Comparative genomic hybridization analysis of craniopharyngiomas

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Object. Craniopharyngioma is the most common childhood brain tumor and is thought to arise from embryonic remnants of the Rathke pouch. Some craniopharyngiomas are monoclonal in origin and hence presumably harbor somatic genetic alterations, although the precise molecular mechanisms involved in craniopharyngioma development are unknown. The goal of this study was to identify genetic alterations in craniopharyngiomas.

Methods. To gain insight into the molecular mechanisms involved in development of these tumors, the authors analyzed nine adamantinomatous craniopharyngiomas by using comparative genomic hybridization. Six tumors (67%) displayed at least one genomic alteration, and three had six or more alterations. Only two tumors displayed a decrease in DNA copy number, and in all others an increase in DNA copy number was noted.

Conclusions. The authors conclude that a subset of craniopharyngiomas consists of monoclonal tumors arising from activation of oncogenes located at specific chromosomal loci.

KEY WORDS • craniopharyngioma • monoclonal tumor • somatic mutation • oncogene

10 craniopharyngiomas were analyzed using a trypsin G banding method, have been published.6,8,13 These studies detailed a number of varying chromosomal abnormalities, including translocation, deletion, and increase of DNA copy. Although a consistent pattern of abnormality was not observed between the different craniopharyngiomas examined, two tumors exhibited translocation between chromosomes 2 and 12, but with different breakpoints. Because preparation of metaphase chromosomes for classic cytogenetic analysis is difficult in craniopharyngiomas, previously reported studies are usually biased in favor of the mitotically most active tumor cells.

To obtain an overview of the genetic events leading to the development of craniopharyngiomas and to identify chromosomal regions that may contain genes important in tumor initiation and/or progression, the CGH method can be applied.3,4 This method is based on hybridization of differentially labeled tumor DNA and normal DNA to compare them with normal metaphase cells. Analysis of the tumor DNA/normal DNA fluorescence intensity ratios along the target chromosomes makes it possible to detect copy number changes throughout the tumor genome in a single hy-
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Hybridization. The CGH study allows us to map the genetic changes on normal chromosomes and thereby enables identification of regions that may contain important genes. Chromosomal regions that are consistently lost may highlight locations of putative tumor suppressor genes, whereas gains or amplification may identify chromosomal regions where oncogenes that are implicated in tumor development and progression are located. In this study, craniopharyngiomas were analyzed using the CGH method to define the somatic genomic pattern of alterations.

Materials and Methods

Clinical Material and Tumor DNA Extraction

Tumor tissues were frozen immediately after resection in liquid nitrogen and were stored at −80°C until the time of analysis. High-molecular-weight DNA was isolated from the samples of craniopharyngiomas according to standard procedures. For control experiments and as reference DNA for CGH experiments, genomic DNA was prepared from the blood of healthy male donors following a standard protocol and using the Gentra Systems kit (Gentra Systems, Inc., Minneapolis MN) according to the manufacturer’s instructions.

Comparative Genomic Hybridization

Hybridization of differentially labeled tumors and normal DNA to compare the results with normal metaphase chromosomes was performed essentially as previously described. Briefly, normal lymphocyte metaphase preparations were denatured at 70°C for 2 minutes in a denaturant solution (70% formamide in 2 × SSC, pH 7) and dehydrated in a graded ethanol series (70, 80, and 100%). Tumor DNAs were labeled with Spectrumgreen deoxyuridine triphosphate, and normal DNAs with Spectrumred deoxyuridine triphosphate by using nick translation (Vysis, Downers Grove, IL). After labeling, the size range of the DNA fragments was 300 to 600 bp. One milligram each of labeled tumor and normal DNA were precipitated in ethanol with 30 ml of unlabeled Cot-1 DNA, dissolved in 14 ml hybridization buffer. The CGH study allows us to map the genetic changes in normal chromosomes and thereby enables identification of regions that may contain important genes. Chromosomal regions that are consistently lost may highlight locations of putative tumor suppressor genes, whereas gains or amplification may identify chromosomal regions where oncogenes that are implicated in tumor development and progression are located. In this study, craniopharyngiomas were analyzed using the CGH method to define the somatic genomic pattern of alterations.

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Digital Image Analysis

Digital image analysis was used to facilitate the identification of chromosomal regions that had abnormal fluorescence ratios. Images of the hybridized metaphases were evaluated using a digital image analysis system based on a fluorescence microscope (Zeiss, Oberkochen, Germany) and a cooled charge-coupled device camera (Photometrics, Inc., Tucson, AZ) interfaced to a Cytovision Image Analysis System (Applied Imaging, Newcastle upon Tyne, UK). Three-color images (green [Spectrumgreen] for the tumor DNA, red [Spectrumred] for the normal reference DNA, and blue [DAPI] for the DNA counterstain) were acquired from 10 to 15 metaphases per specimen. Chromosome identification was based on the DAPI banding pattern. Calculation of the green/red (tumor/normal) fluorescence ratios was done using Cytovision software. Briefly, chromosomes were segmented and interchromosomal backgrounds subtracted from the chromosomal fluorescence. The green/red fluorescence ratio (tumor/normal copy number) was integrated across each chromosome along its medial axis from the p telomere to the q telomere and displayed as a green/red ratio profile. Data from 10 to 15 metaphases (20–30 observations/chromosome) were combined to obtain plots of the mean ratio for all chromosomes. The ratio profiles were standardized so that the overall green/red ratio for the entire metaphase was set to 1. Copy number changes in the tumor were expressed relative to this baseline ratio. All or partial chromosome gains were defined as having a green/red ratio greater than 1.2 and all or partial chromosome losses were defined as having a green/red ratio less than 0.8. Heterochromatic regions were excluded from the analysis.

Results

Tumor tissues were obtained in nine patients (five female and four male patients; age range 9–77 years), with tumors diagnosed in four individuals ages 9 through 16 years (mean 12 ± 3.5 years) and tumors diagnosed in five patients older than 40 years (range 42–77 years, mean 56.4 ± 13.6 years). Tumors ranged in size from 17 to 45 mm, and none showed any invasion into surrounding structures or the walls of the third ventricle. All patients underwent transphenoidal or transcranial surgery, and no recurrence was noted at a mean follow up of 37.6 ± 5.7 months (range 28–48 months). Histologically, all craniopharyngiomas were of the adamantinomatous subtype, according to the World Health Organization nomenclature.

In three tumors no genetic abnormality was detected with the CGH methodology. In contrast, six tumors (67%) exhibited at least one genetic alteration, and in three of these more than six alterations involving different chromosomal regions were displayed; the results are summarized in Table 1. Only two tumors showed a decrease in the DNA copy number, and in all others an increase in the DNA copy number was detected.

Discussion

Craniopharyngiomas are thought to be congenital tumors, with the majority being diagnosed during childhood and puberty. Despite their histologically benign nature, treatment of craniopharyngiomas can be difficult. Their difficult location and adherence to surrounding normal tissue often hinder radical surgical removal, and total resection is not always possible. When only partially removed, craniopharyngiomas are prone to recurrence. Adjuvant therapies are therefore desirable and may be more effective after a better understanding of the molecular mechanisms underlying craniopharyngioma growth is attained. Surprisingly, there have been very few studies directed at determining the molecular defects that might be involved in the genesis of craniopharyngiomas. We therefore applied CGH to a series of craniopharyngiomas to help determine whether genetic
defects, in particular tumor suppressor genes or oncogenes, may play a role in craniopharyngioma tumorigenesis. Our results provide compelling evidence of genetic abnormalities in the majority of these neoplasms, giving support to a recently published report in which it was demonstrated that a subset of craniopharyngiomas is monoclonal in origin. Having a monoclonal origin implies that tumorigenesis evolves by acquisition of somatic genetic alterations, and thus monoclonal craniopharyngiomas should be regarded as bona fide tumors, rather than a maldevelopmental event or a polyclonal disorder. Our results indicate that the most common genetic alterations in this subset of craniopharyngiomas are a gain in the DNA copy number. This is usually taken as indirect evidence of the involvement of oncogenes in the tumorigenic process. There are no known oncogenes that have been conclusively shown to be involved in the pathogenesis of these tumors, and analysis of two presumptive candidate oncogenes (gsp and gip) showed no somatic mutations. Although there are numerous genes that might serve as possible candidates in cases in which the entire chromosome is amplified (for example, chromosomes 17, 19, and 20), the limited pattern of DNA gain on 1p32-tel, 9q22-tel, 12q22-tel, and 22q12-tel may help in pinpointing the relevant oncogenes. Examples include \( TAL1 \) and \( AF1p \) (1q32), \( ABL \) (9q43.1),mast-cell growth factor (12q22), \( ALDH2 \) (12q24.2), and platelet-derived growth factor (22q12.3-1q13.1). This latter gene is of particular interest, because it is known to be involved in the pathogenesis of infiltrative skin tumors of intermediate malignancy, akin to the usually benign nature of craniopharyngiomas.

To define the specific genes that are involved in craniopharyngioma development more clearly, gene chip technology seems to be best suited. This technique facilitates analysis of the expression levels of thousands of genes in a single experiment, or even the detection of the level of DNA amplification of specific genes. Indeed, the application of this novel technology to craniopharyngiomas would unravel the specific genes that are involved in the tumorigenesis of these neoplasms.

Conclusions
A subset of craniopharyngiomas appears to consist of monoclonal tumors whose pathogenesis involves nonrandom genetic alterations and possibly activation of oncogenes located in defined chromosomal regions.

References


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