Genetic Aberrations in Gliomatosis Cerebri Support Monoclonal Tumorigenesis

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Abstract. Gliomatosis cerebri is a rare condition in which the brain is infiltrated by an exceptionally diffusely growing glial cell population involving at least 2 lobes, though often more extensive, sometimes even affecting infratentorial regions. The neoplastic proliferation may have a monoclonal origin, or alternatively, reflect progressive neoplastic change of an entire tissue field (“field cancerization”). The presence of an identical set of genetic aberrations throughout the lesion would point to monoclonality of the proliferation. In contrast, the finding of non-identical genetic changes in widely separated regions within the neoplasm would support the concept of field cancerization.

In the present study, a unique autopsy case of gliomatosis was available to verify one of these hypotheses. Tissue samples were randomly taken from 24 locations throughout the brain and used for genetic investigation. In all samples the histology showed an identical picture of slightly elongated astrocytic cells, typical for gliomatosis. TP53 exon 5–8 mutation analysis was performed on all samples. Genome-wide screening for chromosomal aberrations was accomplished by comparative genomic hybridization (CGH). In addition, loss of heterozygosity analysis for polymorphic markers on chromosomal regions of the 2 most frequently observed DNA deletions was carried out. The most widespread genetic aberration was mutation of exon 7 of TP53, which was detected in 20 of 24 samples. Bidirectional sequencing revealed a mutation in codon 234 (TAC234TGC), resulting in an amino acid substitution Tyr-Cys. CGH analysis revealed losses on 2q11-q31 in 13 of 24 samples and losses on 19q13-pter in 10 of 24 samples from both left and right hemispheres. Allelic imbalances for markers on 2q (2q14.3 and 2q22.1) and 19q (both 19q13.2) were demonstrated in 10 of 24 and 18 of 24 samples, respectively. Other widespread chromosomal aberrations included losses on 3q13-pter and 16q22-pter and gains on 7q22-pter. The wide distribution of a particular set of genetic aberrations in this case supports the concept of monoclonal tumor proliferation. The results point to involvement of TP53 mutation in the tumorigenesis of gliomatosis cerebri.

Key Words: Chromosomal aberrations; Chromosome 2q; Chromosome 19q; Genetics; Glioma; Gliomatosis cerebri; TP53 mutation.

INTRODUCTION

The term gliomatosis cerebri is used for a rare disease in which the brain is diffusely infiltrated by neoplastic glial cells involving at least 2 lobes, often crossing the midline and in most cases also present in deep cerebral structures (1). The proliferation typically consists of slightly elongated glial cells with bipolar cell processes. The term gliomatosis cerebri was coined by Nevin in 1938, although the condition had been described earlier as schwannosis, glial hyperplasia, or glioblastomatosis (2). The cell density and proliferation index are low and there is no endothelial proliferation or necrosis associated with high-grade glioma (3, 4). By neuroimaging the brain appears diffusely swollen with preservation of anatomic structures and no circumscribed tumor center can be identified. Because of the obvious limitations of taking biopsies from the various affected areas of the brain, it is clear that the clinical diagnosis of gliomatosis cerebri largely depends on the radiological presentation of the lesion (5).

In cases of gliomatosis the brain is far more extensively affected than is usually seen in cases of diffuse glioma. The tumorigenesis of gliomatosis cerebri is of considerable interest. Although one might regard this entity as a glioma with extremely diffuse infiltration, the condition has long triggered the idea that effects of field cancerization might be operative (6). In field cancerization a tissue is genomically unstable and therefore prone to additional genetic hits giving rise to polyclonal tumors. These tumors will arise independently in time and place and will harbor non-identical genetic aberrations. In patients with multiple cancers in the head and neck region, the involvement of field cancerization is widely acknowledged (7). So far, no studies have been undertaken to verify a possible effect of field cancerization in gliomatosis cerebri. The question of whether field cancerization is operative in gliomatosis cerebri can be resolved by carrying out a genetic analysis of widely separated areas taken from the affected brain in such cases (8, 9).

For the present study, a unique autopsy case of gliomatosis cerebri was available, enabling histologic and genetic investigations of the entire brain. Since mutation of TP53 is found in the majority of diffuse gliomas (10), the status of TP53 was explored in the tissue samples taken from various regions of the tumor. The presence of a mutation consistently found in the same exon throughout the brain would be strongly indicative of monoclonality. The most frequently mutated exons 5–8 of the p53 gene were screened for the presence of mutations using PCR-SSCP. Comparative genomic hybridization (CGH) is a suitable technique for genome-wide screening for additional chromosomal aberrations. CGH analysis of
samples taken from multiple locations throughout this autopsy brain was carried out in order to reveal the distribution of chromosomal changes. Two of the most widely encountered chromosomal losses were further evaluated by analysis of polymorphic loci (LOH).

MATERIALS AND METHODS

Clinical History

A 37-yr-old woman was admitted because of partial epileptic insults. A CT scan showed a diffuse hypodensity of white matter without mass effect in right and left parts of the brain. The diagnosis of gliomatosis cerebri was made. The epilepsy was treated with anti-epileptic drugs and gradually disappeared over the course of the next 10 yr. At the age of 48 she was admitted with slight loss of strength and coordination of the arms and legs and right-sided anesthesia. She also suffered from progressive fatigue and headaches and became progressively irritable and aggressive. Physical examination did not reveal any systemic disease. Neurologic examination showed only slight paresis of the right hand but no loss of sensibility; there were no signs of raised intracranial pressure. A CT scan demonstrated white matter changes in frontal, parietal, and occipital lobes and the left temporal lobe. Six months later, partial epileptic insults reappeared and the patient became progressively dystarthric. Slight paresis of arms and legs was present but no ataxia. Neuropsychological examination showed impaired cognitive function and memory. A CT scan revealed diffuse aberrations, again compatible with gliomatosis cerebri (Fig. 1). At this point, a stereotactic biopsy was taken from the left frontal region. No treatment was given. The postoperative course was uneventful. The clinical condition of the patient gradually deteriorated over the following year. She died at the age of 49 from raised intracranial pressure due to edema and subsequent cerebral herniation 12 yr after onset of the first symptoms.

Autopsy

The brain weighed 1605 g and was fixed in 10% buffered formalin for 4 wk. There were signs of edema and herniation of the cerebellar tonsils. The arachnoidal membrane appeared to be normal. Transverse cuts showed swollen tissue with blurred delineation between the grey and white matter. No difference in size between the left and right hemisphere was noticed. Grossly, no clear tumor center could be recognized. A total of 24 samples randomly chosen from the entire brain were taken for CGH analysis (Fig. 2, upper panels). Subsequently, the brain was routinely processed for neuropathologic examination.

Immunohistochemistry

Immunohistochemistry for glial fibrillary acidic protein (GFAP) was performed using polyclonal rabbit anti-GFAP serum (Dako, Glostrup, Denmark), dilution 1:500, using the peroxidase-anti peroxidase (PAP) method for visualization. Immunostaining for the Ki-67 antigen was done by using the MIB-1 antibody (Immunotech, Marseille, France), dilution 1:50 and PAP method for visualization.

Comparative Genomic Hybridization of Archival Material

The very same DNA extracts of the tissue samples in this study were used for CGH, LOH analysis, and TP53 mutation analysis. Isolation of DNA from the formalin-fixed, paraffin-embedded tumor material was performed as described by Alers et al (11). Isolation of DNA was performed using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). High molecular weight tumor DNA was labeled with biotin by nick translation (Nick Translation System, Gibco BRL, Gaithersburg, MD). Male reference DNA (Promega, Madison, WI) was labeled by nick translation with digoxigenin (Boehringer Mannheim, Indianapolis, IN). The reaction time and the amount of DNase were adjusted to obtain a matching probe size for reference and tumor DNA. Probe sizes were between 0.7 and 1.4 kb. The labeled DNA was hybridized onto normal male metaphase chromosomes (Vysis Inc. Downers Grove, IL). CGH analysis was accomplished with Quips XL software from Vysis (version 3.1.1). Loss of DNA sequences was defined as chromosomal regions where the mean green:red ratio was below 0.85 and gain of DNA as chromosomal regions where the ratio was higher than 1.15. These threshold values were based on a series of normal controls. Chromosome X was excluded from analysis since this chromosome showed a sex mismatch, serving as an internal control.
Fig. 2. Distribution of the 5 most frequently occurring genetic aberrations detected by CGH. Upper panels: the numbers correspond to the sites from which tissue samples were randomly taken and to the sample numbers in the Table. Lower cuts containing limbic areas, cerebellum, pons, or brainstem are not shown. Lower panels: the 5 most frequently occurring aberrations were all present in sample 15. Losses on 2q and 19q were found in both hemispheres, indicative of diffuse tumor cell infiltration.
LOH and \( p53 \) Mutation Analysis

Since losses on 2q and 19q were the most frequent identical genetic aberrations detected by CGH in the present analysis, subsequent LOH analysis for markers on these chromosome arms was performed in order to verify and possibly extend these results. For LOH analysis the highly polymorphic dinucleotide repeat markers D2S347 (mapped to 2q14.3) and D2S1326 (mapped to 2q22.1) (http://genome.ucsc.edu) and D19S217 and D19S408 (both mapped to 19q13.2) (http://genome.ucsc.edu) were amplified with primers from GDB. Exons 5–8 of the \( TP53 \) gene were investigated by PCR-SSCP. Each exon was amplified in 2 overlapping fragments. LOH and \( TP53 \) PCR-SSCP analyses were performed as described previously (12). For LOH as well as for SSCP analysis the brain tissue DNA samples were compared with normal DNA isolated from the same patient’s liver and spleen. PCR was performed with 100 ng isolated DNA.

For SSCP analysis the brain tissue DNA samples were analyzed by standard dideoxy-method according to Sanger. Mutation found by SSCP was performed on 5 samples by standard dideoxy-method according to Sanger.

RESULTS

Histology and Immunohistochemistry

The frontal, parietal, and occipital lobes together with the left temporal lobe were bilaterally affected by a low cell-dense glial proliferation (Fig. 3A, B). A similar histopathology was found in all affected brain parts. The tumor cells had slightly pleomorphic nuclei and, to a variable extent, cell processes stained positive for GFAP (Fig. 3D). A smaller proportion of the cells had elongated nuclei without noticeable cell processes. Staining for the proliferation-related antigen Ki-67 (MIB-1 antibody) revealed a very low labeling index throughout the lesion, varying from 1% to 2% of nuclei, compatible with low-grade glioma.

\( TP53 \) Mutation Analysis

PCR-SSCP analysis revealed a mutation in exon 7 of \( TP53 \) in 20 of the 24 samples (Fig. 4). Bidirectional sequencing revealed a mutation in codon 234 (TAC234TGC) resulting in an amino acid substitution of Tyr-Cys.

Comparative Genomic Hybridization (CGH)

Genetic aberrations were revealed in 18 of the total of 24 samples (Table). The distribution of the set of 5 most frequently found aberrations is plotted in Figure 2. Chromosomal gains and losses were found in both frontal and parietal lobes and in the left temporal and occipital lobes (Table; Fig. 2). Most aberrations were found in the left side of the brain. Losses on 2q were present in most samples (13/24) (Fig. 5), followed by losses on 19q (10/24) (Fig. 5); gains on 7q (7/24); losses on 3q and 16q (4/24), in decreasing order of frequency (Table; Fig. 2). In sample 13, loss on chromosome arm 2q was evident but did not reach the threshold value (data not shown). In samples 2, 8, 15, and 24, losses on chromosome arm 19q remained below the threshold for loss. In sample 14, all 5 frequently occurring aberrations were seen (Fig. 2). Losses on 1q, 3p, 5q, and 10q were each found in 3 samples in close proximity to each other (Table). Losses on 13q were also found in 3 samples, although one of the samples was at a considerable distance from the other two (Table). Losses on 2p and 4q were each found in 2 closely positioned samples (Table). Additional aberrations found in single samples included gains on 1p, 4p, 10p, 10q and 19p, and losses on 5p, 10q, 11q, 12q and 19q (Table, last column). The losses on 2q, 13q and 19q were found in both the left and right hemispheres. Samples 4 and 5 contained the most genetic aberrations (Table). For the frequently found losses on 2q, 19q, 3q, and 16q, and the gains on 7q, minimal regions of overlap were 2q14.1-q21; 19q13.2; 7q31-q32; 3q25; and 16q23. All samples with DNA aberrations detected by CGH or LOH analysis also demonstrated the \( TP53 \) aberration.

Loss of Heterozygosity (LOH)

LOH for polymorphic markers D2S347 and D2S1326 was detected in 10 of 24 samples and LOH for the polymorphic markers D19S217 and D19S408 in 18 of 24 samples (Table; Fig. 6). In all samples showing LOH, the same (upper) allele was deleted (Fig. 6). In 79% of samples the results of LOH analysis for markers on 2q corresponded with the losses detected by CGH, and in 67% of samples the losses on 19q were found by both CGH and LOH analysis. A correspondence of 83% was found between CGH and LOH on 19q if the 4 samples showing shifts towards CGH loss were taken into account.

DISCUSSION

In the present study, various genetic changes in a unique autopsy case of gliomatosis cerebri were topographically mapped out in order to obtain evidence for either monoclonal or polyclonal tumor cell proliferation. Due to the presence of a relatively high percentage of normal cells in the test samples, molecular techniques...
Fig. 3. A, B: Histology of the cell proliferation as found in samples with large distance from each other, namely, sample 4 (A) and sample 21 (B). The histological picture is essentially identical: a low cell-dense neoplastic glial proliferation without vascular proliferation. The tumor cells are only slightly pleomorphic and there are no mitoses or necrosis (H&E; ×100). C: The tumor cells have oval nuclei (arrows) and the cells are seen in white matter tracts (H&E; ×400). D: Tissue sample immunostained for GFAP. There is slight pleomorphism of nuclei, compatible with low-grade glioma. The cytoplasmic processes are immunopositive for GFAP, indicative of astrocytic lineage of the cells (GFAP; ×400).

may fail to reveal genomic changes. CGH and LOH analysis may be less sensitive methods for detecting genetic aberrations as compared to SSCP. The latter technique is capable of detecting mutations in samples containing less than 20% tumor cells. The TP53 mutation found in widely separated regions of this brain serves as the strongest evidence for monoclonal origin of the tumor cells. Genome-wide screening of chromosomal aberrations was
accomplished by performing CGH on the samples taken from this brain. The set of widespread chromosomal aberrations found by CGH also pointed to monoclonal tumor cell proliferation in this case of gliomatosis. Two of the most widely distributed chromosomal aberrations found by CGH, i.e. losses on 2q and 19q, were confirmed by the detection of allelic imbalance (LOH) on the respective chromosome arms. The results of LOH analysis for all 4 markers showed that in all samples taken from the brain, an identical allele had been lost, again indicative of clonal tumor cell proliferation. The LOH analysis was more sensitive for finding losses on 19q as compared to the CGH analysis, but this was not true for the losses on 2q (Table). The percentages of concordance between the results of CGH and LOH analysis are comparable with those found in the literature (13, 14).

Taken together, the finding of identical TP53 mutations and identical regions of loss on 2q and 19q at different sites throughout the brain is indicative of monoclonal tumor proliferation, and these aberrations may be considered as early genetic events in the genesis of gliomatosis cerebri. The various additional regionally located alterations (Table) would reflect genetic divergence of the tumor rather than being the result of field cancerization. The findings strengthen the hypothesis that gliomatosis cerebri is an extreme diffusely infiltrating, low-grade glioma. The results are corroborated by those obtained from another case of gliomatosis cerebri in the literature in which identical karyotypic abnormalities also pointed to a monoclonal origin of the lesion (15). In common gliomas like low-grade astrocytoma and glioblastoma, monoclonal tumor spread has been established in a comparable way (16). In gliomatosis cerebri, the neoplastic infiltration has no radiologically discernible tumor center. In some cases of gliomatosis cerebri with adequate radiological follow-up, the development of a discernible tumor center was identified by the investigation of successive scans (17–19). In one instance, transition into a multifocal glioblastoma was recorded (20). In recent literature, cases with noticeable tumor centers are no longer rejected as true gliomatosis cerebri cases but referred to as “type 2 gliomatosis” (21). The question of whether a circumscribed tumor center would ultimately appear in all cases of gliomatosis remains unanswered. Although radiology of the present case did not reveal any tumor center, the largest number of genetic aberrations were found in the first left frontal gyrus (samples 4–6, 11, and 14 in Fig. 2; Table), perhaps the origin of the neoplastic clone.

TP53 exon 5–8 SSCP analysis, followed by bidirectional sequencing, revealed a mutation at codon 234.

Fig. 4. Results of TP53 exon 7 SSCP analysis. The lanes marked by an arrowhead contain aberrant migrating bands (arrows). N1 and N2 are normal DNA samples from the same patient. Sample 20 is not shown.
TABLE

Results of TP53 Mutation Analysis (SSCP); Chromosomal Gains and Losses (CGH); Additional LOH for the 2 Most Frequently Involved Chromosome Arms

<table>
<thead>
<tr>
<th>Left</th>
<th>TP53</th>
<th>LOH 2q</th>
<th>LOH 19q</th>
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<tbody>
<tr>
<td>1</td>
<td>m</td>
<td>2q12-q31</td>
<td>LOH</td>
</tr>
<tr>
<td>2</td>
<td>m</td>
<td>2q12-q31</td>
<td>LOH</td>
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<tr>
<td>3</td>
<td>m</td>
<td>2q12-q31</td>
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<tr>
<td>7</td>
<td>m</td>
<td>2q12-q31</td>
<td>LOH</td>
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<tr>
<td>8</td>
<td>m</td>
<td>2q13-q21</td>
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<tr>
<td>10</td>
<td>m</td>
<td>2q11-q24</td>
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<td>11</td>
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<td>15</td>
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<td>16</td>
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<td>2q14.2-q23</td>
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<td>17</td>
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<td>19</td>
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<td>2q14.2-q23</td>
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<td>20</td>
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<td>2q14.1-q22</td>
<td>LOH</td>
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<td>2q14.1-q22</td>
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<td>24</td>
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The numbers refer to the corresponding sample sites (Fig. 2, upper panels). The aberrations listed in the columns are ordered in decreasing frequency from left to right. The last column contains aberrations found only once. Gains are indicated in bold. The first column lists the TP53 mutations (m), the third and fifth columns list the findings with polymorphic markers on the chromosome arms 19q and 2q, respectively. # = Shift in CGH profile but no significant loss.

Fig. 5. Ideograms and corresponding digitized fluorescent images of the 2 most frequently occurring chromosomal aberrations detected by CGH: losses on 2q and 19q. Red bars indicate loss of specific regions.

(TAC-TGC), resulting in the substitution of tyrosin by cysteine. The mutation appears to be in a well-known mutational hotspot of the TP53 gene and has been described as germline mutation in Li-Fraumeni cases as well (p53 database; http://perso.curie.fr/tsoussi). The losses on 19q13 and 2q were the two most widespread aberrations in the present case (Table; Fig. 2). Loss on 19q13 is a frequent finding in astrocytic and oligodendroglial tumors (22–25). Losses on 2q have only incidentally been reported in high-grade gliomas (26). Losses on 3q were found in only a few studies on gliomas (26, 27). In contrast, gain of chromosome arm 7q has been a frequent finding in genomic surveys of astrocytic tumors (28, 29). The losses on 16q were only reported in primitive neuroectodermal tumors (PNETs) but not in glial tumors (30, 31).

GFAP immunopositivity found in the cells of this gliomatosis case illustrates the astrocytic lineage of the tumor cells. Some cases of gliomatosis reported in the literature consisted of cells with oligodendroglial or
gemistocytic phenotype (32, 33), strengthening the hypothesis that gliomatosis cerebri seems to be an extraordinarily diffusely infiltrating glioma rather than a separate tumor entity. In the present study, the histopathology did not differ significantly from one area to the other and appeared to be compatible with the spindle-shaped cells classically described in gliomatosis cerebri. It has been speculated that the spindle shape of the tumor cells reflects their exceptional motility (34). In 1940 it was proposed that the shape of neoplastic glial cells may change as a result of migration through pre-existing structures such as white matter tracts (6). At this point, correlations between cellular morphology, cell motility, and genetic aberrations have yet to be established.

In conclusion, the genetic dissection of this autopsy case of gliomatosis cerebri has revealed compelling evidence for clonal tumor proliferation, suggesting that gliomatosis be classified as the epitome of diffuse infiltrating low-grade glioma.

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