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Detection of Gains and Losses in 18 Meningiomas by Comparative Genomic Hybridization

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ABSTRACT: *Comparative genomic hybridization (CGH) was used to examine gains and losses in 18 meningioma tumors that had been previously analyzed for loss of heterozygosity (LOH) at 22q12. Partial or complete losses were seen by CGH in only 9 of 18 cases on chromosome 22. This compares with 11 of 18 losses of single or more loci by LOH. The discrepancy in these results is probably explained by the increased sensitivity of LOH by using microsatellite markers that are able to detect small deletions, whereas losses on the order of 10–15 megabases are required for confident identification by CGH. There was no consistent pattern of gains or losses by CGH, including those tumors that lacked LOH at 22q12. In one tumor of interest in which CGH and LOH studies failed to demonstrate loss on chromosome 22, CGH identified an area of amplification at 17q22-23.* © *Elsevier Science Inc., 1998*

INTRODUCTION

Meningiomas are frequently occurring primary tumors of the central nervous system and account for 13–19% of all primary adult brain tumors. They arise from the arachnoid layer of the meninges and, although most are benign, some have anaplastic or malignant features [1, 2]. The NF2 gene, which maps to 22q12 [3, 4], acts as a tumor suppressor gene involved in the etiology of meningiomas. Partial or complete loss of chromosome 22 is found in only 50–70% of cases [5–8]. LOH and mutational studies using markers around the NF2 region have also confirmed losses in only 60% of cases [6–11]. Other nonrandom cytogenetic abnormalities reported include complete or partial losses in chromosomes 1, 6, 11, 13, 14, 18, 19, X, and Y and may be involved in tumor etiology or progression [2, 12–14]. In addition, putative tumor suppressor genes have been implicated on chromosomes 1, 9, 10, 14, and 17 by LOH studies [12–14]. There has been no reported case of gene amplification in meningiomas. Other genes may therefore be involved in the etiology of the 40% of meningioma with either normal karyotype or no LOH on chromosome 22 or no mutations of the NF2 gene.

In this study, we applied CGH [15] to detect chromosomal imbalance across the entire genome in 18 meningiomas that had been previously examined by LOH and mutation analysis of the NF2 region [7].

MATERIALS AND METHODS

Nonfamilial meningioma tumor tissues were obtained from 18 patients and frozen at the time of operation. None of the tumors demonstrated histological malignant features. A paired blood sample was drawn from each patient and used as a control. High-molecular-weight DNA was extracted from the tumor and from the patient's lymphocytes by using standard methods.

Degenerate Oligonucleotide Primed Polymerase Chain Reaction (DOP-PCR)

DOP-PCR was used to uniformly amplify the DNA as previously described [16] by using the primer 6MW (5′CCGACTCGAGNNNNNNATGTGG3′). Probes were generated in a secondary labeling DOP-PCR reaction by incorporation of rhodamine-4-dUTP or fluorescein-11-dUTP (Amersham).

Metaphase Preparations

Phytohemagglutinin-stimulated male whole blood was cultured in medium for high-resolution synchronized metaphase spread production by using standard published protocols. Fresh slides were used for each CGH experiment. Each slide was inspected for the presence of long

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chromosomes that were evenly spread and had at least 10 good metaphases. The slides were fixed and dehydrated by using standard protocols. They were then denatured for 3 minutes in 70% formamide/2 × SSC at 70°C, fixed in ice-cold ethanol for 2 minutes, treated with proteinase K for 7.5 minutes, and dehydrated by using an ethanol series.

Hybridization

Cot-1 DNA (10 μg) was added to 30 μL of each of the PCR labeling reactions. The mixture was ethanol precipitated and the pellet dissolved in 25 μL of hybridization buffer (5.5 mL formamide, 1 g dextran sulfate, 0.5 mL 20 × SSC). Equal aliquots (10 μL) of the rhodamine probe were mixed with its paired fluorescein-labeled probe, denatured at 75°C for 10 minutes, applied to the metaphase spread, and covered with a glass cover slip. Slides were hybridized for 3 days at 37°C.

Washing

Slides were washed twice in 50% formamide/2 × SSC for 5 minutes and twice in 2 × SSC for 5 minutes at 42°C. The metaphases were counterstained with and mounted in Citifluor AF1 antifade.

Image Acquisition and Analysis

Good metaphases were identified on the basis of length, evenness of hybridization, and adequate spreading of the chromosomes. The metaphases were viewed by using a Zeiss Axioskop fluorescent microscope with excitation filters for DAPI. Rhodamine and FITC were mounted in a motorized filter wheel, by using a triple band-pass dichroic mirror block (Chroma Technology). Digital fluorescence images were acquired by using a cooled charged couple device camera (KAF1400, Photometrics, Tucson, AZ, USA) with a resolution of 1317 × 1035 pixels.

Computer processing and analysis were performed by using three software packages: IPLab Spectrum (Signal Analytics Corporation), Smart Capture (Digital Scientific), and Quips XL (Vysis). Each chromosome was identified on the bassi of enhanced DAPI banding. The fluorescent intensity along each chromosome was measured for both red and green probes.

The LOH results for 22q12 were used to determine the thresholds for gains and losses. The loss threshold was set as the minimum value required to corroborate the LOH results on the majority of the tumors. The gain threshold was set as the inverse of this. Thresholds were therefore set at 1.11 for gains and 0.9 for losses.

Color-Reversal Experiments

These experiments were done to minimize bias produced by differential labeling or hybridization of a particular fluorochrome. Such bias has, for example, been reported in GC-rich regions of the genome [17]. In the first set of experiments, tumor DNA was labeled green and normal DNA red. A second set of identical experiments were performed in parallel with reversal of the colors; that is, the tumor DNA was labeled red and normal DNA green. Equal numbers of metaphases (range 3–6) per experimental condition for each tumor were combined for analysis.

Mean Fluorescent Ratio Calculations

Conventionally, CGH analysis results have been based on an arithmetic mean of the ratio profiles obtained from individual chromosomes. Here we have taken the geometric means of the ratio profiles because they afford a more accurate measure of the mean when labeling artifacts are present and color-reversed data are combined. For example, consider a region where there is no gain or loss but a labeling artifact causes a ratio other than 1.0. In a forward (green:red) experiment, the ratio measured is, say, r. In the reverse experiment (red:green), the same bias will produce a ratio of $1/r$. The arithmetic mean of r and $1/r$ is not 1.0 unless $r = 1.0$. However, the geometric mean of r and $1/r$ is always 1.0. Taking the geometric mean is equivalent to taking the arithmetic mean of the logarithm of the ratios. For this method to satisfactorily compensate for bias, approximately equal numbers of chromosomes should be measured with forward and reverse hybridization, as we have done here.

The $\log_e$ (tumor fluorescent intensity/control fluorescent intensity ratios) = $\log_e$ (tf/cf) were calculated for each pair of experiments (tumor green vs. normal red and tumor red vs. normal green) for each tumor. A graph of $\exp\{\log_e (tf/nf)\}$ is calculated and plotted along each chromosome for each tumor by a modified version of the Quips XL software.

RESULTS

Complete losses were seen for 9 of 18 cases on chromosome 22, all of which were concordant with the LOH studies (Fig. 1). Discordant results in which CGH failed to confirm losses by LOH were seen in only two tumors (cases 5 and 11).

Other losses for two or more cases included loss of 1p36, 4q13–22, 6q11–22, 6q27, 8q11–21, 13q22–24, 17p12–13, 18q, 19p13, 22q12, Xp22.1–22.3, Xq11–13,

Figure 1 Summary ideogram of losses and gains for 18 meningiomas. Comparative genomic hybridization data for 18 meningiomas are plotted in ideogram form. The 22 autosomal chromosomes and the 2 sex chromosomes are represented by ideograms showing G-banding. To minimize bias produced by differential labeling or hybridization of a particular fluorochrome, tumor was labeled green and normal red in one set of experiments and, in a second set of identical experiments performed in parallel, the tumor was labeled red and normal green. Equal numbers of metaphases (range 3–6) per experimental condition for each tumor were combined for analysis. Each region of DNA copy number is represented by a thin solid line parallel to the chromosomal region where it occurs, as judged by computerized tumor:control fluorescent ratios. Each copy number increase is represented by a line to the right and losses by a line to the left of the chromosome, and case numbers are indicated. The legend below the lines represents results of previously published LOH of 22q12 and mutational studies of the NF2 gene [7].

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LOH22q
LOH22q, Mut.

Xq26–27, and Y. In two cases (1 and 15), loss of 22 appeared to be the sole abnormality. Gains in two or more cases include 1p36, 3q, 12q11–21, 17q22–23, and 19p (Fig. 1).

In those tumors with no LOH or mutation of NF2 or loss of 22, there were no consistent patterns of gains or losses (losses for two or more cases included 1p36, 17p12–13, and 19p13; gains for two or more cases were 12q11–21 and 17q22–23).

In one tumor (case 3) in which CGH and LOH studies failed to demonstrate loss on chromosome 22, with CGH an area of possible amplification was seen of 17q22–23 (Fig. 2).

DISCUSSION

The etiology of meningioma exemplifies the paradigm for Knudson's two-hit hypothesis [18]. The initial event in the majority of cases is a mutation of the NF2 gene followed by loss of the other allele either by complete or partial loss of chromosome 22 [6, 8, 19–21]. The NF2 gene maps to 22q12 [22–24] and has been recently cloned. The gene product is merlin (moesin-ezrin-radixin-like protein), a 590 amino acid protein [3, 4] that shares 45–47% identity with the cytoskeleton-associated proteins from which its name is derived. The weight of evidence therefore supports the hypothesis that the majority of meningiomas arise from the inactivation of both copies of NF2.

Some meningiomas display LOH on 22q but have an intact NF2 gene, raising the possibility of other tumor suppressor genes on 22q that may play a role in the etiology of these and other CNS tumors [25]. Recent molecular studies have tentatively mapped another candidate meningioma tumor suppressor gene between MEFH and EWS on 22q in close proximity to but seemingly distinct from the NF2 gene [8, 26–28]. In addition, multipoint linkage analysis in a case of familial meningioma supported the existence of a familial meningioma locus distinct from NF2 locus [29]. Deletions in chromosomes other than 22, such as 1p, 10, and 14 are thought to lead to aggressive behavior or disease progression [13, 14, 30–32]. Approximately 30–40% of meningiomas have no evidence of loss of 22 or 22q or LOH of 22q12, arguing for the existence of other genes outside chromosome 22 involved in the etiology of these tumors. In an attempt to search for these genes, we used CGH [15] to investigate a group of benign meningiomas that already had extensive LOH and mutational analysis of NF2 region.

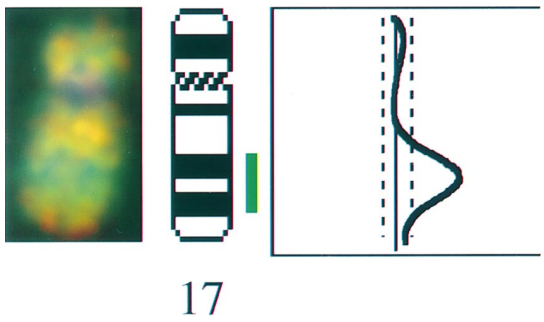

Figure 2 CGH data of chromosome 17 for case 3. An example color image is shown with a chromosome 17 ideogram. Five metaphases were analyzed in which tumor DNA was labeled green and normal DNA red, and five metaphases were analyzed in a second set of identical experiments performed in parallel where the tumor DNA was labeled red and normal DNA green. The results were combined, giving a total of 20 chromosomes analyzed. A possible area of amplification is seen on 17q22–23.

We have shown that meningioma is a remarkably "silent" tumor with very few areas of gains or losses identified by CGH. This finding is in keeping with its benign clinical behavior. We have shown partial or complete losses for 9 of 18 cases on chromosome 22 of which all 9 cases were concordant with LOH studies. Discordant results in which CGH failed to confirm losses seen by LOH (cases 5 and 11) could be due to the increased sensitivity of LOH with the use of microsatellite markers that will detect losses of regions smaller than the minimum size detected by CGH, which is on the order of 10–15 megabases.

In those tumors that did not show loss of 22 by CGH, there was no consistent pattern of losses or gains. In two cases (1 and 15), loss of 22 appeared to be the sole abnormality. In one tumor of interest (case 3) in which both CGH and LOH did not show loss on chromosome 22, an area of amplification was seen at 17q22–23 (Fig. 2). To date, there have been no reported areas of amplification in meningiomas. Although this result was observed in only a single case, it would be interesting to study a group of malignant meningiomas to see if this is a recurrent finding perhaps associated with disease progression. Interestingly, this area has been shown to be amplified in breast cancers and therefore could be a site of a putative oncogene [32–36].

Cancer develops as a result of the genetic instability of any of a number of important regulatory genes, and particular tissues appear to be prone to their own particular molecular mutations that predispose them to malignant transformation. In the meninges surrounding the brain, the most frequent mutation that leads to the development of meningioma appears to be the loss of both NF2 genes. The lack of a consistent pattern of CGH abnormality in tumors without alterations of NF2 suggests that there may be several alternate pathways that lead to malignant transformation into cancer.

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