OBSERVATION

Addressing Diffuse Glioma as a Systemic Brain Disease With Single-Cell Analysis

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Objective: To analyze infiltration patterns of IDH1 mutant diffuse gliomas into the brain by identification of single tumor cells applying an antibody specific to mutant IDH1 R132H protein.

Design: Immunohistochemical analysis.

Setting: University hospital.

Patients: Whole-brain and hemisphere sections of 4 patients diagnosed with diffuse glioma.

Results: Tumor cells were identified in areas that appeared inconspicuous macroscopically and at histological analysis with respect to cellularity, cellular pleomorphism, or mitotic activity in all cases.

Conclusion: Detection of single tumor cells throughout the brain demonstrates diffuse glioma to represent systemic brain disease.


DETERMINATION OF INFILTRATION EXTENT IN DIFFUSE GLIOMA has been a diagnostic problem ever since pathological analysis of this disease. When Virchow coined the term glioma in 1863, he already stated that "most gliomas do not show a clear border to brain tissue at all."[1] Also Bailey and Cushing[2] mentioned the difficulty of identifying a tumor border on both macroscopic and microscopic levels in their seminal work on classification of brain tumors in 1926. In 1940, Scherer[3] most precisely described the growth pattern of malignant glioma. He reported that infiltrative growth must be regarded as a hallmark of most gliomas and discussed in detail the limitations of microscopic determination of tumor borders. Until present, all approaches have been limited by the fact that single neoplastic glial tumor cells frequently cannot be distinguished from normal or reactive cells on grounds of morphology or immunocytochemical profile.[4]

Recently, heterozygous mutations in the cytosolic isocitrate dehydrogenase 1 gene (IDH1) have been shown to constitute the most frequent alteration in diffuse astrocytoma and oligodendroglioma of World Health Organization grades II and III and in secondary glioblastoma World Health Organization grade IV.[5-8] Approximately 70% to 80% of these tumors exhibit such mutations, whereas their frequency in primary glioblastoma ranges between 5% and 10%. More than 90% of the IDH1 mutations in glioma are characterized by exchange of an arginine by a histidine residue in position 132 of the protein (R132H), and a mutation-specific monoclonal antibody recognizing the R132H mutation (H09) has been generated.[9-11] H09 detects single tumor cells and reliably distinguishes neoplastic from other cells.[11,12]

Herein, we applied this antibody on paraffin-embedded whole-brain and hemisphere sections from 4 patients with glioma to comprehensively analyze infiltration patterns.

METHODS

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Formalin-fixed, paraffin-embedded large tissue sections from 9 patients were obtained from the archives of the Department of Neuropathology, Institute of Pathology, University Hospital Heidelberg, Heidelberg, Germany, and the Institute of Neuropathology, Academic Medical Centre, Amsterdam, the Netherlands, and analyzed for IDH1 R132H mutation by immunohistochemical analysis. Specimens from 4 patients were identified to harbor an IDH1 R132H mutation and were therefore subjected to further analysis. Patient data are compiled in the Table.
Table. Case Characteristicsa

<table>
<thead>
<tr>
<th>Case No./Sex/Age at Death, y</th>
<th>Year of First Presentation</th>
<th>Therapy (Year, Dose)</th>
<th>First Diagnosis (Initial Resection)</th>
<th>Survival, y Cause of Death</th>
<th>Diagnosis (Autopsy Specimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/28</td>
<td>1983</td>
<td>Radiotherapy (1985, 60 Gy)</td>
<td>No surgery</td>
<td>2</td>
<td>Respiratory failure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anaplastic astrocytoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiotherapy (1974, 60 Gy)</td>
<td>No surgery</td>
<td></td>
<td>Glioblastoma</td>
</tr>
<tr>
<td>3/M/76</td>
<td>1993</td>
<td>Radiotherapy (1998, 28 Gy)</td>
<td>No surgery</td>
<td>5</td>
<td>Cerebral herniation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Anaplastic astrocytoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Radiotherapy (1988, 66 Gy)</td>
<td>No surgery</td>
<td></td>
<td>Anaplastic astrocytoma</td>
</tr>
</tbody>
</table>

All cases received antiedematous treatment, either steroids (cases 1, 3, and 4) or mannitol (case 2). Radiotherapy was focused to the tumor mass in cases 1, 2, and 4; case 3 underwent a lower irradiation dose as a palliative concept. Diagnosis of the autopsy specimen is given according to the retrieved medical reports. Applying the current World Health Organization classification,4 reevaluation of cases 1 and 2 yielded secondary glioblastoma as the diagnosis of the autopsy specimen.

SI conversion factor: To convert gray to rad, multiply by 100.

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Results

Immunohistochemical analysis with H09 (Dianova GmbH) was applied applying previously published conditions.11 Luxol fast blue/periodic acid–Schiff staining was performed following standard protocols.

CASE 1

We analyzed a horizontal whole-brain section for case 1. Luxol fast blue/periodic acid–Schiff staining, magnetic resonance imaging, and macroscopic appearance demonstrated the tumor bulk in the frontal midline affecting both hemispheres (Figure, 1A and B). Immunohistochemical analysis with H09 revealed dense tumor cell infiltration in the left internal capsule (Figure, 1C), left external capsule, anterior commissure, and left hippocampus (Figure, 1). Sparse infiltration was detected along the outer ends of the anterior commissure (Figure, 1D) and along the left lateral ventricle (Figure, 1).

CASE 2

We analyzed a coronal whole-brain section for case 2. Luxol fast blue/periodic acid–Schiff staining showed the tumor bulk in the left frontal lobe. Additional tumor cells were found in high density at the macroscopic border of the tumor (Figure, 2G and 2H) and in the septum pellucidum, corpus callosum, and opposite frontal lobe. A low density of tumor cells was observed in the white matter of the frontal lobe surrounding the tumor bulk as well as in cortical structures of the left and right insula (Figure, 2I), left cingulated gyrus and sulcus, right gyrus rectus (Figure, 2J), and left middle frontal gyrus as well as both the left and right basal ganglia.

CASE 3

We analyzed a coronal hemispheric section of the temporoparietal lobe for case 3. Immunohistochemical analysis revealed a rather steep gradient of tumor cells at the macroscopic tumor border (Figure, 3K) while single tumor cells of low and focally high density could be detected up to 3 cm in the adjacent brain (Figure, 3L).

CASE 4

We analyzed a coronal hemisphere section of the left parieto-occipital lobe for case 4. Tumor cells migrated from the site of the resected tumor through the white matter to a new tumor bulk. Single tumor cells of low density were also found in the cortex (Figure, 4).

Comment

Applying the mutation-specific antibody H09, we were able to unequivocally detect tumor cells where no certain discrimination between reactive and malignant cells could be drawn by means of histopathological analysis. Single tumor cells were identified in areas that appeared inconspicuous macroscopically and at histological analysis with respect to cellularity, cellular pleomorphism, or mitotic activity. Specificity of the antibody to reliably detect neoplastic cells and to distinguish them from reactive cells has been extensively demonstrated by different research groups.9,15 The presence of single tumor cells in those apparently unaffected areas was expected and discussed in previous studies but could not be proven in situ so far. However, evidence for the presence of glioma cells in areas without microscopic proof of neoplasia was provided by successful cultivation of tumor cells from areas in which histological analysis failed to detect such cells.15 Since an additional tumor bulk beyond the primary lesion is seen neither on hematoxylin-eosin sections nor on immunohistochemical analysis, and all detected cells harbor the same IDH mutation, it is most likely that these tumor cells have migrated from the main lesion and do not represent independent neoplasms.

Our finding of tumor cells present in virtually most areas of the diseased brain raises the question of why glioma usually recurs in proximity to the area of initial resection. One explanation discussed might be the development of a tumorigenic milieu in areas with higher density of residual cells.16 Also, rapidly migrating glioma cells do not proliferate and vice versa, that proliferating glioma cells tend not to migrate, which might favor a lo-
cal recurrence. However, the dynamics of this "go-or-grow" paradigm in vivo have not been examined.

Glioma cells preferentially invade brain tissue by following white matter tracts. This pattern, along with angiotro-
pism (Figure, 2H) and perineuronal satellitosis of tumor cells, was termed secondary structures of glioma invasion by Scherer.3 These observations also match with our results. However, in 1 patient (case 2) we also detected an accumulation of tumor cells in the cortex far from the tumor center. Possibly, these tumor cells used the white matter tracts for invasion and ran up against the leptomeningeal border, preventing further migration. Our analysis unfortunately does not allow observing the dynamics of infiltration as it is limited to end-stage gliomas. Thus, the status of infiltration at first presentation of disease cannot be determined. Presence of mutant protein or 2-hydroxyglutarate, which is excessively produced by the mutant protein, might offer a target for non-invasive detection by high-resolution imaging in the future. The fact that mutant protein enables us to identify the tumor cells results in the limitation that only 4 of our 9 cases were suitable for this study, which showed an IDH1 R132H mutation. Therefore, no conclusions can be drawn for IDH wild-type tumors, such as nonmutant diffuse glioma grades II and III and especially primary glioblastoma.

The findings of the present study also challenge the current definition of gliomatosis cerebri (GC). Gliomatosis cerebri is defined as extensive glioma infiltration into the brain affecting at least 3 cerebral lobes. These lesions have been subdivided into primary GC, where extensive infiltration is seen at the time of diagnosis, and secondary GC, developing from a focal glioma.4,18 Assessment of the required affection of 3 lobes usually is based on imaging. Our findings demonstrate infiltration of tumor cells into the entire cerebrum in cases 1 and 2, thus fulfilling the criteria for the diagnosis of GC. However, in both cases the lesion was diagnosed as focal glioma. Therefore, the frequency of GC, especially of secondary GC, is likely to be much higher on analysis with markers specific for single tumor cells. This enormous invasion potential of glioma cells also explains the previous observation of IDH1 mutations in 42% of secondary GC.18 Our findings therefore suggest restricting the term GC to patients with initial presentation of diffuse disease in 3 or more lobes and without focal tumor. In turn, diagnosis of secondary GC should be avoided because patients with secondary GC cannot unequivocally be distinguished from patients with focal glioma but extensive infiltration such as our cases 1 and 2.

In conclusion, the present analysis demonstrates for the first time, to our knowledge, the extent of infiltration of diffuse glioma on the single-cell level and underlines the concept of addressing glioma not as a focal but a systemic disease of the entire brain. Further studies should investigate whether mutated IDH1 protein allows specific targeting of all tumor cells.

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Author Contributions: All of the authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Sahm, Capper, Paulus, and von Deimling. Acquisition of data: Sahm, Jeibmann, Habel, Troost, and von Deimling. Analysis and interpretation of data: Sahm, Capper, Jeibmann, and von Deimling. Drafting of the manuscript: Sahm and von Deimling. Critical revision of the manuscript for important intellectual content: Capper, Jeibmann, Habel, Paulus, and Troost. Statistical analysis: von Deimling. Obtained funding: von Deimling. Administrative, technical, and material support: Sahm, Capper, Jeibmann, Paulus, Troost, and von Deimling. Study supervision: Sahm, Capper, and von Deimling.

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REFERENCES