Prognostic value of WT1 protein expression level and MIB-1 staining index as predictor of response to WT1 immunotherapy in glioblastoma patients

Abstract The use of Wilms’ tumor 1 (WT1) immunotherapy is considered to be an innovative approach for the treatment of malignant gliomas. Because of its novelty, tools that can accurately predict response to this therapy are still lacking. In this article, we investigated the role of WT1 protein expression level (score 1–4) and MIB-1 staining index in predicting survival outcome after therapy in patients with recurrent or progressive glioblastoma multiforme. Tumor samples from 37 patients enrolled in a phase II clinical trial on WT1 immunotherapy were immunohistochemically analyzed for WT1 levels and MIB-1 index. Results showed that median progression-free survival (PFS) was longer in the WT1 high expression group (score 3 and 4) compared with that of the low expression group (score 1 and 2) (20.0 weeks vs. 8.0 weeks; \( P = 0.022 \)), and that the median overall survival (OS) was likewise longer in the former compared to the latter group (54.4 weeks vs. 28.4 weeks; \( P = 0.035 \)). Furthermore, within the WT1 high expression group, tumors with intermediate staining intensity (WT1 score 3) have both the longest median PFS and OS, 24.4 weeks and 69.4 weeks, respectively. On the other hand, no significant correlation was noted between MIB-1 staining index and survival. In conclusion, our study has shown that WT1 protein expression level, not MIB-1 staining index, can be used as a prognostic marker to foretell outcome after immunotherapy, and that patients whose tumors have intermediate WT1 expression have the best survival outcome.

Key words Glioblastoma · Wilms’ tumor 1 · Immunotherapy · MIB-1 staining index · Prognostic value

Introduction

Our understanding of the biological behavior of cancers has expanded through the years. With therapeutic strategies available, both standard and “nonstandard” modalities, finding a suitable treatment for a patient has become increasingly difficult. Oncologists have been constantly on the lookout for tools that can accurately predict response to therapy.

A novel approach for the treatment of glioblastoma multiforme (GBM) is with the use of Wilms’ tumor 1 (WT1) immunotherapy. Studies have shown that the WT1 gene is highly expressed in this disease and that it is absent in normal astrocytes.1,2 Similarly, it has been found to play a significant role in gliomagenesis.1 A preliminary report on WT1 immunotherapy has demonstrated survival outcome that is equal or superior to chemotherapy in patients suffering from recurrent or progressive GBM.3 Consequently, this information has stirred up interest among glioma specialists on finding reliable indicators that can help predict treatment response in this category of patients. One promising marker is the level of tumor expression of WT1. As it is tagged as one of the major culprits in glioma pathogenesis, this choice seemed rational. Another potential one, implicated in the proliferative nature of the disease, is the MIB-1 staining index. Up to the present, there have been no substantial studies that specifically involved patients who underwent WT1 immunotherapy to address this concern.
In this article, we assessed the expression of WT1 levels, as well as MIB-1 staining index, in tumor samples of GBM patients before WT1 immunotherapy and correlated it with survival outcome. As an adjunct, we also analyzed the same parameters in tumor samples of postvaccine patients who failed therapy.

### Materials and methods

#### Patients and treatments

Patients were enrolled in a phase II clinical trial of WT1 immunotherapy for recurrent or progressive GBM. Details of the trial were described previously. Patients who were eligible received intradermal injections of 3.0 mg HLA-A*2402-restricted modified 9-mer WT1 peptide emulsified with Montanide ISA51 adjuvant weekly for 12 consecutive weeks. Response was determined by measuring the change in the size of the target lesions on magnetic resonance (MR) imaging, labeled as either complete response, partial response, stable disease, or progressive disease, based on the RECIST criteria.

If an effect was observed after 12 vaccinations, WT1 immunotherapy was further given at 2-week intervals until disease progression was noted. The progression-free survival (PFS) period was defined as the day of the first WT1 immunotherapy to the day of the last image before the detection of disease progression. The overall survival (OS) period was defined as the time from the day of the first WT1 immunotherapy to death.

Tumor specimens were taken at the time of the initial diagnosis of GBM before WT1 immunotherapy, as well as during the second operation after failed immunotherapy (if available).

All patients provided written informed consent, and the study was approved by the ethics review board of the Osaka University Faculty of Medicine.

#### Immunohistochemical analysis

**WT1 expression level**

Formalin-fixed tissue sections were prepared from resected tumors of eligible patients. Sections were microwaved in citrate buffer for antigen retrieval and incubated with anti-human WT1 mouse monoclonal antibody 6F-H2 (diluted 1:50, DAKO North America, Carpinteria, CA, USA). The WT1 reaction was visualized with the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) and diaminobenzidine (WAKO, Osaka, Japan). Sections were then counterstained with hematoxylin. The level of WT1 expression was classified, based on a scale proposed by Izumoto and colleagues, as follows: 1, slightly increased staining in some tumor cells compared with that in normal glial cells; 2, staining at intermediate intensity in some tumor cells; 3, strong staining in some tumor cells and intermediate staining in almost all tumor cells; and 4, greatly increased staining in almost all tumor cells compared with that in normal glial cells (Fig. 1).

Samples were scored independently by three competent authors (Y.C., N.H., and N.K.). A score agreed upon by at least two of them was deemed acceptable.

#### MIB-1 staining index

The same serial sections used for WT1 immunohistochemical evaluation were utilized for MIB-1 staining. Antibody against the Ki-67 antigen (DAKO) was diluted 1:50. The staining index was determined by calculating the percentage of positively stained tumor cell nuclei of 1000 seen in areas with the greatest degree of immunostaining. A cutoff of 20 was arbitrarily chosen to divide the group into a high and a low MIB-1 labeling group.

#### Statistical analysis

To assess the relationship of WT1 levels and MIB-1 staining index with survival outcome after treatment in the study population, the Kaplan–Meier technique was utilized. PFS and OS were estimated using the Kaplan–Meier curves and were compared using the log-rank test. A P value < 0.05 was considered statistically significant, and all statistical computation was performed using StatMate III software (ATMS, Tokyo, Japan).

### Results

#### Patient population

Table 1 shows the characteristics of the 37 patients (23 men and 14 women; mean age, 49.0 years; range, 20–75 years) included in the study. Of the patients, 51% had right-sided lesions, most of which were in the frontal region, whereas 41% had lesions involving the left lobe.

#### Table 1. Characteristics of 37 patients

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<th>Sex</th>
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<tbody>
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<table>
<thead>
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<th>Age (in years)</th>
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<table>
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<th>Temporal lobe</th>
<th>Parietal lobe</th>
<th>Occipital lobe</th>
<th>Basal ganglia</th>
<th>Left side</th>
<th>Frontal lobe</th>
<th>Temporal lobe</th>
<th>Parietal lobe</th>
<th>Occipital lobe</th>
<th>Basal ganglia</th>
<th>Cerebellum</th>
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