Comparative genetic patterns of glioblastoma multiforme: Potential diagnostic tool for tumor classification

Rodney N. Wiltshire, B.K. Ahmed Rasheed, Henry S. Friedman, Allan H. Friedman, and Sandra H. Bigner[1]

Departments of Pathology (R.N.W., B.K.A.R., H.S.F., S.H.B.), Surgery (H.S.F., A.H.F.), and Pediatrics (H.S.F.), Duke University, Durham, NC 27710

Cytogenetic and molecular genetic studies of glioblastoma multiforme (GBM) have shown that the most frequent alterations are gains of chromosome 7, losses of 9p loci and chromosome 10, and gene amplification, primarily of the epidermal growth factor receptor (*EGFR*) gene. Although this profile is potentially useful in distinguishing GBM from other tumor types, the techniques used tend to be labor intensive, and some can detect only gains or losses of genetic loci. Comparative genomic hybridization (CGH) is a powerful technique capable of identifying both gains and losses of DNA sequences. The present study compares the CGH evaluation of 22 GBM with classic cytogenetics, loss of heterozygosity by allelotyping, and gene amplification by Southern blot analysis to determine the reliability of CGH in the genetic characterization of GBM. The CGH and karyotypic data were consistent in showing gain of chromosome 7 accompanied by a loss of chromosome 10 as the most frequent abnormality, followed by a loss of 9p in 17 of 22 GBM cases. Loss of heterozygosity of chromosomes 10 (19/22) and 9p (9/22) loci confirmed the underrepresentation by CGH. Genomic amplifications were observed by CGH in 5 of the 10 cases where gene amplification was detected by Southern blot analysis. The data show that CGH is equally reliable, compared with the more established genetic methods, for recognizing the prominent genetic alterations associated with GBM and support its use as a plausible adjunct to glioma classification. *Neuro-Oncology 2, 164-173, 2000 (Posted to Neuro-Oncology [serial online], Doc. 00-015, June 20, 2000. URL <neuro-oncology.mc.duke.edu>)*

Received 2 March 2000, accepted 24 April 2000.

[1]Address correspondence and reprint requests to Sandra H. Bigner, Duke University Medical Center, Department of Pathology, PO Box 3712, Durham, NC 27710.

[2]Abbreviations used are as follows: CGH, comparative genomic hybridization; EGFR, epidermal growth factor receptor; GBM, glioblastoma multiforme; LOH, loss of heterozygosity; PCR, polymerase chain reaction.

The most malignant and common astrocytic neoplasm is GBM.[2] Due to its large size and accessibility by surgical resections, it has been exhaustively studied over the years, and consistent genetic alterations have been identified using various techniques. Classic cytogenetic studies have reported a gain of chromosome 7 accompanied by a loss of chromosome 10 in more than 60% of GBM samples and a net loss of 9p loci usually resulting from chromosomal rearrangements in 30% of the tumors (Bigner et al., 1988a, 1990). Moreover, gene amplifications represented by double minutes were reported in approximately 50% of glioblastomas (Bigner et al., 1990, 1998). Southern blot analyses have shown a positive correlation between the double minutes and *EGFR* gene amplification, implicating involvement of this gene in the etiology of the disease (Bigner et al., 1987). LOH studies have corroborated the cytogenetic findings of a reduction of chromosomes 9 and 10 loci (Bello et al., 1994; Fults et al., 1990; James et al., 1988; Olopade et al., 1992; Rasheed et al., 1992). Subsequent mutational analyses identified homozygous deletions and other mutations in specific genes, including phosphatase and tensin homolog (*PTEN*, 10q23) and cyclin kinase-dependent inhibitor-2 (*CDKN2*, 9p21) (Giani and Finocchiaro; 1994; Jen et al., 1994; Li et al., 1997; Rasheed et al., 1997; Steck et al., 1997; Ueki et al., 1994; Walker et al., 1995; Wang et al., 1997). Combined, these data provide a genetic profile of GBM that is distinct from lower grade astrocytomas and oligodendrogliomas.

Despite the wide acceptance of the World Health Organization grading system, histologic complexity and heterogeneity of brain neoplasms have often made classi-

fication subjective and controversial within and between different tumor types. Known genetic alterations can be used to aid in classification. However, the methodologies used to obtain the genetic data tend to be cumbersome and time consuming and can be difficult to interpret, making them inefficient for routine tumor classification. Therefore, using another technique that recognizes distinct genetic patterns among different histologic grades would be clearly beneficial in brain tumor characterization. CGH is such a technique that detects numeric changes in genomic DNA (Kallioniemi et al., 1992, 1994). The fidelity and accuracy of CGH have been proven by the invariable detection of DNA sequence copy number alterations in a variety of tumors, including those of the CNS (Bigner et al., 1999; Kallioniemi et al., 1992, 1994; Kros et al., 1999; Schrock et al., 1994; Wiltshire et al., 1995; Zitzelsberger et al., 1997). CGH results have shown a reasonable correlation with the simple karyotypes usually found with hematologic neoplasia (Bentz et al., 1995; Gribble et al., 1999; Karhu et al., 1997; Larramendy et al., 1998; Wilkens et al., 1998, 1999). However, few studies have been reported directly comparing CGH profiles with more complex karyotypes of solid tumors or molecular genetic analyses of solid tumors (Bigner et al., 1999; Persson et al., 1999). Thus far, it has been demonstrated that CGH can efficiently detect numeric genomic imbalances in neoplasms, but the possibility of using CGH as a diagnostic tool for analyzing solid tumors has not been sufficiently explored.

In an effort to evaluate the diagnostic potential of CGH in assisting histologic brain tumor classification, we directly compared CGH data with that obtained from independent studies using established genome scanning techniques on a series of 22 GBM biopsies. The CGH patterns were consistent with published reports and correlated with conventional cytogenetic and molecular genetic studies on the most frequent chromosomal changes. In conclusion, this report demonstrates that CGH is an efficient tool that provides a simple recognizable pattern of prominent genetic alterations for glioblastomas and supports its utility for helping to classify neoplasms of the CNS.

Materials and Methods

Tumor Samples

A series of adult brain tumors that were routinely resected and histologically characterized according to World Health Organization classifications (Kleihues et al., 1993a, 1993b) were collected and examined. Twenty-two tumors were classified as GBM, including 1 variant gliosarcoma (Table 1). A portion of each tumor was cultured shortly after being resected and analyzed for cytogenetic alterations. The remaining portion of the specimens was preserved in liquid nitrogen and stored at –135°C. Examination of cryostat sections confirmed that each sample had at least 70% neoplastic cells. The DNA was extracted from the frozen blocks according to conventional procedures and examined with molecular genetic techniques and CGH.

Cytogenetic Analysis

Cells from a portion of each freshly resected tumor were grown in short-term culture and examined for cytogenetic alterations as previously described (Bigner et al., 1988a). Briefly, the tumor biopsies were cultured from 1 to 16 days prior to karyotypic analysis. The modal number of each case was calculated from a numerical examination of approximately 30 cells, and a composite karyotype was discerned from the examination of at least 3 karyotypes (Table 1). Karyotypes for cases H311, H315, H332, and H342 were previously published (Bigner et al., 1988a), but were reevaluated for this study. The cytogenetic designations conformed to the ISCN 1995 notations (Mitelman, 1995).

Molecular Genetic Analyses

DNA was extracted from the frozen tumor biopsies and studied for LOH and gene amplification. As previously described, a series of 200 PCR allelotype microsatellite markers, spaced an average distance of 25 centimorgans and spanning the autosomes, were selected for the LOH analysis (Blaeker et al., 1996). Loss of an amplified band, or at least a 50% visual reduction in band intensity of the tumor DNA compared with the patient's normal DNA, indicated hemizygosity. The association of LOH with 1 primer set was sufficient to indicate a loss of the chromosome arm. More comprehensive chromosome 10 LOH studies were previously performed on these samples (Rasheed et al., 1992, 1994, 1995). However, in reevaluating the cases for this study, fewer chromosome 10 PCR microsatellite primers were used. The samples were also tested with conventional Southern blot analysis for known gene amplifications, including *EGFR, GLI, MDM2, CDK4,* and *MYC*. The data from this analysis have already been reported for all of the samples except the *CDK4* analysis (Rasheed et al., 1994, 1997).

Comparative Genomic Hybridization

The samples were assayed for DNA sequence copy number alterations using CGH (Kallioniemi et al., 1992, 1994). The procedure was carried out according to the Vysis protocol (Vysis, Downers Grove, Ill.). Briefly, 1 μg of tumor DNA extracted from the frozen portion of the specimens was labeled with SpectrumGreen dUTP, whereas 1 μg of normal male or female peripheral blood DNA was labeled with SpectrumRed dUTP by nick translation. Approximately 400 ng of each labeled DNA and 10 μg of human Cot-1 DNA were hybridized to normal metaphase spreads prepared from an Epstein-Barr virus–transformed lymphoblast cell line. The hybridized metaphases were washed, counterstained with 0.1 μg/ml 4,6-diamino-2-phenylindole (DAPI) in an antifade solution, and viewed on a Zeiss Axioplan microscope equipped with 4,6-diamino-2-phenylindole, fluorescein isothiocyanate, and Texas Red fluorescent filters (Chroma Technology, Brattleboro, Vt.). Images were captured with the SmartCapture software, and at least 6 metaphases were analyzed with the Quips XL image analysis program (Vysis, Downers Grove, Ill.). An aver-

Table 1. Comparison of cytogenetic and comparative genomic hybridization analyses

Case H#	Sex/ age	Composite karyotype	Comparative genomic hybridization: Gains	Comparative genomic hybridization: Losses
311	F/53	44-47,XX,add(5)(q35),**+7,-10**,-11,-16,-18,**+19,+20,**1~3mar[cp7]/NI[a], 20~100dmin[cp4]	**7p[d],19p,20p**	**9p[d],10p[d],10q[d]**
315	M/69	NI	7	10q,13,14,18
332	F/68	NI,1~10dmin[cp4]	1p[d],2q[d],5q[d],7[e],8q[d],12q[d]	6,8p[d],10,11q[d], 13q[d],16,19,22
342	M/62	46,XY[cp5]	3q[d]	1q[d],13q[d]
365	F/67	44-46,XX,**+dic(1;9)(p21;p21),**add(2)(p21),**+7**,-8[4],-9,**-10**[cp12]	**1q**,**7**,Xq[d]	**10**
368	M/67	69-71,XXY,+1,**+2,-6,+7,+7,+7,-8,-9,-10,-11**,+12,**-14,-15,-16,-17**,+1-7 mar[cp4]	**2p,2q[d],7[c]**	**6,8,9,10,11,14, 15q[d],16,17q[d]**,Y
395	F/60	47-50,XX,+5,**+7**,-8,der(8)t(8;9)(q13;q12),**-9**,**der(9)t(9;14)(p13;q13)**, -10,**+12**,-14,-15,**-16**,-17,+der(19)t(19;?)(q13.3;?),+20,+mar1-2[3], +1-6mar[cp7]	**7p[d],7q,12p[d],12q[d]**	**9p,16q,**21,X
397	M/53	44-46,XY,del(2)(q33),add(4)(p16),**+7**,**del(9)(p13)**,der(11)t(11;14)(p15; q13),-13,-14,-14,-15,-17,add(17)(p13),+mar1,+1-5mar[cp6]	**7[e]**,17p[d],17q[d]	**9p[d]**,10,11,22
398	M/75	45,XY,**del(6)(q23),+7,del(9)(p13),-10,-13**,~3dmin[cp6]/45,X,**-Y**[cp3]	**7p,7q[d]**	1p[d],**6q[d],9p[d],**9q[d]**, 10,13,**16p,17p, 19,22,Xp[d], Xq[d],**Y**
399	M/62	NI	18q[c]	Y
409	M/57	48-50,XY,**+Y,+7,-10**,del(10)(p11.2)[3],+19,**+20**,+2-5mar,dmin[cp9]	**7,20,Y**	1p[d],9q[d],**10**,17
423	M/51	NI,3~15dmin[cp6]	5p[d],5q[d],7p[d],7q[d],11,13q[d] 17p,17q[d]	1p[d],10,Y
450	M/64	46,XY[cp5]/45-44,X,**-Y**[cp4]/45-47,XY,der(6)t(2;6)(q21;q27),**+7,-10**, 20~100dmin[cp4]	2q[d],**7[e]**,20q[d]	**10**,14,17,**Y**
457	M/39	46,XY,**+7,del(9)(p13),-10**,10~30dmin[cp3]	**7p[e,d],7q**	**9p,10,**22,Y
471	F/81	45,X,-X[cp4]	7	5q[d]
475	F/59	46, XX [cp5]	7p[d],7q[d]	9,10,14q[d],16q
493	F/60	45,X,-X[cp4]/46,XX,[cp4]	7[e],13q[d],14q[d],20p[c],20q	1p[d],3p,3q[d],6, 9p,10,16,19
509	M/63	46,XY,**+Y**,add(4)(p16),**+7,-10,del(11)(q13),-13**,-16,**-17,-17**,-18,+21, +22,+mar[cp4]	5q[d],**7**,20p,**Y**	1p[d],**10,11,13, 17p[d],17q**,22
534	M/63	44,XY,**-6,+7,der(9)t(6;9)(p21.1;p21),-10**,der(12)t(12;?)(q13;?),**-13,-15**, +mar1[cp9]	**7**,12q[d]	**6q[d]**,**9p[d]**,**10**,12q[d], **13q[d],15**
542	F/45	NI	9p[d],12q[d]	1p[d],19
566	M/69	42-47,X,-Y[3],-4,der(6)t(6;?)[2],**+7**,del(9)(p21),**-10**,-13,-15[4],**-17**, 5~16dmin[cp8]	1q[d],5q[d],**7p[d],7q[d]**,20p	1p[d],**10,17**,19,22
502[b]	M/77	47,XY,-1,del(2)(p21),+der(5)t(5;?)(q35;?),del(6)(q21),**der(8)t(7;8) (q11.23;q24.3)**,der(9)t(8;9)(q22;p22),**del(9)(p22)**,der(17)t(1;17) (q31;q11.2),+20,[cp3]/91-94,XX,-Y,-Y,-1,-1,del(2)(p21)x2,+der(5) t(5;?)(q35;?)x2,del(6)(q21)x2,der(8)t(7;8)(q11.23;q24.3)x2,der(9)t (8;9)(q22;p22)x2,del(9)(p22)x2,der(17)t(1;17)(q31;q11.2)x2,+20, +20,+mar1[cp6]	1q[d],6q[d],7p[d],**7q[d]**,8q[d]	6p[d],8p,**9p[d]**,11q[d]

Bold type indicates similar numeric alterations detected by cytogenetic and comparative genomic hybridization analyses.

[a]Metaphase spreads were not informative.

[b]Gliosarcoma.

[c]High level gain of DNA sequences.

[d]Partial gain and loss of chromosome arms.

[e]Comparative genomic hybridization amplification including the *EGFR* locus.

age ratio profile of green-to-red signal intensities was plotted along the length of each chromosome, and values of 0.85 and 1.20 indicated loss and gain of DNA sequences, respectively. Profile ratios of 1.20 were considered simple gains, and those 1.45 and higher were suggestive of high-level gain and amplified DNA sequences. Amplification was implied in profiles with sharp rising peaks relative to the rest of the chromosomal profile. Gains and losses detected solely at centromeric, heterochromatic, and telomeric regions are unreliable due to incomplete blocking of the repetitive sequences by Cot-1 DNA and inconsistent hybridizations at the ends of the chromosomes (Kallioniemi et al., 1994). These regions were excluded on a case-by-case basis after a careful examination of the metaphase chromosomes and signal intensities.

Table 2. Summary of most frequent numerical alterations and gene amplifications

Case No.	Karyotypes	CGH	LOH	Gene amplification
H311	+7, –10, dmin	+7p[d],–9p[d],–10p[d],–10q[d]	10	*EGFR*
H315	NI[a]	+7, –10q	9q,10q	na[f]
H332	NI, dmin	7[e],–10	10	*EGFR*
H342	Normal[b]	—	10	na
H365	+7, –9, –10	+7,–10	9p,10q	na
H368	+7, –9, –10	+7, –9,–10	9,10	na
H395	+7, –9p, –10	+7p[d],+7q,–9p	9p,10q	na
H397	+7, –9p	+7[e],9p[d],10	9p,10	*EGFR*
H398	+7, –9p, –10, dmin	+7p,+7q[d],9p[d],10	10q	na
H399	NI	—	—	na
H409	+7, –10, dmin	+7,–10	9p,10	*EGFR*
H423	NI, dmin	+7p[d],+7q[d],–10	9,10	*GLI*
H450	+7, –10, dmin	+7[e],–10	9p,10	*EGFR*
H457	+7, –9p, –10, dmin	+7p[d,e],+7q,–9p,–10	9p,10	*EGFR*
H471	—[c]	+7	10	na
H475	Normal	+7p[d],+7q[d],–9,–10	9,10q	na
H493	—	+7[e],–9p,–10	9p,10	*EGFR*
H509	+7, –10	+7,–10	10	na
H534	+7, –9p, –10	+7,–9p[d],–10	9p,10	*CDK4*, *MDM2*
H542	NI	—	—	na
H566	+7, –9p, –10, dmin	+7p[d],+7q[d],–10	10	*MYC*
H502	–9p	+7p[d],+7q[d],–9p[d]	—	na

[a]Metaphase spreads were not informative.

[b]Normal karyotypes were obtained.

[c]Alterations other than +7, –9, and –10 were detected.

[d]Partial gain or loss of chromosome arms.

[e]CGH amplification including the *EGFR* locus.

[f]No amplification detected.

Results

Cytogenetic Analysis

Short-term cultures of the 22 tumor biopsies were studied for chromosomal alterations. Abnormal karyotypes were obtained from 15 (68%) cases, whereas 2 tumors showed normal karyotypes and 5 were not informative for analyzable metaphase spreads (Table 1). The aberrant karyotypes showed a complex array of near-diploid to hypertetraploid clonal populations with various numerical and structural abnormalities. A net gain of chromosome 7 (13/15), followed by net losses of chromosomes 10 (11/15) and 9 (9/15) were the most frequent cytogenetic aberrations associated with these tumors (Tables 1 and 2). Further scrutiny of the composite karyotypes revealed that gain of the entire chromosome 7 was accompanied by loss of the entire chromosome 10 in 11 of the gliomas. No consistent structural rearrangements or partial gains or losses were observed with these chromosomes. On the other hand, chromosome 9 was involved in both numerical and structural alterations. Loss of the entire chromosome 9 was identified in 2 cases, whereas structural alterations were found in 8 (Table 1). The alterations resulted mainly in a net loss of 9p sequences, and consistent breakpoints involving chromosomal bands 9p13 and 9p21 were observed in 4 and 3 cases, respectively. The smallest common region lost was 9pter→p22, seen in case H502 (Table 1). The other cytogenetic alterations were far less common. Loss of chromosomes 13, 15, and 17 was seen in 5 cases each, whereas loss of chromosome 16 and gain of chromosome 20 sequences was found in only 4 cases. No alterations involving chromosome 3 were detected, and aberrations involving the remaining chromosomes appeared in no more than 20% (3/15) of the abnormal cases. Loss of chromosome X was recorded as the sole abnormality in the 2 remaining cases with aberrant karyotypes. Irrespective of analyzable metaphases, double minutes were detectable in 36% (8/22) of the samples (Table 1).

Molecular Genetic Analysis

All 22 samples were informative for the microsatellite PCR markers. The most frequent LOH was associated with chromosome 10 in 19 (86%) of the 22 GBM samples; 5 of these cases depicted LOH exclusively on 10q (Fig. 1). Chromosome 9 had the second highest LOH frequency in this study, with 12 (54%) samples showing hemizygosity, primarily on the short arm (Fig. 1 and Table 2). LOH was detected on chromosomes 6, 22, and the short arm of

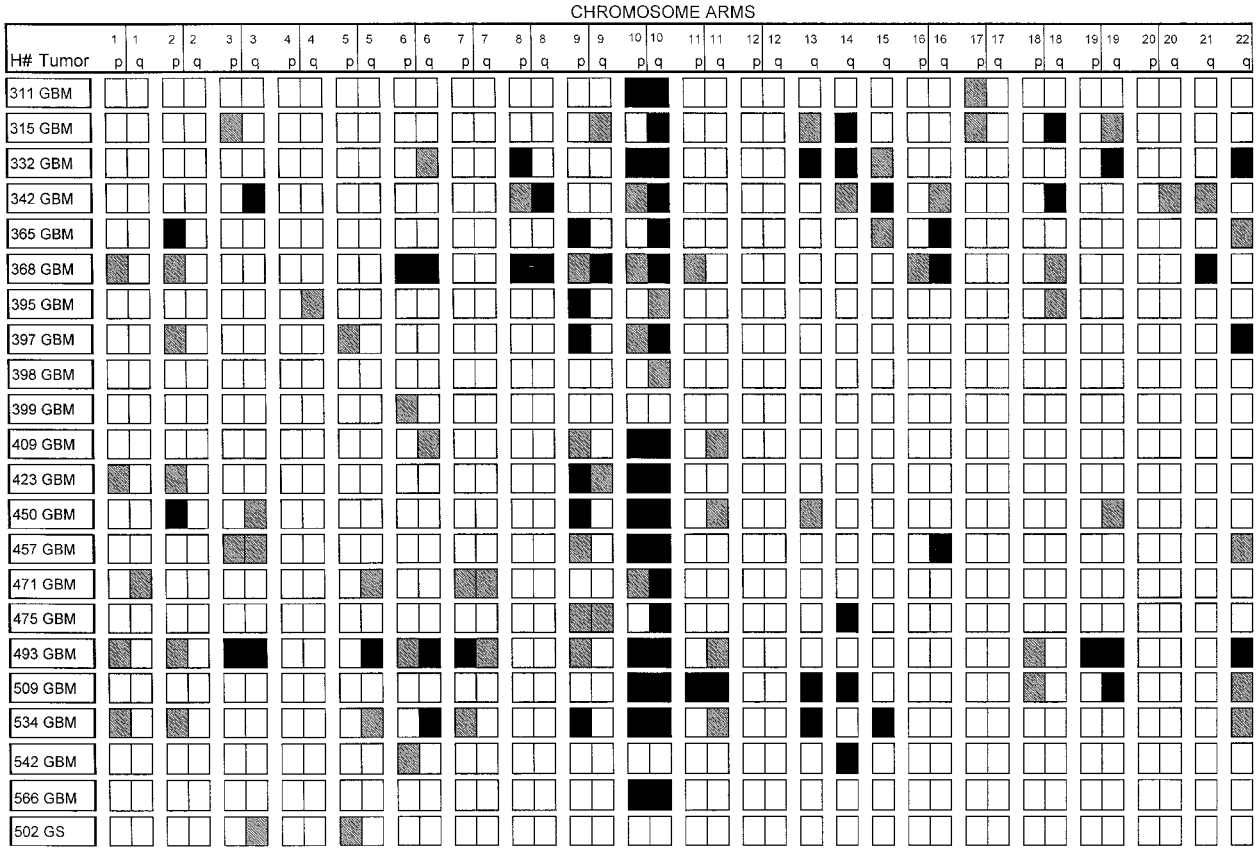

Fig. 1. Summation of microsatellite analysis of 22 glial tumors. GBM, glioblastoma multiforme; GS, gliosarcoma. Solid squares, loss of heterozygosity was detected for multiple PCR markers; shaded squares, loss of heterozygosity was detected for 1 PCR marker; white squares, no loss of heterozygosity was detected.

chromosome 2 in 7 (32%) biopsies. The reduction of 1 allele was consistently seen with the remaining autosomes in no more than 6 (27%) samples (Fig. 1).

The tumors had previously been investigated for gene amplifications, using specific gene probes against Southern blots (Rasheed et al., 1994). Briefly, amplifications were detected in 10 (45%) of the tumors (Table 2). The *EGFR* gene was amplified in 7 of these GBM cases, whereas the *GLI* and *MYC* genes were solely amplified in H423 and H566, respectively. The remaining case, H534, had coamplifications of *MDM2* and *CDK4*.

Comparative Genomic Hybridization

The same DNA used for the molecular analyses was examined by the CGH protocol for copy number alterations. Similar to the karyotypic and microsatellite analyses, the CGH profile composite revealed the highest copy number changes on chromosomes 7 and 10, followed by the short arm of chromosome 9 (Table 1 and Fig. 2). First, an overrepresentation of chromosome 7 sequences was seen in 86% (19/22) of the samples, and in 10 cases this involved the entire chromosome. One case, H368, revealed a high-level gain of chromosome 7. The smallest consistent region of simple gain was localized to 7q32→q34 (Fig. 2). Amplification spanning the chromosomal region 7p15→q11 was detected in 5 cases, and the smallest region was limited to 7p13→p10 (Table 1 and Fig. 2). Second, all but 3 cases, H395, H471, and H502, with a gain of chromosome 7 loci, were associated with a loss of the entire chromosome 10 (Fig. 2 and Table 1). The smallest overlapping region of loss was 10q10→q22 (Fig. 2). Third, underrepresentation of chromosome 9 and 9p loci was recorded in 45% (10/22) of the GBM samples, with 9p21 as the smallest common region lost (Table 1 and Fig. 2).

Numeric changes of DNA sequences were seen on the other autosomes at lower frequencies. Underrepresentation of chromosomes 1p and 22 sequences were each detected in 7 (32%) GBM samples, whereas loss of the remaining loci were observed in no more than 6 cases (Table 1 and Fig. 2). The second most commonly gained region was on chromosome 20 in 6 GBM cases. Furthermore, a high level of gain was discovered on the long arm of chromosome 18 in case H399, and on 20p in H493 (Fig. 2). Few changes were detected with the X chromosome, but 43% (6/14) of the male GBM patients indicated a loss of the Y chromosome (Fig. 2).

Comparison of Cytogenetic, CGH, and Molecular Genetic Results

Data from these independent studies revealed consistent chromosomal alterations in each case. As depicted in

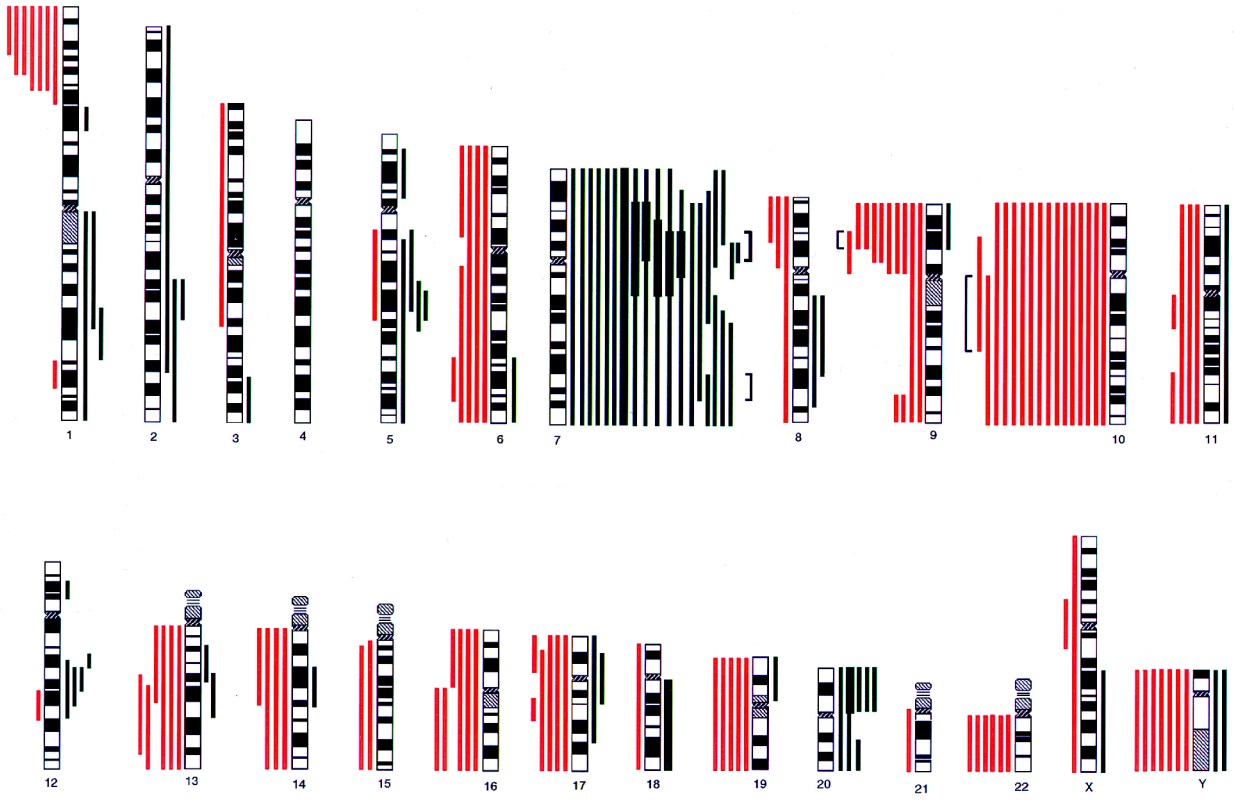

Fig. 2. Composite of CGH profiles from 22 GBM cases. Each line depicts the chromosomal region lost (red) and gained (green) in a single case. Thick lines indicate amplification and high-level gain of a region. Thin brackets indicate smallest overlapping regions of simple gains or losses of DNA sequences. Thick bracket marks smallest overlapping region of amplification.

Table 1, 87% (13/15) of the cases with complex aberrant karyotypes had at least 2 numerical alterations in common with the CGH profiles. For example, the cytogenetic alterations in H457, +7, del(9)(p13), –10, were also detected by CGH (Fig. 3). Similarly, 73% (11/15) of the karyotypes detected at least 2 deletions consistent with the LOH data. Finally, 82% (18/22) of the GBM cases had the same chromosomal losses detected by both LOH and CGH, an average of 3 common deletions per case.

Specific comparisons showed that samples with a cytogenetic gain of chromosome 7 (13/22) also depicted an overrepresentation of the chromosome by CGH (Table 2). Additionally, CGH detected a gain of chromosome 7 sequences in 7 other cases that were either not informative for karyotypic analysis, had normal karyotypes, or showed a missing X chromosome as the only alteration (Table 2). The 11 cases with cytogenetic loss of chromosome 10 also had LOH for 10 and 10q loci, whereas 10/11 showed loss by CGH. Of the 5 cases without analyzable metaphase chromosomes, 3 depicted loss of these loci by LOH and CGH, but the other 2, H399 and H542, were equally noninformative (Table 2). LOH for chromosome 10 or 10q loci was observed in 5 of the 6 remaining karyotypes that did not show a loss of chromosome 10 material, and CGH detected underrepresentation in 3 cases (Table 2). Loss of chromosome 9 or 9p loci was seen in 9 cases by cytogenetic analysis. Five of these cases were corroborated by both CGH and LOH, whereas 2 (H398, H502) were consistent with CGH and 1 (H365) with LOH alone. The remaining case, H566, revealed loss of 9p exclusively by karyotypic analysis (Table 2). Both CGH and LOH analyses identified loss of chromosome 9 loci in 2 cases, H475 and H493, that did not show loss by cytogenetics. On the other hand, loss of 9p was observed in H311 solely by CGH, and 4 other cases (H315, H409, H423, and H450) only showed LOH for chromosome 9 loci (Table 2). In total, 10 cases showed underrepresentation of 9p by CGH, and 12 cases showed hemizygosity by LOH compared with the 9 composite karyotypes with a net loss of 9p loci.

Seven of the 8 cases with double minutes had amplification of the *EGFR* (5 cases), *MYC* (1 case) and *GLI* (1 case) genes (Table 2). Three of the 4 cases (H332, H450, and H457) with *EGFR* amplification and double minutes also showed a sharp CGH profile peak in the region corresponding to the location of this gene, which is indicative of genomic amplification (Fig. 3). *EGFR* gene amplification was suggested solely by CGH in 2 other cases, H493 and H397, and confirmed by Southern blot analysis (Table 2). In this study, cases with *MYC* and *GLI* amplifications were not detectable by CGH, but were associated with double minutes.

Discussion

This study was designed to directly compare conventional genetic techniques with CGH in order to ascertain the potential of CGH in aiding routine classification of brain neoplasms. A series of 22 histologically character-

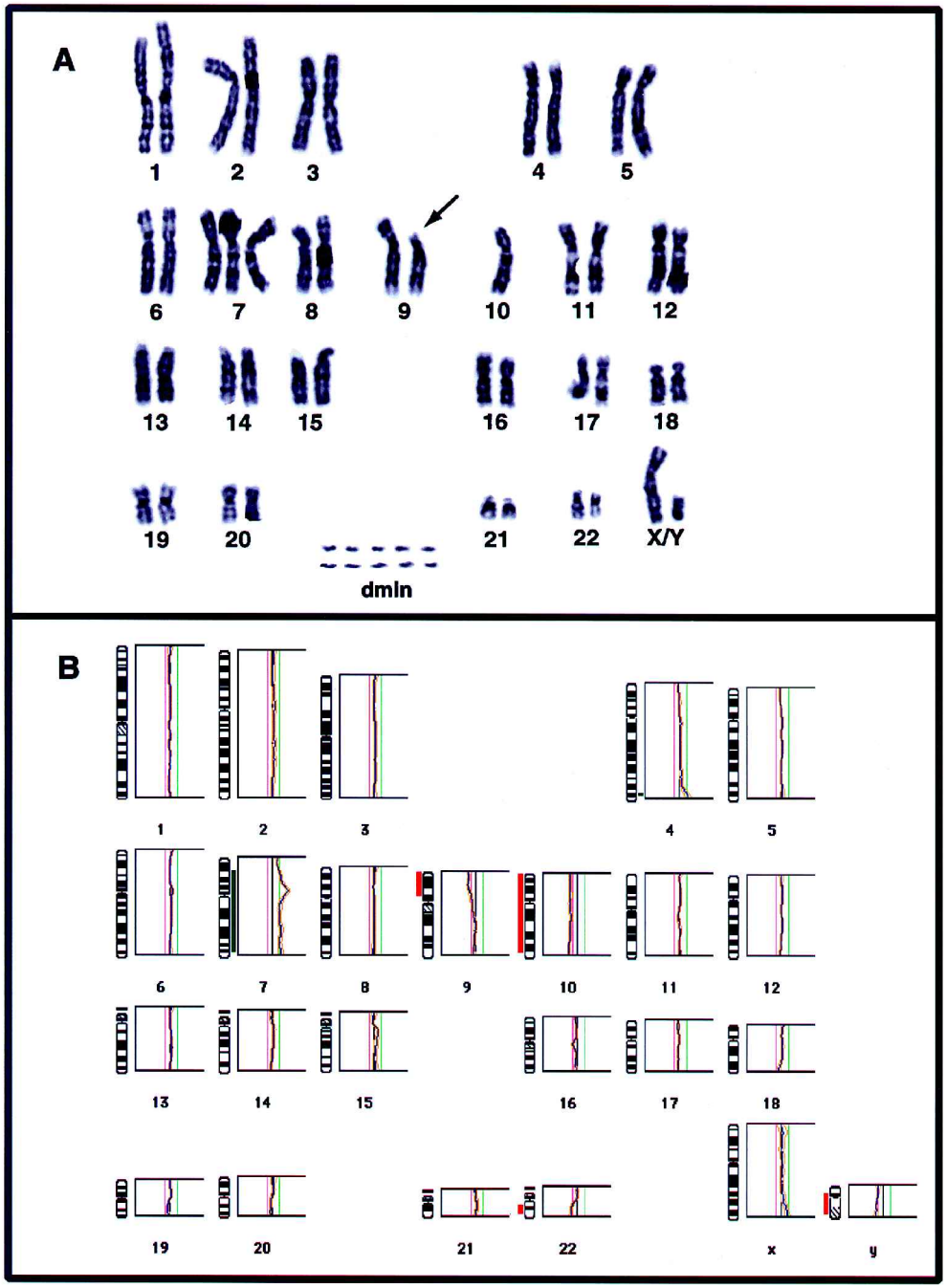

Fig. 3. Karyotype and CGH profile of a typical GBM case, H457. A. Representative karyotype 46,XY,+7,del(9)(p13),-10,10dmin. Arrow points to chromosome del(9)(p13). B. CGH profile indicating gain of chromosome 7, losses of chromosomes 9p, 10, 22, and Y. Green and red lines represent the 1.20 and 0.85 ratio thresholds, respectively. The green and red bars along the ideograms denote the regions that are gained and lost, respectively. Amplification is implicated by the sharp peak on chromosome 7 profile. The gain seen on the telomeric portion of chromosome 4q was an artifact.

ized adult astrocytic tumors were independently examined using classic cytogenetics, LOH analysis with PCR microsatellite allelic markers, Southern blot analysis for gene amplification, and CGH analysis.

Masked among the complex array of genetic alterations and histologic heterogeneity, a pattern emerged using classic cytogenetic and CGH methodologies for GBM involving chromosomes 7, 9, and 10. A cytogenetic gain of chromosome 7 was observed in 13/15 (86%) cases, and 11 (73%) of these were accompanied by a full loss of chromosome 10. Chromosomal rearrangements involving chromosome 9 primarily resulted in a loss of 9p loci in 9/15 (60%) GBM samples (Table 1). These data were reminiscent of published cytogenetic reports (Bigner et al., 1988a, 1990, 1998; Debiec-Rychter et al., 1995; Jenkins et al., 1989). The pattern was reproduced by CGH: an overrepresentation of the entire chromosome 7 was seen in 19 of 22 (86%) GBM biopsies and accompanied by a loss of the whole chromosome 10 in 16 (72%) cases. The third most common alteration was also the loss

of the short arm of chromosome 9 in 10 (45%) biopsies (Fig. 2 and Table 2). These findings were also consistent with published CGH studies on gliomas (Giollant et al., 1996; Kim et al., 1995; Mohapatra et al., 1995, 1998; Nishizaki et al., 1998; Schlegel et al., 1996; Schrock et al., 1994; Venkatraj et al., 1998; Weber et al., 1996a, 1996b). On a case-by-case comparison, the prominent net numerical changes identified by cytogenetics were also recognized by CGH (Table 1 and Fig. 3). Specifically, there was 100% correlation with the gain of chromosome 7, 91% correlation with loss of chromosome 10, 67% correlation with a loss of chromosome 9 loci, but only 35% correlation with the other cytogenetic alterations (Table 1). As illustrated in Fig. 3, the prominent changes, +7, –10, –9p, and gene amplification were readily seen in the karyotype and the CGH profile, but –22 and –Y are observed only with CGH. These discrepancies are due partly to the nature of the 2 techniques. Only actively dividing cells are analyzed by cytogenetics, causing some cells to be excluded from the analysis. On the other hand, CGH invariably analyzes the DNA from all the cells in the tumor sample and discerns a genetic profile of the most prominent changes in the tumor. Therefore, it is reasonable to assume that both cytogenetic and CGH analyses would have a good correlation in identifying the most common alterations, compared with the less frequent changes.

The LOH investigation was also in agreement with the reduction of genetic loci identified by CGH. Hemizygosity of chromosomes 10 and 9 were observed in 86% (19/22) and 54% (12/22) of the GBM samples, respectively, which was concurrent with published data (Bello et al., 1994; Bigner et al., 1998; Fults et al., 1990; James et al., 1988; Rasheed et al., 1995; Ueki et al., 1994). All of the cases showing underrepresentation of chromosome 10 loci by CGH also had LOH for the chromosome. Only 3 cases, H342, H395, and H471, with LOH of chromosome 10 did not indicate loss by CGH. CGH and LOH detected loss of chromosome 9p loci in a similar percentage of cases: 45% (10/22) and 55% (12/22), respectively. On the other hand, only 46% of the LOH detected on other chromosomes were illustrated by CGH.

Gene amplification is seen in about 60% of gliomas and in conjunction with the altered DNA sequence copy number pattern, this can be an important indicator for GBM classification. The *EGFR* gene is amplified in approximately 40% of glioblastomas, and its amplification is often associated with double minutes (Bigner et al., 1987, 1988b; Collins, 1995a, 1995b; Kleihues and Cavenee, 1997). Other genes, including *CDK4* and *SDS* (11%-15%), *MDM2* (5%), and *MYC* (<1%), have been amplified but at comparably modest frequencies (Bigner et al., 1988b; Fischer et al., 1994; He et al., 1994; Kinzler et al., 1987; Reifenberger et al., 1994; Schmidt et al., 1994; Trent et al., 1986). In this investigation, 10 cases revealed specific gene amplification by Southern blot analysis (Table 2). As expected, the *EGFR* gene amplification was found in the most samples, 32% (7/22), and *GLI*, *MDM2*, *CDK4*, and *MYC* were each detected in 1 sample (Table 2). The CGH profiles depicted amplified genomic sequences spanning the *EGFR* locus (7p12) in 71% (5/7) of the cases in which *EGFR* amplification was detected by Southern blot analysis. However, CGH profiles did not implicate amplifications in the regions housing *CDK4*, *GLI*, *MDM2* (12q13-q15), and *MYC* (8q24) genes (Table 2 and Fig. 2). When the data from this study are included with other reported CGH studies, amplification of the *EGFR* locus has been detected in 22% (52/232) of the cases, compared with 40% by other molecular methods (Giollant et al., 1996; Kim et al., 1995; Mohapatra et al., 1995, 1998; Nishizaki et al., 1998; Schlegel et al., 1996; Schrock et al., 1994; Venkatraj et al., 1998; Weber et al., 1996a, 1996b). Based on the LOH and gene amplification data, it appears that although CGH is sensitive enough to detect the major chromosome arm alterations, the limits of resolution make it difficult to identify a single gene locus. The specific marker or gene must be part of a larger region (at least 5 megabases) to be efficiently detected. Therefore, some LOH or gene amplifications will be below the level of CGH detection.

Together, classic cytogenetic, LOH, and Southern blot analyses provide a distinct genetic pattern for glioblastoma that is clearly reproducible by CGH. A gain of chromosome 7 accompanied by a loss of chromosome 10 is the most prominent pattern, followed by loss of 9p loci and *EGFR* amplification. This pattern was recognized in 73% (16/22) of the cases examined by CGH (Table 2). Two cases, H395 and H502, showed a gain of chromosome 7 loci and a loss of 9p without chromosome 10 involvement. Three other cases, H342, H399, and H542, did not have any recognizable pattern of chromosomal changes, and case H471 had only a gain of chromosome 7. In addition to the technical difficulties associated with CGH, such as unequal hybridization and degraded DNA, poor or differential sampling of the biopsies also could be the reason for the different genetic patterns. Variants of GBM, as with the gliosarcoma (H502), might be associated with slightly different genetic patterns.

Comparing the CGH profiles of glioblastoma with those of other brain tumors shows different patterns of copy number changes. Anaplastic astrocytomas show gains of chromosome 7 and losses of chromosome 10, but in a lower proportion of cases and without gene amplification (Mohapatra et al., 1995; Nishizaki et al., 1998; Schrock et al., 1994; Weber et al., 1996a). Furthermore, work done in this and other laboratories has shown loss of chromosomes 1p and 19q as the most frequent alterations in oligodendrogliomas (Bigner et al., 1999; Kros et al., 1999). The recognition of distinct copy number changes in glial tumors can be potentially important for more precise tumor classification. CGH is ideal for this because it pinpoints the prominent gross numeric changes of chromosomal arms without the increased complexity of genetic heterogeneity as seen with classic cytogenetics (Table 1). The number of genes altered in specific glial tumors is growing, but none have been identified at a high enough frequency to be useful for routine classifications. Therefore, CGH is an attractive alternative to an array of LOH allelic markers and Southern blot probes and is currently needed to assess the genetic distinction of glial tumors.

Acknowledgments

A special appreciation is extended to Annie Lloyd, Linda Cleveland, and David Lister for their technical contributions to this project.

References

Bello, M.J., de Campos, J.M., Vaquero, J., Kusak, M.E., Sarasa, J.L., Pestana, A., and Rey, J.A. (1994) Molecular and cytogenetic analysis of chromosome 9 deletions in 75 malignant gliomas. *Genes Chromosomes Cancer* **9**, 33-41.

Bentz, M., Dohner, H., Huck, K., Schutz, B., Ganser, A., Joos, S., du Manoir, S., and Lichter, P. (1995) Comparative genomic hybridization in the investigation of myeloid leukemias. *Genes Chromosomes Cancer* **12**, 193-200.

Bigner, D.D., McLendon, R.E., and Bruner, J.M. (Eds.) (1998) *Russell and Rubinstein's Pathology of Tumors of the Nervous System.* London: Arnold.

Bigner, S.H., Wong, A.J., Mark, J., Muhlbaier, L.H., Kinzler, K.W., Vogelstein, B., and Bigner, D.D. (1987) Relationship between gene amplification and chromosomal deviations in malignant human gliomas. *Cancer Genet. Cytogenet.* **29**, 165-170.

Bigner, S.H., Mark, J., Burger, P.C., Mahaley, M.S., Jr., Bullard, D.E., Muhlbaier, L.H., and Bigner, D.D. (1988a) Specific chromosomal abnormalities in malignant human gliomas. *Cancer Res.* **48**, 405-411.

Bigner, S.H., Burger, P.C., Wong, A.J., Werner, M.H., Hamilton, S.R., Muhlbaier, L.H., Vogelstein, B., and Bigner, D.D. (1988b) Gene amplification in malignant human gliomas: Clinical and histopathologic aspects. *J. Neuropathol. Exp. Neurol.* **47**, 191-205.

Bigner, S.H., Mark, J., and Bigner, D.D. (1990) Cytogenetics of human brain tumors. *Cancer Genet. Cytogenet.* **47**, 141-154.

Bigner, S.H., Matthews, M.R., Rasheed, B.K.A., Wiltshire, R.N., Friedman, H.S., Friedman, A.H., Stenzel, T.T., Dawes, D.M., McLendon, R.E., and Bigner, D.D. (1999) Molecular genetic aspects of oligodendrogliomas including analysis by comparative genomic hybridization. *Am. J. Pathol.* **155**, 375-386.

Blaeker, H., Rasheed, B.K., McLendon, R.E., Friedman, H.S., Batra, S.K., Fuchs, H.E., and Bigner, S.H. (1996) Microsatellite analysis of childhood brain tumors. *Genes Chromosomes Cancer* **15**, 54-63.

Collins, V.P. (1995a) Gene amplification in human gliomas. *Glia* **15**, 289-296.

Collins, V.P. (1995b) Genetic alterations in gliomas. *J. Neurooncol.* **24**, 37-38.

Debiec-Rychter, M., Alwasiak, J., Liberski, P.P., Nedoszytko, B., Babinska, M., Mrozek, K., Imielinski, B., Borowska-Lehman, J., and Limon, J. (1995) Accumulation of chromosomal changes in human glioma progression: A cytogenetic study of 50 cases. *Cancer Genet. Cytogenet.* **85**, 61-67.

Fischer, U., Wullich, B., Sattler, H.P., Gottert, E., Zang, K.D., and Meese, E. (1994) DNA amplifications on chromosomes 7, 9 and 12 in glioblastoma detected by reverse chromosome painting. *Eur. J. Cancer* **30A**, 1124-1127.

Fults, D., Pedone, C.A., Thomas, G.A., and White, R. (1990) Allelotype of human malignant astrocytoma. *Cancer Res.* **50**, 5784-5789.

Giani, C., and Finocchiaro, G. (1994) Mutation rate of the CDKN2 gene in malignant gliomas. *Cancer Res.* **54**, 6338-6339.

Giollant, M., Bertrand, S., Verrelle, P., Tchirkov, A., du Manoir, S., Ried, T., Mornex, F., Dore, J.F., Cremer, T., and Malet, P. (1996) Characterization of double minute chromosomes' DNA content in a human high grade astrocytoma cell line by using comparative genomic hybridization and fluorescence in situ hybridization. *Hum. Genet.* **98**, 265-270.

Gribble, S.M., Sinclair, P.B., Grace, C., Green, A.R., and Nacheva, E.P. (1999) Comparative analysis of G-banding, chromosome painting, locus-specific fluorescence in situ hybridization, and comparative genomic hybridization in chronic myeloid leukemia blast crisis. *Cancer Genet. Cytogenet.* **111**, 7-17.

He, J., Allen, J.R., Collins, V.P., Allalunis-Turner, M.J., Godbout, R., Day, R.S., III, and James, C.D. (1994) CDK4 amplification is an alternative mechanism to p16 gene homozygous deletion in glioma cell lines. *Cancer Res.* **54**, 5804-5807.

James, C.D., Carlbom, E., Dumanski, J.P., Hansen, M., Nordenskjold, M., Collins, V.P., and Cavenee, W.K. (1988) Clonal genomic alterations in glioma malignancy stages. *Cancer Res.* **48**, 5546-5551.

Jen, J., Harper, J.W., Bigner, S.H., Bigner, D.D., Papadopoulos, N., Markowitz, S., Willson, J.K., Kinzler, K.W., and Vogelstein, B. (1994) Deletion of p16 and p15 genes in brain tumors. *Cancer Res.* **54**, 6353-6358.

Jenkins, R.B., Kimmel, D.W., Moertel, C.A., Schultz, C.G., Scheithauer, B.W., Kelly, P.J., and Dewald, G.W. (1989) A cytogenetic study of 53 human gliomas. *Cancer Genet. Cytogenet.* **39**, 253-279.

Kallioniemi, A., Kallioniemi, O.P., Sudar, D., Rutovitz, D., Gray, J.W., Waldman, F., and Pinkel, D. (1992) Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* **258**, 818-821.

Kallioniemi, O.P., Kallioniemi, A., Piper, J., Isola, J., Waldman, F.M., Gray, J.W., and Pinkel, D. (1994) Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer* **10**, 231-243.

Karhu, R., Siitonen, S., Tanner, M., Keinanen, M., Makipernaa, A., Lehtinen, M., Vilpo, J.A., and Isola, J. (1997) Genetic aberrations in pediatric acute lymphoblastic leukemia by comparative genomic hybridization. *Cancer Genet. Cytogenet.* **95**, 123-129.

Kim, D.H., Mohapatra, G., Bollen, A., Waldman, F.M., and Feuerstein, B.G. (1995) Chromosomal abnormalities in glioblastoma multiforme tumors and glioma cell lines detected by comparative genomic hybridization. *Int. J. Cancer* **60**, 812-819.

Kinzler, K.W., Bigner, S.H., Bigner, D.D., Trent, J.M., Law, M.L., O'Brien, S.J., Wong, A.J., and Vogelstein, B. (1987) Identification of an amplified, highly expressed gene in a human glioma. *Science* **236**, 70-73.

Kleihues, P., and Cavenee, W.K. (Eds.) (1997) *Pathology and Genetics of Tumours of the Nervous System.* Lyon, France: International Agency for Research on Cancer.

Kleihues, P., Burger, P.C., and Scheithauer, B.W. (1993a) The new WHO classification of brain tumours. *Brain Pathol.* **3**, 255-268.

Kleihues, P., Burger, P.C., and Scheithauer, B.W. (1993b) *Histological Typing of Tumours of the Central Nervous System.* Second edition. New York: Springer-Verlag.

Kros, J.M., Van Run, P.R., Alers, J.C., Beverloo, H.B., van den Bent, M.J., Avezaat, C.J.J., and Van Dekken, H. (1999) Genetic aberrations in oligodendroglial tumours: An analysis using comparative genomic hybridization (CGH). *J. Pathol.* **188**, 282-288.

Larramendy, M.L., Huhta, T., Vettenranta, K., El-Rifai, W., Lundin, J., Pakkala, S., Saarinen-Pihkala, U.M., and Knuutila, S. (1998) Comparative genomic hybridization in childhood acute lymphoblastic leukemia. *Leukemia* **12**, 1638-1644.

Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S.I., Puc, J., Miliaresis, C., Rodgers, L., McCombie, R., Bigner, S.H., Giovanella, B.C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M.H., and Parsons, R. (1997) PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275**, 1943-1947.

Mitelman, F. (Ed.) (1995) *ISCN 1995: An International System for Human Cytogenetic Nomenclature (1995).* New York: S. Karger.

Mohapatra, G., Kim, D.H., and Feuerstein, B.G. (1995) Detection of multiple gains and losses of genetic material in ten glioma cell lines by comparative genomic hybridization. *Genes Chromosomes Cancer* **13**, 86-93.

Mohapatra, G., Bollen, A.W., Kim, D.H., Lamborn, K., Moore, D.H., Prados, M.D., and Feuerstein, B.G. (1998) Genetic analysis of glioblastoma multiforme provides evidence for subgroups within the grade. *Genes Chromosomes Cancer* **21**, 195-206.

Nishizaki, T., Ozaki, S., Harada, K., Ito, H., Arai, H., Beppu, T., and Sasaki, K.

(1998) Investigation of genetic alterations associated with the grade of astrocytic tumor by comparative genomic hybridization. *Genes Chromosomes Cancer* **21**, 340-346.

Olopade, O.I., Jenkins, R.B., Ransom, D.T., Malik, K., Pomykala, H., Nobori, T., Cowan, J.M., Rowley, J.D., and Diaz, M.O. (1992) Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. *Cancer Res.* **52**, 2523-2529.

Persson, K., Pandis, N., Mertens, F., Borg, A., Baldetorp, B., Killander, D., and Isola, J. (1999) Chromosomal aberrations in breast cancer: A comparison between cytogenetics and comparative genomic hybridization. *Genes Chromosomes Cancer* **25**, 115-122.

Rasheed, B.K., Fuller, G.N., Friedman, A.H., Bigner, D.D., and Bigner, S.H. (1992) Loss of heterozygosity for 10q loci in human gliomas. *Genes Chromosomes Cancer* **5**, 75-82.

Rasheed, B.K., McLendon, R.E., Herndon, J.E., Friedman, H.S., Friedman, A.H., Bigner, D.D., and Bigner, S.H. (1994) Alterations of the TP53 gene in human gliomas. *Cancer Res.* **54**, 1324-1330.

Rasheed, B.K., McLendon, R.E., Friedman, H.S., Friedman, A.H., Fuchs, H.E., Bigner, D.D., and Bigner, S.H. (1995) Chromosome 10 deletion mapping in human gliomas: A common deletion region in 10q25. *Oncogene* **10**, 2243-2246.

Rasheed, B.K., Stenzel, T.T., McLendon, R.E., Parsons, R., Friedman, A.H., Friedman, H.S., Bigner, D.D., and Bigner, S.H. (1997) PTEN gene mutations are seen in high-grade but not in low-grade gliomas. *Cancer Res.* **57**, 4187-4190.

Reifenberger, G., Reifenberger, J., Ichimura, K., Meltzer, P.S., and Collins, V.P. (1994) Amplification of multiple genes from chromosomal region 12q13-14 in human malignant gliomas: Preliminary mapping of the amplicons shows preferential involvement of CDK4, SAS, and MDM2. *Cancer Res.* **54**, 4299-4303.

Schlegel, J., Scherthan, H., Arens, N., Stumm, G., and Kiessling, M. (1996) Detection of complex genetic alterations in human glioblastoma multiforme using comparative genomic hybridization. *J. Neuropathol. Exp. Neurol.* **55**, 81-87.

Schmidt, E.E., Ichimura, K., Reifenberger, G., and Collins, V.P. (1994) CDKN2 (p16/MTS1) gene deletion or CDK4 amplification occurs in the majority of glioblastomas. *Cancer Res.* **54**, 6321-6324.

Schrock, E., Thiel, G., Lozanova, T., du Manoir, S., Meffert, M.C., Jauch, A., Speicher, M.R., Nurnberg, P., Vogel, S., and Janisch, W. (1994) Comparative genomic hybridization of human malignant gliomas reveals multiple amplification sites and nonrandom chromosomal gains and losses. *Am. J. Pathol.* **144**, 1203-1218.

Steck, P.A., Pershouse, M.A., Jasser, S.A., Yung, W.K., Lin, H., Ligon, A.H., Langford, L.A., Baumgard, M.L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D.H., and Tavtigian, S.V. (1997) Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat. Genet.* **15**, 356-362.

Trent, J., Meltzer, P., Rosenblum, M., Harsh, G., Kinzler, K., Mashal, R., Feinberg, A., and Vogelstein, B. (1986) Evidence for rearrangement, amplification, and expression of c-myc in a human glioblastoma. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 470-473.

Ueki, K., Rubio, M.P., Ramesh, V., Correa, K.M., Rutter, J.L., von Deimling, A., Buckler, A.J., Gusella, J.F., and Louis, D.N. (1994) MTS1/CDKN2 gene mutations are rare in primary human astrocytomas with allelic loss of chromosome 9p. *Hum. Mol. Genet.* **3**, 1841-1845.

Venkatraj, V.S., Begemann, M., Sobrino, A., Bruce, J.N., Weinstein, I.B., and Warburton, D. (1998) Genomic changes in glioblastoma cell lines detected by comparative genomic hybridization. *J. Neurooncol.* **36**, 141-148.

Walker, D.G., Duan, W., Popovic, E.A., Kaye, A.H., Tomlinson, F.H., and Lavin, M. (1995) Homozygous deletions of the multiple tumor suppressor gene 1 in the progression of human astrocytomas. *Cancer Res.* **55**, 20-23.

Wang, S.I., Puc, J., Li, J., Bruce, J.N., Cairns, P., Sidransky, D., and Parsons, R. (1997) Somatic mutations of PTEN in glioblastoma multiforme. *Cancer Res.* **57**, 4183-4186.

Weber, R.G., Sabel, M., Reifenberger, J., Sommer, C., Oberstrass, J., Reifenberger, G., Kiessling, M., and Cremer, T. (1996a) Characterization of genomic alterations associated with glioma progression by comparative genomic hybridization. *Oncogene* **13**, 983-994.

Weber, R.G., Sommer, C., Albert, F.K., Kiessling, M., and Cremer, T. (1996b) Clinically distinct subgroups of glioblastoma multiforme studied by comparative genomic hybridization. *Lab. Invest.* **74**, 108-119.

Wilkens, L., Tchinda, J., Nolte, M., Werner, M., and Georgii, A. (1998) Analysis of hematologic diseases using conventional karyotyping, fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH). *Hum. Pathol.* **29**, 833-839.

Wilkens, L., Burkhardt, D., Tchinda, J., Busche, G., Werner, M., Nolte, M., Ganser, A., and Georgii, A. (1999) Cytogenetic aberrations in myelodysplastic syndrome detected by comparative genomic hybridization and fluorescence in situ hybridization. *Diagn. Mol. Pathol.* **8**, 47-53.

Wiltshire, R.N., Duray, P., Bittner, M.L., Visakorpi, T., Meltzer, P.S., Tuthill, R.J., Liotta, L.A., and Trent, J.M. (1995) Direct visualization of the clonal progression of primary cutaneous melanoma: Application of tissue microdissection and comparative genomic hybridization. *Cancer Res.* **55**, 3954-3957.

Zitzelsberger, H., Lehmann, L., Werner, M., and Bauchinger, M. (1997) Comparative genomic hybridisation for the analysis of chromosomal imbalances in solid tumours and haematological malignancies. *Histochem. Cell Biol.* **108**, 403-417.