Chromosomal imbalances in primary oligodendroglial tumors and their recurrences: clues about malignant progression detected using comparative genomic hybridization

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Object. Despite the rapid increase in knowledge concerning the genetic basis of malignant progression in astrocytic tumors, progression of oligodendroglial tumors (including both pure oligodendrogliomas and mixed oligoastrocytomas) is still poorly understood. The aim of the present study is the elucidation of chromosomal imbalances involved in the progression of oligodendroglial tumors toward malignancy.

Methods. Using comparative genomic hybridization (CGH) on snap-frozen tumor tissue, the tumor genomes of five primary oligodendroglial tumors and associated recurrent tumors were screened for chromosomal imbalances. This information was correlated with clinical data (including follow-up data) and histopathological malignancy grade.

In all cases an increase in chromosomal imbalances was detected in the recurrent tumor, indicating genetic progression. In three of the five cases this correlated with malignant progression detected at the histopathological level. The results indicate that, similar to what occurs in astrocytic tumors, chromosomal imbalances harboring genes involved in the cell proliferation control mechanism at the G1–S border are involved in the progression of oligodendroglial tumors. Additionally, although gains of genetic material on chromosome 7 and losses on chromosome 10 are most frequently detected in the course of malignant progression of astrocytic tumors, either or both of these can also occur during malignant progression of typical oligodendrogliomas that contain losses involving chromosome 1p and/or chromosome 19q.

Conclusions. When performed on optimally preserved material from a small set of primary oligodendroglial tumors and associated recurrent tumors, CGH detects chromosomal aberrations that potentially play a mechanistic role in the malignant progression of these tumors.

KEY WORDS • oligodendroglioma • oligoastrocytoma • tumor recurrence • progressive disease • comparative genomic hybridization • histological study

Abbreviations used in this paper: CGH = comparative genomic hybridization; GBM = glioblastoma multiforme; HGOD = high-grade oligodendroglioma; HGODA = HG oligoastrocytoma; LGOD = low-grade OD; LOH = loss of heterozygosity.
and in half of pure HGODs, whereas the other half of the HGODs contained a loss of genetic material on chromosome 10 and a gain of material on chromosome 7. A combination of these aberrations occasionally has been detected in oligodendroglial tumors. To obtain better insight into aberrations involved in malignant progression of oligodendroglial tumors, we performed CGH on five primary oligodendroglial tumors and associated recurrent tumors.

### Materials and Methods

Surgical specimens of tumors selected for the present study were obtained from patients treated at the University Medical Center Nijmegen and the Canisius Wilhelmina Hospital. During surgery, a portion of the tumor was snap frozen in liquid nitrogen and stored at −80°C. One patient (Case 5) underwent surgery for an ODA four times, but snap-frozen tissue samples were only available from the third (LGODA) and fourth (HGODA) biopsies. In this paper, we will regard these samples as primary and recurrent tumor, respectively.

Hematoxylin and eosin–stained paraffin sections of tumor were examined by an experienced neuropathologist and classified according to the most recent World Health Organization classification. Oligodendroglial tumors without prominent astrocytic differentiation (< 5% of tumor cells) were diagnosed as pure ODs, whereas tumors containing more prominent astrocytic features were classified as ODAs. Tumors were diagnosed as being of high grade if at least three of the following criteria were present: high cellularity, nuclear polymorphism, marked mitotic activity (> 10/2 mm²), necrosis, and florid microvascular proliferation.

Comparative genomic hybridization was performed in the exact manner previously described by using biotin– or digoxigenin–deoxyuridine triphosphate for labeling in nick translation and streptavidin–fluorescein isothiocyanate and sheep antidigoxigenin–tetramethylrhodamine (Roche Diagnostics Nederland BV, Almere, The Netherlands), respectively, for detection. Analysis was performed with the aid of CGH computer software (QUIPS; Applied Imaging International Ltd., Newcastle upon Tyne, UK) and the standard thresholds determined for gains (1.2) and losses (0.8) of genetic material were used. Aberrations of 1.4 and 0.6 were called clear-copy number changes.

Based on our previous CGH studies on 29 pure ODs and 39 mixed ODAs, the tumors were subtype as “−1p/−19q” (characterized by loss of genetic material on chromosome 1p and/or chromosome 19q), “+7/−7” (characterized by a gain of genetic material on chromosome 7 and/or a loss on chromosome 10, but no loss on chromosome 1p or 19q), “−1p/−10” (characterized by loss of genetic material on chromosome 1p and/or chromosome 19q in combination with a gain on chromosome 7 and/or a loss on chromosome 10), or “other” (tumors without the previously mentioned aberrations).

### Results

The clinical data obtained in patients and the histopathological diagnoses of the tumors are summarized in Table 1. Because astrocytic features were not prominent in Tumors 1a, 1b, 3a, and 3b, these tumors were classified as pure ODs, whereas Tumors 2a, 2b, 4a, 5a, and 5b, were classified as ODAs. All primary tumors as well as the recurrent tumors in Cases 1 and 2 were diagnosed as low-grade tumors. The other three recurrences were classified high-grade tumors because, in contrast to the primary tumors, they showed marked nuclear polymorphism (Tumor 3b), more than 10 mitoses/2 mm² (Tumors 3b and 4b), necrosis

### Table 1

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Patient Age (yr)</th>
<th>Tu-</th>
<th>KPS Score</th>
<th>Postop Therapy</th>
<th>Survival Interval</th>
<th>WHO Grade</th>
<th>CGH-Detected Chromosome</th>
<th>No. of Aberrations</th>
<th>Original Sample</th>
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<tr>
<td>1</td>
<td>37.9</td>
<td>1a</td>
<td>100</td>
<td>RT</td>
<td>10.3A</td>
<td>7.5</td>
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<td>1p, 15q, 19q††, 19q‡‡, 22</td>
<td>−1p/−19q</td>
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<tr>
<td></td>
<td>45.4</td>
<td>1b</td>
<td>90</td>
<td>CP</td>
<td>2.8A</td>
<td></td>
<td>LGOD</td>
<td>1p, 15q, 19q, 15q18, 15q19, 19q22</td>
<td>−1p/−19q</td>
</tr>
<tr>
<td>2</td>
<td>48.7</td>
<td>2a</td>
<td>100</td>
<td>none</td>
<td>6.4D</td>
<td>5.8</td>
<td>LGODA</td>
<td>3q11−24</td>
<td>+7/−10</td>
</tr>
<tr>
<td></td>
<td>54.4</td>
<td>2b</td>
<td>70</td>
<td>none</td>
<td>0.6D</td>
<td></td>
<td>LGODA</td>
<td>3q13−24, 5q31, 9p, 10, 13q11−31, 14q11−24, 18p</td>
<td>+7/−10</td>
</tr>
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<td>3</td>
<td>46.5</td>
<td>3a</td>
<td>100</td>
<td>none</td>
<td>5.1A</td>
<td>1.6</td>
<td>LGOD</td>
<td>1p, 15q, 19q††, 19q‡‡, 19q22, X</td>
<td>−1p/−19q</td>
</tr>
<tr>
<td></td>
<td>48.1</td>
<td>3b</td>
<td>90</td>
<td>CP</td>
<td>3.5A</td>
<td></td>
<td>LGOD</td>
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<td>−1p/−19q</td>
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<tr>
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<td>4a</td>
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<td>none</td>
<td>3.0D</td>
<td>2.3</td>
<td>LGODA</td>
<td>3p21−q21, 11p−q14, 14q14−24, 12q21−23, 19q13, 22q13</td>
<td>1p/−19q</td>
</tr>
<tr>
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<td>27.8</td>
<td>4b</td>
<td>70</td>
<td>RT</td>
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<td>HGODA</td>
<td>3p21−q21, 10, 11p−q14, 14q14−qter, 12p, 12q21−23, 13q11−31, 19q13, 21, 22q13</td>
<td>1q, 1p, 8q, 9q, X</td>
</tr>
<tr>
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<td>23.1</td>
<td>5a</td>
<td>90</td>
<td>none</td>
<td>3.6A</td>
<td>2.5</td>
<td>HGOD</td>
<td>1p11−31, 19q13.2–qter</td>
<td>−1p/−19q</td>
</tr>
<tr>
<td></td>
<td>25.6</td>
<td>5b</td>
<td>60</td>
<td>RT</td>
<td>1.1A</td>
<td></td>
<td>HGODA</td>
<td>1p11−31, 10q24–qter, 14q13.2–qter, 22</td>
<td>−1p/−19q</td>
</tr>
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</table>
Chromosomal imbalances involved in oligodendroglial tumor progression

(Tumors 4b and 5b), and/or florid microvascular proliferation (Tumors 4b and 5b). Representative areas of hematoxylin and cosin-stained paraffin sections of all primary and recurrent tumors are shown in Fig. 1. Additionally, the chromosomal imbalances on chromosomes 1, 7, 10, and 19 detected by CGH are depicted in this figure. The chromosomal imbalances detected by CGH are summarized in Table 1 and shown for each in Fig. 2. The genetic subtyping of the tumors is also indicated in Table 1. Overall, CGH detected 23 aberrations (17 losses and six gains) in the primary tumors and 54 aberrations (36 losses and 18 gains) in the recurrent tumors. Although the majority of the aberrations detected in the primary tumors were also detected in the recurrent tumors, some aberrations (indicated in italics in Table 1) were not present in the corresponding recurrent tumor. In addition, all recurrent tumors harbored newly acquired chromosomal imbalances. Some of these aberrations were already present in the primary tumors (underlined in the table), but for these aberrations the thresholds were not crossed. In Fig. 3, representative examples of ratio profiles of such aberrations and corresponding standard deviations for one couple of primary and recurrent tumors (12–15 chromosomes were analyzed) are shown.

Discussion

Genetic and Histopathological Progression in Oligodendroglial Tumors

To investigate chromosomal imbalances involved in progression of oligodendroglial tumors, we analyzed five pairs of primary tumors and associated recurrent tumors by performing CGH. Because DNA isolated from paraffin-embedded tissue becomes degraded and crosslinked during fixation (depending on the fixation time and fixative used), we only used snap-frozen tumor samples to exclude artifacts that could arise from differences in processing of paraffin-embedded tissue between each biopsy sample in a patient. In all cases an increase in chromosomal imbalances was detected in the recurrent tumor, indicating genetic progression. Interestingly, some of these aberrations were already present in the primary tumor but did not cross the thresholds, implying that these aberrations were present in only a limited percentage of cells in the primary tumors. A few aberrations detected in a primary tumor were absent in the recurrent tumor. This may have been caused by clonal expansion of a cell population without this aberration or by sampling, because only a small portion of the tumor was analyzed using CGH. In three of the five patients (Cases 3–5), recurrent tumors displayed histopathological features of increased malignancy when compared with the primary tumors (histopathological progression). In samples from Case 1 an increase in chromosomal imbalances was found; however, no aberrations reported to be involved in the malignant progression of gliomas, such as losses of genetic material on chromosomes 9p, 13q, or 10 or gains of material on chromosomes 12q or 7q11 (see later discussion), were acquired in the recurrent tumor. This patient is still alive and well 2.8 years after biopsy of the recurrent tumor. Thus histopathological, genetic, and clinical investigations agree on the diagnosis of the recurrent tumor in Case 1 as an LGOD. In Case 2, on the other hand, histopathological progression was absent, but CGH detected the accumulation of aberrations known to be involved in malignant progression of gliomas (losses of chromosomes 9p and 13q11–31 and a gain of chromosome 7p11–15). This patient died as a result of tumor growth 0.6 years after biopsy of the recurrent tumor and, hence, this tumor may indeed have been more malignant than expected on the basis of the histopathological diagnosis. In addition to histopathological examination genetic analysis might have been useful in predicting malignant progression in this case, implying a shorter duration of survival for the patient.

Oncogenesis and Genetic Subtypes of Oligodendroglial Tumors

In a previous CGH study we found that 29 tumors determined to be pure ODs could be subtyped as either −1p/−19q tumors or as +7/+10 tumors.14 A recent CGH analysis of 39 ODAs revealed two additional genetic subtypes, that is, “intermediate” (−1p and/or −19q in combination with +7 and/or −10) and “other” (tumors without −1p, +7, −10, or −19q).10 Oligodendroglial tumors reported in other CGH studies can also be subclassified according to these four genetic subtypes.2,14,27 Because losses of genetic material on chromosomes 1p and 19q are early events in the oncogenesis of these tumors, whereas a gain on chromosome 7 and a loss on chromosome 10 are regarded as late events in glioma oncogenesis,14,16,18,20,23,24 we hypothesize that intermediate oligodendroglial tumors progressed from −1p/−19q tumors. In the current study, we show that intermediate tumors indeed can progress from −1p/−19q tumors and, therefore, it is more appropriate to designate them “intermediate −1p/−19q tumors.” To our knowledge no one has attributed a gain of chromosome 7 and/or a loss of chromosome 10 to −1p/−19q oligodendroglial tumors during malignant progression, a phenomenon that is well known in astrocytic tumors.

Histopathological delineation of GBMs with or without oligodendroglial differentiation and HGODAs is difficult. The 10% of GBMs that previously have been reported to have had a loss of genetic material on chromosome 1p and/or chromosome 19q10 may very well represent tumors that are classified as −1p/−19q or intermediate −1p/−19q HGODAs in the present study. Simply diagnosing these tumors as GBMs implies that they will not be included in chemotherapy protocols for oligodendroglial tumors, although they harbor the positive predictor for chemosensitivity, LOH on chromosome 1p.4 Although our results show that a gain of genetic material on chromosome 7 and a loss on chromosome 10 are late events in the oncogenesis of oligodendroglial tumors, the intermediate −1p/−19q subtype has also been identified in five of 33 low-grade oligodendroglial tumors.5,14,25 Whether identification of this genetic subtype in these tumors is important for prognosis should be further analyzed.

Other Chromosomal Imbalances Involved in Oligodendroglial Tumor Progression

Loss of heterozygosity involving chromosome 9p is reported to be associated with high-grade gliomas,3,4,6,7,11,13,15,16,18,20,23 We previously hypothesized that a loss of genetic material on chromosome 9p in LGODs should, therefore, be considered an early marker for malignant progression.11 Our current results support this hypothesis: in Case 3 the
Fig. 1. Photomicrographs showing histological findings and ideograms depicting chromosomal imbalances for chromosomes 1, 7, 10, and 19 of all primary (a) and recurrent (b) tumors. Circed numbers indicate case numbers. Histological features indicated by arrows are as follows: Tumor 1b, calcium deposition; Tumor 2a, microcystic change; Tumor 3b, mitoses; Tumor 4a, entrapped neuron; Tumor 4b, necrosis; and Tumor 5b, florid microvascular proliferation. Chromosomal imbalances for chromosomes 1, 7, 10, and 19 are also shown. The chromosome number is shown under the appropriate ideogram. Imbalances are indicated by lines on the left and/or right of each chromosome ideogram, which indicate losses and gains of these chromosomal regions, respectively. H & E, original magnification × 250.
LGOD, which contained a loss of chromosome 9p, progressed to become an HGOD, whereas both low-grade tumors without this chromosomal loss (Cases 1 and 2) did not display malignant histopathological progression. A loss of chromosome 9p, however, appears not to be essential for malignant progression of oligodendroglial tumors because it was detected in only some recurring high-grade tumors by CGH or LOH analysis (case reports and those of others). The products of these genes are involved in the cell proliferation control mechanism at the G1–S border. Although CGH analysis only reveals imbalances of chromosomal regions without gene-specific information, the same mechanism was detected by CGH in GBMs, showing a gain on 12q (CDK4), a loss on chromosome 9p (CDKN2A), and a loss on chromosome 13q (RB1) to be complementary. Our CGH results indicate that this mechanism is also involved in malignant progression of oligodendroglial tumors; one of the two high-grade tumors that had no loss on chromosome 9p contained a loss of chromosome 13q11–31 including the RB1 region (Tumor 4b).

Previously, it was suggested that progression of LGODs to HGODAs is associated with deletions on chromosomes 3p, 14q, and 15q. In the present study, loss of 14q was acquired in the recurrent HGODA obtained in Case 5; however, loss of genetic material on chromosome 14q was also acquired in the recurrent low-grade tumors of Cases 1 and 2. Additionally, it was suggested that a gain of chromosome 7q is an early event in the initiation of astrocytic tumors, whereas a gain of chromosome 7p is predictive of short survival times in patients with astrocytic tumors. In the patient in Case 2, the gain of 7p11–15 might indeed be considered as an indicator of short survival (0.6 years after detection), in contrast to a gain in chromosome 7q31–qter.
Our results show accumulation of chromosomal imbalances in oligodendrogial tumors over time. In three of five cases this was accompanied by histopathological signs of progression. Although within the context of gliomas, the loss of chromosomes 1p and 19q are characteristic early events in the oncogenesis of oligodendrogial tumors, we detected similarities in malignant progression between these tumors and astrocytic tumors. Although a gain of chromosome 7 and a loss of chromosome 10 are most frequently detected in GBMs, they are also involved in the oncogenesis of oligodendroglial tumors, either in tumors in which there is no loss of genetic material on chromosome 1p or 19q (+7/−10 tumors) or in combination with loss on chromosome 1p or 19q (intermediate −1p/−19q tumors). Additionally, chromosomal imbalances harboring genes involved in the cell proliferation control mechanism at the G1–S border (CDKN2A [−9p21], CDK4 [+12q13.3−q14], RB1 [−13q14.3]) can be involved in progression of oligodendrogial tumors and can be regarded as early signs of progression in low-grade tumors. In summary, CGH performed on optimally preserved material of a small set of primary oligodendrogial tumors and associated recurrent tumors revealed chromosomal aberrations that are involved in malignant progression of these tumors. Investigation of a larger group of oligodendrogial tumors is necessary to analyze further the chromosomal imbalances and their potential target genes involved in malignant progression and to establish the additional value of genetic analysis of these aberrations in predicting malignant progression, which may be helpful to guide therapeutic decisions.

Conclusions

References

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