ERBB1 Is Amplified and Overexpressed in High-grade Diffusely Infiltrative Pediatric Brain Stem Glioma


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ABSTRACT

Purpose: This study was conducted to investigate the incidence of ERBB1 amplification and overexpression in samples of diffusely infiltrative (WHO grades II–IV) pediatric brain stem glioma (BSG) and determine the relationship of these abnormalities to expression and mutation of TP53 and tumor grade.

Experimental Design: After central pathology review, the incidence of ERBB1 amplification and overexpression was determined in 28 samples (18 surgical biopsy and 10 postmortem specimens) of BSG using quantitative PCR and immunohistochemistry, respectively. Mutation and expression of TP53 were also determined in these same samples by direct sequence analysis of microdissected tumor material and immunohistochemistry, respectively. All experimental procedures were performed blind to tumor grade.

Results: Twelve, 9, and 7 tumors were classified as WHO grades II, III, and IV, respectively. A significant increase in ERBB1 expression was observed with increasing tumor grade (P < 0.001). Two grade IV tumors displayed intense membranous ERBB1 expression in 90% of tumor cells in association with high-level ERBB1 gene amplification. One grade III tumor also contained low-level amplification of ERBB1. Six tumors demonstrated TP53 nuclear immunoreactivity, and six contained a mutation in TP53. No correlation was observed between abnormalities in TP53 and either tumor grade or amplification and overexpression of ERBB1.

Conclusions: These data suggest that ERBB1 signaling is important for the development of childhood BSG and is worthy of study as a therapeutic target in this disease. Our data also indicate that the genetics of childhood BSG are complex and include both grade-dependent amplification and overexpression of ERBB1 and grade-independent expression and mutation of TP53.

INTRODUCTION

Over 90% of children with diffusely infiltrative BSG3 succumb to their disease within 2 years of diagnosis (1). These tumors are not amenable to surgical management (2, 3), and there is no evidence that radiation therapy, including hyperfractionated and accelerated regimens (4–6), or chemotherapy (1, 7) improves patient survival. The failure of conventional treatment to reduce the mortality of children with BSG has intensified the search for novel therapeutic approaches for this disease.

The PBTC in the United States recently commenced a Phase I/II study of ZD1839 (Iressa), an inhibitor of the EGFR (ERBB1) tyrosine kinase, in children with high-grade supratentorial astrocytoma and non disseminated diffuse intrinsic BSG. There is considerable evidence that inhibitors of the ERBB1 tyrosine kinase might have therapeutic efficacy against high-grade gliomas. ERBB1 is amplified and overexpressed in up to one-half of adult high-grade gliomas (8–10) and overexpressed, usually in the absence of gene amplification, in ~30% of pediatric nonbrain stem high-grade gliomas (11–14). Furthermore, up-regulation of ERBB1 cell signaling promotes gliomagenesis in mice (15, 16) and an invasive (17, 18) and radioresistant phenotype in human glioma cells (19). Although these data indicate that ZD1839 might be a useful therapy for children with supratentorial high-grade glioma, there are no direct data to support its use in BSG. Therefore, in this study, the PBTC analyzed the incidence of ERBB1 amplification and expression in 28 diffusely infiltrative (WHO grades II–IV) pediatric BSGs. Our data show that ERBB1 is amplified and overexpressed in a significant proportion of high-grade pediatric BSG and support the rationale for treating children with this disease with inhibitors of the ERBB1 tyrosine kinase.

PATIENTS, MATERIALS, AND METHODS

Patients and Tumor Material. With Institutional Review Board approval, formalin-fixed tumor material was collected from 43 children (≤17 years) with a radiological or

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3 The abbreviations used are: BSG, brain stem glioma; PBTC, Pediatric Brain Tumor Consortium; qPCR, quantitative PCR; EGFR, epidermal growth factor receptor; PA, pilocytic astrocytoma; IHC, immunohistochemical.
ERBB1 amplification in tumors was detected using a qPCR-based assay described previously (24). Briefly, DNA was extracted from 10-μm-thick tumor sections by xylene dewaxing and digestion with Proteinase K. Serial m-thick tumor sections were then submitted for further analysis by investigators who were blinded to tumor grade.

**qPCR Analysis.** ERBB1 amplification in tumors was detected using a qPCR-based assay described previously (24). Briefly, DNA was extracted from 10-μm-thick tumor sections by xylene dewaxing and digestion with Proteinase K. Serial m-thick tumor sections were then submitted for further analysis by investigators who were blinded to tumor grade.

**RESULTS**

ERBB1 is amplified and overexpressed in high-grade diffusely infiltrative pediatric BSG. Twelve, 9, and 7 tumors were classified as grade II–IV, respectively (Fig. 1). One oligodendroglioma was identified among the grade II tumors. No significant age or sex difference was observed among patients with different grades of tumor. A significant increase in ERBB1 expression was observed with increasing tumor grade (P < 0.001 for both intensity and percentage of cell ERBB1 expression; Fig. 1). ERBB1 expression was detected at low levels in only 2 of 12 grade II tumors (#4 and 12). In keeping with these expression data, no grade II tumor contained an amplified ERBB1 gene (Fig. 1). In contrast, ERBB1 protein was detected in 7 of 9 grade III tumors, including 4 with moderate levels of protein expression in 10–30% of tumor cells (#14, 16, 18, and 21) and three with low-level expression in 30–90% of tumor cells (#13, 15, and 17). Tumor #15 also contained low-level ERBB1 gene amplification (ERBB1:NACH = 2.8). ERBB1 protein was detected in all grade IV tumors; three of these displayed intense membranous ERBB1 immunoreactivity (#24–26). In two of these cases, ERBB1 was expressed in 90% of tumor cells in association with high-level ERBB1 gene amplification (ERBB1:NACH > 5; Figs. 1 and 2).

Together, these data demonstrate that around one-half of diffusely infiltrative pediatric BSGs are high-grade (grade III or IV) tumors and that a significant proportion of these contain amplification and/or overexpression of ERBB1. Although the six grade I BSGs that were identified during central pathology review were excluded from the formal study, none of these cases contained detectable ERBB1 amplification or expression. These data also support the hypothesis that ERBB1 amplification and overexpression is restricted to high-grade BSG.

TP53 Is Frequently Mutated in Pediatric BSGs. Mutations in TP53 are relatively uncommon in primary adult high-grade
grade gliomas, but high-level expression and/or mutation of TP53 affects over one-third of supratentorial and brain stem high-grade gliomas in children (11, 14, 22, 26). Six tumors in the current study demonstrated TP53 nuclear immunoreactivity (Fig. 1). A trend toward an increase in TP53 protein expression was observed among grade III and IV BSGs, although the small numbers of cases involved precluded formal statistical analysis. Mutations in TP53 were detected in 6 of 16 analyzed tumors. These included two grade II tumors (#6, del exon 6; #7, Arg175His), three grade III tumors (#13, del exon 5; #15, Leu252Phe; #18, Tyr126Cys), and one grade IV tumor (#24, Arg248Trp).

**DISCUSSION**

With an incidence of \( \sim 7 \times 10^6 \) person years, BSGs account for 20% of all childhood brain tumors (27). This disease has been subclassified as either focal or diffusely infiltrative (21). The latter carries a particularly dismal prognosis, with patients rarely surviving beyond 2 years (1, 4–6, 28). Efforts to identify causative molecular abnormalities that might serve as therapeutic targets in BSG have been severely restricted by a lack of tumor material. Here, we report the largest molecular analysis of diffusely infiltrative BSG performed to date and show that ERBB1 amplification and TP53 mutation were observed concurrently in two tumors (#15 and 24).

**Fig. 1** BSG clinical and genetic data. Cases are numbered consecutively (1–28) through three groups separated by tumor grade (WHO II-IV). Clinical variables include: sex (M, male; F, female), age (years), method of tumor retrieval (B, surgical biopsy; P, postmortem), and pathology (A, diffuse astrocytoma; O, oligodendroglioma; AA, anaplastic astrocytoma; GB, glioblastoma). The results of ERBB1 IHC are shown by intensity of immunoreactivity (■, negative; ±, +; +, ++; +++, +++++) and graphically as the percentage of immunopositive tumor cells. The results of qPCR analysis of ERBB1 amplification (Amp) are summarized as □, normal; ■, amplified. The results of TP53 IHC are summarized on a scale of 1–4 (see “Patients, Materials, and Methods” section; 0 or 1; 2, 3, 4). The results of TP53 mutational analysis (Mut) are summarized as □, normal; ■, mutant. Tumors with insufficient material for analyses are shown as missing squares.

The hypothesis that ERBB1 signaling is important for the development of childhood BSG and is worthy of further study as a therapeutic target in this disease.

Genetic abnormalities in adult high-grade gliomas vary among a number of distinct tumor subgroups (29). Primary high-grade gliomas (primary glioblastoma) arise de novo in older patients and frequently contain an amplified and rearranged ERBB1 locus, deletion of INK4A/ARF, loss of PTEN, and intact TP53 (8–10, 30–34). In contrast, secondary glioblastomas that progress from lower grade tumors in younger patients rarely contain an amplified ERBB1 locus but frequently contain mutations in TP53 (30–32, 34, 35). Tumors with combinations of these abnormalities, including some containing concurrent mutation of TP53 and amplification of ERBB1, have also been described (11, 34). Far less is known about genetic abnormalities in pediatric glioma. Some studies indicate that nonbrain stem pediatric high-grade gliomas are genetically similar to adult secondary glioblastoma. In this regard, although ERBB1 amplification is rarely detected in pediatric high-grade gliomas (\( n = 2 \) of 119 cases in the literature), mutation of TP53 occurs relatively frequently (11, 13, 14, 36). However, ERBB1 overexpression has been identified in a significant proportion of childhood high-grade gliomas (12, 13), and the precise pattern of genetic abnormalities within pediatric gliomas remains to be determined. Our study suggests that the genetics of pediatric BSG are complex and include grade-dependent amplification...
and overexpression of ERBB1 and grade-independent expression and mutation of TP53. The ERBB1 primers and antibody used in our study do not distinguish between the wild-type and truncated, constitutively active ERBB1 (EGFRvIII). EGFRvIII is present in 50% of adult glioblastomas that contain an amplified ERBB1 locus (9, 10). Therefore, additional analyses are required to establish whether the EGFRvIII mutation also occurs in pediatric BSG.

Although the precise role of ERBB1 in gliomagenesis remains to be established, evidence indicates that cell signaling via this receptor promotes an aggressive phenotype, e.g., ERBB1 mediates glioma cell resistance to radiation and chemotherapies (19, 37–39) and increases the motility and invasive capacity of glioma cells (18, 40). Taken together with the results of the current study, these data suggest that inhibitors of the ERBB1 tyrosine kinase represent an attractive new therapeutic approach for childhood BSG.

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