MOLECULAR CARCINOGENESIS 36:6–14 (2003)

ARTICLES

Chromosomal Abnormalities in Human Glioblastomas: Gain in Chromosome 7p Correlating With Loss in Chromosome 10q

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Various genomic alterations have been detected in glioblastoma. Chromosome 7p, with the epidermal growth factor receptor locus, together with chromosome 10q, with the phosphatase and tensin homologue deleted in chromosome 10 and deleted in malignant brain tumors-1 loci, and chromosome 9p, with the cyclin-dependent kinase inhibitor 2A locus, are among the most frequently damaged chromosomal regions in glioblastoma. In this study, we evaluated the genetic status of 32 glioblastomas by comparative genomic hybridization; the sensitivity of comparative genomic hybridization versus differential polymerase chain reaction to detect deletions at the phosphatase and tensin homologue deleted in chromosome 10, deleted in malignant brain tumors-1, and cyclin-dependent kinase inhibitor 2A loci and amplifications at the cyclin-dependent kinase 4 locus; the frequency of genetic lesions (gain or loss) at 16 different selected loci (including oncogenes, tumor-suppressor genes, and proliferation markers) mapping on 13 different chromosomes; and the possible existence of a statistical association between any pair of molecular markers studied, to subdivide the glioblastoma entity molecularly. Comparative genomic hybridization showed that the most frequent region of gain was chromosome 7p, whereas the most frequent losses occurred on chromosomes 10q and 13q. The only statistically significant association was found for 7p gain and 10q loss.

Key words: phosphatase and tensin homologue deleted in chromosome 10; deleted in malignant brain tumors-1; cyclin-dependent kinase inhibitor 2A; epidermal growth factor receptor; comparative genomic hybridization

INTRODUCTION

Glioblastoma (GBM) is the most frequent and aggressive kind of brain tumor. GBMs have been studied extensively at the molecular level. Usually, only a few loci are explored per study [1–9]. Genome-wide scans also have been performed to detect the genetic lesions linked to GBM development, by the use of comparative genomic hybridization (CGH), a technique that shows the gained and lost chromosomal regions in tumor DNA in a single experiment [10–13]. In our study we first evaluated the genetic changes in 32 GBMs by a CGH approach. We specifically explored 16 loci, most of them documented so far to be involved in GBM development: 11 correspond to oncogenes and loci related to cell proliferation (epidermal growth factor receptor [*EGFR*], platelet-derived growth factor receptor [*PDGFR*], *myc*, cyclin-dependent kinase 4 [CDK4], PCNA, and the [E2F] transcription factor family of genes, E2F1–E2F6) and five tumor-suppressor gene–like loci (phosphatase and tensin homologue deleted in

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Received 16 January 2002; Revised 16 August 2002; Accepted 2 October 2002

Abbreviations: GBM, glioblastoma; CGH, comparative genomic hybridization; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; CDK4, cyclin-dependent kinase 4; PTEN, phosphatase and tensin homologue deleted in chromosome 10; DMBT1, deleted in malignant brain tumors-1; CDKN2A, cyclin-dependent kinase inhibitor 2A; PCR, polymerase chain reaction; LOH, loss of heterozygosity.

DOI 10.1002/mc.10085

chromosome 10 [PTEN], deleted in malignant brain tumors-1 [DMBT1], cyclin-dependent kinase inhibitor 2A [CDKN2A], tumor protein [TP] 53, and retinablastoma [RB]).

MATERIALS AND METHODS

Tumor samples were obtained from 32 patients with GBM who underwent surgery at the Departments of Neurosurgery of the General Hospital of Tianjin Medical University, Tianjin, People's Republic of China (22 patients); Hospital de Navarra, Pamplona, Spain (eight patients); and Hospital Miguel Servet, Zaragoza, Spain (two patients). A case of pilocytic astrocytoma (grade I) was included as a negative control. Genomic DNA from all tumors was extracted by standard methods. CGH analysis was performed according to the standard protocol, with slight modifications [14]. Differential polymerase chain reaction (PCR) assays were designed to test for homozygous deletions at the loci *CDKN2A* (9p21), *PTEN* (10q23.31), and *DMBT1* (10q25.3-q26.1) [15] and for gene amplification at the locus *CDK4* (12q14) [3,16]. A methylation-specific PCR strategy to detect *CDKN2A* promoter hypermethylation also was applied [17]. Statistical analysis to study the hypothetical correlation between frequencies of lesions at any pair of damaged loci was by the chi-square test, including Fisher's exact test.

RESULTS AND DISCUSSION

Genetic Profile of GBM

The chromosomal regions with DNA copy number alterations (losses and gains) identified in all 31 GBMs by CGH are illustrated in Figure 1 and Table 1. Overall, gains occurred slightly more frequently than deletions (59 of 109 total aberrations). The most common gains occurred on chromosome 7 (65%). The next most common relative gains in genetic material involved chromosomes 20 (39%), 8 (35%), and 9 (26%). Finally, chromosomes 1 and 5, showing gains in 23% of GBMs, were among the chromosomes on the borderline of 25% gains. The most common losses in this series of GBMs involved chromosome 10, with 58% of cases affected. Regions of chromosomes 13 (35%) and 6 (26%) also were lost in these tumors.

Gains of genetic material

The frequency of gains of genetic material at 7p has been reported to range from 25% to 70% in different studies [11,12]. Candidates for oncogenes on 7p include *EGFR*, which is assigned to 7p12. The *EGFR* gene is the most frequently amplified oncogene in astrocytic tumors; it is amplified in about one-third of GBMs and in a few anaplastic astrocytomas [4].

We found gains on chromosome 20 in 36% of GBM cases. Similar results have been published elsewhere [13]. Candidate oncogenes on 20q are hematopoietic cell kinase (20q11); *AIB1*, a steroid receptor coactivator; and *BTAK*, a serine/threonine kinase that has been found to be amplified and overexpressed in breast cancer. The *PTPN1* gene, located at 20q12, is a nonreceptor tyrosine phosphatase involved in growth regulation and recently has been reported to be overexpressed in 72% of breast carcinomas. Another candidate gene is MYBL2, at 20q13, which encodes a transcription factor and plays an important role in cell-cycle progression. Furthermore, the human cellular apoptosis susceptibility gene (CAS) has been mapped to this same region [18].

It has not been reported that any of these oncogenes is amplified in gliomas, which suggests the presence of other oncogenes amplified at 20q that have yet to be identified. PCNA [19] and E2F1 [20], both of which act as proliferating factors in numerous tumors, also map to chromosome 20. The next most frequent gains were noted at chromosome 8. The main candidate oncogene on 8q is *myc* (8q24). Amplification of *myc* in gliomas has been reported [21]. Mohapatra et al. [22] found five GBMs with gains at 9q. One candidate oncogene at 9q is TRKB (9q22.1). This oncogene has been found to be expressed in gliomas [23].

Losses of genetic material

The most frequent loss found in this study was on chromosome 10 (58%). This finding agrees with those of previous studies [22]. At least three separate regions on chromosome 10 have been implicated in GBM [5]: one in the telomeric region of 10p and the others in the telomeric and centromeric regions of 10q. Our results showed that the common region of loss on chromosome 10 was 10q23-qter, which suggests inactivation of tumor-suppressor genes in the telomeric region of 10q. It has been found that the *PTEN* gene, on 10q23.31, is mutated in various malignant tumors, including GBM [24,25]. *DMBT1*, on 10q25.3-q26.1, has been reported to carry intragenic homozygous deletions in about 23% of GBMs [26].

Other losses were seen frequently on chromosomes 13q and 6q. Inactivation of the retinoblastoma gene on 13q in gliomas has been reported previously [6]. The loss on 9p probably is related to the genes encoding p15 and p16 [7]. Structural rearrangements involving 9p are believed to target homozygous deletion of p16 and/or p15 in 35–40% of high-grade GBMs [7]. Only 23% of GBMs in our series had loss on 9p. This low frequency may be related to the limited sensitivity of CGH, which depends on both the copy number and the size of the region, whereas other methods, such as differential PCR, depend only on copy number. If the size of the region at 9p is below the sensitivity of CGH, a deletion may still be detected by other methods.

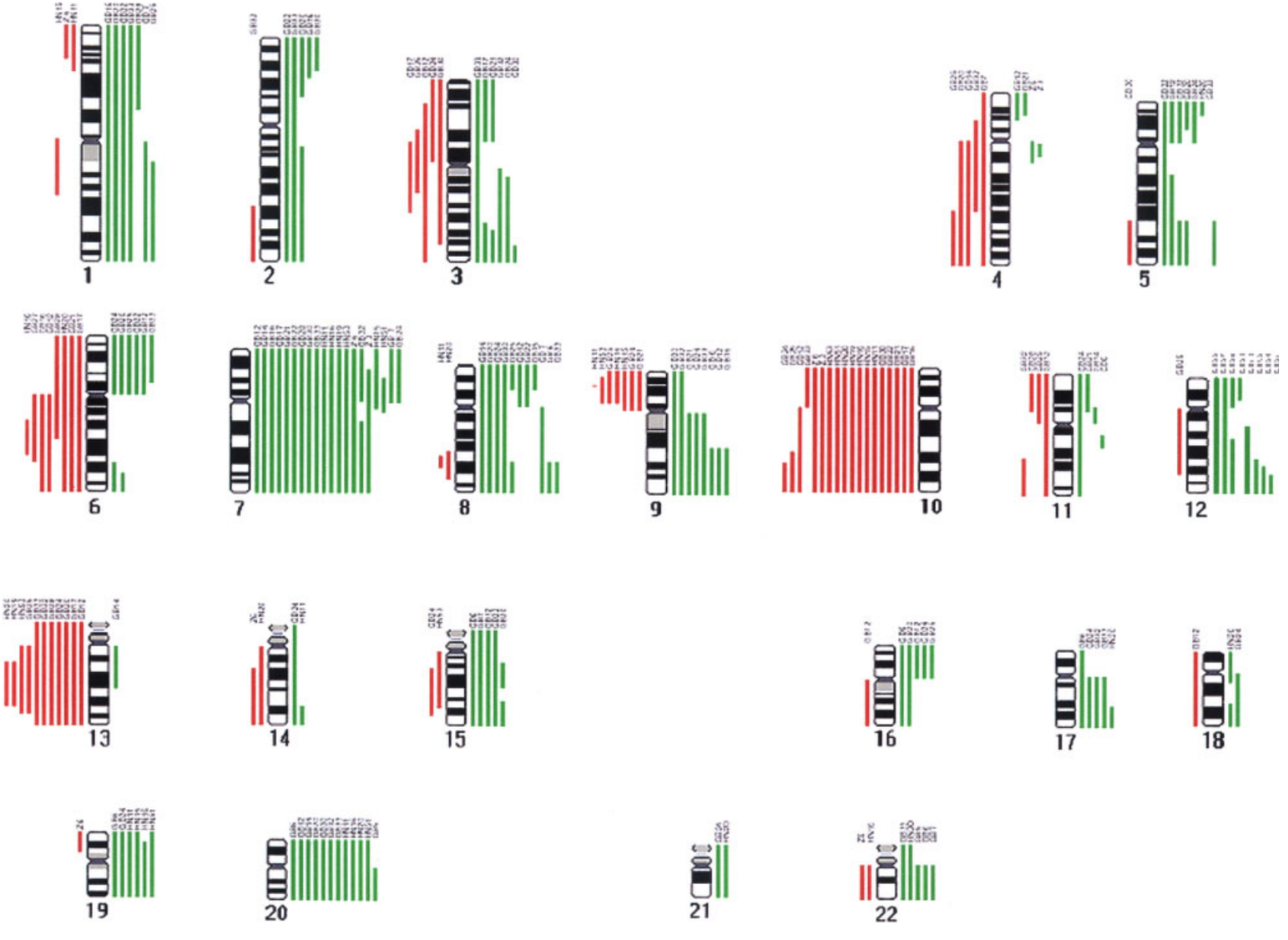

Figure 1. Summary of all DNA copy number changes detected by CGH in 32 glioblastomas. Each line illustrates the affected chromosome region in a single tumor sample. The vertical lines on the right side of the chromosome ideograms indicate gains, and the lines on the left represent losses.

Table 1. Genetic Profiles of 32 Patients With Glioblastoma

	Regions of DNA copy number changes		Number of changes		
No.	Loss	Gain	Losses	Gains	All
GB5		11q13, 20q, 22q	0	3	3
GB6		8q23-qter, 9q22-qter, 15, 16, 17, 19, 20, 22q	0	8	8
GB7	4	1q, 7p, 8q, 15, 22q	1	4	5
GB9			0	0	0
GB10			0	0	0
GB12	3p22-qter, 6q, 10q, 11, 13, 16q, 18	4p14-pter, 5p, 5q14-qter, 6p, 7, 8p, 9q22-qter, 15, 16p, 20	7	9	16
GB14	4q, 21	2p21-pter, 7, 8, 11p11-p12, 13q11-q21, 20	2	6	8
GB15		8p21-pter, 12q13-qter	0	2	2
GB16	6q, 10	1, 2p21-pter, 7, 9q22-qter	2	4	6
GB17	3p13-q23, 6, 10, 13	3p14-pter, 3q25-qter, 4q27-qter, 7	4	4	8
GB19			0	0	0
GB20	4q, 13	1, 2p14-pter, 2q14-qter, 2q35-qter, 5p14-pter, 5q31-qter, 6p, 6q25-qter, 8, 12p, 12q15-qter, 15q14-q21, 15q24-qter, 16, 20	2	14	18
GB21	6, 9p, 10, 13	3p14-pter, 3q26-qter, 4p15-pter, 7, 9q, 11p12-pter, 12q24-qter	4	7	11
GB22	10	2, 5, 7, 8p, 12, 15	1	6	7
GB24	3p11-pter, 9p, 10q24-qter, 13, 15q11-q24	3q12-qter, 5p, 6p, 6q24-qter, 7p, 8, 9q, 11, 12q23-qter, 14, 16p, 17q, 18q, 19, 21	5	11	16
GB25	3p14-q13, 4q27-qter, 9p 13-pter, 10q23-qter, 11p, 12q11-q24, 13q11-q32	1q21-q23, 6p, 8p21-pter, 8q23-qter, 12p12-pter, 16p	7	6	13
GB27	6q11-q23	12	1	1	2
GB28	6p12-q21, 11p13-qter, 13	1p22-pter, 7	3	2	5
GB29			0	0	0
GB30	3pter-q26, 5q31-qter, 10, 21	2p22-pter, 3q27-qter, 5q31-pter, 7, 8, 9, 20	4	5	9
GB32	2q32-qter, 4p14-q27, 11q21-qter, 13	1, 3q, 5p, 5q31-qter, 6p, 7p, 7q21-qter, 9, 12q22-qter, 17q, 20	4	9	13
GB33	10p, 13	1, 2, 3, 5q31-qter, 6p21-pter, 7, 8q23-qter, 9q, 17q, 20, 22	2	11	13
HN11	1p32.2-pter, 8q22.3-q23.2, 9p22, 10	4q24-qter, 7, 14q31-qter, 19, 20	4	5	9
HN15	1q11-q25, 9p, 10	7pter-q11.22, 19,	3	2	5
HN16	6q16-q22, 9p13-pter, 10, 22q	7, 19p 13.2-qter, 20	4	3	7
HN19	10, 13q14-q31	7	2	1	3
HN20	6, 8q22-q24, 9q12-13, 10, 13q, 14q	5p15-pter, 17q23-qter, 18pter-q11, 1821.3-qter, 20, 21, 22	5	6	11
HN26			0	0	0
HN51	10	7p14-q11.22, 20, 19	1	3	4
HN53	9p13-p23, 10, 13q, 15q15-q25	1q21-q23, 7	4	2	6
Z3	10	4q11-q13.1, 7p15-qter	1	1	2
Z6	1p34-pter, 10, 14q21-qter, 19p13-pter, 22q	4q11-q13, 7	5	2	7
Total			50	59	109

Differential PCR Versus CGH to Detect Deletions or Amplifications at Genomic Loci

To compare the very sensitive molecular technique differential PCR [3,15], which aims to show deletion or amplification of genomic material at a particular chromosomal locus, with CGH [27], which shows a panoramic overview of all chromosomal loci, presenting losses and gains in a single experiment, we decided to examine by both methods the 22 GBMs from the General Hospital of Tianjin Medical University. First, tumor DNA was subjected to differential PCR as previously described [3,15]. Cases with homozygous deletions of *CDKN2A*,

PTEN, and *DMBT1* and amplification of CDK4 are shown in Table 2. Then CGH was performed on the same 22 cases. As shown in Table 2, CDKN2A showed homozygous deletions in six of 22 (27%) GBMs. Two of the 22 cases (9%) (GB6 and GB28) were found to contain *p16* promoter hypermethylation when the methylation-specific PCR technique was performed [15]. Hypermethylation of the *CDKN2A* promoter can explain a loss of function of the p16 protein in the absence of deletions of the chromosomal locus. In contrast, in samples GB15, GB17, GB21, GB24, GB30, and GB33, the *CDKN2A* locus was found to be homozygously deleted, which caused an obvious loss of function of p16.

Only two of the six samples (GB21 and GB24) showed chromosomal loss after CGH analysis. Samples GB15, GB17, GB30, and GB33 might have suffered a small deletion in the *CDKN2A* locus not detected by CGH. CGH revealed a loss of 9p13-pter in sample GB25, but concordance could not be established with the molecular study. This sample, in fact, might have harbored loss of heterozygosity (LOH) at the locus *CDKN2A* (shown by CGH but not by differential PCR). It would have been necessary to conduct an LOH study to clarify the genetic lesion, but we did not have blood DNA from the patients, which is required for LOH.

Table 2. Deletions and Amplifications by PCR and CGH

	Differential PCR	CGH
Deletions of *CDKN2A*		
GB21	+	+
GB24	+	+
GB25	−	+
GB15	+	−
GB17	+	−
GB30	+	−
GB33	+	−
GB6-met*	−	−
GB28-met*	−	−
Deletions of *PTEN*		
GB16	+	+
GB30	+	+
GB12	−	+
GB17	−	+
GB21	−	+
GB22	−	+
GB25	−	+
Deletions of *DMBT1*		
GB16	−	+
GB30	−	+
GB12	−	+
GB17	−	+
GB21	−	+
GB22	−	+
GB24	−	+
GB25	−	+
Amplification of *CDK4*		
GB15	+	+
GB27	+	+
GB28	+	−

*met, CDKN2A promoter methylation, as detected by a methylation-specific PCR technique.

Table 2 shows the results for *PTEN*. Two of 22 cases of GBMs (9%)—GB16 and GB30—showed homozygous deletions of *PTEN*, but seven of 22 (32%) GBMs showed deletions by CGH. *DMBT1* results also are presented in Table 2, showing no concordance at all between the molecular and the cytogenetic techniques in eight cases of GBM. It seems from the *PTEN* and *DMBT1* results that 10q was a frequent target of deletions in GBM (eight of 22, or 36%), but the corresponding pattern of homozygous deletions of *PTEN* and/or *DMBT1*, two important tumor-suppressor genes, did not occur. This might indicate that a loss at 10q, detected by CGH and perhaps corresponding to a 10q− cytogenetic event, is more common than homozygous deletion itself. This could mean that the second allele at the PTEN or DMBT1 locus (or both) might undergo a different genetic event to fulfil Knudson's two-hit hypothesis. The second lesion might correspond to point mutations or even promoter hypermethylation of those genes.

Table 2 also shows that only three of 22 cases had amplification of *CDK4* [3] at chromosome 12q. In two cases (GB15, and GB27), there was a corresponding CGH result. After comparison of differential PCR and CGH to detect gains and losses at specific loci, we concluded that losses at the chromosome 9 *CDKN2A* locus corresponded to small interstitial deletions not usually detected by CGH. CGH proved to be a more appropriate technique for the detection of deletions on chromosome 10 than differential PCR, although this might have been due to a higher frequency of LOH versus homozygous deletions at 10q in GBMs.

Gains or Losses at 16 Selected Loci in GBM

Once we had results from the analysis of 32 GBMs by the CGH technique (Table 1 and Figure 1), we studied alterations in 16 different chromosomal loci selected on the basis of their known or supposed role in gliomagenesis. Eleven corresponded to oncogene-like loci (EGFR, PDGFR, myc, CDK4, and such proliferation markers as *PCNA* and the E2F transcription factor family of genes, E2F1–E2F6) and five to tumor-suppressor genelike loci (*PTEN*, *DMBT1*, *CDKN2A*, *TP53*, and *RB*). The selected loci placed at their specific chromosomes and the typical lesion by which they are affected are shown in Figure 2. A summary of gains and losses at those specific loci in the different patients is presented in Table 3.

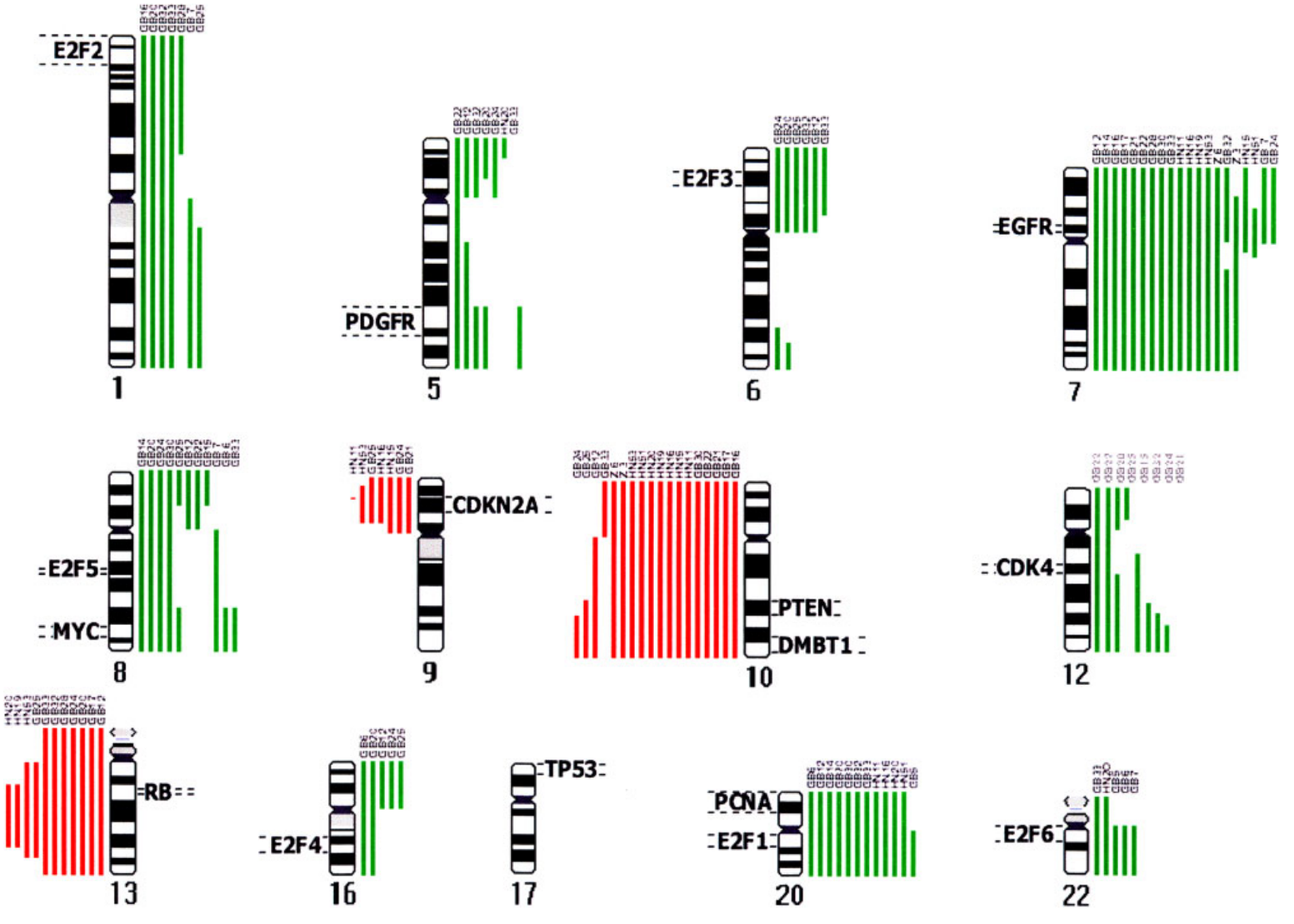

Figure 2. Summary of DNA copy number changes detected by CGH in 32 glioblastomas. Only 16 selected loci from 13 different chromosomes were considered for analysis. Each line illustrates the affected chromosome region in a single tumor sample. The vertical lines on the right side of the chromosome ideograms indicate gains, and the lines on the left represent losses.

Table 3. Gains and Losses Determined in 32 Glioblastoma at 16 Different Loci From 13 Chromosomes*

	E2F2 1p36	*PDGFR* 5q31-q32	*E2F3* 6p22	*EGFR* 7p12	*E2F5* 8q21.13	*myc* 8q24.12-q24.13	*CDKN2A* 9p21	*PTEN* 10q23.3	*DMBT1* 10q25.3-q26.1	*CDK4* 12q14	*RB* 13q14.2	*E2F4* 15q21-q22	*TP53* 17p13.1	*PCNA* 20pter-p12	*E2F1* 20q11.2	*E2F6* 22q11
GB5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	G	G
GB6	—	—	—	—	—	G	—	—	—	—	—	G	G	G	G	G
GB7	—	—	—	G	G	G	—	—	—	—	—	—	—	—	—	G
GB9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GB10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GB12	—	G	G	G	—	—	—	L	L	—	L	L	—	G	G	—
GB14	—	—	—	G	G	G	—	—	—	—	G	—	—	G	G	—
GB16	—	—	—	—	—	—	—	—	—	G	—	—	—	—	—	—
GB16	G	—	—	G	—	—	—	L	L	—	—	—	—	—	—	—
GB17	—	—	L	G	—	—	—	L	L	—	L	—	—	—	—	—
GB19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GB20	G	G	G	—	G	G	—	—	—	—	L	G	—	G	G	—
GB21	—	—	L	G	—	—	L	L	L	—	L	—	—	—	—	—
GB22	—	G	—	G	—	—	—	L	L	G	—	—	—	—	—	—
GB24	—	—	G	G	G	G	L	—	L	—	L	—	—	—	—	—
GB25	—	—	G	—	—	L	L	L	L	L	L	—	—	—	—	—
GB27	—	—	—	—	—	—	—	—	—	G	—	—	—	—	—	—
GB28	G	—	—	G	—	—	—	—	—	—	L	—	—	—	—	—
GB29	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GB30	—	G	—	G	G	G	G	L	L	—	—	—	—	G	G	—
GB32	G	G	G	G	—	—	G	—	—	—	L	—	—	G	G	—
GB33	G	G	G	G	—	G	—	—	—	—	L	—	—	G	G	G
HN11	G	—	—	G	—	—	—	L	L	—	—	—	—	G	G	—
HN15	—	—	—	G	—	—	L	L	L	—	—	—	—	—	—	—
HN16	—	—	—	G	—	—	L	L	L	—	—	—	—	G	G	L
HN19	—	—	—	G	—	—	—	L	L	—	L	—	—	—	—	—
HN20	—	—	L	—	—	L	—	L	L	—	L	—	—	G	G	G
HN26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
HN51	—	—	—	G	—	—	—	L	L	—	—	—	—	G	G	—
HN53	—	—	—	G	—	—	L	L	L	—	L	—	—	—	—	—
Z3	—	—	—	G	—	—	—	L	L	—	—	—	—	—	—	—
Z6	L	—	—	G	—	—	—	L	L	—	—	—	—	—	—	L

*G = gain; L = loss; — = no gain or loss. HN20, secondary glioblastoma. HN26, pilocytic astrocytoma (grade I).

Only gain of chromosome 7p (containing *EGFR*) significantly associated with 10q deletions (containing *PTEN* and/or *DMBT1* among the most frequent targets of deletion; $p = 0.003$). In our study, 75% of cases with a gain of 7p had a simultaneous loss of chromosome 10 and 88% of cases with loss of chromosome 10 had gains at 7p. Other studies showed that EGFR amplification occurs at similar frequencies in GBMs with or without homozygous deletions or mutations of the *PTEN* gene, located on 10q23 [8], although about 20% of primary GBMs show both *EGFR* amplification and *PTEN* mutations [9]. This might imply that other oncogenes on 7p have some kind of regulatory association with suppressor genes on chromosome 10.

In conclusion, our data showed that CGH was a comprehensive and rapid approach for the analysis of global and locus-specific genomic alterations in glial tumors. Therefore, it may serve as an additional tool to define subsets of human GBMs exhibiting different genetic pathways. Nevertheless, owing to its sensitivity, CGH should be combined with other, more sensitive techniques, such as differential PCR, to guarantee detection of small deletions.

ACKNOWLEDGMENTS

We thank Oskar Sáez for his help and advice with the artwork. This research was supported in part by grants from the Departamento de Salud del Gobierno de Navarra, Fondo de Investigación Sanitaria (FIS 99/1265), Fundación Científica de la AECC, and Ministerio de Ciencia y Tecnología (PROFIT), Madrid, Spain. María del Mar Inda and Jorge Muñoz are predoctoral fellows from the Ministerio de Educación, Cultura y Deporte, Madrid, Spain. Xing Fan received funds from the Agencia Española de Cooperación Internacional, and Ministerio de Ciencia y Tecnología, Madrid, Spain.

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