Loss of heterozygosity for DNA polymorphisms mapping to chromosomes 10 and 17 and prognosis in patients with gliomas

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Abstract

Twenty nine patients with gliomas were investigated for loss of heterozygosity for 40 DNA polymorphisms in tumour DNA, particularly concentrating on those mapping to chromosomes 10 and 17. Eight of 18 grade IV gliomas showed loss of sequences from chromosomes 10, 17, or both. The data suggested total loss of one copy of chromosome 10, but there were interstitial deletions of the short arm of chromosome 17 in three of five tumours. Heterogeneous interstitial deletions of chromosome 17 were also found in two lower grade astrocytomas and one benign oligodendroglioma. The striking finding of this study was that patients with high grade gliomas whose tumours exhibited loss of heterozygosity for chromosomes 10, 17, or both survived significantly longer after surgery (median 17.4 months) than those whose tumours did not show loss of these chromosomes (median 6.7 months). These findings suggest that there is a subset of particularly aggressive high grade gliomas with no currently known molecular genetic abnormalities.

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Gliomas are the most common primary tumours of the nervous system and there is evidence that the genetic mechanisms involved in their pathogenesis are complex. They exhibit various cytogenetic abnormalities, including polyploidy and aneuploidy, but also a tendency to show specific chromosomal loss, particularly for chromosome 10.¹ It is possible to detect loss of DNA sequences in tumours by assessing the maintenance or loss of heterozygosity (LOH) in tumour DNA compared with that from the patient's blood. This can be most easily demonstrated if genomic DNA from the patient is heterozygous for a given restriction fragment length polymorphism (RFLP), but one allele is missing from tumour DNA. With this approach, which is more sensitive for small chromosomal deletions, it has been shown that LOH for sequences mapping to chromosome 10 is common in glioblastomas.²⁻⁸ Losses of chromosome 17, particularly the short arm, occur in all grades of astrocytomas.⁹⁻¹⁴ Other chro-

mosomal regions lost in some adult astrocytomas include 9p, 13q, 19q, and 22q.^{2,4,15-18} It has been suggested that survival time is better in patients whose gliomas have normal karyotypes on cytogenetic analysis than those with tumours showing clonal abnormalities,¹⁹ but the relation, if any, between prognosis and molecular genetic abnormalities has not been reported. In this study we have investigated a total of 29 gliomas for LOH of DNA sequences, particularly concentrating on chromosomes 10 and 17. In addition, we have correlated the presence or absence of LOH with clinical outcome.

Patients and methods

Samples of tumours were obtained from 29 patients with gliomas of a variety of histological grades at the time of debulking surgical procedures performed between February and October 1988. These samples were adjacent to those analysed histologically. DNA was extracted from carefully dissected and washed tumour tissue and simultaneously obtained blood samples by standard methods. Approximately 3 µg of DNA from each sample was digested separately with *MspI*, *TaqI*, *PvuII*, *PstI*, *EcoRI*, and *HindIII*. The digested DNA was electrophoresed through horizontal agarose gels, with tumour and blood DNA samples from each patient in adjacent lanes, and transferred to nylon membranes (Hybond-N, Amersham) by Southern blotting before hybridisation to probes labelled with ³²P. These included eight mapping to chromosome 10, 13 (detecting 14 RFLPs) mapping to 17p, and one to 17q (figure). RFLPs were also studied with a further 18 probes mapping to 15 other chromosomes: D1S7 (1p35-33), MST1 (3p21), D4S10 and D4S95 (4p16.3), D5S43 (5q35-qter), pHLA1.1 (6p21.3), D7S23 (7q31-32), D9S10 (9q34-33), NRASL1 (9p12-21), D11S97 (11q13.1), GLI (12q13), RB1 (13q14.3), D15S86 (15q26), D16S85 (16p13.3), LDLR (19p13.2), D19S9 (19q13.1), D20S26 (20q), and D22S164 (22q).²⁰ After hybridisation filters were exposed to Fuji-XR film for 24 hours to seven days at -70°C; hybridisation patterns of tumour and blood DNA were compared on the resulting autoradiographs.

The tumour sections were graded histologically by one neuropathologist (JFG), as oligodendroglioma and astrocytoma grades I-IV as defined by Daumas-Duport and colleagues.²¹

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Chromosome 10 loci and patterns of loss of heterozygosity

		Tumour number							
Band	Locus	49	35	20	36	33	23	32	2
p15	D10S92	●	▲	●	●	▲	●	▲	▲
	D10S17	ND	ND	▲	ND	ND	●	ND	ND
q11.2	D10S15	▲	▲	ND	ND	▲	▲	▲	▲
q21.1	D10S22	▲	ND	▲	ND	ND	●	ND	●
q21.1-22	D10S19	●	▲	▲	▲	▲	ND	▲	▲
q23.3	GLUD	ND	▲	▲	▲	●	▲	▲	▲
q26	D10S25	ND	ND	ND	▲	ND	●	ND	▲
	D10S90	●	●	▲	▲	●	ND	●	●

Chromosome 17 loci and patterns of loss of heterozygosity

		Tumour number/type							
Band	Locus	49	35	33	50	12	Olig	Astro I	Astro III
p13.3	D17S5	●/▲	○	●/▲	▲/▲	ND/▲	▲	▲	●/▲
	D17S28	●	○	●	▲	▲	▲	○	▲
pter-12	D17S126	▲	ND	ND	▲	▲	○	▲/▲	▲
	D17S68	ND	○	ND	ND	ND	▲	●	▲
	D17S66	▲	▲/▲	ND	▲	ND	▲	▲/V	▲
p13.1	TP53	▲	▲	ND	▲	ND	▲	○	▲
pter-p12	D17S67	ND	ND	ND	ND	ND	ND	○	ND
p12-11.2	D17S124	ND/V	●	ND	ND	ND	○	▲	▲/V
	D17S125	○	▲	ND	●/V	ND	●/▲	○	○
	D17S122	○	ND	ND	○	ND	▲	ND	▲
	D17S71	○	▲	ND	▲	▲	▲	▲	▲
	D17S61*	▲	▲	ND	○	ND	▲/V	ND	▲
p11.2-cen	D17S58	▲	▲	ND	▲	●	○	○	▲
q11.2-12	THRA1	▲	▲/V	ND/V	▲	▲/V	▲	○	▲

Figure Chromosomes 10 and 17 polymorphisms and patterns of loss of heterozygosity in different tumours. Locus order²⁰ is from distal short arm (top) to distal long arm (bottom). Loci in the same box have not been mapped relative to each other; *not mapped relative to other loci in the p12-11.2 group. Tumour numbers are as in the table. ND = not done; ● = LOH, ▲ = not informative; ○ = heterozygosity maintained; / and V = possible upper and lower limits of deletions respectively; these extend to, but do not include, immediately adjacent loci. Olig = benign oligodendroglioma; Astro I and III = astrocytomas grades I and III.

Statistical comparisons were performed with the Mann-Whitney *U* test, Spearman's rank correlation, and χ^2 analysis with Yates' correction for 2 × 2 tables.

Results

Eighteen patients had grade IV astrocytomas.

Table Clinical and molecular genetic features of patients with grade IV astrocytomas

Patient no	Age (y)	Site of tumour	Karnofsky score	Onset to surgery (months)	Surgery to death (months)	Radiotherapy	Chemotherapy	LOH
2	61	RP-O	90	2	13	+	-	10
4	59	RO	60-70	2	13	+	+	-
11	68	RF-P	60	6	5	+	-	-
12	54	RF	80	1	13	+	+	17*
20	38	R F-P	80	3	23	+	+	10
21	30	RT	40	6 (y)	12	-	-	-
22	54	RP	50	1	7	+	+	-
23	65	RT	50	1	8	+	-	10
24	55	RF	30	2	3	-	-	-
32	56	RF	40	1	9	+	+	10
33	57	LT-P	80	1	14	+	-	10, 17
35	44	LP	40	1	18	+	+	10, 17
36	39	RP	80	3	40	+	+	10
44	64	LP	40-50	1	1	-	-	-
47	50	LF	40	3	3	+	+	-
48	58	LT	20-30	1	10	+	-	-
49	65	RT	30	1	6	+	+	10, 17
50	26	LF	90	4	60†	+	+	17*

*Not informative for chromosome 10 markers; †alive; L = left; R = right; F = frontal; P = parietal; T = temporal; O = occipital.

All but two of these were informative for one or more loci on chromosome 10 and all were informative for one or more chromosome 17 loci. The table shows the numbers of tumours with 10 or 17 losses. The pattern of LOH of chromosome 10 sequences indicated that the whole of this chromosome was lost in these tumours (figure); there was no evidence for maintenance of heterozygosity for some chromosome 10 markers and loss for others in any of them. By contrast, data for chromosome 17 indicate partial loss of the short arm in some tumours (figure). Eleven lower grade tumours were investigated: one grade I, six grade II, and, one grade III astrocytomas, and three oligodendrogliomas. The grade I astrocytoma showed partial loss of heterozygosity for chromosome 17, as did the grade III astrocytoma and one benign oligodendroglioma (figure).

LOH for RFLPs mapping to other chromosomes was detected in four tumours. One of these, a grade IV astrocytoma (case 49), had lost heterozygosity for sequences from chromosomes 19p and 22q. The other losses were in the grade III astrocytoma (1p), and two grade IV astrocytomas (case 33; 4p and case 12; 9q). One grade IV tumour (case 11) showed amplification of the *gli* oncogene,

demonstrated by a marked increase in hybridisation signal.

There were too few patients with low grade tumours in each category of malignancy, or with chromosomal losses, to permit correlation between molecular genetic data and prognosis. In patients with grade IV astrocytomas, table 2 shows the relevant clinical features. Most were treated by radiotherapy, and in many cases adjuvant chemotherapy, after surgery. Only one patient is still alive, 60 months after her operation.

In patients whose tumours showed LOH for chromosomes 10, 17, or both, median survival after surgery was 17.4 (range 6–60) months, compared with 6.75 (1–13) months in patients whose tumours did not show loss of sequences from these chromosomes ($p = 0.01$). There was still a significant difference in survival if the two patients with tumours uninformative for chromosome 10 markers were excluded ($p < 0.05$). Seventeen of the 18 patients with high grade gliomas had surgery within six (usually within three) months of onset of symptoms (table 2). The exception (patient 21) presented with seizures six years previously and had a temporal lobectomy followed by radiotherapy two years later. Median duration of symptoms before surgery was one (range 1–4) month in the LOH group and two (range 1–72) months in the patients with no LOH ($p > 0.05$). There was no difference in the age distribution of patients whose tumours showed LOH (median 55, range 26–65 years), compared with those whose did not (median 56.5, range 30–68 years, $p > 0.05$). The LOH group had higher Karnofsky scores at presentation (median 80, range 30–90, compared with 42.5, range 25–65), although this difference was not statistically significant ($p > 0.05$). Therapeutic decisions reflected this; all the patients with LOH had postoperative radiotherapy, with or without chemotherapy, whereas three of the other eight had neither (table 2; $\chi^2 = 2.2$, $p = 0.14$). There was no correlation between age and Karnofsky score in all the patients with grade IV tumours ($r = -0.18$, $p = 0.46$). There was a significant inverse correlation between age and survival ($r = -0.56$, $p = 0.016$), and a positive correlation between Karnofsky score and postoperative survival ($r = 0.59$, $p = 0.01$).

Discussion

Our molecular genetic data are in agreement with those of others in that LOH for chromosome 10 loci was restricted to grade IV gliomas, in eight of 16 informative samples (50%). The frequency of this finding has varied substantially, from 51% to 95% in previous studies.^{2,3,5-8,10} This presumably reflects differences in number, informativeness, and localisation of probes used, and some variation in pathological classification. As was the case in this series, evidence for partial, as opposed to total, loss of chromosome 10 is relatively rare. Some authors have described occasional LOH for only 10p or 10q, or reten-

tion of only the distal part of 10q.^{4,8} Fujimoto and colleagues³ defined a common region of loss on chromosome 10 in three tumours with interstitial deletions, between the middle of the short arm and band 10q23. These data are compatible with observations in two tumours retaining part of distal 10q,⁴ suggesting the presence of a tumour suppressor gene in this region that relates to high grade malignancy in gliomas.

By contrast with chromosome 10, partial loss of chromosome 17, particularly involving the distal short arm (17p11.1-pter) is common and occurs in all grades of astrocytoma, as found here. We found LOH for chromosome 17 in eight of 24 (33%) informative astrocytomas within the range of frequencies reported previously, 11–50%.⁸⁻¹⁴ All three informative grade IV gliomas with LOH for chromosome 17 had also lost sequences from chromosome 10; this combination is known to occur in 25% to 50% of grade IV tumours with LOH for chromosome 10, and suggests that these tumours may represent a subset that arises through progression from lower grade astrocytomas.^{8,9}

James and colleagues¹¹ suggested that LOH for chromosome 17 was confined to tumours of astrocytic differentiation, but one benign oligodendroglioma in this series had an interstitial deletion of one copy of 17p (figure). Relatively few molecular genetic data concerning oligodendrogliomas have been reported; James and coworkers studied six oligodendrogliomas but used fewer chromosome 17 probes than in the present study. LOH for 19q sequences has been described in four of seven oligodendrogliomas.⁷

The deletions of chromosome 17p seen in astrocytomas often involve the p53 tumour suppressor gene. This gene has been implicated in the pathogenesis of a wide variety of cancers, and mutations of p53 are found in about 30% of diffuse fibrillary astrocytomas, usually accompanied by loss of the other copy of chromosome 17p, which is to be expected given the recessive nature of tumour suppressor genes¹⁴; however, p53 point mutations have been described without loss of the other 17p.^{12,14,21} and some losses of 17p do not involve the p53 locus.¹³ The chromosome 17 deletions in six of our tumours may include p53, but one (a grade I astrocytoma) maintained heterozygosity for p53 with LOH for a more distal 17p locus (figure). These findings suggest that there is another, more telomeric, tumour suppressor gene on chromosome 17p, in this case between p53 and D17S28. The data from the oligodendroglioma indicate yet a further deleted region of 17p between DS58 and DS124 (figure). We did not investigate other chromosomes systematically, but found LOH in three tumours for chromosomes 1p, 4p, or 9q. Occasional losses of 1p and 9q, and other chromosomal regions, have been noted before,^{4,9,18} and are probably not of any pathogenetic relevance. We did not find any tumours with LOH for RB1, which has previously been reported in four of nine glioblastomas,¹⁷ or 19q or 22q, seen in apparently

non-random proportions of other series of gliomas.^{22 23} One grade IV tumour showed evidence of amplification of *gli*, an oncogene that maps to chromosome 12q; this has been reported in only occasional gliomas to date.^{23 24}

The striking finding in this study is the association between LOH and longer postoperative survival, more than twice as long as those without LOH, in patients with high grade gliomas, despite the small numbers. This may well be explained by the tendency for patients whose tumours showed LOH to have higher Karnofsky scores at presentation, although this was not statistically significant. There was no difference in duration of preoperative symptoms. There was a correlation between Karnofsky status and survival in this series of grade IV tumours overall, as has been noted previously.²⁵ Although age is known to be a major determinant of survival in patients with malignant gliomas^{25 26} and was inversely correlated with survival in this series, there was no difference in age between the two groups of patients defined by presence or absence of LOH. One possible explanation for longer survival in the LOH group is that slightly, but not significantly, more had postoperative radiotherapy, and often adjuvant chemotherapy, than in those without LOH. This was largely determined by Karnofsky status.

The explanation for this finding is not clear. It is unexpected, given recent observations made by von Deimling and colleagues.⁸ Based on a series of 67 patients with glioblastomas, these authors suggested that two main genetic types could be defined. One showed LOH for chromosome 17 and sometimes for chromosome 10, but did not exhibit amplification of the epidermal growth factor receptor (EGFR) gene. These tumours, which may be derived from lower grade astrocytomas, tended to occur in younger female patients. Amplification of EGFR was only found in tumours with LOH for chromosome 10, but not all of them, and rarely in those with additional LOH for chromosome 17. Patients with this second type of tumour exhibiting EGFR amplification were, on average, older and more likely to be male. Given that age is a major determinant of prognosis in glioblastoma, it might be expected that patients with tumours showing LOH for chromosome 10 would have a particularly poor prognosis. Von Deimling and coworkers⁸ did not analyse demographic data from their patients without LOH for chromosomes 10 or 17, who had a particularly bad prognosis in our series, although the age range of these patients, like ours, was wide. Our findings, which need to be confirmed, suggest that there is a subset of high grade gliomas, with currently no known molecular genetic substrate, which is particularly aggressive.

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