Identification of a Microsatellite Instability Phenotype in Meningiomas

Mark J. Pykett, Maureen Murphy, Peter R. Harnish, and Donna L. George

Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-6145

Abstract
To better understand the molecular mechanisms responsible for meningioma tumorigenesis we previously utilized subtractive hybridization protocols to identify genes the expression or structure of which is altered in these common brain tumors. Here we show that a CA dinucleotide repeat element present in one complementary DNA isolated by this approach has undergone a contraction in size in a meningioma cell line. Extension of this initial observation has revealed widespread genetic alterations affecting simple repeat sequences in this and other meningiomas. These data indicate that genetic instability may play a previously unrecognized role in the etiology of meningiomas.

Introduction
Meningiomas represent one of the most common tumor types of the central nervous system, comprising approximately 20% of intracranial and 25% of intraspinal neoplasms (1). Despite their frequency little is known about the molecular mechanisms underlying the development and progression of these tumors. To gain a better understanding of such mechanisms we previously used subtractive hybridization protocols to identify genes the expression or structure of which is altered in meningiomas relative to their normal precursor cells (2). These efforts led to the isolation of several cDNAs that detect altered RNA hybridization patterns in meningioma tumor samples. In the present report our analysis of one of these clones has revealed a CA dinucleotide repeat element, located within the 3' nontranslated region, that is altered in size in the meningioma cell line utilized in the subtractive hybridization procedures, compared to normal cells from the same individual. This finding prompted us to extend the examination of additional simple repeat sequences in meningioma cell lines and primary meningiomas, an analysis that revealed the presence of a microsatellite instability phenotype in meningiomas. Recently, the presence of such widespread mutations in simple repeat sequences has been associated with defective mismatch repair processes in certain colorectal and endometrial carcinomas (3-7). The data presented in this report provide initial evidence that genomic instability, likely resulting from faulty DNA repair, plays an important role in meningioma tumorigenesis.

Materials and Methods
Tumor Specimens and DNA. Meningioma cell lines T2898 and T2866 and matched fibroblast controls were kindly provided by K. Zang (University of Saarland) and were cultured as described previously (2). DNA from 14 primary meningiomas and corresponding normal blood samples was generously provided by L. Jacoby, V. Ramesh, and J. Gusella (Molecular Neurogenetics Unit, Massachusetts General Hospital). The 14 tumor-control pairs were designated MN001-MN007 and MN101-MN107.

PCR Analysis of Microsatellites. PCR analysis of the CA dinucleotide repeat in PMC 1 was performed using 100 ng DNA and the primers PMC 1 mutfor (5'-CTATCCACAGAGATTGTTAATGT-3') and PMC 1 mutrev (5'-ACTAAACCCAAAACCCCTTTTGTA-3'). Standard amplification reactions were carried out using [γ-32P]ATP 5' end-labeled PMC 1 mutfor primer in a 100 ng DNA stock volume under the following conditions: 30 cycles at 94°C, 58°C, and 72°C for 1 min each, followed by 10 min at 72°C. PCR of humAR CAG trinucleotide repeat was carried out using 100 ng DNA and primers ARfor (5'-TCCAGAATCTGGTCCAGAGCCGTGC-3') and ARrev (5'-GCTGTGAAGGTTGCTGTTCCTCAT-3'), both kindly obtained from K. Fishbeck (University of Pennsylvania) (8). Standard reactions were carried out in 50-μl reaction volumes using [γ-32P]ATP 5' end-labeled ARfor under the following conditions: 30 cycles at 94°C, 60°C, and 72°C each, followed by 10 min at 72°C. PCR analysis of other microsatellites was carried out using 10 ng DNA and primers for the following loci: D17S933, D17S999, D17S942, D17S796, D19S177, CTT16, DHFRP2, and FES (all from Research Genetics, Huntsville, AL); and D18S59, D18S46, D19S210, D19L1PE, and D19S199, kindly provided by R. Spielman (University of Pennsylvania). PCR using these primers was performed with 0.2 μCi of [32P]dCTP incorporated into a 15-μl reaction mixture. PCR cycles consisted of the following: a total of eighteen consecutive cycles of 94°C denaturation, followed by annealing at consecutive temperatures of either 72°C, 71°C, 70°C, 69°C, 68°C, 67°C, 66°C, 65°C, 64°C, 63°C, 62°C, 61°C, 60°C, 59°C, 58°C, 57°C, 56°C, or 55°C (1 round at each temperature); and extension at 72°C, each temperature for 1 min, followed by 30 cycles of 94°C, 55°C, and 72°C for 1 min each, followed by 10 min at 72°C. PCR products were denatured in sequencing stop solution for 10 min at 94°C, run on 6% polyacrylamide gels, and visualized by autoradiography. PCR products were cloned in the TA vector (Invitrogen) following the recommendations of the supplier.

Results
A Transcribed CA Dinucleotide Repeat Is Altered in Size in a Meningioma Cell Line. In work reported previously (2) we utilized subtractive hybridization protocols to isolate cDNA clones representing genes the expression of which is altered in meningiomas relative to cultures of normal meningeal cells. One clone (mac PI) isolated from a subtraction cDNA library detects a 3-kilobase transcript in normal meningeal cells. Analysis of RNA from the meningioma cell line used to generate the subtraction library, designated T2898, revealed a slightly smaller transcript. For additional details see Ref. 2. Library screening with this clone identified a larger cDNA termed PMC 1, which detects the same altered RNA pattern in the T2898 meningioma cell line. To examine regions of PMC 1 for sequence alterations, we utilized genomic PCR assays to analyze overlapping regions of the PMC 1 clone. One PCR product generated from T2898 tumor DNA was reproducibly smaller than that obtained in constitutional DNA from the same individual and in DNA from other sources (PMC 1; Fig. 1a). This PCR product comprised part of the 3' nontranslated region of PMC 1 and was found to contain a CA dinucleotide repeat element. DNA sequence analysis of several clones from independent PCR assays revealed that products amplified from tumor DNA contained 17 or 21 CA repeats, while products from constitu-
Fig. 1. Identification of microsatellite instability in the meningioma cell line T2898. 

a. PAGE of PCR products from representative microsatellite markers in genomic DNA from the meningioma cell line T2898 (T) and from the corresponding fibroblast line (N). The PMC 1 locus contains a 46-base pair CA dinucleotide repeat; D17S942 and D17S796, loci containing dinucleotide repeats; C7776, locus containing a trinucleotide repeat; and DHRFP2, locus containing a tetranucleotide repeat. Arrows, products of altered size in the tumor relative to normal cells from the same individual. 

b. Analysis of the CAG repeat of the human androgen receptor gene in DNA from the T2898 meningioma cell line (T) and its corresponding control (N). Arrows, PCR products of increased size in the T2898 meningioma cell line relative to the control. 

c. Sequence of the androgen receptor (CAG) repeat region in representative PCR clones from b, confirming an alteration in the number of CAG repeats in the T2898 meningioma cell line. Numbers to the left of each sequence represent the number of CAG repeat units.

T2898 tumor cells relative to DNA from corresponding normal cells. The loci analyzed were mapped to six different chromosomes and consisted of di-, tri-, and tetranucleotide repeats. The results of this analysis were striking; the tumor DNA exhibited size alterations in 13 of the 14 loci examined. Representative results are shown in Fig. 1a.

One of the markers analyzed in these studies encompasses the CAG trinucleotide repeat within the coding region of the humAR. PCR products generated with primers flanking this CAG repeat were found to be larger in tumor DNA than in DNA from matched normal cells (Fig. 1b). DNA sequence analysis of representative clones confirmed that the size alterations of the PCR products reflected changes in the

tional DNA contained 24 or 25 CA repeats. No other consistent changes in the PMC 1 sequence were identified in the tumor DNA.

Recent studies have revealed that various tumor types, particularly certain hereditary and sporadic colorectal carcinomas, are characterized by instability of simple repeat sequences. Therefore, we extended our studies to explore the possibility that the reduction in the size of the CA dinucleotide repeat identified within the PMC 1 transcript was indicative of a more widespread microsatellite instability phenotype in T2898 meningioma cells.

Widespread Microsatellite Instability Detected in the T2898 Meningioma Cell Line. Genomic PCR assays were used to compare the sizes of 13 other microsatellite markers in DNA from
Microsatellite Instability Occurs at High Frequency in Meningiomas. To extend these studies we examined DNA from 1 additional meningioma cell line (designated T2966), and from 14 primary meningioma tumor samples and their matched normal controls for evidence of genetic instability at 4 microsatellite markers from different chromosomes (humAR, D19S210, DHFRP2, and C17'16). While no changes were detected in the sizes of PCR products generated with DNA from the T2966 meningioma cell line (data not shown), three of the primary meningiomas exhibited expansions or contractions of microsatellite length at two or more DNA markers (see Fig. 2). Specifically, primary tumor sample MN03 exhibited alterations at microsatellite markers humAR and DHFRP2; MN004 exhibited alterations at humAR and CTT16, and MN007 had alterations affecting DNA markers humAR and D19S210, and DHFRP2. Thus, of the 16 tumors examined, 4 (25%) exhibited a microsatellite instability phenotype. These findings suggest that a defect in one or more repair processes is associated with meningioma tumorigenesis.

Discussion

Somatic alterations in simple repeat sequences, or microsatellites, have been detected in several types of human cancer, particularly in colorectal tumors and other carcinomas with hereditary nonpolyposis colon cancer (3–7). Important clues to understanding the origin of such microsatellite instability phenotypes resulted from the discovery of mutations in either of two putative DNA mismatch repair genes (hMSH2 and hMLH1) in tumors of some hereditary nonpolyposis colon cancer patients, as well as in sporadic colorectal tumors (9–12). Deficiencies in mismatch repair also have been detected in colorectal and endometrial cancer cell lines with a microsatellite instability phenotype (13). These findings support the hypothesis that mutations affecting one or more DNA repair pathways likely contribute to the development or progression of certain human cancers.

The frequency and nature of the alterations affecting simple repeat sequences in the meningiomas that we examined here resemble those reported previously for certain other kinds of sporadic tumors. For example, recent studies have demonstrated a microsatellite instability phenotype in 17–22% of sporadic endometrial carcinomas (6, 14), in 22% of esophageal adenocarcinomas (15), and in 23–39% of gastric carcinomas (16, 17). The alterations of microsatellites detected in meningiomas, which included expansions and contractions of di-, tri-, and tetranucleotide repeats, are characteristic of those that arise in cells with defective mismatch repair (3–7, 13). It has been proposed that accumulation of such somatic mutations could contribute to the multistep process that results in cancer, although the mechanism by which such mutations mediate transformation is currently unclear. One possibility is that while genetic instability is conveniently measured at microsatellites, repair defects lead to quantitative or qualitative changes in genes predisposing to cancer. In our sample of affected meningiomas, we detected microsatellite alterations not only in nontranscribed DNA but also in the coding region of the human androgen receptor gene and in the noncoding region of the PMC 1 cDNA. Further studies are needed to determine whether alteration of either of these genes, both of which are expressed in meningiial cells, contributes to the transformed properties of these cells.

Meningiomas comprise a heterogeneous group of tumors, both histologically and clinically (1). Although the molecular basis of this diversity is currently unclear, different meningioma subtypes could arise through the alteration of different tumor suppressor genes or differentiation specific genes. Recently, evidence has been obtained that the functional inactivation of the NF2 tumor suppressor gene on chromosome 22 contributes to the formation of some meningiomas (18, 19). However, we and others have shown that a significant percentage of meningiomas fail to exhibit alterations of the NF2 tumor suppressor gene (18–20). Moreover, linkage studies indicate that the NF2 gene is not allelic to the putative predisposition locus for familial meningiomas (21). Other reports indicate that loci on chromosome 22, distinct from the NF2 gene, as well as loci outside chromosome 22 are important in the development of meningiomas (22, 23). Together, these findings support the conclusion that the development of many meningiomas involves mechanisms distinct from or in addition to mutation of the NF2 gene. Our data now indicate that mutations associated with a genetic instability phenotype represents an important, and previously unrecognized, factor in meningioma tumorigenesis. The results presented here provide the basis for subsequent investigations to determine the origin of the genetic instability in meningiomas and its relationship to the clinical and histological diversity of these common brain tumors.

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References


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