Clinical and Molecular Prognostic Factors for Long-Term Survival of Patients with Glioblastomas in Single-Institutional Consecutive Cohort

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■ OBJECTIVE: **The purpose of this study was to clarify the clinical and molecular characteristics associated with long-term survival in patients with glioblastoma.**

■ METHODS: **We analyzed the characteristics of 96 glioblastoma patients. Long-term survivors (LTSs) were classified into moderate LTSs (mLTSs), who survived >3 years, and LTSs, who survived >5 years, and compared with short-term survivors (STSs). Clinical and molecular factors were investigated.**

■ RESULTS: **Younger age, better recursive partitioning analysis class, lack of subventricular zone (SVZ) involvement, promoter methylation of the O6-methylguanine-DNA methyltransferase *(MGMT)* gene, and loss of 19q were associated with mLTSs as compared with STSs. After adjustment for these factors, younger age and *MGMT* methylation remained independently associated with mLTSs. Younger age, better recursive partitioning analysis class, lack of SVZ involvement, and loss of 19q were associated with LTSs as compared with STSs. After adjustment, younger age and better preoperative Karnofsky performance scale (KPS) score remained independently associated with LTSs. Kaplan-Meier analyses revealed that younger age (<50 years), better preoperative KPS score (≥70), lack of SVZ involvement, and loss of 19q were associated with longer overall survival. In the multivariate analysis, only age was significantly associated with overall survival.**

■ CONCLUSIONS: **Younger age and better preoperative KPS score were the characteristics associated with LTSs as compared with STSs. *MGMT* promoter methylation was associated with mLTSs, but not with LTSs. In addition, lack of SVZ involvement and loss of 19q might be prognostic for longer survival.**

INTRODUCTION

Despite recent advances in medical oncology and surgical technology, the majority of patients with glioblastoma (GB) survive <2 years. However, there are rare cases of patients surviving for comparatively long periods of >3 or 5 years. Investigation of such long-term survivors (LTSs) may help to identify potential LTSs and could improve management of patients with GB. As such, many investigators have studied the factors associated with LTSs, and the reported characteristics of LTSs include younger age, better Karnofsky performance scale (KPS) score, female sex, and promoter methylation of the *O6-methylguanine-DNA methyltransferase* (*MGMT*) gene.[1-6] However,

Key words
- Age
- Glioblastoma
- Long-term survival
- Loss of 19q
- *MGMT*
- Subventricular zone involvement

Abbreviations and Acronyms
CAN: Copy number aberration
CGH: Comparative genomic hybridization
DNA: Deoxyribonucleic acid
FFPE: Formalin-fixed paraffin-embedded
GB: Glioblastoma
IDH: Isocitrate dehydrogenase
KPS: Karnofsky performance scale
LTS: Long-term survivor
MGMT: O6-methylguanine-DNA methyltransferase
OS: Overall survival
RPA: Recursive partitioning analysis
STR: Subtotal removal
SVZ: Subventricular zone
TERT: Telomerase reverse transcriptase
WHO: World Health Organization

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the definition of long-term survival varies among studies (>3 vs. 5 years) and, except for younger age, there remains no consensus about the characteristics of LTSs.

In the present study, we aimed to elucidate the clinical and molecular characteristics of the LTSs with GB. Because the number of patients who survive >3 years is increasing since the introduction of the Stupp protocol,[7] LTSs were classified into moderate LTSs (mLTSs), who survived >3 years, and LTSs, who survived >5 years, and compared with short-term survivors (STSs), who survived <2 years. In addition to the known clinical and molecular prognostic factors in GB patients, including mutations in the isocitrate dehydrogenase (*IDH*) genes and *MGMT* promoter methylation, chromosomal copy number aberrations (CNAs) as a genome-wide screen and mutations in the promoter region of the telomerase reverse transcriptase (*TERT*) gene, a potential novel molecular prognostic marker in GB, were also investigated.

MATERIALS AND METHODS

Patients and Clinical Data

Pathology records of the patients with brain tumors treated at the Department of Neurosurgery, Keio University Hospital, from April 1990 through December 2011 were reviewed, and patients who fulfilled the following criteria were included: 1) histopathologic diagnosis of GB, World Health Organization (WHO) grade IV[8-10]; 2) no history of lower-grade gliomas; 3) no prior radiotherapy to the skull; and 4) written informed consent or, for the deceased patients and the patients who underwent surgical intervention before December 2000, opt-out consent via website.

This study was conducted as part of the molecular–clinical translational research program approved by the Institutional Review Board at Keio University School of Medicine and named "Towards molecular classification and personalized treatment of brain tumors" (approval number 20050002). Clinical data including age at diagnosis, sex, KPS before initial surgery, maximum tumor diameter, extent of resection, presence or absence of subventricular involvement (SVZ), initial postoperative therapy, treatment at recurrence, and date of death or last contact were obtained from patients' records. Survival data were updated via telephonic communication.

The extent of resection was estimated by comparison of preoperative and postoperative T1-weighted magnetic resonance imaging with contrast enhancement. In cases in which magnetic resonance images were not available for review, computed tomography images with contrast enhancement or surgical records were used as a substitute. Accordingly, extent of resection was classified into subtotal resection (STR) defined by >90% resection (GTR is included here), partial resection defined by ≤90% resection, and biopsy.

Tumors were judged as involving SVZ if the shortest distance between the contrast-enhancing lesion and a ventricle was ≤10 mm.[1]

Tissues and Deoxyribonucleic Acid (DNA) Extraction

Tumor tissues were obtained from the archives of the Department of Neurosurgery at Keio University Hospital. Tumor DNA was extracted from microdissected pieces of formalin-fixed paraffin-embedded (FFPE) tissue as described previously.[11,12] Microdissection was performed using hematoxylin-eosin (HE) staining of consecutive sections with exclusion of the intermixed non-neoplastic glial or vascular cells, as well as hemorrhagic or necrotic regions.

Comparative Genomic Hybridization

Chromosomal CNAs were assessed using metaphase comparative genomic hybridization (CGH) as described previously.[13,14] In brief, crude tumor DNA extracted from FFPE sections was amplified via degenerate oligonucleotide primed–polymerase chain reaction and labeled with another degenerate oligonucleotide primed–polymerase chain reaction using digoxigenin (DIG)-11-dUTP (Roche, Mannheim, Germany). The reference DNA was amplified from 50 ng of normal male or female DNA and labeled with biotin-dUTP (Roche). The probe mixture was denatured and hybridized to normal metaphase spreads (Vysis, Downers Grove, Illinois, USA). After the unhybridized probes were washed, the metaphase spread was incubated with fluorescein isothiocyanate–conjugated anti-DIG-antibody (Roche) and rhodamine-conjugated avidin (Roche). The preparations were washed and counterstained with 4,6-diamino-2-phenylinodole in antifade solution. Red, green, and blue images were acquired, and the ratios of fluorescence intensity along the chromosomes were quantitated using the CytoVision analysis system (Applied Imaging, San Jose, California, USA).

Screening for *IDH* Mutations

Because non-R132H *IDH1* and *IDH2* mutations are nearly absent in primary GB,[15,16] screening for mutations of *IDH* genes was carried out using immunohistochemical analysis with an anti-IDH1R132H antibody (Dianova, Hamburg, Germany) on FFPE sections. For the patients who survived >3 years (mLTSs/LTSs), direct sequencing of *IDH1/2* genes was performed following negative R132H *IDH1* on immunohistochemical analysis.

Assessment of *MGMT* Promoter Methylation

The promoter methylation status of the *MGMT* gene was assessed using methylation-specific PCR with the EZ DNA Methylation-Direct kit (Zymo Research Corp., Orange, California, USA) as described previously.[17]

Assessment of Mutations in *TERT* Promoter Region

To allow amplification of DNA extracted from the archival tissues, the 163bp promoter region of the TERT gene flanking the known 2 mutation hot spots was amplified using the previously published primer pairs (forward primer: 5′CAGCGCTGCCTGAAACTC; reverse primer: 5′GTCCTGCCCCTTCACCTT) with the GC-RICH PCR System kit (Roche).[18] The PCR conditions consisted of initial denaturation at 95°C for 3 minutes, 40 cycles of 95°C for 30 seconds, 60°C for 25 seconds, and 72°C for 30 seconds, and a final extension step at 72°C for 10 minutes. PCR products were separated on NucleoSpin gel (Macherey-Nagel, Düren, Germany) and purified with the PCR Clean-up kit (Macherey-Nagel) according to the manufacturer protocols. Sequencing was performed bidirectionally.