Clinical Neurology and Neurosurgery 151 (2016) 31–36

Contents lists available at ScienceDirect

Clinical Neurology and Neurosurgery

journal homepage: www.elsevier.com/locate/clineuro

IDH mutation and MGMT promoter methylation are associated with the pseudoprogression and improved prognosis of glioblastoma multiforme patients who have undergone concurrent and adjuvant temozolomide-based chemoradiotherapy

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ARTICLE INFO

Article history:
Received 3 August 2016
Received in revised form 4 October 2016
Accepted 9 October 2016
Available online 12 October 2016

Keywords:
Glioblastoma
Chemotherapy
Pseudoprogression
Molecular genetics
IDH mutation
MGMT

ABSTRACT

Purpose: This study aimed to investigate the potential association between IDH mutation and O^6–methylguanine methyl transferase (MGMT) gene promoter methylation and pseudoprogression disease (psPD) in glioblastoma multiforme (GBM) patients after concurrent temozolomide (TMZ)-based chemoradiotherapy.
Methods: A total of 157 GBM patients who received concurrent TMZ-based chemoradiotherapy were included in this retrospective study. The association between psPD and a number of demographic and genetic factors, including IDH mutation and MGMT promoter methylation, were analyzed based on logistic regression, Cox regression, and multivariate analysis.
Results: Of the 157 GBM patients, 145 (92.36%) patients, including 38 patients with psPD, 38 patients with early progression (ePD), and 69 patients with non-progression (non-PD), were followed up for six to 56 months. We identified a higher rate of MGMT promoter methylation and IDH1 mutation in psPD patients compared with ePD patients ($P = 0.002$). In addition, MGMT promoter methylation and IDH1 mutation predicted a high probability of psPD development in GBM patients ($P = 0.001$ and $P < 0.001$, respectively). MGMT promoter methylation, IDH1 mutation, Karnofsky performance score (KPS) $\geq$70, and psPD were associated with a significantly longer overall survival of GBM patients ($P = 0.001$, 0.001, 0.002, and $P < 0.001$, respectively). Both of MGMT promoter methylation and IDH mutation had a cumulative effect on the OS of GBM patients. GBM patients with psPD (39.2 ± 2.1 months, $P < 0.001$) had a longer median survival (MS) than GBM patients with ePD (11.9 ± 1.1 months) or with non-PD (24.4 ± 2.4 months).
Conclusion: MGMT promoter methylation and IDH1 mutation were associated with PsPD and predicted a longer median survival in GBM patients after TMZ-based chemoradiotherapy. Genetic analyses of the MGMT promoter and IDH1 may allow us to effectively treat GBM patients.

1. Introduction

Glioblastoma multiforme (GBM), which accounts for approximately 54% of all gliomas, is one the most fatal diseases with a limited survival time. Only one-third of GBM patients survive for one year and less than 5% of GBM patients live more than five years [1]. Concurrent and adjuvant temozolomide (TMZ)-based chemoradiotherapy, followed by six months of TMZ maintenance therapy is the current standard strategy for the treatment of GBM patients up to 70 years of age [2,3]. However, it has been recently reported that GBM patients who have undergone concurrent and adjuvant TMZ-based chemoradiotherapy have a high likelihood of developing pseudoprogression disease (psPD) [4,5]. In previous reports, the incidence of pseudoprogression in GBM patients undergone adjuvant TMZ-based chemoradiotherapy ranged from 19% to 33% [5–9].

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http://dx.doi.org/10.1016/j.clineuro.2016.10.004
0303-8467/

Therefore, the prediction and early diagnosis of psPD are important for the prognosis of patients with GBM.

PsPD exhibits contrast enhancement, which is similar to tumor progression based on magnetic resonance imaging (MRI), leading to the misdiagnosis of tumor recurrence and/or radiation necrosis [4]. According to the response assessment in Neuro-Oncology criteria [10], tumor progression can only be diagnosed if the majority of the new contrast enhancement is outside of the radiation field or if there is pathologic confirmation of progressive disease (PD) within the first 12 weeks after completion of radiotherapy. The incidence of psPD at different times after chemoradiotherapy remains controversial [8]. When contrast enhancement was detected by radiological examination, GBM patients frequently underwent unnecessary salvage surgery or biopsy due to misdiagnosis. Therefore, it is critically important to identify novel methods that can better discriminate psPD from early progression disease (ePD) to avoid unnecessary and potentially harmful surgical interventions.

Some molecular markers may be useful for the evaluation and prediction of psPD in patients with GBM. It has been reported that promoter methylation of the O^6–methyl-guanine methyl transferase (MGMT) gene is associated with improved overall survival (OS) of GBM patients and the development of psPD [5,11]. Overexpression of P53 [12] and IDH1 mutation [13] have also been associated with the development of psPD. Typically, a number of demographic values and genetic factors, such as young age, secondary GBM, and 1p/19q loss, are associated with a longer OS of patients with GBM. It has been reported that GBM patients with psPD have a longer OS than those without psPD [5,8,9,14]. However, it remains unclear whether and what demographic values or molecular markers can be used predict the occurrence of psPD in patients with GBM.

This aims of this cohort study were to identify and evaluate genetic markers to predict development of psPD in GBM patients who had undergone concurrent and adjuvant TMZ-based chemoradiotherapy. Our results suggest that IDH mutation and promoter methylation of the MGMT gene can be used to predict the development of pseudoprogression and an improved prognosis of glioblastoma multiforme patients who have undergone concurrent and adjuvant TMZ-based chemoradiotherapy.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Medical Ethics Committee of Beijing Tiantan Hospital of the Capital Medical University, and Navy General Hospital. The experiments followed the principles of the Declaration of Helsinki and the rules of Good Clinical Practice. Informed consent was provided by all patients included in the study.

2.2. Demographic characteristics of the patients

Three hundreds and eighty-seven patients with histopathologically diagnosed GBM in the Department of Neurosurgery, Beijing Tiantan Hospital and Navy General Hospital, between 2010 and 2013 were recruited for this study. After surgical resection of tumors, all patients received a standard Stupp regimen: concurrent chemoradiotherapy with daily TMZ (75 mg/m^2/d), followed by six cycles of TMZ maintenance therapy (150 to 200 mg/m^2 for five days every 28 days). The first magnetic resonance imaging (MRI) was performed one month after the surgery if no clinical symptoms related to tumor progression were observed. Recent contrast enhancement after total resection or volume-increased contrast enhancement after subtotal resection based on MRI performed within three months was observed in patients that included pseudoprogression (psPD) and early real progression (ePD). Radiological stabilization within three months was defined as non-progression disease (non-PD). The radiological enhancement, which was stable in subsequent follow-ups or pathologically confirmed as necrosis by biopsy of multiple areas, was defined as psPD. Otherwise, radiological enhancement was considering ePD. Patient age, sex, MGMT promoter methylation, epidermal growth factor receptor (EGFR) amplification, phosphatase and tensin homolog (PTEN) deletion, IDH mutation, loss of 1p19q, presence or absence of pseudoprogression, PFS, and OS were collected for data analysis.

2.3. Determination of IDH1/2 mutation and MGMT promoter methylation

IDH1/2 mutations were identified by direct DNA sequencing according to a previous report [15]. MGMT promoter methylation was analyzed by methylation-specific polymerase chain reaction (MSP), as described in a previous study [16].

2.4. Immunohistochemical staining

5 μm sections were prepared. After deparaffinization and rehydration, the slides were digested with pepsin at 37 °C for 10 min or boiled in 10 mM citrate buffer (pH 6.0) for 12 min to expose the antigen epitope of EGFR, PTEN, respectively. Endogenous peroxidase was blocked with 3% aqueous hydrogen peroxide. The sections were incubated with primary antibody at 4 °C overnight. Then the sections were washed with PBS for 3 times and incubated with the secondary antibody at 37 °C for 20 min. The antibodies were then detected with diaminobenzidine as a chromogen. The slides were counterstained with hematoxylin. The immunohistochemical staining results were evaluated using Image Pro-Plus software (version 6.1) as described in the previous study [17]. The expression levels of a number of proteins were evaluated based on the percentage of immunopositive cells. Negative staining was defined as positive staining of <30% tumor cells, and positive staining was defined as positive staining of ≥30% tumor cells (Fig. 1). The primary antibodies used for immunohistochemical staining were anti-EGFR (1:100, Invitrogen, CA, USA) and anti-PTEN (1:150, Lab Vision, CA, USA).

2.5. Statistical analyses

SPSS 13.0 (SPSS Inc., Chicago, Illinois) was used for the statistical analyses. Categorical variables were reported as percentages. Continuous variables were presented as means ± standard deviations. Tumor progression was defined according to MacDonald's Criteria as a 25% increase in the tumor size or the appearance of new lesions. Overall survival (OS) was measured from the initial pathological diagnosis of GBM to disease progression or death, or the date of last follow-up. Data for patients who survived until the end of the observation period were censored at the final follow-up. The Kaplan-Meier method was employed to compare OS between psPD, ePD and non-psPD. The Chi-square test was used to evaluate differences in molecular genetics among patients with psPD, ePD, and non-PD. Logistic regression was used to analyze the influential factors associated with psPD. A Cox regression model was used to determine the risk factors in the survival analysis. A P value less than 0.05 was considered to indicate a statistically significant difference.