomeningeal disease, multifocal disease, ependymal dissemination, or gliomatosis cerebri. There was a trend toward increased ependymal involvement in the tumors expressing EGFR (46%) and EGFRvIII (48%) compared with tumors not expressing EGFR (34%), but this was not statistically significant. This may be largely because our study patients were deemed to be surgical candidates and patients with leptomeningeal disease, gliomatosis cerebri, ependymal dissemination, or multifocal disease are less likely to be considered surgical candidates. The incidence of leptomeningeal disease, gliomatosis cerebri, ependymal propagation, or multifocal disease may be more common in patients with EGFR- and EGFRvIII-expressing tumors that are biopsied or subtotally resected, and this has not been

Table 3 Survival of glioblastoma multiforme patients based on EGFR expression category of the tumor

	Median survival (95% CI), y		
Variable	EGFR negative	EGFRwt positive only	EGFRvIII positive
Overall survival	0.96 (0.81-1.11)	0.98 (0.68-1.28)	1.07 (0.98-1.16)
Overall survival in patients surviving for 1 year or more	2.03 (0.50-3.56)	2.02 (1.58-2.46)	1.21 (1.06-1.36)
Ependymal involvement			
No	1.00 (0.83-1.17)	1.63 (1.24-2.02)	1.07 (1.02-1.12)
Yes	0.90 (0.72-10.8)	0.65 (0.54-0.76)	0.95 (0.62-1.28)
KPS score			
>80	1.27 (0.91-1.63)	1.46 (0.85-2.07)	1.09 (0.87-1.31)
≤80	0.75 (0.65-0.85)	0.56 (0.38-0.74)	1.06 (0.88-1.24)
Age (y)			
≤40	3.48 (1.92-5.04)	2.22 (0.24-4.20)	1.82 (1.32-2.32)
41-64	1.00 (0.77-1.23)	1.46 (0.90-2.02)	1.07 (0.88-1.26)
≥65	0.66 (0.41-0.90)	0.56 (0.24-0.88)	1.04 (0.74-1.34)
Necrosis			
No	1.31 (1.00-1.62)	2.22 (0.00-4.55)	1.53 (0.64-2.42)
Yes	0.84 (0.66-1.02)	0.79 (0.45-1.13)	1.07 (1.01-1.13)

Abbreviations: wt, wild type; vIII, vIII mutant.

addressed in the subset of patients studied. This is an additional explanation to the lack of prognostic effect of the overexpressed EGFR or the EGFRvIII on overall survival compared with other studies. Variables previously established as prognostic for glioblastoma multiforme patients such as KPS score, age, and radiographically visualized necrosis were confirmed to be independent prognostic indicators in this study as well.

In conclusion, within the subcategory of patients with glioblastoma multiforme who can undergo gross total resection, the presence of EGFRvIII as a single mutation does not account for poor prognosis; however, simultaneous mutations with prognostic significance have not been addressed in this study. In the subset of these patients who survive for $\geq$1 year, the presence of EGFRvIII is a significant independent negative prognostic indicator.

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