Chromosomal Abnormalities in Glioblastoma Multiforme by Comparative Genomic Hybridization: Correlation with Radiation Treatment Outcome

Stephen L. Huhn, Gayatry Mohapatra, Andrew Bollen, Kathleen Lamborn, Michael D. Prados, and Burt G. Feuerstein

ABSTRACT
Glioblastoma multiforme (GM) is the most common and most malignant astrocytoma in adults. After surgery, radiation therapy extends patient survival; however, in vivo response to radiation therapy is variable. The purpose of this investigation was to determine whether the cytogenetic abnormalities of GM differ according to patient response to radiation therapy. Radiation response was defined by either progression [radiation-resistant (RR)] or resolution [radiation-sensitive (RS)] of tumor at the first postradiation radiographic imaging evaluation. Twenty RR and 10 RS frozen tissue specimens were subjected to cytogenetic analysis by comparative genomic hybridization. RS and RR specimens had different cytogenetic aberrations that mapped predominantly to chromosomes 7, 9, 10, 13, and 19. Relative gain of 7 occurred in 70% of the RR and 30% of the RS cases and was the most significant difference involving a single change between the two groups (P = 0.06). RR and RS specimens also differed in their patterns of simultaneous cytogenetic aberrations. A simultaneous gain of chromosomes 7 and 19 was found in 30% of the RR cases but was absent in the RS group. Concurrent loss of 9p23–24 and 13q14 regions was absent in the RS cohort but occurred in 30% of the RR series. This latter cytogenetic pattern was also associated with older age. Amplifications were more common in the RR series, but the difference did not reach statistical significance. The data suggest that GM with different in vivo responses to radiation therapy also differ cytogenetically.

INTRODUCTION
Cytogenetic aberrations are an established factor in the evaluation of many hematological malignancies and are beginning to emerge as significant in the investigation of solid tumors (1). Recent studies of neuroblastoma have revealed the prognostic importance of NMYC amplifications (2), allelic loss of chromosome 1p (3), and expression of the MDRI gene (4). Gains of chromosome 3q correlates with transition from severe dysplasia to invasive carcinoma of the uterine cervix (5), and loss of 9p is associated with shorter recurrence-free survival in renal cell carcinoma (6). For primary central nervous system tumors, however, the relationships between cytogenetic abnormalities and outcome are uncertain (7–10).

GM (WHO grade IV astrocytoma) is the most common and most malignant astrocytoma (11, 12). With few 5-year survivors, overall prognosis is poor. Brain tumor study group trials have shown that external-beam radiation is the only treatment after surgery that extends survival for patients with GM (13–15). Radiation response in vivo is quite variable; some cases clearly demonstrate improvement, whereas others show definite tumor progression. This variability has independent prognostic value (16). Thus, the spectrum of response to radiation therapy among patients with GM suggests that sensitivity to radiation is an important biological variable. Furthermore, studies of radiation response have suggested that mutations in genes such as TP53 have effects on cellular susceptibility to radiation (17). Thus, identification of genomic regions associated with radiation sensitivity may help to clarify whether genetic mechanisms are associated with patient radiation response and may allow clinicians to determine who will benefit from radiation therapy.

In this study, radiation response (i.e., treatment outcome) is defined as changes in enhancing tumor volume as depicted by MRI and/or CT examination immediately following completion of radiation therapy, and the cytogenetic abnormalities for each tumor genome are determined by CGH. The aim of this investigation was to determine cytogenetic differences between GM specimens with variable radiation treatment outcomes, thereby identifying chromosomal regions involved in radiation response.

CGH measures cytogenetic aberrations by relative gains
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and losses of DNA across the entire tumor genome (18–20). Test (tumor) and reference (normal) DNA labeled with different fluorescent tags are simultaneously hybridized to normal metaphase target chromosomes. The number of copies of tumor DNA is reflected in the ratio of labeled test (tumor) DNA to labeled reference (normal) DNA that hybridize to the target chromosome. Thus, if signal from tumor DNA has a relatively higher intensity than signal from normal DNA, the tumor DNA has a relative gain in copy number compared to normal DNA. Results of CGH analysis in glioma cell lines and tumors have been reported by several investigators (21–23), and alterations in DNA copy number detected by CGH have been confirmed by other methods (24).

Here, we summarized the CGH results from an archival series of adult GM specimens selected specifically by radiation treatment outcome. The cytogenetic aberrations of cases with definitive progression after radiation (i.e., RR) were compared to the aberrations in cases with clear improvement after radiation (i.e., RS). Patient age and BrdUrd labeling index (25) were considered in the analysis.

MATERIALS AND METHODS

Patient Selection. Cases were obtained from the Tissue Bank of the Brain Tumor Research Center at UCSF. There were 207 samples (166 primary and 41 recurrent) of GM collected at the time of surgery at UCSF and stored as frozen tissue specimens from May 8, 1984, through June 8, 1994. Specimen numbers were cross-referenced with patient records maintained by the Neuro-Oncology Service in the UCSF Department of Neurological Surgery. Of the 207 cases, 131 could be readily sorted by radiographic imaging status or score at the time of first postradiation assessment. The image scoring system used by the Neuro-Oncology service for evaluating changes in tumor status depicted by MRI and/or CT has five levels, as follows: +1, >50% reduction in contrast enhancement; +2, between 25% and 50% reduction; 0, between 25% reduction and 25% increase; −1, between 25% and 50% increase; and −2, >50% increase in contrast enhancement. We defined images assigned a +2 value as “sensitive” to intervening therapy because of a reduction in contrast enhancement and images with a −2 value as “resistant” because of an increase in the enhancing volume. The scores of −2 and +2 represented the most divergent changes in MRI/CT contrast enhancing volume and therefore, upon conclusion of radiation therapy, signified the most dramatic changes in tumor activity. From the 131 patients with postradiation evaluations, 28 cases with a score of −2 and 15 cases with a score of +2 were identified. Chart reviews of these patients were conducted, and the following information was recorded: age at diagnosis, sex, date of surgical diagnosis, subtotal or gross total resection, BrdUrd labeling index, dates of radiation therapy, radiation fractionation, total radiation dose (in cGy), use of radiation-sensitizing agents, and the date of the first postradiation imaging examination. Additional clinical data included adjuvant therapy (such as additional surgery, brachytherapy, or chemotherapy), date of tumor progression, salvage therapy, and date of death.

Because resolution of postoperative changes or radiation effects can complicate interpretation of images, whenever possible, the preoperative, postoperative, preradiation, and postradiation scans were reviewed again. Cases felt to have postradiation scan changes secondary to possible postoperative artifact or radiation effects were excluded. Cases were also excluded if a delay of >6 weeks occurred between surgery and the initiation of radiation or if the first postradiation imaging assessment was prolonged to >45 days after the last treatment date. A total of 13 cases were excluded from analysis for reasons noted above, leaving a final sample of 20 cases with −2 tumor progression and 10 cases with +2 tumor response imaging scores immediately following the conclusion of radiation therapy.

Specimen Collection. Tissues from the 30 GM tumors selected were obtained as frozen tissue samples from the Brain Tumor Research Center. Specimens were collected at the time of initial resection and stored in liquid nitrogen at −80°C. Histology slides were prepared from sections adjacent to the tumor specimen used for DNA isolation and reviewed by the Division of Neuropathology to confirm the presence of tumor. Each case was confirmed to contain >50% tumor. One case (RR case 4) contained atypical astrocytes but no distinct neoplastic cells. Because this could represent brain with early tumor infiltration, the specimen was included for analysis (see “Results”).

DNA Extraction. Genomic DNA was extracted and purified by a previously described method (26). Briefly, tumor cells were incubated in a buffer of 0.1 mg/ml proteinase K, 0.5% SDS, and 25 mM EDTA for 12 h at 50°C. DNA was isolated with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated in 100% ethanol-7.5 M ammonium acetate. DNA was quantitated fluorometrically (model TKO 100; Hoefer Scientific Instruments, San Francisco, CA). Reference male and female DNAs were isolated from leukocytes of normal donors.

Metaphase Preparation. Target metaphase spreads were prepared on glass slides from phytohemagglutinin-stimulated peripheral blood lymphocytes from a normal donor using conventional procedures.

DNA Labeling. Tumor DNA and normal DNA were labeled by nick translation with fluorescein-2-dUTP and Texas Red-5-dUTP (DuPont, Wilmington, DE) respectively for the “forward” hybridization (Bionick Labeling Kit; Life Technologies, Inc., Bethesda, MD). DNase I concentration was altered (from 0.0075 to 0.0225 units/µg DNA) to optimize double-stranded probe fragment sizes from 200 to 2000 bp. The “reverse” hybridization was performed with tumor DNA labeled in Texas Red-5-dUTP and reference DNA labeled in fluorescein-2-dUTP.

CGH. Target metaphase chromosomes were denatured in 70% formamide and 2× SSC (0.3 M NaCl-0.03 M sodium citrate, pH 7) for 5 min at 75°C; dehydrated in a succession of 70, 80, 90, and 100% ethanol; and air-dried at 37°C on a slide warmer. Two hundred ng each of tumor and sex-matched reference DNA, along with 20 ng of human Cot-1 DNA (Life Technologies, Inc.), were precipitated in ethanol. The DNA was resuspended in 50% formamide, 10% dextran sulfate, and 2× SSC; denatured for 5 min at 75°C; and hybridized to the target metaphase chromosomes for 48 h at 37°C.

Slides were washed at 45°C three times in 50% formamide-2× SSC (pH 7), twice in 2× SSC; and once in 0.1× SSC for 10 min each. Additional washings were conducted at room temperature in 4× SSC, 4× SSC with 1% Triton, 4× SSC, and 4× SSC with 50% formamide-2× SSC (pH 7), twice in 2× SSC; and once in 0.1× SSC for 10 min each. Additional washings were conducted at room temperature in 4× SSC, 4× SSC with 1% Triton, 4× SSC, and
phosphate buffer for 10 min each. Slides were counterstained with 0.2 mM 4,6-diamino-2-phenylindole in an antifade solution and kept refrigerated until microscopic viewing.

**Image Analysis.** A Zeiss fluorescence microscope equipped with a double band pass filter and a ×63 objective (Chroma Technology, Brattleboro, VT) was used to visualize the chromosomes and probe signals. The microscope was attached to a Photometrics CH25D camera (Photometrics Ltd., Tucson, AZ) and linked to a SUN 4/330 work station (SUN Microsystems, Inc., Mountain View, CA). Red, green, and blue fluorescence images were acquired and analyzed from individual metaphase spreads using this Quantitative Image Processing System and SCIL-image software program cghprofstats (27). In each case, at least eight metaphase spreads were acquired, and four were analyzed. Chromosomes with inadequate hybridization were excluded from analysis. 4,6-Diamino-2-phenylindole-banded images were used to identify individual chromosomes.

The software produced an image of relative DNA copy number for each chromosome and displayed the mean and SDs of the normalized fluorescence intensity ratios. Normalized ratios of 1.0 signified no relative gains or losses in tumor DNA. In the forward hybridization, a ratio of >1.0 indicated a relative increase in tumor DNA copy number because more tumor DNA hybridized at the locus compared to normal reference DNA. A ratio of <1.0 indicated a decrease in tumor DNA copy number. Amplifications were scored by inspection when a relative gain was confined to a small subchromosomal region. CNAs were scored if ratio values exceeded 2.67 SDs evaluated in 20 normal-normal hybridizations. The average SD was 0.075 for the 20 normal-normal hybridizations. The absolute cutoffs were ratios of 1.2 and 0.8. Each CNA was also confirmed by visual inspection. Blocking repeat sequences with Cot-1 DNA prevented interpretation of ratio changes at centromeres and heterochromatic regions. Hybridizations with excessive SDs were repeated, and questionable changes in forward hybridizations were confirmed by testing with the reverse hybridizations.

**Statistical Analysis.** Differences in frequency of abnormalities between RR and RS patients were tested using Fisher’s exact test. Tests for differences in patient groups with respect to BrdUrd labeling index and age were based on the Wilcoxon rank sum test. Differences in survival between groups were tested using the log-rank test.

**RESULTS**

**Patient Data**

A total of 20 patients with postradiation tumor progression (−2) and 10 patients with tumor response (+2) were selected for study. The cohort of patients with tumor progression were called RR, and the cohort of patients who responded to radiation were called RS. Table 1 lists the clinical characteristics of the 30 patients. All patients underwent primary surgery at UCSF between July 12, 1988, and August 12, 1994. The majority had
subtotal surgical resections; one patient in each group had a gross total resection. No patients were classified as having biopsy only. The postoperative scan, which served as the preradiation baseline study, was available for review in all 30 cases. Twenty-eight of 30 postradiation scans were reviewed; the remaining two cases (one in each group) had detailed chart notes regarding the postradiation study by one of the investigators (M. D. P.). Patients were evaluated by the Neuro-Oncology service after surgery, and 90% (9 of 10) of the RS group and 65% (13 of 20) of the RR group entered institutional protocols. The postoperative scan was obtained within 3 days after surgery in 80% (24 of 30) of cases and within 4 days in 97% (29 of 30) of cases. Scans obtained beyond 3–4 days after surgery may reflect enhancement secondary to granulation changes rather than actual tumor. In both groups, 90% of patients started radiation therapy within 30 days after surgery. The remaining patients started treatment on postoperative days 31, 32, and 44. Total radiation dose delivered at single fractions per day varied between 5800 and 6100 cGy for 18 of 20 in the RR group and 7 of 10 in the RS group. Two patients in the resistant group and three patients in the sensitive group had hyperfractionated radiation therapy (total doses of 7040–7240 cGy). Radiation sensitizers, α-difluoromethylornithine, and/or hydroxyurea were given per protocol to 60% (12 of 20) of patients in the RR group and 70% (7 of 10) of the RS group. Posttreatment MRI or CT examinations were performed within 30 days of completing radiation in 90% (27 of 30) of cases, and most (66%) were performed within 7 days. MRI was used for both the pre- and postradiation scans in 70% of patients. Thus, RR and RS groups were similar with regard to when they began and completed radiation therapy, their radiation dose, when they were assessed for radiation response, and the proportion that received hyperfractionation, radiation sensitizers, α-difluoromethylornithine, and hydroxyurea. Thus, these factors are unlikely to account for genetic differences we may find in RS and RR groups.

Table 2 illustrates the median age, BrdUrd labeling index, and survival for the two patient groups. BrdUrd labeling index was calculated in all cases, except for RR case 20. There was no significant difference in the median age of the RR group (47 years) when compared to the RS group (45 years). Likewise, no significant difference was found between the median BrdUrd labeling index for the RR group (7.4%) and the RS group (6.3%). Only one patient (case 15) in the RR group was lost to follow-up at 16 months. No statistically significant difference in median survival was found between the two groups, although a trend for longer survival was noted in the RS group as compared to the RR group (462 versus 384 days, respectively).

CGH Analysis

**RR Cases.** Forward CGH was performed in all 20 cases and repeated in 10 cases. Four cases were confirmed by reverse CGH. CNAs were found in 19 of the 20 cases. One case (RR case 1) had no CNAs, despite having histologically confirmed tumor present in the sample and being analyzed by repeat forward and reverse CGH. In contrast, histological review of another case (RR case 4) revealed no definitive tumor, but upon CGH analysis, gain of 3q26.3–29 and amplification of 12q13.2–15 were demonstrated.

Table 3 outlines the CNAs for each case of the RR group. Losses occurred as both whole and partial chromosomal deletions. The most common losses of whole chromosomes (“whole losses”) were of chromosomes 10 (65%), 13 (35%), 14 (15%), 15 (15%), 9 (10%), 22 (10%), and Y (10%). The most frequent subchromosomal deletions (“partial losses”) involved 9p (50%) and 13q (25%). The 9p deletions varied in length, and the most commonly lost region was 9p21. When both whole and partial losses are considered, the 9p21 band was lost in 60% of cases. Likewise, 13q had interstitial deletions that were clustered around a common region covering bands 14.1–21.1. This region was lost in 60% (12/20) of RR cases when both the partial and whole chromosomal losses are combined. Less common partial deletions were observed on 3q, 4q, 5q, 8q, 10q, 14q, and 18q.

Gain of chromosome 7 was the most frequent whole chromosome gained in the RR series. It was observed in 70% (14 of 20) of cases. Two cases had partial gains of 7p and 7q. There were frequent whole gains of chromosomes 19 (35% of cases), 20 (20%), and 17 (15%). There were less frequent whole gains of chromosomes 1 (1 of 20), 4 (1 of 20), 12 (1 of 20), 16 (1 of 20), and X (1 of 20). Partial gains were less common and involved 1p, 3q, 6p, 19p, and 20q. Gains at band 12p13 were seen in three cases. Amplifications in the RR series were seen on 1p, 1q, 2q, 7p, 7q, 12p, and 12q. The two most common amplifications were at 4q12 (three cases) and 7p11.2–13 (three cases).

**RS Cases.** Table 4 lists the CNAs for the RS cases. All 10 cases had CNAs detected by CGH.

Whole chromosome losses occurred for chromosomes 10 (four cases), 22 (three cases), and 13 (two cases). Whole loss of chromosomes 14, 15, and Y each occurred once. The most common partial losses were on 9p, 10q, and 13q. As in the RR series, the 9p deletions commonly encompassed the 9p21 band (six cases). The 10q deletions were clustered toward the q26 band in 50% of the RS series. The losses seen on 13q most frequently involved the q32 band (five cases).

Whole gains most commonly involved chromosome 7 (30% of cases), 17 (20%), 19 (20%), and 20 (20%). Gains of chromosomes 15, 16, and Y were each seen once. Partial gains occurred on 1q, 3p, 3q, 12p, 12q, 19p, and 19q. Amplifications were restricted almost without exception to chromosome 7. In the RS series, amplifications on 7p, 7q, and 7qter were found in four cases. The only other amplification in the RS series occurred on 17q.

**RR/RS Comparisons.** The CNAs found in the 20 RR and 10 RS samples are illustrated by chromosome in Figs. 1 and 2, respectively. The majority of shared abnormalities map to chromosomes 7, 9, 10, 13, and 19.
There were both similarities and differences between whole chromosomal gains and losses in the RR and RS specimens. There was no significant difference between the frequencies of whole losses of chromosomes 10 or 13 or frequencies of whole gains of chromosome 19 in the two cohorts, although in each case, the frequency of the whole chromosome aberration was higher in the RR cohort. The frequency of whole gain of chromosome 7 differed in the RR and RS cohorts. Whole gains of 7 were identified in 14 of 20 cases (70%) in the RR group and in 3 of 10 in the RS group (30%). Although whole chromosome changes occurred more often in the RR group, the higher frequency of chromosome 7 gain was the only whole chromosome aberration that approached statistical significance (P = 0.06).

Frequencies of simultaneous CNAs at separate chromosome locations in single cases differed in the two patient groups (Table 5). Whole gain of 7 together with whole gain of 19 occurred in six cases in the RR group, but it did not occur in the RS cohort (P = 0.07). Furthermore, simultaneous partial gains on 7q and 19p occurred in 50% (10 of 20) of the RR but in only 10% (1 of 10) of the RS cases (P = 0.05). There were also simultaneous copy number losses at 9p23–24 and 13q14. This pattern was identified in 30% (6 of 20) of the RR series but was absent among the RS cases (P = 0.07). This simultaneous loss at 9p23–24 and 13q14 was also associated with older age. Patients with both CNAs had a median age of 61 years, whereas those without it had a median age of 43 years. Finally, whole gains of 7 and whole loss of 10 occurred in 9 of 20 (45%) of the RR cases but only 1 of 10 (10%) of the RS cases. Although the combination of gain of 7 and loss of 10 occurred more frequently in the RR group, this difference was not statistically significant.

Amplifications were present in both cohorts, but their po-

Table 3 Summary of chromosomal regions with changes in DNA sequence copy number by CGH: RR cases

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Regional change</th>
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<tr>
<td>1</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>−5p, (5)(q13–31), (5)(q34–35), −6q, +7, −9, −10, +19p, +20, −22</td>
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<tr>
<td>3</td>
<td>+7, + (7)(p11.2–13), −9, −10, −13, +20</td>
</tr>
<tr>
<td>4</td>
<td>+ (3)(q23–29), + (12)(q13.2–15)</td>
</tr>
<tr>
<td>7</td>
<td>+4, +5p, +7, +12, + (12)(p13), +X, −Y</td>
</tr>
<tr>
<td>8</td>
<td>+7, −8, −(9)(p21–pter), −10, −13, +19, +20</td>
</tr>
<tr>
<td>9</td>
<td>+7, −(8)(q22–23.2), −(9)(p21–22), + (12)(pter–q22), −(13)(q14–21.1), +19</td>
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<tr>
<td>10</td>
<td>+7, +8, −10, −15, −Y</td>
</tr>
<tr>
<td>12</td>
<td>+ (1)(p35–36.2), −6, +7, −(8)(q21–23), −(9)(p21–22), −10, −13, −14, + (15)(q22–qter), −16, +17, +19, +20q</td>
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<tr>
<td>13</td>
<td>+ + (1)(q32), + + (4)(q12), −(6)(p11.2–12), +7, −(9)(p21–pter), −(13)(q14-32), +19</td>
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<td>14</td>
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<tr>
<td>16</td>
<td>+ (4)(q12), +7, −10, −(13)(q14–31), +17, +19p</td>
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<td>17</td>
<td>+7, −10, −15, +19</td>
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<td>18</td>
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<tr>
<td>19</td>
<td>+7, − (9)(p21–pter), −10, −14, − (22)(q12–qter)</td>
</tr>
<tr>
<td>20</td>
<td>−(1)(p36), + (3)(q21–qter), +6p, −6q, −9p, −10, −13, −14, −(17)(q23–qter), −22</td>
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</tbody>
</table>

Table 4 Summary of chromosomal regions with changes in DNA sequence copy number by CGH: RS cases

<table>
<thead>
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<th>Case no.</th>
<th>Regional change</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>+7, + (7)(p11.2–12), − (9)(p21–22), −10, −(11)(q23.3–24), + (12)(p13)</td>
</tr>
<tr>
<td>3</td>
<td>−6q, −7q, + (7)(p11.2–13), + (7)(q11.2), − (9)(p21–22), −10, +20</td>
</tr>
<tr>
<td>5</td>
<td>−1p, +1q, +3p, −4, −9, +15, +16, +17, +19p, −19q</td>
</tr>
<tr>
<td>6</td>
<td>+7, −(9)(p21–22), − (10)(p–q21), − (10)(q23–qter), −18, −22, +Y</td>
</tr>
<tr>
<td>8</td>
<td>+ (7)(p11.2–12), − (9)(p21–pter), − (10)(q23–qter), +19, +20p</td>
</tr>
<tr>
<td>9</td>
<td>+ (3)(q13.2–23), +7, + (7)(q34.36), − (10)(p–q11.2), − (10)(q21.2–25), −13</td>
</tr>
<tr>
<td>10</td>
<td>− (3)(p13–26), −6q, + (7)(pter–q22), −8, − (9)(p21–22), −10, −13, −14, −15, +19, +20, + (21)(q22), −22, −Y</td>
</tr>
</tbody>
</table>
sitions differed in the RR and RS tissues. Of the eight amplifications seen in the RS series, only one occurred outside chromosome 7. In contrast, of the 12 amplifications in the RR series, 6 were found on chromosome 7, but 6 were found on chromosomes 1, 4, and 12. This trend indicates that amplifications occur more commonly at sites outside chromosome 7 in patients with RR clinical courses.

No association was demonstrated in this series between the CGH results and either BrdUrd labeling index or survival. The only CNAs associated with age was the concurrent loss of 9p23–24 and 13q14, as described above.

DISCUSSION

This investigation shows that GM specimens from RR and RS cohorts are cytogenetically different. Radiation response was defined in this study as a change in volume of contrast enhancement by MRI/CT following completion of radiation and prior to any other intervention. Factors that influence response to radiation, such as tumor hypoxia and intercellular contact (28), obviously could not be controlled in this investigation. In addition, radiation sequelae cannot always be reliably separated from tumor growth, although suspect cases were excluded in this study. Because a large number of chromosomal aberrations were compared statistically, there is a risk that the differences between the two cohorts could reflect the number of observations tested. Despite these considerations, differences between the RR and RS groups centered on chromosomes 7, 9p, and 13q, each of which have been implicated in the pathogenesis of astrocytomas (29–33).

Our investigation failed to uncover associations between cytogenetic abnormalities detected by CGH and proliferative potential (BrdUrd labeling index) or survival. Although the RR cohort had a shorter median survival (384 versus 462 days), the difference was not statistically significant ($P > 0.10$). Similarly, the RR cohort had a greater median BrdUrd labeling index (7.4 versus 6.3%), but this was not significant. Other investigators, using CGH, have failed to link DNA copy number changes with time to radiographic tumor progression in a series of 20 patients with GM (34). In that report, the chromosomal alterations had no association with either tumor progression or overall survival. Questions regarding associations between cytogenetic abnormalities and proliferative potential or attempts to link a cytogenetic pattern with survival will require larger sample sizes without skewed patient selection criteria.

Gains of chromosome 7 represented the most striking difference between the RR and RS specimens. In this study, whole gains of chromosome 7 occurred more frequently in the RR cohort when compared to the RS group (70 versus 30%; $P =
Simultaneous whole gains of 7 and 19 were also more common in the RR series ($P = 0.07$), and further analysis revealed an even higher frequency of concurrent 7q and 19p gains specifically in the RR group ($P = 0.05$). In general, the significance of extra copies of chromosome 7 in astrocytomas remains uncertain. Although trisomy 7 is the most frequent cytogenetic abnormality in astrocytomas (30, 32, 35, 36), gains of 7 have been reported in other human tumors and in short-term cell cultures of nonneoplastic brain tissue (37, 38). In contrast, polysomy of chromosome 7 is less frequent in low-grade than in high-grade astrocytomas (39), and some have proposed that trisomy 7 is a marker for neoplasia (7). That whole gains of chromosome 7 occurred more frequently in the RR cohort is intriguing and suggests that gains of genetic material on chromosome 7 affects in vivo radiation response.

A second pattern of CNAs that was unique to the RR cohort was the simultaneous loss of 9p23 and 13q14. This combination occurred exclusively in RR cases ($P = 0.05$). In addition, this specific CNA pattern correlated with older age. The significance of the concurrent loss of 9p23–24 and 13q14 in the RR group and its relationship to older age is unknown. But it is interesting that the $Rb$ susceptibility gene located at 13q14 has been proposed to play a role in the malignant progression of astrocytomas (40, 41). Loss of chromosome 13, which incorporated the 13q14 region, occurred more commonly in the RR group, but the difference was not significant. Likewise, chromosome 9p loss is well recognized in astrocytomas (29, 30, 32, 35). However, current focus is on the 9p21–22 region and sites of multiple candidate tumor suppressor genes, including $CDKN2A$ and $CDKN2B$ (42–46). One group has reported an inverse relationship between $CDKN2A$ and $Rb$ alterations in glioblastomas (47). Thus, the more telomeric loss of 9p23–24 suggests another 9p locus of interest that, in association with 13q14 loss, may be involved in radiation response.

This study suggests that GM with different responses to radiation differ cytogenetically and that associations exist between chromosomal alterations and in vivo radiation response. In this study, CNAs were always more frequent in the group

<table>
<thead>
<tr>
<th>Chromosome abnormality</th>
<th>RR group (no. of cases/ % of total)</th>
<th>RS group (no. of cases/ % of total)</th>
<th>$P$</th>
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<tbody>
<tr>
<td>7q gain and 19p gain</td>
<td>10/50</td>
<td>1/10</td>
<td>0.05</td>
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<tr>
<td>Whole gain of 7</td>
<td>14/70</td>
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</tr>
<tr>
<td>Whole gains of 7 and 19</td>
<td>6/30</td>
<td>0/0</td>
<td>0.07</td>
</tr>
<tr>
<td>9p23–24 loss and 13q14 loss</td>
<td>6/30</td>
<td>0/0</td>
<td>0.07</td>
</tr>
</tbody>
</table>

* Fisher’s exact test, two-tailed $P$. 

Fig. 2 CNAs for RS cases. Regional losses and gains are drawn adjacent to the normal metaphase chromosome. Lines signify extent of loss or gain in single cases; numbers indicate the numbers of cases with that specific abnormality. Losses are represented to the left of the chromosome, and gains are shown to the right. Thick lines, regions of amplifications.
with radiation treatment failure. The data indicate that CNAs occur more commonly in the RR cohort, although the small sample size and large number of cytogenetic associations analyzed reduce the significance of this association. The meaning of the specific genetic loci involved and the higher frequency of CNAs in the RR series is uncertain. One possible interpretation is that more frequent CNAs in the RR population reflect a greater degree of genetic instability. It is possible that GMs unaffected by radiation are genetically less stable and are, therefore, able to produce a phenotype that can withstand radiation therapy. Although a unique genetic locus corresponding to radiation response was not identified in this pilot study, the results do suggest an association between radiation response and particular CNAs and justify further investigation of radiation response (treatment outcome) and genetic abnormalities in GM.

ACKNOWLEDGMENTS

We thank the Tissue Bank at the Brain Tumor Research Center, University of California, San Francisco, for their contribution.

REFERENCES


Chromosomal Abnormalities in Glioblastoma Multiforme by Comparative Genomic Hybridization: Correlation with Radiation Treatment Outcome

Stephen L. Huhn, Gayatry Mohapatra, Andrew Bollen, et al.


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