Morphological and flow cytometric analysis of cell infiltration in glioblastoma: a comparison of autopsy brain and neuroimaging

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Abstract Even when we successfully perform a total extirpation of glioblastoma macroscopically, we often encounter tumor recurrence. We examined seven autopsy brains, focusing on tumor cell infiltration in the peripheral zone of a tumor, and compared our findings with the MR images. There has so far been no report regarding mapping of tumor cell infiltration and DNA histogram by flow cytometry, comparing the neuroimaging findings with the autopsy brain findings. The autopsy brain was cut in 10-mm-thick slices, in parallel with the OM line. Tissue samples were obtained from several parts in the peripheral zone (the outer area adjacent to the tumor edge as defined by postcontrast MRI) and then were examined by H&E, GFAP, and VEGF staining. We defined three infiltrating patterns based on number of infiltrated cells as follows: A zone, 100%–60% of the cells infiltrated tumor cells compared with tumor cell density of the tumor mass; B zone, 60%–20%; C zone, 20%–0%. In the autopsy brain, the tumor was easily identified macroscopically. We found that (1) the tumor cells infiltrated the peritumoral area; and (2) tumor cell infiltration was detected over an area measuring from 6 to 14 mm from the tumor border in the A zone. When performing surgery on glioblastoma, a macroscopic total extirpation of the tumor as defined by the contrast-enhanced area in MRI is therefore considered to be insufficient for successfully reducing tumor recurrence.

Key words Glioblastoma · Autopsy · Infiltration · MRI · Flow cytometry

Introduction

Neurosurgical treatments have made remarkable advances in their respective fields, and improvements in treatment outcomes have been recognized. However, treatment outcomes have not yet yielded improvements for glioblastoma, with an average prognosis of 12 to 18 months and a 3-year survival rate of 11.4%, thus making it a disease with a very poor prognosis. For glioblastoma, although the amount of surgical extirpation greatly affects prognosis, its strong infiltrating nature into normal brain tissue causes unfavorable results. Even if radiation therapy and chemotherapy are performed following extirpation that is as surgically complete as possible, we clinically experience recurrence from the infiltrated area in most cases. Gaspar et al. described that 96% of locally recurrent glioblastoma was found within 2 cm outside the range to be imaged with magnetic resonance imaging (MRI) or computed tomography (CT) scan and that all recurrences confined to the brain were found within 4 cm of the enhancing tumors, as seen on CT scan.

Regarding infiltration, various methods are being used to reveal its mechanism. However, there have been no reports evaluating morphological cell infiltration by comparing autopsy brains with images and also using flow cytometry. We herein report that we compared autopsy brains with living images and thus examined the infiltration range of tumor cells by mapping the tumor cells, in addition to examining the cell kinetics using flow cytometry.

Materials and methods

Cases

Among the patients who were diagnosed with glioblastoma in our hospital from 1992 to 2008 and whose fully informed consent was obtained from their families in the end stage, seven cases whose images and autopsy brains could be compared were included as subjects (Table 1). There were five men and two women, with a mean age of 60.4 years and a mean period of 18.3 months from definitive diagnosis to death: five cases of primary glioblastoma and two cases of secondary glioblastoma. Following a definitive diagnosis, no posttherapy was performed in one case because of poor...
general condition and refusal of following chemotherapy and radiation therapy by the patient’s family. Four cases underwent radiation therapy (locally expanded, 60 Gy) and interferon administration (three times a week). Two cases underwent radiation therapy similar to the aforementioned protocol and a 42-day administration of temozolomide (75 mg/m²). After the patients’ deaths were confirmed, specimens were extirpated by the pathologist in charge at our university hospital within 24 h. Using the slicer invented in Montefiore Hospital, brain cutting was performed in 10-mm slices, parallel to the OM (otitis media) line. After the specimens were fixed in formalin, specimens were obtained with numbered labels attached to the respective sites centering around the tumor site.

Comparison between images and autopsy brains

The latest MRI or CT images within 1 month before death were used. The autopsy brains and images were selected in corresponding slices and adjusted to size by photoprinting. Macroscopically, the outer edge of the tumor was defined to be the outer edge of enhanced area. The position of the outer edge was measured using a vernier caliper from the midline, brain surface, and definite anatomical landmark. Microscopically, the vertical and horizontal distances were measured under a microscope. We defined a site where 100%–60% tumor cells existed as compared to the number of cells in the peripheral zone as the A zone, a site with less than 60%–20% existence as the B zone, and a site with less than 20% existence as the C zone: we analyzed at least three sites of cells in the peripheral zone as the A zone, a site with 100%–60% tumor cells existed as compared to the number measured under a microscope. We defined a site where the proliferative capacity of tumors, the DNA index (DI) and proliferating index (PI) were measured. The DNA index (DI) is obtained by measuring the ratio of the peak value of lymphocytes “diploid” to the first peak value of the tumor cells: [(channel numbers of G0/G1 peak in specimen) / (channel numbers of G0/G1 peak in normal diploid)]. In our past studies, it has been proven that the more malignant a tumor cell is, the higher is the DI. The proliferating index (PI) shows the percentage of tumor cells in S-, G2-, and M-phases among all the counted tumor cells: [(cell numbers of S + G2 + M phase) / (all cell numbers)]. It has been said that the more proliferative a tumor is, the higher the PI value that is observed. Both DI and PI were used as indices for evaluating the existence range of tumor cells.

Table 1. Summary of surgery and treatment of the seven cases of glioblastoma

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Location</th>
<th>Surgery</th>
<th>Therapy</th>
<th>Survival</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27</td>
<td>M</td>
<td>Left thalamus</td>
<td>Partial</td>
<td>IAR + IFN</td>
<td>2.0 years</td>
<td>Secondary (anaplastic astrocytoma)</td>
</tr>
<tr>
<td>2</td>
<td>68</td>
<td>M</td>
<td>Left thalamus</td>
<td>Biopsy</td>
<td>(–)</td>
<td>4 months</td>
<td>Primary</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
<td>F</td>
<td>Corpus callosum</td>
<td>Subtotal</td>
<td>IAR + IFN</td>
<td>1.3 years</td>
<td>Primary</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>F</td>
<td>Left thalamus</td>
<td>Subtotal</td>
<td>IAR + IFN</td>
<td>3.4 years</td>
<td>Secondary (anaplastic astrocytoma)</td>
</tr>
<tr>
<td>5</td>
<td>70</td>
<td>M</td>
<td>Left thalamus</td>
<td>Biopsy</td>
<td>IAR + TMZ</td>
<td>5 months</td>
<td>Primary</td>
</tr>
<tr>
<td>6</td>
<td>72</td>
<td>M</td>
<td>Left occipital</td>
<td>Subtotal</td>
<td>IRA + IFN</td>
<td>2.8 years</td>
<td>Primary</td>
</tr>
<tr>
<td>7</td>
<td>63</td>
<td>M</td>
<td>Left occipital</td>
<td>Biopsy</td>
<td>IAR + TMZ</td>
<td>8 months</td>
<td>Primary</td>
</tr>
</tbody>
</table>

IAR, intracranial radiation; IFN, interferon; TMZ, temozolomide

Results

Comparison of the size between the autopsy brains and radiologic images

The autopsy brains and radiologic images were almost identical in their morphology. It was easier to identify brain tumors in the fresh brain than in the formalin-fixed brain. Furthermore, the outer edge of the tumor in the image was almost identical with the slice of autopsy brain. The formalin-fixed brain showed some atrophy, but the morphology became almost identical by evenly scaling the image; as a result, it was revealed that atrophy resulting from formalin fixation with the autopsy brain does not differ greatly depending on the sites.

Cell infiltration pattern according to the morphology

Case 2, a 68-year-old man, left thalamic glioblastoma

MRI image: In T2WI 1 month before death, high signals were extensively detected in the periphery (Fig. 1A), and ringlike contrast enhancement was demonstrated (Fig. 1B).

Autopsy brain: The tumor was poorly defined in the fresh brain (Fig. 2A), but the color tones of the tumor were obviously different from the outer parts. On the other hand, the formalin-fixed brain had no great difference in color tones from the normal brain. The respective sites in the