American Journal of Pathology, Vol. 155, No. 2, August 1999
Copyright © American Society for Investigative Pathology

Acquisition of the Glioblastoma Phenotype during Astrocytoma Progression Is Associated with Loss of Heterozygosity on 10q25-qter

Hironori Fujisawa,* Michael Kurrer,† Rui M. Reis,* Yasuhiro Yonekawa,‡ Paul Kleihues,*† and Hiroko Ohgaki*

From the International Agency for Research on Cancer, Lyon, France; and the Departments of Pathology† and Neurosurgery,‡ University Hospital, Zürich, Switzerland*

Loss of heterozygosity on chromosome 10 (LOH#10) is the most frequent genetic alteration in glioblastomas and occurs in more than 80% of cases. We recently reported that *PTEN (MMAC1)* on 10q23.3 is mutated in approximately 30% of primary (*de novo*) glioblastomas but rarely in secondary glioblastomas that progressed from low-grade or anaplastic astrocytomas. Because secondary glioblastomas also show LOH#10, tumor suppressor genes other than *PTEN* are likely to be involved. We analyzed LOH on chromosomes 10 and 19, using polymorphic microsatellite markers in microdissected foci showing histologically an abrupt transition from low-grade or anaplastic astrocytoma to glioblastoma, suggestive of the emergence of a new tumor clone. When compared to the respective low-grade or anaplastic astrocytoma of the same biopsy, deletions were detected in 7 of 8 glioblastoma foci on 10q25-qter distal to D10S597, covering the *DMBT1* and *FGFR2* loci. Six of 8 foci showed LOH at one or two flanking markers of *PTEN* but did not contain *PTEN* mutations. LOH on 10p and 19q was found in only one case each. These data indicate that acquisition of a highly anaplastic glioblastoma phenotype with marked proliferative activity and lack of glial fibrillary acidic protein expression is associated with loss of a putative tumor suppressor gene on 10q25-qter. *(Am J Pathol 1999, 155:387–394)*

Glioblastoma (WHO Grade IV) is the most frequent and malignant type of human brain tumor, occurring at an incidence of 2 to 3 new cases per 100,000 population annually for most European and North American countries.[1] Despite progress in surgical and adjuvant therapy, the mean survival of patients with this neoplasm is still less than 1 year.[2,3] Glioblastomas may develop rapidly, with a short clinical history *de novo* (primary glioblastoma), or more slowly, through progression from low-grade (WHO Grade II) or anaplastic (WHO Grade III) astrocytomas (secondary glioblastoma).[4,5] There is increasing evidence that these glioblastoma subtypes constitute distinct disease entities that are manifested in different age groups and develop through different genetic pathways. Primary glioblastomas occur in older patients and are characterized by *EGFR* amplification/overexpression, *PTEN* mutations, *p16* deletion, or *MDM2* overexpression, whereas secondary glioblastomas occur in younger patients and contain *p53* mutations as a genetic hallmark.[4,5]

Loss of heterozygosity (LOH) on chromosome 10 (LOH#10) is the most frequent genetic alteration in glioblastomas and occurs in approximately 80% of cases.[6–14] It is less frequent (40%) in anaplastic astrocytomas[6–10,12–14] and absent or rare in low-grade astrocytomas.[6,8–10,12–14] Most glioblastomas appear to have lost an entire copy of chromosome 10.[6–12] In the remaining cases, three commonly deleted loci have been identified, suggestive of the presence of several tumor suppressor genes. These regions include 10p14-pter, observed in about 80% of cases,[8,9,12–15] 10q23–24, in about 80% of cases,[6,7,10–14,16] and 10q25-qter, in about 90% of cases.[6,7,10–14]

The *PTEN* tumor suppressor gene identified on 10q23.3[17,18] has been found to be mutated in glioblastomas at frequencies of about 20%.[11,16,19–23] We recently reported that *PTEN* mutations are common (32%) in primary glioblastomas but rare (4%) in secondary glioblastomas,[24] which corroborates the observation of a significant reciprocal correlation between *p53* and *PTEN* mutations in glioblastomas.[23] Because more than 50% of glioblastomas with *p53* mutations have been reported to show LOH#10,[25,26] these neoplasms are likely to exhibit LOH#10 at loci other than *PTEN*.

The objective of this study was to identify tumor suppressor loci on chromosome 10 that are involved in the genetic pathway leading to secondary glioblastomas. We analyzed LOH#10 using polymorphic microsatellite markers in DNA from microdissected areas showing histologically an abrupt transition from low-grade or anaplastic

Supported by a grant from the Foundation for Promotion of Cancer Research, Japan.

Accepted for publication April 13, 1999.

Address reprint requests to Dr. Hiroko Ohgaki, Unit of Molecular Pathology, International Agency for Research on Cancer, 69372 Lyon Cedex 08, France. E-mail: ohgaki@iarc.fr.

astrocytoma to glioblastoma, which suggests the emergence of a new tumor clone. Instead of using normal DNA as a control, microsatellite analysis was carried out for each glioblastoma focus in comparison with genomic DNA from the respective, less malignant precursor lesion, ie, low-grade or anaplastic astrocytoma. We also analyzed LOH on chromosome 19, which may contain one or more putative tumor suppressor loci involved in astrocytoma progression.[27–32]

The results of this study provide evidence that acquisition of genetic alteration during progression from low-grade or anaplastic astrocytoma to a highly anaplastic glioblastoma phenotype, with marked proliferative activity and lack of glial fibrillary acidic protein (GFAP) expression, is associated with loss of a putative tumor suppressor gene on 10q25-qter.

Materials and Methods

Tumor Selection and DNA Extraction

Five secondary glioblastoma cases were selected that histologically showed an abrupt transition from low-grade or anaplastic astrocytoma to glioblastoma. These biopsies were from the Departments of Neurosurgery at University Hospital, Zürich, Switzerland (Cases 1–4) and Tübingen, Germany (Case 5). Tumors were fixed in formalin, embedded in paraffin, and classified according to the WHO grading system.[33] Under light microscopic observation, the regions showing different malignancy grades (Grades II-IV) were marked and scraped off into an Eppemdorf tube. Genomic DNA was extracted from each area as described previously.[34]

Case 1

This 28-year-old female patient was diagnosed with a left frontal anaplastic astrocytoma (WHO Grade III), which was surgically resected. One and a half years later, the tumor recurred at the same site and was diagnosed as glioblastoma (WHO Grade IV). In the second biopsy, an abrupt transition from anaplastic astrocytoma to glioblastoma was observed histologically. After partial resection of the glioblastoma, the patient received whole brain radiation therapy with a boost on tumor bed and margin. Nine months later, the patient showed massive cervical lymphadenopathy. Histological examination of a supraclavicular lymph node revealed the presence of a glioblastoma metastasis. The patient underwent cervical irradiation and chemotherapy but died 1 year later. Areas of anaplastic astrocytoma, glioblastoma, and adjacent normal brain tissue from the second biopsy, as well as a lymph node metastasis, were microdissected for LOH analysis. (Figure 1, #1).

Case 2

This 49-year-old female patient presented with generalized seizures. A right temporo-occipital tumor was subtotally removed and histologically diagnosed as low-grade fibrillary astrocytoma (WHO Grade II). Two and a half years later, the tumor recurred as a cystic lesion with ventricular infiltration and was diagnosed as glioblastoma (WHO Grade IV). Subsequently the patient received cranial radiation therapy but died 3 months later. The second biopsy contained areas with low-grade astrocytoma features and others that fulfilled the histological criteria for the diagnosis of glioblastoma. Both areas and adjacent normal brain tissue were microdissected and analyzed.

Case 3

This 48-year-old male patient presented with persisting headache and partial complex seizures. A left parieto-occipital tumor was extirpated and histologically diagnosed as low-grade fibrillary astrocytoma (WHO Grade II). Four years later, the tumor recurred as glioblastoma. The patient did not receive radiation therapy and died 4 months after the second operation. Two different areas of glioblastoma foci (IV1 and IV2) were microdissected from two tumor blocks taken at the second biopsy. Histologically, the IV1 area consisted of polymorphic glioblastoma cells, whereas the IV2 area showed a more homogeneous pattern (Figure 1, #3). Three different areas of low-grade astrocytoma, one adjacent and the others farther away from the area with glioblastoma histology (IV1), were microdissected.

Case 4

Presenting with a history of generalized seizures, this 40-year-old male patient underwent surgery for a right frontal tumor, which was macroscopically well delineated. This tumor showed histologically the features of low-grade astrocytoma with a gemistocytic component (WHO Grade II). Two years later, the tumor recurred as an invasive lesion that infiltrated the ventricular walls and the corpus callosum. The tumor was resected and diagnosed as glioblastoma. Subsequently the patient received radiation therapy. One year later, he displayed low back and leg pain due to spinal dissemination of the glioblastoma. He died of extensive tumor dissemination after 3 months. Areas of low-grade astrocytoma and glioblastoma identified in the second biopsy were microdissected for LOH analysis.

Case 5

This 69-year-old female patient presented with severe headache and left hemiparesis that had commenced 3 weeks before admission. A right frontal cystic tumor was totally resected. The patient subsequently received radiotherapy. The biopsy showed a focally abrupt transition from low-grade astrocytoma to glioblastoma. The glioblastoma focus showed brisk mitotic activity and lacked GFAP expression (Figure 1, #5, center). One year later the tumor recurred at the same site with invasion of the basal ganglia and deep white matter. The patient under-

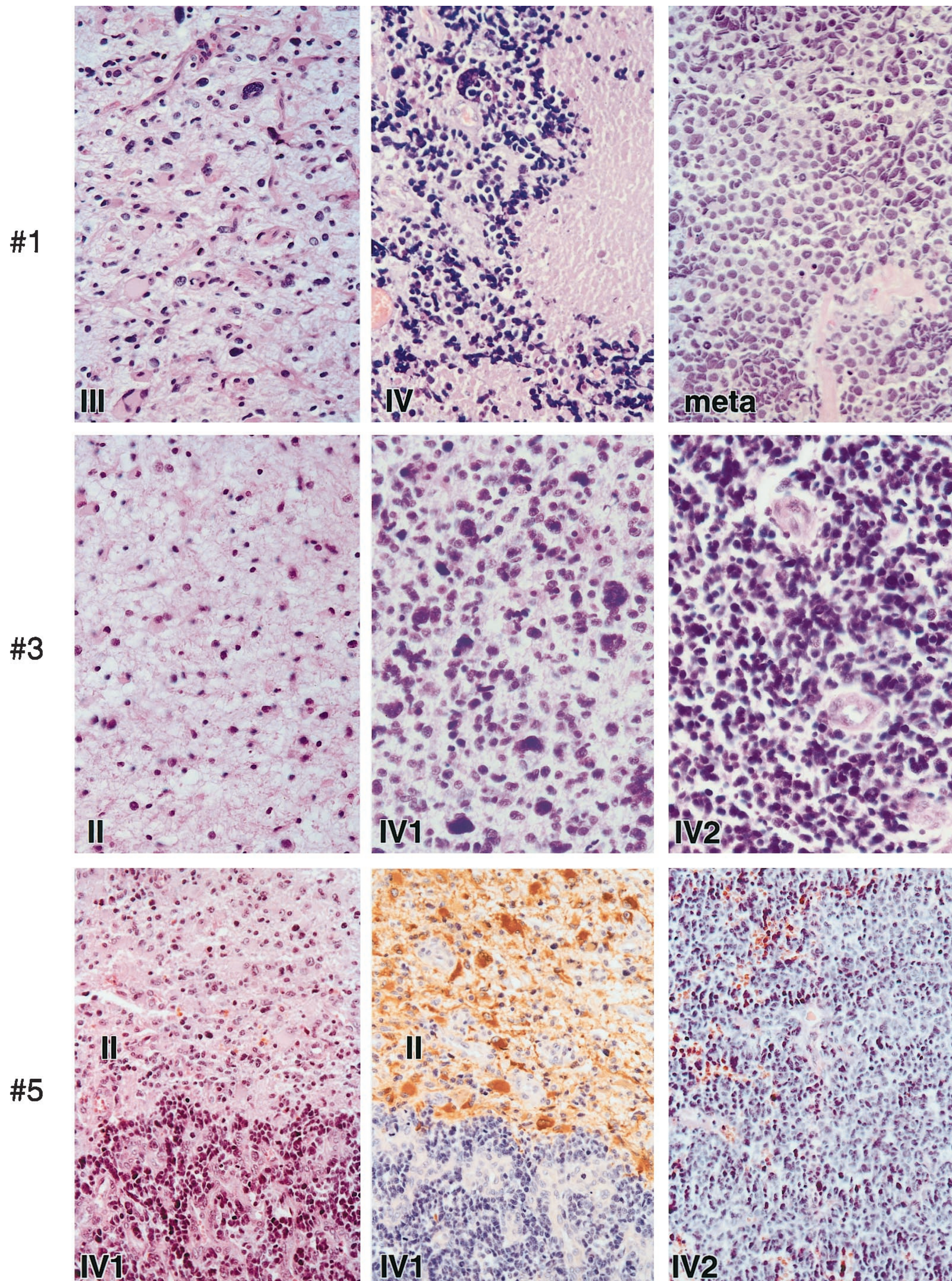

Figure 1. Photomicrographs of microdissected tumor areas. In Case 1 (#1), two tumor areas, a glioblastoma focus (IV, center) and its cervical lymph node metastasis (meta, right), were compared with an area of anaplastic astrocytoma (III, left). In Case 3 (#3), tumor cells of one glioblastoma focus (IV1, center) displayed highly polymorphic, often multinucleated, while other areas (IV2, right) showed a more isomorphic but highly cellular tissue pattern. The same biopsy contained areas of a differentiated low-grade fibrillary astrocytoma (II, left). In Case 5 (#5), a sharply delineated focus of glioblastoma (IV1, left) was present on the background of a low-grade astrocytoma (II) with a gemistocytic component. The glioblastoma focus completely lacked GFAP expression (IV1, center) and showed marked mitotic activity. A second glioblastoma focus showed similar histological features (IV2, right). All sections were stained with H&E, except Case 5, center (GFAP).

went a second operation and received a second cycle of radiotherapy but showed no clinical improvement. Treatment was continued at another hospital and further clinical follow-up data are not available. For LOH analysis, areas of glioblastoma (IV1) and low-grade astrocytoma (II) and another more distant area of glioblastoma (IV2) in the first biopsy were microdissected.

Analyses of LOH on Chromosomes 10 and 19 Using Microsatellite Markers

LOH on chromosomes 10 and 19 was studied by polymerase chain reaction (PCR)-based microsatellite analysis. Microsatellite loci on each chromosome were selected to fully cover reported common deletions on 10p14-pter,[8,9,12–15] 10q23–24,[6,7,10–14] 10q25-qter,[6,7,10–14] 19p13.2-pter,[30] and 19q13.2–13.4.[27–29,31,32] Thirty-seven and nine microsatellite markers were used for analyses on chromosomes 10 and 19, respectively. All microsatellite markers were purchased from Research Genetics (Huntsville, AL). They were dinucleotide repeats except for D10S1435, D10S527, and D19S246 (tetranucleotide repeats). The size range and heterozygosity of each marker was obtained from the Genome Database (*http://gdbwww.gdb.org/*). Genetic maps and distances of chromosomes 10 and 19 were obtained from the enhanced location databases at *ftp://cedar.genetics.soton.ac.uk/pub/chrom 10/gmap* and *ftp://cedar.genetics.soton.ac.uk/pub/chrom 19/gmap*, respectively.[35]

The allelic losses for each primer set were determined by comparing the electrophoretic patterns of PCR product of glioblastomas and their respective precursor lesions (low-grade or anaplastic astrocytoma areas). PCR was performed according to the instructions of Research Genetics with minor modification. Briefly, 1 μl of DNA solution was subjected to PCR with 2 μl of 5$\times$ PCR buffer, 200 μmol/L of each dNTP, 6 pmol each of forward and reverse primer, 0.5 μCi of [α-^{33}P]-dCTP (ICN Biomedicals, specific activity 3000 Ci/mmol), 0.225 units of *Taq* polymerase (Sigma, St. Louis, MO) and 1.5 mmol/L of $MgCl_2$ in a final volume of 10 μl. After an initial cycle of 95°C for 2 minutes, 35 cycles of 94°C for 45 seconds, 57°C for 45 seconds, and 72°C for 1 minute were followed by a final extension 72°C for 7 minutes in a Genius DNA Thermal Cycler (Techne, Cambridge, UK). PCR products were mixed with an equivalent volume of the denaturing solution containing 95% formamide, 20 mmol/L EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue. Immediately after heating at 95°C for 5 minutes, 4 μl of the mixture was loaded onto a 6 or 7% polyacrylamide/7 mol/L urea sequencing gel. Gels were run at 70W for 3–5 hours, depending on the length of the products, dried at 80°C, and autoradiographed for 48–96 hours. The signal intensity of each allele on the X-ray film was measured by densitometry (Bio-Rad model GS-670). LOH was assumed when the signal intensity of the allele in the glioblastoma focus was less than 50% of that in the reference DNA (normal brain, low-grade, or anaplastic astrocytoma).

PCR-Single-Strand Conformation Polymorphism (SSCP) Analysis and Direct DNA Sequencing for p53 *and* PTEN *Mutations*

Prescreening for mutations was carried out by PCR-SSCP analysis[34] of exons 5–8 of the *p53* gene for all cases and of exons 1–9 of the *PTEN* gene for cases 1–4.[36] *PTEN* mutations were not analyzed in Case 5 because of the limited amount of tumor tissue available. Samples that showed mobility shifts in the SSCP gels were further analyzed by direct DNA sequencing as previously described.[34]

Results

LOH on Chromosome 10

Using 37 microsatellite markers, we examined a total of 285 polymorphic loci on chromosome 10 and obtained 178 informative results (62%). Loss of an entire copy of chromosome 10 was not found in any of the glioblastomas analyzed. Among 8 glioblastoma foci analyzed in biopsies from 5 patients, 7 showed partial LOH#10 at 10q25-qter distal to D10S597, covering the *DMBT1*[37] and *FGFR2* loci[38] (Figure 2). Six of the 8 glioblastoma foci showed LOH at one or two flanking markers for *PTEN*, ie, D10S215 and D10S541.[17] LOH on 10p was found in only 2 glioblastoma foci from one patient (Figure 2, Case 5).

In Case 1, normal brain tissue and an anaplastic astrocytoma focus were used as a reference. Only 3 markers (D10S215, D10S187, and D10S216) had already been lost in the anaplastic astrocytoma but an additional 12 loci were lost during progression from anaplastic astrocytoma to glioblastoma. The glioblastoma focus and its metastasis to cervical lymph nodes (Case 1) showed an identical pattern of LOH#10 (Figure 2).

In Case 2, an area of peritumoral normal brain was also microdissected and analyzed. The allelic patterns of normal brain tissue were identical to those of the low-grade astrocytoma.

In Case 3, one glioblastoma focus (IV1) showed LOH on 10q25-qter, whereas another glioblastoma focus (IV2) did not show LOH with any of the microsatellite markers used (Figures 2 and 3). Three areas of low-grade astrocytoma analyzed in Case 3 all showed the same allelic patterns.

LOH on Chromosome 19

Using 9 microsatellite markers, a total of 68 polymorphic loci were examined, covering chromosomal regions 19p13.2-pter and 19q13.2–13.4 and yielding 49 informative results (72%). Except for one glioblastoma focus (Case 3, IV1), there was no apparent LOH in any of the regions analyzed.

Mutations in the p53 *and* PTEN *Genes*

PCR-SSCP followed by direct DNA sequencing revealed two *p53* missense mutations and one deletion. In all

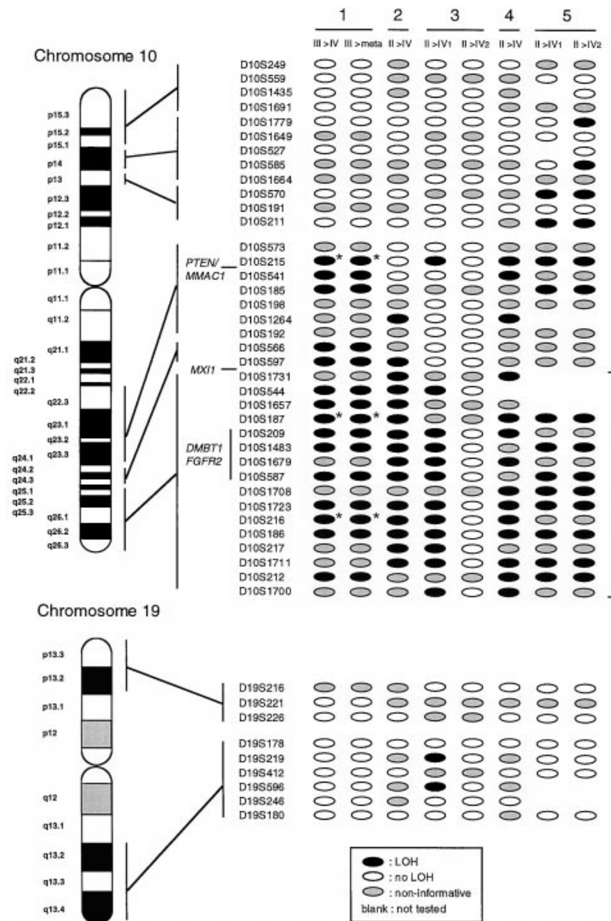

Figure 2. Deletion mappings of chromosomes 10 and 19 in 8 glioblastoma foci from 5 secondary glioblastoma patients. Case numbers are indicated at the top of each column. Glioblastoma foci were compared with low-grade astrocytoma foci (Grade II, Cases 2–5). In Case 1, normal brain tissue and an anaplastic astrocytoma focus were used as a reference. Only 3 markers (D10S215, D10S187, and D10S216) had already been lost in the anaplastic astrocytoma (marked as *), but an additional 12 loci were lost during progression from anaplastic astrocytoma to glioblastoma. The chromosomal region most commonly deleted is indicated by the bracket on the right.

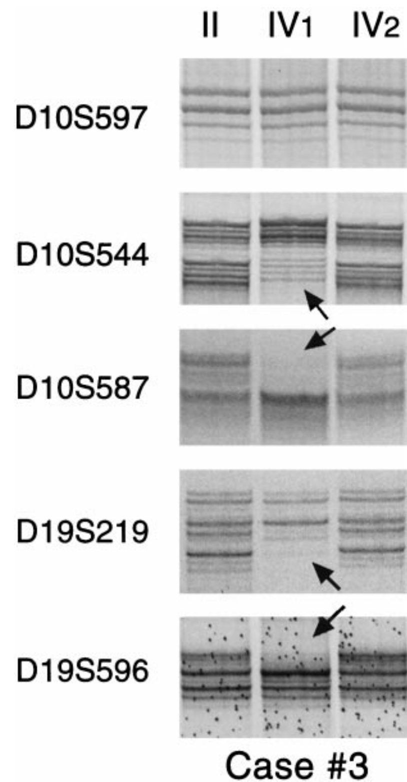

Figure 3. Representative results of LOH analysis on chromosomes 10 and 19 in Case 3. Microsatellite markers are indicated on the left side of each panel. Allelic loss is indicated by arrows.

cases, the mutations were already detected in the first biopsy. The mutations had the following locations: codon 275 (TGT->TAT, Cys->Tyr, glioblastoma focus and metastasis of Case 1), codon 278 (CCT->ACT, Pro->Thr, Case 2) and codon 275–279 (15-bp deletion, Case 4). The mutation data of Cases 2 and 4 have previously been published.[34] PCR-SSCP analysis for *PTEN* mutations (exons 1–9) revealed no mobility shift in any of the tumor foci analyzed.

Discussion

The phenotypic changes associated with astrocytoma progression are usually diffuse and continuous, with a gradual increase in nuclear atypia, mitotic activity, cellularity, and, eventually, the appearance of microvascular proliferation and necrosis.[1,39] However, biopsies occasionally show a sudden transition to the glioblastoma phenotype[40,41] in the form of well delineated foci that show brisk mitotic activity and complete loss of GFAP expression (Figure 1, #5, center). Such foci resemble the focus-in-focus lesion or carcinoma-in-adenoma phenomenon described in experimental hepatocarcinogenesis[42–44] and human liver tumors[45] and are likely to be caused by the acquisition of an additional genetic alteration. We observed five such cases and used microsatellite analysis to determine whether this sudden change of morphology is due to LOH on chromosomes 10 and 19. Rather than comparing the allelic pattern with that of normal DNA, we aligned the microsatellite patterns of the glioblastoma foci with those of the adjacent, less malignant precursor lesion, ie, low-grade or anaplastic astrocytoma.

The results obtained provide initial evidence that acquisition of the glioblastoma phenotype during astrocytoma progression is associated with loss of a putative tumor suppressor gene on 10q25-qter, covering the *DMBT1*[37] and *FGFR2* loci.[38] The *DMBT1* gene was originally identified from a homozygously deleted region in a medulloblastoma cell line and showed homology to the scavenger receptor cysteine-rich superfamily.[37] Although allelic losses and homozygous deletions at *DMBT1* were detected in 59% and 23% of glioblastomas, respectively,[37] no mutation has been reported so far. *FGFR2* encodes fibroblast growth factor receptors and is considered a potential tumor suppressor gene because decreased expression of its two isoforms, FGFR2c (*BEK*) and FGFR2b (*KGF-R*),[46] have been demonstrated in glioblastomas[47] and transitional cell carcinomas of the

bladder.[48] However, *FGFR2* mutations have not yet been identified in human neoplasms.

Loss of an entire copy of chromosome 10 has been observed in a majority of glioblastomas.[6–12] However, none of the glioblastoma foci analyzed in this study showed loss of an entire copy of chromosome 10. The possibility exists that partial deletion on chromosome 10 is typical for secondary glioblastomas progressing from low-grade or anaplastic astrocytoma, whereas large deletions and loss of the entire chromosome are prevalent in primary *(de novo)* glioblastomas.[5] Alternatively, newly emerged glioblastoma foci may ultimately become the prevailing tumor cell fraction and may, in the process, develop larger chromosomal deletions due to increased genetic instability.

LOH at the *PTEN* locus has been found in up to 80% of glioblastomas,[6,7,10–13,16] whereas *PTEN* mutations have been detected in less than 25% of cases.[11,16,19–23] In the present study, none of the 6 glioblastoma foci analyzed contained a *PTEN* mutation, although 6 of 8 glioblastoma foci showed LOH at one or two of the flanking markers of *PTEN* (Figure 2). This supports the observation that mutational loss of *PTEN* function is rarely involved in the genetic pathway to secondary glioblastoma.[24] However, other mechanisms of *PTEN* inactivation, eg, promoter hypermethylation or homozygous *PTEN* deletion, cannot be ruled out. *PTEN* promoter hypermethylation has not been found in human tumors including prostate cancer, bladder cancer, and renal cell carcinomas,[49,50] but data on brain tumors are not available. Homozygous *PTEN* deletion appears to be rare in glioblastomas.[16,19,21,24]

Deletion on 19q13.2–13.4 has been found in a variety of gliomas, including low-grade astrocytomas ($<$20%), anaplastic astrocytomas ($\sim$40%), and glioblastomas ($\sim$30%),[27,28,31,32] as well as in oligodendrogliomas ($\sim$70%) and mixed oligo-astrocytomas ($\sim$70%).[27,28,31,32,51] There is limited evidence for the occurrence in gliomas of LOH at a second locus on 19p13.2-pter.[30] In this study, LOH on 19q13.2–13.4 was observed in only 1 of 8 glioblastoma foci analyzed (Case 3, IV1, Figure 2), suggesting that 19q13.2–13.4 and 19p13.2-pter loci are not typically involved in the acquisition of the glioblastoma phenotype during progression from low-grade or anaplastic astrocytoma. It remains to be shown whether LOH on chromosome 19 occurs at a later stage than LOH on chromosome 10 in secondary glioblastomas, or whether LOH on chromosome 19 is more typically associated with the evolution of primary *(de novo)* glioblastomas.

Little is known about the effect of radiation therapy on genetic alterations in glioblastomas. Hulsebos et al[52] showed that no additional LOH at p16 and Rb loci was found after irradiation. In many other cases, additional genetic alterations were detected in the absence of radiotherapy.[52] The present study is largely noninformative because, except in Case 3, all patients received radiotherapy only after the histological diagnosis of glioblastoma. The lymph node metastasis of Case 1 was resected 9 months after cranial irradiation and showed the same LOH pattern on chromosomes 10 and 19 as that of the parent glioblastoma.

Glioblastomas are histologically and biologically heterogeneous, but it is largely unknown whether this is due to a polyclonal development of glioblastomas from less malignant, monoclonal precursor lesions or to genetic instability after the acquisition of the glioblastoma phenotype. In two cases of this study (Cases 3 and 5), we analyzed two glioblastoma foci separately. In Case 3, one glioblastoma area showed LOH at 14 markers on 10q and 19q, but the other glioblastoma area retained heterozygosity at all of these loci, suggesting that progression from low-grade astrocytoma to glioblastoma can be polyclonal. In the biopsy of Case 5, one glioblastoma area showed LOH on 10p13, 10q23, and 10q25-qter, whereas another glioblastoma area showed an additional deletion on 10p14. This could signify that both glioblastoma foci originated from the same tumor clone, but that an additional deletion occurred in only one area (Figure 2). Similar examples of genetic heterogeneity have been demonstrated on chromosome 8 during prostate cancer progression.[53]

In conclusion, the present study on secondary glioblastomas with a histologically sudden transition from low-grade or anaplastic astrocytoma indicate that the acquisition of the glioblastoma phenotype is typically associated with LOH at 10q25-qter but not on 10p, 10q23, or 19.

Acknowledgments

We thank Dr. F. Canzian and Dr. M. Stark of the Unit of Genetic Cancer Susceptibility, International Agency for Research on Cancer, Lyon, France, for valuable discussions.

References

1. Lantos PL, VandenBerg SR, Kleihues P: Tumours of the nervous system. Greenfield's Neuropathology. Edited by DI Graham, PL Lantos. London, Arnold, 1996, pp 583–879
2. Leenstra S, Oskam NT, Bijleveld EH, Bosch DA, Troost D, Hulsebos TJM: Genetic sub-types of human malignant astrocytoma correlate with survival. Int J Cancer 1998, 79:159–165
3. Galanis E, Buckner JC, Dinapoli RP, Scheithauer BW, Jenkins RB, Wang CH, O'Fallon JR, Farr G, Jr: Clinical outcome of gliosarcoma compared with glioblastoma multiforme: North Central Cancer Treatment Group results. J Neurosurg 1998, 89:425–430
4. Kleihues P, Ohgaki H: Genetics of glioma progression and the definition of primary and secondary glioblastoma. Brain Pathol 1997, 7:1131–1136
5. Kleihues P, Ohgaki H: Primary and secondary glioblastoma: from concept to clinical diagnosis. Neuro-Oncology 1999, 1:44–51
6. Rasheed BK, McLendon RE, Friedman HS, Friedman AH, Fuchs HE, Bigner DD, Bigner SH: Chromosome 10 deletion mapping in human gliomas: a common deletion region in 10q25. Oncogene 1995, 10: 2243–2246
7. Albarosa R, Colombo BM, Roz L, Magnani I, Pollo B, Cirenei N, Giani C, Conti AMF, DiDonato S, Finocchiaro G: Deletion mapping of gliomas suggests the presence of two small regions for candidate tumor-suppressor genes in a 17-cM interval on chromosome 10q. Am J Hum Genet 1996, 58:1260–1267
8. Voesten AMJ, Bijleveld EH, Westerveld A, Hulsebos TJM: Fine mapping of a region of common deletion on chromosome arm 10p in human glioma. Genes Chromosomes Cancer 1997, 20:167–172
9. Kon H, Sonoda Y, Kumabe T, Yoshimoto T, Sekiya T, Murakami Y:

Structural and functional evidence for the presence of tumor suppressor genes on the short arm of chromosome 10 in human gliomas. Oncogene 1997, 16:257–263
10. Maier D, Comparone D, Taylor E, Zhang Z, Gratzl O, Van Meir EG, Scott RJ, Merlo A: New deletion in low-grade oligodendroglioma at the glioblastoma suppressor locus on chromosome 10q25–26. Oncogene 1997, 15:997–1000
11. Fults D, Pedone CA, Thompson GE, Uchiyama CM, Gumpper KL, Iliev D, Vinson VL, Tavtigian SV, Perry WL: Microsatellite deletion mapping on chromosome 10q and mutation analysis of MMAC1, FAS, and MXI1 in human glioblastoma multiforme. Int J Oncol 1998, 12: 905–910
12. Ichimura K, Schmidt EE, Miyakawa A, Goike HM, Collins VP: Distinct patterns of deletion on 10p and 10q suggest involvement of multiple tumor suppressor genes in the development of astrocytic gliomas of different malignancy grades. Genes Chromosomes Cancer 1998, 22:9–15
13. Karlbom AE, James CD, Boethius J, Cavenee WK, Collins VP, Nordenskjöld M, Larsson C: Loss of heterozygosity in malignant gliomas involves at least three distinct regions on chromosome 10. Hum Genet 1993, 92:169–174
14. Sonoda Y, Murakami Y, Tominaga T, Kayama T, Yoshimoto T, Sekiya T: Deletion mapping of chromosome 10 in human glioma. Jpn J Cancer Res 1996, 87:363–367
15. Kimmelman AC, Ross DA, Liang BC: Loss of heterozygosity of chromosome 10p in human gliomas. Genomics 1996, 34:250–254
16. Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R: Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res 1997, 57:4183–4186
17. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R: PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997, 275:1943–1947
18. Steck PA, Pershouse MA, Jasser SA, Yung WKA, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DHF, Tavtigian SV: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997, 15:356–362
19. Boström J, Cobbers JM, Wolter M, Tabatabai G, Weber RG, Lichter P, Collins VP, Reifenberger G: Mutation of the PTEN (MMAC1) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm 10q. Cancer Res 1998, 58:29–33
20. Chiariello E, Roz L, Albarosa R, Magnani I, Finocchiaro G: PTEN/MMAC1 mutations in primary glioblastomas, and short-term cultures of malignant gliomas. Oncogene 1998, 16:541–545
21. Duerr E-M, Rollbrocker B, Hayashi Y, Peters N, Meyer-Puttlitz B, Louis DN, Schramm J, Wiestler OD, Parsons R, Eng C, von Deimling A: PTEN mutations in gliomas, and glioneuronal tumors. Oncogene 1998, 16:2259–2264
22. Maier D, Zhang Z, Taylor E, Hamou MF, Gratzl O, Van Meir EG, Scott RJ, Merlo A: Somatic deletion mapping on chromosome 10 and sequence analysis of PTEN/MMAC1 point to the 10q25–26 region as the primary target in low-grade and high-grade gliomas. Oncogene 1998, 16:3331–3335
23. Rasheed BK, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD, Bigner SH: PTEN gene mutations are seen in high-grade but not in low-grade gliomas: Cancer Res 1997, 57: 4187–4190
24. Tohma Y, Gratas C, Biernat W, Peraud A, Fukuda M, Yonekawa Y, Kleihues P, Ohgaki H: PTEN (MMAC1) mutations are frequent in primary glioblastomas (de novo) but not in secondary glioblastomas. J Neuropath Exp Neurol 1998, 57:684–689
25. Lang FF, Miller DC, Koslow M, Newcomb EW: Pathways leading to glioblastoma multiforme: a molecular analysis of genetic alterations in 65 astrocytic tumors. J Neurosurg 1994, 81:427–436
26. Fults D, Brockmeyer D, Tullous MW, Pedone CA, Cawthon RM: p53 mutation and loss of heterozygosity on chromosomes 17 and 10 during human astrocytoma progression. Cancer Res 1992, 52:674–679
27. von Deimling A, Louis DN, von Ammon K, Petersen I, Wiestler OD, Seizinger BR: Evidence for a tumor suppressor gene on chromosome 19q associated with human astrocytomas, oligodendrogliomas, and mixed gliomas. Cancer Res 1992, 52:4277–4279
28. von Deimling A, Nagel J, Bender B, Lenartz D, Schramm J, Louis DN, Wiestler OD: Deletion mapping of chromosome 19 in human gliomas. Int J Cancer 1994, 57:676–680
29. Rubio MP, Correa KM, Ueki K, Mohrenweiser HW, Gusella JF, von Deimling A, Louis DN: The putative glioma tumor suppressor gene on chromosome 19q maps between APOC2 and HRC. Cancer Res 1994, 54:4760–4763
30. Ritland SR, Ganju V, Jenkins RB: Region-specific loss of heterozygosity on chromosome 19 is related to the morphologic type of human glioma. Genes Chromosomes Cancer 1995, 12:277–282
31. Rosenberg JE, Lisle DK, Burwick JA, Ueki K, von Deimling A, Mohrenweiser HW, Louis DN: Refined deletion mapping of the chromosome 19q glioma tumor suppressor gene to the D19S412-STD interval. Oncogene 1996, 13:2483–2485
32. Yong WH, Chou D, Ueki K, Harsh GR, von Deimling A, Gusella JF, Mohrenweiser HW, Louis DN: Chromosome 19q deletions in human gliomas overlap telomeric to D19S219, and may target a 425 kb region centromeric to D19S112. J Neuropathol Exp Neurol 1995, 54:622–626
33. Histological typing of tumours of the central nervous system. World Health Organization International Histological Classification of Tumours. Edited by Kleihues P, Burger PC, Scheithauer BW: Berlin, Springer Verlag, 1993
34. Watanabe K, Tachibana O, Sato K, Yonekawa Y, Kleihues P, Ohgaki H: Overexpression of the EGF receptor and p53 mutations are mutually exclusive in the evolution of primary and secondary glioblastomas. Brain Pathol 1996, 6:217–224
35. Collins A, Frezal J, Teague J, Morton NE: A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 1996, 93:14771–14775
36. Watanabe K, Peraud A, Gratas C, Wakai S, Kleihues P, Ohgaki H: p53, and PTEN gene mutations in gemistocytic astrocytomas. Acta Neuropathol 1998, 95:559–564
37. Mollenhauer J, Wiemann S, Scheurlen W, Korn B, Hayashi Y, Wilgenbus KK, von Deimling A, Poustka A: DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3–26.1 is deleted in malignant brain tumours. Nat Genet 1997, 17:32–39
38. Moschonas NK, Spurr NK, Mao J: Report of the first international workshop on human chromosome 10 mapping 1995. Cytogenet Cell Genet 1996, 72:100–112
39. Burger PC, Scheithauer BW: Tumors of the Central Nervous System. Washington, DC, Armed Forces Institute of Pathology, 1994
40. Schmitt HP: Rapid anaplastic transformation in gliomas of adulthood: "selection" in neuro-oncogenesis. Pathol Res Pract 1983, 176:313–323
41. Pathology and Genetics of Tumours of the Nervous System. Edited by Kleinhues P and Cavenee WK. Lyon, International Agency for Research on Cancer, 1997
42. Scherer E: Neoplastic progression in experimental hepatocarcinogenesis. Biochim Biophys Acta 1984, 738:219–236
43. Scherer E: Relationship among histochemically distinguishable early lesions in multistep-multistage hepatocarcinogenesis. Arch Toxicol Suppl 1987, 10:81–94
44. Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K: The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med 1998, 4:1065–1067
45. Oda T, Tsuda H, Sakamoto M, Hirohashi S: Different mutations of the p53 gene in nodule-in-nodule hepatocellular carcinoma as a evidence for multistage progression. Cancer Lett 1994, 83:197–200
46. Miki T, Bottaro DP, Fleming TP, Smith CL, Burgess WH, Chan AML, Aaronson SA: Determination of ligand-binding specificity by alternative splicing: two distinct growth factor receptors encoded by a single gene. Proc Natl Acad Sci USA 1992, 89:246–250
47. Yamaguchi F, Saya H, Bruner JM, Morrison RS: Differential expression of two fibroblast growth factor-receptor genes is associated with malignant progression in human astrocytomas. Proc Natl Acad Sci USA 1994, 91:484–488
48. Gil Diez de Medina S, Chopin D, El Marjou A, Delouvee A, LaRochelle WJ, Hoznek A, Abbou C, Aaronson SA, Thiery JP, Radvanyi F: Decreased expression of keratinocyte growth factor receptor in a subset of human transitional cell bladder carcinomas. Oncogene 1997, 14: 323–330

49. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D: Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res 1997, 57:4997–5000
50. Cairns P, Evron E, Okami K, Halachmi N, Esteller M, Herman JG, Bose S, Wang SI, Parsons R, Sidransky D: Point mutation and homozygous deletion of PTEN/MAC1 in primary bladder cancers. Oncogene 1998, 16:3215–3218
51. Reifenberger J, Reifenberger G, Liu L, James CD, Wechsler W, Collins VP: Molecular genetic analysis of oligodendroglial tumors shows preferential allelic deletions on 19q and 1p. Am J Pathol 1994, 145:1175–1190
52. Hulsebos TJM, Oskam NT, Troost D, Leenstra S, Bijleveld EH: Dynamics of genetic alterations associated with glioma recurrence. Genes Chromosomes Cancer 1998, 23:153–158
53. Macintosh CA, Stower M, Reid N, Maitland NJ: Precise microdissection of human prostate cancers reveals genotypic heterogeneity. Cancer Res 1998, 58:23–28