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Published in:
Journal of Neurology, Neurosurgery and Psychiatry

DOI:
10.1136/jnnp.2003.025031

Citation for published version (APA):

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J. Neurol. Neurosurg. Psychiatry 2004;75;723-726
doi:10.1136/jnnp.2003.025031

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Molecular-genetic characterisation of gliomas that recur as same grade or higher grade tumours

T J M Hulsebos, D Troost, S Leenstra

INTRODUCTION

Gliomas are the most common primary neoplasms of the central nervous system. Their capacity to invade surrounding normal brain prevents complete removal of the tumour. Despite intensive radio- or chemotherapy, there is almost always recurrence of the tumour. Gliomas usually recur as a same grade or as a higher grade lesion. However, the time to recurrence and the type of the recurrent tumour are highly variable and, in general, cannot be predicted from the histopathological and clinical characteristics of the primary tumour.

Glioma tumourigenesis is thought to be the consequence of the accumulation of genetic changes that confer growth advantage to a glial cell. Important genetic changes in gliomas are loss of heterozygosity (LOH) at 1p, 9p, 10p, 10q, 13q (with RB1), 17p, 19q, and 22q, EGFR amplification, homozygous deletion of CDKN2A/B on 9p and DMBT1 on 10q, and mutation of PTEN on 10q and TP53 on 17p. Earlier, we studied genetic changes associated with glioma recurrence in a limited series of cases with same grade or higher grade recurrences. We concluded that glioma recurrence is characterised by the increased involvement of tumour suppressor genes or gene regions, even in those cases in which the primary and the recurrent tumour were of the same (high) malignancy grade. These series did not allow us to draw conclusions about possible preferences with regard to the type of genetic changes involved in glioma recurrence or possible differences in accumulation rate of these genetic changes between the two types of recurrence. To investigate this, we extended our analyses by the inclusion of a substantial number of new cases of both types of recurrence. We specifically studied LOH at 1p36, 19q13, 10p15, 10q23 with PTEN, and 10q25 with DMBT1, LOH and mutation of TP53, and EGFR amplification as indicators of genetic instability because these are well known genetic changes that occur rather frequently in gliomas. In addition, we analysed LOH at 22q13 because we, and others, have recently given evidence for the presence of a tumour suppressor gene in that region that might be involved in astrocytoma progression.

METHODS

Tumour samples and clinical data

Tumour samples were obtained from 40 pairs of primary gliomas and one or more recurrences. Of these, 18 have been analysed for genetic changes previously. All tumour samples were classified and graded according to the World Health Organization (WHO) criteria. DNA was extracted from frozen samples or from formalin fixed, paraffin embedded sections according to standard methods. The clinical data for the patients are given in table 1.

LOH analysis

Primer sequences for amplification of markers and genes and conditions for polymerase chain reaction (PCR) were taken from the Genome Database (www.gdb.org/). The LOH status of the chromosomal regions was determined by analysing the markers that we used in our previous study and the following additional markers: D1S468/1795, D19S1180/ 1182, PLAG4C, D10S186/212/215/249/541/542/587/597/608/ 1234/1716/1745, PTENCA, D22S270/274/282/418/922/928/ 1141/1149/1151/1169/1170. The LOH status of each region was inferred from the data of at least two informative markers.

Abbreviation: LOH, loss of heterozygosity
Table 1 Genetic changes in cases with same lower grade, glioblastoma, and higher grade recurrences

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/ Sex</th>
<th>Histology</th>
<th>Treatment</th>
<th>Interval (months)</th>
<th>Loss of heterozygosity (LOH)††</th>
<th>LOH</th>
<th>TP53</th>
<th>MUT**</th>
<th>LOH</th>
<th>PTEN</th>
<th>DMBT1</th>
<th>RPE1</th>
<th>EGFR Ampl</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>1p36</td>
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<td>10p15</td>
<td>PTEN</td>
<td>DMBT1</td>
<td>IP53</td>
<td>IP53</td>
<td>22q13</td>
<td>Ampl</td>
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<tr>
<td>2020</td>
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<td>n</td>
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<td>n</td>
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<td>n</td>
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<td>n</td>
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<td>DA/DA</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>y</td>
<td>y</td>
<td>n</td>
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<td>DA/DA</td>
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<td>143</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>n</td>
<td>n</td>
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<tr>
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<td>y</td>
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<td>n</td>
<td>n</td>
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<td>y</td>
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<tr>
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<td>62/M</td>
<td>AO/AO</td>
<td>RT</td>
<td>27</td>
<td>y</td>
<td>n</td>
<td>n</td>
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<td>47/M</td>
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<td>AO/AO</td>
<td>RT, CT</td>
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<td>n</td>
<td>n</td>
<td>y</td>
<td>n</td>
<td>n</td>
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<td>n</td>
<td>n</td>
<td>y</td>
<td>n</td>
<td>n</td>
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<td>n</td>
<td>y</td>
</tr>
<tr>
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<td>52/V</td>
<td>AA/AO</td>
<td>RT</td>
<td>92</td>
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<td>n</td>
<td>y</td>
<td>y</td>
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<td>n</td>
<td>y</td>
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<td>RT</td>
<td>56</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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<tr>
<td>Higher grade recurrences</td>
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<td>AA/AO/AOA</td>
<td>RT/CT</td>
<td>5/53</td>
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<td>n</td>
<td>n</td>
<td>n</td>
<td>y</td>
<td>y</td>
<td>y</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
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<td>29/M</td>
<td>AA/OA/AO</td>
<td>CT</td>
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<td>n</td>
<td>n</td>
<td>y</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
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</tr>
</tbody>
</table>

*Number in parentheses denotes case number in a previous communication
†Age (years) at first operation
$^2$P, primary tumour; R, first recurrence; RR, second recurrence; PA, pilocytic astrocytoma; DA, diffuse astrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma multiforme; GBM-PNET, primitive neuroectodermal tumour-like glioblastoma; GBM-glias, mixed glioblastoma-gliosarcoma; GBM-O, glioblastoma with oligodendrogial features; A/O, (anaplastic) oligodendroglioma; A/OA, (anaplastic) oligoastrocytoma
$^\ddagger$RT, radiotherapy; BR, bromochloroethytherapy; CT, chemotherapy
$^*\ddagger$Interval between operations
**Type and position of TP53 mutation were reported previously or can be obtained from the corresponding author (T.H.)
††See table 2 for details. Imb denotes imbalance.

Table 2 Marker allele diminished or lost in primary tumour (P) or recurrence (R)

<table>
<thead>
<tr>
<th>Case</th>
<th>P/R</th>
<th>Marker</th>
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<tr>
<td>1083</td>
<td>P</td>
<td>(10p15)</td>
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<td></td>
<td>P</td>
<td>bottom dim</td>
</tr>
<tr>
<td>1083</td>
<td>R</td>
<td>(22q13)</td>
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<tr>
<td></td>
<td>R</td>
<td>bottom dim</td>
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<tr>
<td>2111</td>
<td>P</td>
<td>(10p15)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>bottom lost</td>
</tr>
</tbody>
</table>

TP53 mutation and EGFR amplification

Exons 5 and 6 of TP53 were directly sequenced. Exons 7 and 8 were first screened for the presence of mutations by denaturing gradient gel electrophoresis, which was followed by direct sequencing of the mutation-containing exon. Amplification level of EGFR was determined as described previously.

RESULTS

Forty pairs of primary tumour and one or more recurrent tumours were analysed for LOH at 1p36, 19q13, 10p15, the PTEN region in 10q23, the DMBT1 region in 10q25, LOH and mutation of TP53, LOH 22q13, and EGFR amplification. These genetic changes were studied because they frequently occur in gliomas and because they have prognostic and diagnostic significance for certain types of glioma. Except for LOH 22q13, which was newly determined for all cases in this study, the genetic changes for 18 cases were taken from a
probably present, their mixed opposite allelic losses could chromosomal region was lost. Since both subclones were (table 1). In a subsequent step, two subclones would have genetic changes were already present in the primary tumour TP53 inactivation of the primary tumour. In case 1083, an early event would be explained by the presence of more than one subclone in between primary and recurrent tumour that we noted might affect genetic changes that confer growth advantage to a cell.11 In Tumours are thought to develop by the temporal acquisition of genetic changes in the primary tumour, which was no longer present in the first or second recurrent tumour (fig 1D). Outgrowth of one subclone with additional LOH at 1p36 and DMBT1 would then have resulted in the recurrent tumour with almost complete allelic loss at 10p15 and 22q13. The apparently opposite allelic losses for 10p15 markers in primary and recurrent tumour of case 2111 might be explained by assuming that the recurrent tumour was the outgrowth of a subclone that was underrepresented in the primary tumour. In case 2778, the primary tumour was an oligoastrocytoma with clear oligodendrogial and astrocytic elements (not shown). Others have analysed the two components of biphasic oligoastrocytomas and found mutation and overexpression of TP53 in the astrocytic component only.13 Therefore, in our case it might be that the recurrent tumours, which were oligodendrogliomas, were outgrowths of the oligodendrogial component of the primary tumour without TP53 mutation. We conclude that lower grade primary gliomas might be genetically heterogeneous, permitting outgrowth to a higher grade recurrence of a subclone that was not dominantly present in the primary tumour.

**DISCUSSION**

Genetic heterogeneity in the primary tumour

Tumours are thought to develop by the temporal acquisition of genetic changes that confer growth advantage to a cell.1 In a primary tumour, this clonal evolution may result in the development of variant sublines with different sets of genetic changes. The apparent discrepancies in genetic changes between primary and recurrent tumour that we noted might be explained by the presence of more than one subclone in the primary tumour. In case 1083, an early event would be inactivation of TP53 by mutation and deletion, since both genetic changes were already present in the primary tumour (table 1). In a subsequent step, two subclones would have developed in which one or the other—10p15 or 22q13—chromosomal region was lost. Since both subclones were probably present, their mixed opposite allelic losses could have resulted in the allelic imbalances noted for this primary tumour (fig 1A, B, table 2). Outgrowth of one subclone with additional LOH at 1p36 and DMBT1 would then have resulted in the recurrent tumour with almost complete allelic loss at 10p15 and 22q13. The apparently opposite allelic losses for 10p15 markers in primary and recurrent tumour of case 2111 might be explained by assuming that the recurrent tumour was the outgrowth of a subclone that was underrepresented in the primary tumour. In case 2778, the primary tumour was an oligoastrocytoma with clear oligodendrogial and astrocytic elements (not shown). Others have analysed the two components of biphasic oligoastrocytomas and found mutation and overexpression of TP53 in the astrocytic component only.13 Therefore, in our case it might be that the recurrent tumours, which were oligodendrogliomas, were outgrowths of the oligodendrogial component of the primary tumour without TP53 mutation. We conclude that lower grade primary gliomas might be genetically heterogeneous, permitting outgrowth to a higher grade recurrence of a subclone that was not dominantly present in the primary tumour.

**Additional genetic changes in the recurrent tumour**

The total number of additional genetic changes acquired during glioma recurrence was considerably higher for the group of lower grade gliomas with a higher grade recurrence (mean 2.0 genetic changes per case) than for the group of lower grade gliomas with a same grade recurrence (mean 0.6) or the group of glioblastoma recurrences (mean 0.8). In accordance with earlier reports,13 14 we found a remarkable high frequency of TP53 mutation (93%) in the primary tumour of the group of higher grade recurrence cases. Inactivation of TP53 is supposed to induce genetic instability, including specific chromosomal deletions.15 The additional genetic changes acquired during glioma recurrence might be a measure for the latter. However, analysing all 40 cases of glioma recurrence, we did not find a statistically significant association between the presence of TP53 mutation in the primary tumour and the presence of additional genetic.

**Figure 1** Cases with apparent genetic heterogeneity in the primary tumour. (A, B) Microsatellite analysis of the primary (P) and recurrent (R) tumour of case 1083. (A) Diminished intensity of the bottom allele of D10S249 in the primary tumour (open arrowhead) and loss of the top allele in the recurrent tumour (filled arrowhead). (B) Diminished intensity of the top allele of D22S282 in the primary tumour and loss of the bottom allele in the recurrent tumour. (C) Microsatellite analysis of the primary and recurrent tumour of case 2111. Loss of the top allele of D10S1435 in the primary tumour and loss of the bottom allele in the recurrent tumour. (D) DGGE analysis of exon 8 of TP53 of the primary tumour and first and second recurrent tumour of case 2778. Presence of the TP53 mutation in the primary tumour, absence in the first and second recurrent tumour. N, normal DNA from corresponding, leukocytes. See table 1 for tumour abbreviations.

Previous study.1 The 40 cases were subdivided in 14 cases with a same lower grade recurrence, 12 cases with a (variant) glioblastoma recurrence and 14 cases with a higher grade recurrence. The combined data are listed in table 1. As can be concluded from this table, in many cases the investigated genetic changes proved to be already present in the primary tumour and retained in the recurrence. Cases with additional genetic changes in the first or second recurrence were found and these are shown in bold. Apparent discrepancies between the genetic changes in the primary tumour and the corresponding recurrent tumour were noted in three cases of low grade glioma with a higher grade recurrence. Case 1083 displayed allelic imbalance with apparent reduction in intensity of one allele in the primary tumour for markers in 10p15 and 22q13, whereas the other allele was almost completely lost in the recurrent tumour (fig 1A, B, table 2). In case 2111, the lost allele of markers in 10p15 seems to be different in the primary tumour and the recurrent tumour. (fig 1C, table 2). Case 2778 had a TP53 mutation in exon 8 in the primary tumour, which was no longer present in the first or second recurrent tumour (fig 1D).
changes in the recurrence (TP53 mutation yes v no, p = 0.21, χ²). Therefore, it is unlikely that TP53 mutation in the primary tumour is the major cause of the increased accumulation of genetic changes in the higher grade recurrence cases. It remains to be determined what other factors induce the increased accumulation of genetic changes in the latter cases.

Genetic changes in operable recurrent glioblastomas

EGFR amplification in the primary glioblastoma—that is, a glioblastoma at first presentation, was present in only one of 12 (8%) glioblastoma recurrence cases (table 1). This is remarkable considering that unselected primary glioblastomas display EGFR amplification in 34% of cases. It has to be emphasised that all glioblastomas recur but that in only a small percentage of cases the recurrence is operated depending on the presence of prognostically more favourable clinical indices, such as high Karnofsky performance score, low age of the patient, and long interval between primary tumour and recurrence. Others have shown that the time to glioblastoma recurrence is significantly shorter for patients with EGFR amplification than for those without. These data would indicate that primary glioblastomas with EGFR amplification have a small chance to recur as an operable tumour. In our series of glioblastoma recurrences, four cases (33%) displayed complete LOH 10, and five cases (42%) had TP53 mutation in the primary tumour. This is considerably less frequent for LOH 10 and more frequent for TP53 mutation than reported for EGFR amplification in 34% of cases. We found a low frequency of TP53 mutation with or without an operable recurrence, display genetic changes in operable recurrent glioblastomas that are secondary glioblastomas. Secondary glioblastomas typically develop in young patients (less than 45 years of age) and primary glioblastomas in older patients (mean age of 55 years). Indeed, the mean age of the patients in our glioblastoma recurrence group was 45.8 years (table 1), which corresponds more to the age at which patients develop a secondary glioblastoma. Finally, the glioblastomas that recurred as an operable tumour were glioblastomas at first presentation. However, it cannot be excluded that these primary tumours developed from an undetected precursor lesion and were in fact secondary glioblastomas.

In conclusion, unselected primary glioblastomas—that is, with or without an operable recurrence, display EGFR amplification in 34% of cases. We found a low frequency of EGFR amplification (8%) in our series of primary glioblastomas that recur as an operable tumour. Taken together, this would indicate that primary glioblastomas with EGFR amplification would have a small chance to recur as an operable tumour.

ACKNOWLEDGEMENTS

We thank A Ararou, P W A Cornelissen, and S Redeker for expert technical assistance. This work was supported by the Dutch Cancer Society, grant number UVA 2001–2561.

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Competing interests: none declared

REFERENCES


