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Correlation of preoperative seizures with clinicopathological factors and prognosis in anaplastic gliomas: A report of 198 patients from China

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ABSTRACT

Purpose: Seizures are a common manifestation of many diseases and play an important role in the clinical presentation and quality of life (QOL) in patients with gliomas. The purpose of the present study was to investigate the possible correlation between tumor-related seizures and clinicopathological factors that influence preoperative seizure characteristics and relevant survival outcomes.
Methods: We retrospectively investigated the correlation of preoperative seizures with clinicopathological factors and prognosis in a cohort of 198 Chinese patients with anaplastic gliomas. Univariate and multivariate logistic regression analyses were used to identify factors associated with preoperative seizures. Survival function curves were calculated using the Kaplan–Meier method.
Results: Of the 198 patients, 68 (34.3%) patients had preoperative seizures. Among the patients with seizures, 26 (38.2%) had generalized seizures, 38 (55.9%) had simple partial seizures, and four (5.9%) complex seizures. There was a higher proportion of epidermal growth factor receptor (EGFR) amplification, frontal lobe involvement, left cerebral hemisphere involvement, and lower Ki-67 expression in patients with preoperative seizures in both univariate and multivariate analyses. Patients with preoperative seizures had a longer overall survival (OS) time compared with those without (median: 1924 days vs. 923 days, $P = 0.048$).
Conclusion: The current study updates existing information on tumor-related seizures and clinicopathological factors in anaplastic gliomas, and suggests two putative biomarkers for preoperative seizures; Ki-67 expression and EGFR amplification. These factors may provide insights for developing effective treatment strategies aimed at prolonging patient survival.

1. Introduction

The World Health Organization (WHO) classifies gliomas into four grades, of which anaplastic astrocytoma, oligodendroglioma, and oligoastrocytoma constitute the majority of grade III brain tumors.[1] In the United States, glioblastomas account for approximately 60–70% of malignant gliomas, anaplastic astrocytomas for 10–15%, and anaplastic oligodendrogliomas and anaplastic oligoastrocytomas, 10%.[1,2] In China, the percentage of anaplastic gliomas is much higher.[3] Prognosis of anaplastic gliomas is influenced by age, symptom duration, mental health status, and Karnofsky performance status.[4] Based on a large database of clinical trials, the Radiation Therapy Oncology Group (RTOG) defined six prognostic classes. Classes I–III describe anaplastic astrocytoma, while classes IV–VI define glioblastomas.[5,6] A previous analysis illustrated the diversity in outcome of patients diagnosed with anaplastic gliomas.[4]

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Seizures are the most common presenting symptom in patients with gliomas.[7–10] When uncontrolled, tumor-related epilepsy has a significant impact on patients' QOL, causes cognitive deterioration, and may result in significant morbidity.[11–14] The persistence of seizures and the use of anti-epileptic drugs (AEDs) may negatively influence QOL and cognitive function.[11,13] More than one third of patients with anaplastic gliomas experience epileptic seizures at disease onset,[15–19] and not all anaplastic gliomas are associated with seizures, despite having similar histology and tumor location. It is likely that variability in the presence of seizures cannot be explained entirely by tumor-related factors, but instead may be a complex interaction between tumor-related and genetic factors. Although, to date, some susceptibility candidate genes associated with tumor-related seizures have been identified in low-grade gliomas,[20,21] few studies have focused on anaplastic gliomas.

To investigate molecular genetic profiles that might correlate with the presence of seizures, and the possible correlation between tumor-related seizures and survival outcome in Chinese patients with anaplastic gliomas, we retrospectively analyzed a series of 198 gliomas, including 39 anaplastic oligodendrogliomas, 103 anaplastic oligoastrocytomas, and 56 anaplastic astrocytomas, for clinicopathological factors and survival, with respect to tumor-related seizures.

2. Methods

2.1. Patients

We retrospectively identified all patients (>18 years old) with anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic oligoastrocytoma (AOA) from the Chinese Glioma Genome Atlas (CGGA), who underwent surgical resection at the Glioma Treatment Center of Beijing Tiantan Hospital from January 2006 to July 2012. Histological diagnosis was reaffirmed by two independent neuropathologists and graded according to the WHO classification.[1] Cases with discrepancies were re-reviewed by another pathologist until a consensus was reached. Clinical data, including patient's age at diagnosis, sex, presenting symptoms, preoperative Karnofsky performance status (KPS) score, and operation status, were obtained from medical records. OS time, defined as the period from surgery to death, was collected when patients visited the clinics and in phone interviews with patients and/or their relatives. Progression-free survival (PFS) was defined as the time of surgery until radiographic progression (the appearance of a new lesion or an increase in tumor size of ≥25%). Patients who were lost to follow-up or who died of non-primary diseases were excluded. This study was approved by the Ethics Committee of Beijing Tiantan Hospital, and written informed consent was obtained from all patients.

2.2. Preoperative seizure characteristics

Data on seizure characteristics included date of seizure onset and type of seizure (simple partial, complex partial, and generalized seizures). The study population was divided into two groups based on preoperative seizure status.

2.3. Tumor location

Tumor location was considered to be the lobe or region of the brain within which the bulk of the glioma resided. These assignments were based on an evaluation of medical records and imaging study results, mainly MRI characteristics including tumor side (left, right, or bilateral) and specific lobe involvement (frontal, temporal, parietal, occipital, and insula).

2.4. Samples

Tumor tissue samples were obtained by surgical resection before treatment with radiation and/or chemotherapy. Resected specimens were snap-frozen and stored in liquid nitrogen until DNA extraction or paraffin-embedding.

2.5. DNA extraction

Genomic DNA was isolated from frozen tumor tissues using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentration and quality were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Houston, TX, USA).

2.6. Molecular evaluations

All data from the CGGA for which biomaterial was available, IDH mutation status (pyro-sequencing for IDH1/2 mutation), MGMT promoter methylation (DNA pyro-sequencing), and Ki-67 expression level (immunohistochemistry) were assessed according to routine methods.[22,23]

2.7. Detection of 1p/19q deletion by fluorescence in situ hybridization (FISH)

The 1p/19q fluorescent probe kit (Vysis Inc., Downers Grove, IL, USA) was used for FISH. Briefly, 4-mm-thick paraffin slides were deparaffinized, dehydrated, and incubated in 1 mol/L NaSCN for 35 min at 80 °C. Slides were then immersed in pepsin solution (0.65% in protease buffer with 0.01 mol/L HCl) for 10 min at 37 °C, and tissues were fixed with 10% neutral buffered formalin. Then, the specimens were dehydrated in an ethanol series (70%, 85%, and 100%, 2 min in each bath) and air-dried, 20 μl of each probe was added separately, and slides were sealed with rubber cement. After co-denaturation for 10 min at 75 °C, the slides were then placed in a humidified atmosphere with Hybrite (ThermoBrite, Vysis) for 16 h at 37 °C. Slides were immersed first in 2× SSC/0.3% NP-40 for 2 min at RT (room temperature) and then in 2× SSC/0.3% NP-40 for 2 min at 73 °C. After drying, nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and anti-fade compound (p-phenylenediamine). FISH signals for each locus-specific FISH probe were assessed under an Olympus BX51TRF microscope (Olympus, Ina-shi, Nagano, Japan) equipped with a triple-pass filter (DAPI/Green/Orange; Vysis). The assessment and interpretation of FISH results were made according to guidelines defined by the SIOP Europe Neuroblastoma Pathology and Biology and Bone Marrow Group.[24] For each probe, more than 100 non-overlapping nuclei were enumerated per hybridization. Tumors with more than 30% of nuclei showing DNA loss were defined as tumors with chromosomal loss.

2.8. Analysis of EGFR amplification by fluorescence in situ hybridization (FISH)

A probe for the *EGFR* locus (7p11.2), labeled with Texas Red, was used in combination with a fluorescein isothiocyanate-labeled centromeric probe for chromosome 7. The procedure was performed according to the manufacturer's instructions (Cytocell, Cambridge, UK). The tissue section was incubated at 56 °C overnight, deparaffinized in xylene, hydrated in 100% and 70% ethanol, and treated with 0.1 M HCl at RT for 20 min. After incubation in the pretreatment reagent (Abbott Molecular Inc., Des Plaines, IL, USA) at 80 °C for 30 min, the section was digested with pepsin (2 mg/mL in 0.01 M HCl) at 37 °C for 10−20 min and dehydrated in an ethanol series. The probe set