論 文 内 容 要 旨

Multicentric Glioma Develops via a Mutant

IDH1-Independent Pathway:

Immunohistochemical Study of Multicentric Glioma

(多中心性グリオーマの発生機序は IDH1 と無関係であ

る：多中心性グリオーマの免疫組織学的検討)

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Introduction

Multicentric glioma is widely separated brain tumor masses in different lobes or hemispheres without any spreading via commissural or cerebrospinal fluid channels or local metastases. Among gliomas, multicentric glioma is very rare. The pathogenesis has not well established and is different from the solitary gliomas.

New insights on molecular of brain tumors unfold a better understanding of glioma's biological nature. Mutant isocitrate dehydrogenase 1 (IDH1) is the most powerful prognostic factor and is used to classify glioma based on the updated WHO 2016. In addition, some research also classified grade II and III gliomas into subgroups of mutant IDH1 or mutant IDH2 positive and negative.

We profiled immunohistochemical staining of 14 multicentric gliomas and compared them with those of the better-understood solitary tumors. We report that the pathogenesis of multicentric gliomas is mutant IDH1-independent.

Patients and Methods

Our study subjects, 6 males and 8 females, aged from 27 to 75 years. The diagnostic criteria were based on Batzdorf and Malamud. MRI images showed multiple brain tumors with no connecting signal alteration, and the glioma type was confirmed histopathologically. The MR characteristics were defined as FLAIR high (no gadolinium enhancement), focally, diffusely and ring enhanced. In all but two patients the histopathology analyses were based on one biopsy from the primary operation. Two cases of glioblastoma were based on the analyses of 2 different lesions.

Immunohistochemical staining for all antibodies except p53 was performed in an automated immunostainer (Benchmark GX, Ventana). Mutant IDH1, alpha-thalassemia X-linked intellectual disability (ATRX) and epidermal growth factor receptor (EGFR) were detected with Iview detection kit using cell conditioning (CC)-1 at 37ºC. Mutant IDH1 and EGFR were treated with standard CC-1 at 37°C for 1 hr and 32 min, respectively; ATRX with extended CC-1 at 37°C for 32 min with amplification and blocking. The primary antibodies were anti-human IDH1-R132H (1:100, Dianova), anti-ATRX (1:200, Sigma-Aldrich) and anti-human wild-type EGFR (1:50, Dako). Immunostaining for phosphatase and tensin homolog (PTEN) was done with Optiview detection kit with CC-1 at 37°C for 32 min, using anti-human PTEN (1:100, Dako), HQ universal linker and HRP multimer for 8 min without amplification. Antibody for p53

was prepared using anti-human p53 protein (1:100, Dako), and Ki-67/MIB-1 was using anti-human Ki-67 antigen clone MIB-1 (1:25, Dako).

For FISH analysis, sample slides (4 μm in thickness) were deparaffinized and dehydrated. At room temperature, they were pretreated with 0.2 N HCl, washed and incubated at 80°C for 30 min in pretreatment solution (Abbott Molecular Inc., IL, USA). The hybridization was at 75°C for 5 min followed by 37°C for 16 hr with the FISH probe kit for 1p and 19q (Abbott Laboratories, IL, USA). After washing with 0.3% nonionic detergent and DAPI (Life Technology) application, the slides were evaluated under IX81 Olympus fluorescent microscope using Meta Imaging series software (version 7.1).

Results

MRI and histological findings of the 14 patients: 8 were diagnosed as glioblastoma (grade IV), two as anaplastic astrocytoma (grade III), and four as diffuse astrocytoma (grade II). Two patients presented with four and the others with two to three lesions. Half of the patients showed different imaging characteristic among the lesions. In one patient with 4 lesions, one was ring- and the others were diffusely enhanced. In other patients, two to three lesions manifested either as both ring enhanced and FLAIR-high or focally enhanced and FLAIR-high.

Immunohistochemical staining results showed all patients were negative for mutant IDH1 (16 tumor specimens). One patient (7.1%) manifested nuclear positivity for PTEN; it was negative in the cytoplasm of all 16 tumor samples. FISH results for 1p19q co-deletion were negative in all 9 studied cases (11 tumor specimens); p53 was positive in 5 of 14 patients (35.7%; 6 of 16 samples) and ATRX was negative in 4 of 14 patients (28.6%, 6 of 16 specimens). EGFR was over-expressed in 9 of 14 patients (64.3%, 6 of 16 samples). MIB-1 LI ranged between 0.9-15.6% for grade II and III tumors, and in the range of 17.3-52.4% for glioblastoma.

Discussion

We focused on important molecules in the pathogenesis of glioma: mutant IDH1, ATRX, EGFR, p53 and 1p19q co-deletion status. These molecules are commonly used in histopathology diagnosis of glioma. Most of our cases showed different profiles of immunohistochemical staining from the solitary glioma.

IDH1 mutations occur at the early stage of most gliomas and secondary glioblastomas. There are evidences that the mechanism in mutant IDH1 or IDH2 grade II and III glioma is different from the wild-type tumor. Some studies categorized grade II and III gliomas into 3 subgroups: type 1 = mutant IDH1- or mutant IDH2-positive with 1p19q co-deletion; type 2 = mutant IDH1- or mutant IDH2- positive with ATRX and/or p53 mutation, and type 3 = mutant IDH1- or mutant IDH2-negative. Correspondingly, all of our cases were negative for mutant IDH1 staining. Most of them also showed ATRX immunonegativity, no 1p19q co-deletion and negative p53 staining.

In glioblastoma mechanism, EGFR is involved in cellular functions such as cell cycle progression and proliferation. It characteristically strongly expressed in primary glioblastoma. Nuclear PTEN regulates entry into the cell cycle from G1 and mediates cell-cycle arrest and apoptosis. In most of our cases EGFR was also over-expressed and PTEN was negative, indicating high EGFR activity and destabilization of PTEN functions. Futhermore, a study of 258 glioblastoma by Liu et al reported that multicentric glioblastomas were, in fact, dominated by mesenchymal subtype and retained no mutation in both IDH1 and ATRX. They also have poorer survival than the solitary one.

In summary, regardless of the grading, multicentric gliomas' predominant characteristics and pathogenesis are those of primary glioblastoma. This phenomenon differentiates multicentric glioma from the majority of mutant IDH1 immunopositive solitary glioma and glioblastomas. Our findings suggest that the nature of multicentric gliomas is different from the solitary gliomas and might require different management and therapeutic approaches.