Age-Dependent Prognostic Effects of Genetic Alterations in Glioblastoma

Tracy T. Batchelor,1,2 Rebecca A. Betensky,5 J. Matthew Esposito,1 Loc-Duyen D. Pham,2 Molly V. Dorfman,1,2 Nicole Piscatelli,2 Sarah Jhung,1 David Rhee,1 and David N. Louis1,3,4
1Molecular Neuro-Oncology Laboratory and Departments of 2Neurology, 3Neurosurgery, and 4Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, and 5Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts

ABSTRACT

Purpose: Although the genetic alterations in glioblastoma have been well characterized, reports regarding their prognostic effects have been inconsistent.

Experimental Design: In this series of 140 consecutive cases of glioblastoma treated at a single center, we analyzed the frequency, age dependency and prognostic effects of TP53 mutation, CDKN2A/p16 deletion, EGFR amplification, as well as loss of chromosome 1p, chromosome 10q, and chromosome 19q. The complete set of genetic alterations was available on 60 of 140 patients.

Results: In this cohort of glioblastoma cases, TP53 mutation was significantly associated with patient age. The prognostic effects of TP53 mutation, EGFR amplification, CDKN2A/p16 alterations, and loss of chromosome 1p were dependent on the age of the patient.

Conclusions: This is the first observation that the prognostic effects of TP53, 1p, and CDKN2A/p16 alterations are dependent on patient age. These observations concerning the interactions of age and genetic changes in glioblastoma suggest that tumorigenic pathways to glioblastoma vary with the age of the patient and that future molecular marker studies should carefully evaluate the potential age-dependent prognostic effects of these biological variables. The inconsistent or negative prognostic effects of molecular markers reported in prior studies of glioblastoma may be because different effects at different ages may have resulted in a cancellation of an overall effect in the entire cohort.

INTRODUCTION

Gliomas accounted for 44.4% of the 39,550 primary brain tumors diagnosed in the United States in 2002. Glioblastoma was the most common type of glioma accounting for 51.9% of these tumors (1). Despite aggressive management with surgery, radiation, and chemotherapy, the prognosis for patients with malignant gliomas is poor. In a randomized study of radiation alone versus radiation plus chemotherapy in 674 patients with newly diagnosed malignant gliomas, the median survival in each arm was <1 year (2). However, survival duration does vary among patients with glioblastoma, and some patients survive for periods longer than a year. Younger patients and patients with better initial performance status have longer durations of survival. There is also significant genetic heterogeneity among tumor specimens from different patients with glioblastoma (3).

It has been speculated that the genetic differences among these tumors may also contribute to differences in survival. Mutations of the TP53 gene and amplification and rearrangement of the EGFR gene are common genetic alterations in patients with glioblastoma (4). Studies of the relationship of TP53 and EGFR alterations with prognosis have yielded inconsistent results. Shortcomings of these investigations have included small sample sizes, inclusion of different tumor histologies, and lack of uniform treatment. Nevertheless, the studies to date have suggested that the relationship of genetic alterations and prognosis in patients with glioblastoma is complex and may be a function of the age of the patient (5). In this study, we analyzed 140 consecutive patients with glioblastoma treated at a single institution over the past decade for potential associations of survival with a panel of well-characterized genetic alterations.

MATERIALS AND METHODS

Study Base. The study base was a consecutive series of patients with a histological diagnosis of glioblastoma treated at our institution from 1990 to 2001. Patients were identified from an institutional cancer registry or from the electronic database of the Massachusetts General Hospital Brain Tumor Center. There were 140 cases identified for which archival paraffin-embedded tumor tissue could be identified and retrieved. A total of 133 of 140 cases were assessed for EGFR amplification and 134 of 140 cases were analyzed to TP53 mutations, whereas a more complete panel of genetic analyses (EGFR, TP53, CDKN2A/p16, 1p status, 10q status, and 19q status) was conducted in 60 of 140 cases.

Clinical Data. Treatment details and outcomes for all patients were retrieved from the electronic database, the medical record, or a national death index. Clinical parameters that were analyzed as potential markers of prognostic significance in-
Molecular Data. Tumor DNA was extracted from microdissected, formalin-fixed, paraffin-embedded sections; constitutional DNA was extracted from blood lymphocytes or from formalin-fixed, paraffin-embedded sections of adjacent, uninvolved brain or other tissues (6). Allelic chromosomal loss was assessed by loss of heterozygosity assays in constitutional DNA/tumor DNA pairs using microsatellite markers on 1p36.3 (D1S2734, D1S199, and D1S508), 19q13.3 (D19S219, D19S112, D19S412, and D19S596), 10q23-24 (D10S185 and D10S2491, near PTEN), and 10q25-26 (D10S587; Refs. 6, 7). Exons 5 through 8 of TP53 gene were screened for mutation by single-strand conformation polymorphism analysis and direct sequencing (8). Homozygous deletion of the CDKN2A/p16 gene was evaluated by comparative multiplex PCR and EGFR gene amplification by differential PCR (9–11).

Statistical Analysis. In the statistical analysis of this data set, Fisher’s exact test was used to test for associations among discrete variables. The Wilcoxon rank-sum test was used to test for associations among discrete and continuous variables. Two-sided tests were used, and the significance level was taken to be 0.05 for the tests of association among genetic variables and between age and clinical and genetic variables, with subsequent Bonferroni correction for multiple comparisons, where applicable. Cox proportional hazards models were fit to test for associations with survival. In these models, age was categorized into the quartiles of its distribution (<46, 46–60, 60–70, >70). Karnofsky performance score (KPS) was dichotomized as >70 versus ≤70, and EOR was dichotomized as biopsy versus complete or partial resection. To assess possible interactions of each genetic variable with age, we fit multivariate Cox models that included genetic variable, categorical age, interactions of categorical age with the genetic variable, and important clinical variables. We then applied a backward elimination procedure for model selection, with a 0.10 P threshold for elimination. Each model was fit on the entire set of subjects who had complete information available for the included variables. Although we do not formally adjust the resultant Ps for the model selection procedure, we are able to confirm similar results based on the full, unselected models.

RESULTS

Demographic data from this cohort of glioblastoma patients are shown in Table 1. The median age of the entire cohort was 60 years, 96% (135 of 140) of the patients were Caucasian, and 72% (101 of 140) of the subjects were male. All patients underwent biopsy (37%) or resection of the tumor (63%), and 95% of patients received standard, external beam radiation therapy. At least 66% of patients also received adjuvant chemotherapy after the conclusion of radiation. Pathological diagnosis was glioblastoma in all patients. There were 8 of 140 (6%) patients who had been diagnosed with lower grade (8 grade II and 2 grade III) astrocytic tumors before the diagnosis of glioblastoma. Among the 74 subjects with information on initial performance status, 59% had KPS ≥70. There were 120 deaths among the 139 subjects with follow-up information, and the median follow-up time was 4.1 years.

The genetic alterations of interest occurred in the following percentages of glioblastoma tumor specimens: allelic loss of 10q, 74.1%; EGFR amplification, 36.1%; CDKN2A/p16 deletion, 32.8%; loss of 19q, 23.7%; TP53 mutations, 18.7%; and loss of 1p, 12.7%. Potential associations between these different genetic alterations were assessed, and the following significant relationships were observed. Loss of 1p and loss of 19q were positively associated (P = 0.051), as were loss of 10q and EGFR amplification (P = 0.037) and CDKN2A/p16 deletion...
and EGFR amplification (P = 0.006). In contrast, TP53 mutation and EGFR amplification were negatively associated (P = 0.002). Only the latter association is significant at the 0.05 level after Bonferroni correction for multiple comparisons.

Univariate analyses of nongenetic and genetic factors are listed in Table 2. Nongenetic factors that were significantly associated with prognosis included age and EOR. Older age was associated with a significant increased hazard of death. Each 10-year increment in age at diagnosis was associated with a 57% increase in the hazard of death (P < 0.0001). Age > 60 years was associated with a 2.51-fold increase in the hazard of death. Subtotal or gross total resection versus biopsy was significantly associated with improved survival (hazard ratio = 0.61, P = 0.009). KPS > 70 was not found to be associated with improved survival (hazard ratio = 0.86, P = 0.57). On the basis of prior reports of the prognostic significance of both of these factors in patients with glioblastoma, these clinical parameters were initially included in all multivariate models.

The clinical parameter most closely associated with age at diagnosis was KPS; subjects with KPS > 70 tended to be younger at diagnosis than those with lower KPS (P = 0.033). The genetic marker most closely associated with age at diagnosis was TP53 mutation; subjects with TP53 mutation tended to be younger at diagnosis compared to those without TP53 mutation (P = 0.05). Neither of these associations is significant upon Bonferroni adjustment of the significance levels for the four clinical comparisons and eight genetic comparisons.

None of the genetic markers (TP53, EGR, CDKN2A/p16, 1p, 10q, 19q, 1p + 19q, and 10q+EGFR) was associated with survival on univariate analysis in this cohort of subjects with glioblastoma. Loss of heterozygosity (LOH) on chromosome 1p (LOH 1p) came closest to statistical significance (P = 0.18, unadjusted). However, there were only 4 deaths among the 8 of 63 patients with LOH 1p. In addition, no associations between the genetic markers and survival were found when controlling for EOR and KPS.

In multivariate analyses of age and genetic alterations, controlling for EOR and KPS, several genetic markers had differential effects on survival based on the age of the patient. In patients > 70 years, TP53 alterations were associated with reduced survival [hazard ratio = 7.54; 95% confidence interval (CI), 2.38–23.87], whereas the opposite was true in patients < 70 years (hazard ratio = 0.84; 95% CI, 0.49–1.42; Fig. 1). The interaction between TP53 mutations and age < 70 years was highly significant (P = 0.001). In patients < 46 years, EGFR amplification was associated with reduced survival (hazard ratio = 2.19; 95% CI, 0.83–5.61), whereas the opposite was true in patients > 46 years (hazard ratio = 0.74; 95% CI, 0.47–1.16; Fig. 2). There was a significant interaction between age < 46 years and EGFR amplification (P = 0.039). The negative prognostic effect of CDKN2A/p16 was more pronounced in patients > 70 years (hazard ratio = 11.48; 95% CI, 1.97–66.78) than in patients < 70 years (hazard ratio = 1.33; 95% CI, 0.66–2.67; Fig. 3). The interaction between CDKN2A/p16 and age < 70 years was significant (P = 0.024). The good prognostic effect of LOH 1p was more pronounced in patients > 60 years (hazard ratio = 0.10; 95% CI, 0.01–0.78) than in patients < 60 years (hazard ratio = 0.91; 95% CI, 0.27–3.02). The interaction between LOH 1p and age < 60 years was marginally significant (P = 0.071). After the backward elimination model selection procedure, only the multivariate models for EGFR amplification and TP53 mutations retained the clinical variable of EOR. KPS was not retained in any model. The 1p and TP53 results should be interpreted cautiously due to sparseness of the data (e.g., only 2 of the 8 subjects with LOH 1p are >60 years, only 1 of these subjects is a death, and only 4 of the 25 subjects with TP53 mutation are >70 years, but all 4 are deaths).

In confirmation of these results, we examined the original full models, excluding the variable of KPS, because it was not a significant univariate predictor (Table 2) and was consistently the first variable to be eliminated in the model selection. The significant interactions found through repeated model fittings described above were retained with Ps of 0.005 for the interaction between TP53 mutations and age < 70 years, 0.015 for the interaction between age < 46 years and EGFR amplification, 0.089 for the interaction between CDKN2A/p16 and age < 70 years, and 0.041 for the interaction between LOH 1p and age < 60 years. Nonetheless, some of these results are based on small numbers and should still be interpreted with care. These analyses are suggestive of an important interactive role of age and genetics and should be repeated in larger, independent studies.

**DISCUSSION**

In this study of 140 consecutive subjects with glioblastoma treated at our institution over 10 years, we observed age-dependent associations between survival and specific genetic alterations: TP53 mutations; allelic loss of 1p and CDKN2A/p16 homozygous deletion, which had not been previously reported; and EGFR amplification, which had been noted in a prior report. In accordance with other studies, age was an important prognostic factor in our series (5). The frequencies of most genetic alterations observed in this study were comparable with previously reported series (4). However, our figures for TP53 mutations and 10q loss were lower than what has been reported. The
remarkable findings in our series related to the striking age-dependent prognostic effects of specific genetic alterations in glioblastoma. There were statistically significant interactions between age and genetic alterations in TP53, CDKN2A/p16, EGFR, and chromosome 1p. In our series, TP53 mutations and CDKN2A/p16 alterations had negative prognostic effects in older patients, whereas EGFR amplification and LOH 1p had positive prognostic effects in older patients. Moreover, for EGFR and TP53, the effects were in opposite directions in the older versus younger patients. EGFR amplification had negative prognostic effects in younger patients versus positive prognostic effects in older patients, whereas TP53 mutations had positive prognostic effects in younger patients and negative effects on prognosis in older patients.

**Fig. 1** Kaplan-Meier curves comparing survival with respect to TP53 mutation by age at diagnosis <70 and >70 years. (---) represents subjects without a TP53 mutation, and (· · · ) represents subjects with a TP53 mutation.

**Fig. 2** Kaplan-Meier curves comparing survival with respect to epidermal growth factor receptor (EGFR) amplification by age at diagnosis <46 and >46 years. (---) represents subjects without EGFR amplification, and (· · · ) represents subjects with EGFR amplification.
Although there are conflicting results regarding the prognostic significance of TP53 and EGFR amplification in subjects with glioblastoma, many large studies have found no association between these common genetic alterations and survival, consistent with the results of our investigation. However, with rare exception, the possibility of age-dependent prognostic effects of different genetic alterations was not addressed in prior studies. Consistent with the observations from our study, a previous genetic study of 110 glioblastoma subjects demonstrated an age-dependent prognostic effect of EGFR amplification (5). EGFR amplification was associated with better prognosis in older patients and worse prognosis in younger patients.

Age-dependent occurrence and effects of different biological markers have been reported in breast cancer, gastric cancer, and thyroid cancer (12, 13). For example, associations between patient age and tumor grade, mitotic index, Ki-67 (MIB-1) labeling, apoptotic indices, EGFR expression, and ErbB2 expression have been reported in breast cancer. A correlation has also been noted between estrogen receptor positivity and patient age (14), and BRCA1 expression has been found to be age-dependent (15, 16). Older age is associated with slower growth and fewer metastases in patients with breast and prostate carcinoma (12). In contrast to the positive prognostic effect of age in patients with breast and prostate carcinoma, older age is associated with more aggressive clinical behavior in patients with differentiated thyroid carcinoma (13) and glioblastoma. It is conceivable that the age-dependent clinical behavior of these tumors is a reflection of underlying age-dependent genetic and hormonal differences between these tumors.

There has been limited study of the possible age dependency of genetic events in patients with malignant gliomas. In a study of 80 tumor specimens from patients with anaplastic astrocytoma, comparative genomic hybridization was done to assess the relationship between cytogenetic alterations and clinical parameters (17). Age-dependent cytogenetic alterations were observed with +7p, +19, and −4q occurring more commonly in older patients, whereas −11p occurred more frequently in younger patients. Gain of 7p was a poor prognostic marker, regardless of age, and this alteration occurs more frequently in older patients with anaplastic astrocytoma. Thus, the authors suggest that this cytogenetic alteration (+7p) may underlie the clinical observation that the prognosis of anaplastic astrocytoma is worse in older patients. These investigators did not report significant interactions between age and cytogenetic alterations with respect to prognosis (17).

This series of 140 consecutive glioblastoma cases treated at a single institution represents one of the largest series analyzed for genetic alterations. However, there are several potential limitations regarding the generalizability of our results. We had performance status information on only about half of this patient population. This might explain the observation that, in our series, performance status was not strongly associated with survival, whereas this clinical parameter has been associated with survival in other series of patients with glioblastoma. In order for performance status to confound our results, it must be associated with our main predictor of interest (genetic alterations) and the outcome (survival). In fact, in our cohort, performance status was significantly associated only with EGFR status (P = 0.032): 50% of the 42 subjects with performance status > 70 had EGFR amplification, whereas only 30% of the 91 subjects with performance status ≤ 70 had EGFR amplification. Performance status was not significantly associated with any of the other genetic alterations. Performance status may be related to the location of the tumor, but we did not find any
association between tumor location and specific genetic alterations. Another potential limitation of our study, common to virtually all such studies, is that all patients did not receive uniform treatment during the course of the illness. Radiation and chemotherapy were not administered to all patients. At least 66% of the patients in our series received adjuvant chemotherapy and at most 34% did not. It remains controversial whether adjuvant chemotherapy of any type significantly extends survival in patients with glioblastoma. Although it would be ideal to include patients who received identical treatment in a series such as the present one, this is not practical. Although other series have included patients treated as part of clinical trials in which the same chemotherapy was administered, these studies do not control for subsequent treatments (surgery, radiation, chemotherapy) at relapse (5). Radiation prolongs survival in patients with glioblastoma and response to radiation may be partially determined by the underlying genetic profile of the tumor (18). On the basis of this information, we excluded all patients in whom radiation could not be documented (5%) and reran our analyses. After exclusion of these subjects, the primary outcomes cited above were not affected.

In summary, the prognosis of patients with glioblastoma may be at least partially determined by a complex interaction between age and different genetic alterations. It is possible that the failure to consistently identify prognostic effects of specific genetic alterations in prior studies of patients with glioblastoma may be because such studies did not account for the possibility of age-dependent effects in the analysis or that the sample sizes were too small to detect such effects. To further elucidate these relationships, our observations should be confirmed in future large sample cohorts of glioblastoma patients. Moreover, given the age dependency of these genetic effects, it will be intriguing to analyze gene expression patterns in younger versus older glioblastoma patients to ascertain the status of genes involved in EGFR- and p53-mediated genetic pathways. It could be that the differential survival effects observed with age are because of different patterns of pathway activation or inactivation. Finally, it would be ideal to incorporate prospective genetic profiling and complete clinical information into future clinical trials in which treatment is controlled as much as possible. If these and other observations regarding age and genetics are confirmed, future clinical trials could be restricted to specific genetic subsets of glioblastoma. This would reduce the variability associated with sampling a nonhomogeneous patient population and allow more precise interpretation of study results.

REFERENCES