Analysis of Meningiomas by Methylation- and Transcription-based Clonality Assays

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ABSTRACT

The clonal derivation of tumors can be determined by X chromosome inactivation analysis based on differential expression of genes or differential methylation of cytosine residues in CpG islands near polymorphic loci. In this report, we compared a transcription-based RNA analysis with a methylation-based DNA assay to determine clonality of meningiomas. Both clonality assays use PCR-based analysis at the human androgen-receptor gene (HUMARA) on the X chromosome. Among 23 meningiomas from female patients, 19 were informative heterozygotes at this locus (83%). The patterns of X chromosome inactivation in four patients were extremely skewed towards one allele in blood (unequal Lyonization), which precluded clonality determination in the tumor samples. Concordant clonality results with methylation- and transcription-based clonality assays were demonstrated in 9 of 13 informative tumors expressing the androgen receptor. Seven meningiomas were monoclonal, but surprisingly, two pathologically documented cases of meningiomas were polyclonal. There was disparity in 4 of 13 tumor specimens that were polyclonal by the methylation-based assay but monoclonal by the transcription assay. Clonality examination of these meningiomas by the methylation-based phosphoglycerate kinase assay provided identical results to the methylation-based analysis at the HUMARA locus. In addition, loss of heterozygosity (LOH) studies of chromosome 22, which is frequently deleted in meningiomas, showed that four of four informative samples of the six polyclonal tumors had partial LOH in tumor tissues. However, complete LOH was observed in primary cultured cells, which were also monoclonal by the methylation assay. Taken together, these data suggest that the disparity of the two assays in these four cases may be due to differences in the level of expression of the androgen receptor gene in tumors. Therefore, we conclude that: (a) clonal derivation of meningiomas determined by both transcription- and methylation-based clonality assays are in full agreement in many (9 of 13) but not all cases (4 of 13); and (b) most meningiomas (9 of 15) are monoclonal in origin, whereas some meningioma samples (6 of 15) are polyclonal or may contain heterogeneous components.

INTRODUCTION

Characterization of clonal derivation of human neoplasms has provided important information about etiology and pathogenesis and has practical implications for both diagnosis and subsequent studies of disease progression. Analysis of clonality in females heterozygous for specific genes or polymorphic markers on the X chromosome has been widely used for determination of tumor origin (1). The traditional method of clonality analysis developed by Fialkow (2, 3) used isozyme expression of the X chromosome-linked G6PD gene.

Although this method provides valuable and definitive data on a number of diseases, including clonal origin determination of myeloproliferative disorders (4), it is limited by the low frequency of heterozygosity in females of many ethnic populations.

Vogelstein et al. (5) introduced a molecular genetic approach to assess the clonal origin of tumors using DNA RFLP and the differential methylation of nearby cytosine residues on the X chromosomes. Combining RFLPs occurring in the X-linked genes HPRT and PGK, this methylation-dependent method is informative in over 50% of human tumors in females (6). Another clonality assay that uses a highly informative (90%) variable number tandem repeat marker, M27ß at the DXS255 locus, has expanded informativeness to include most females (7). However, hypermethylation at the M27ß locus limits its application (8, 9). In addition, HPRT, PGK, and M27ß techniques are limited by the requirement of large amounts of DNA (5–10 μg) for Southern blot analysis. A modified clonality assay at the PGK locus has been devised to study clonality of myeloproliferative disorders with small numbers of cells by means of PCR (10). The validity of this method was confirmed by van Kamp et al. (11) through comparative clonal analysis of hematopoietic cells by the PCR-based assay with that of Southern blot analysis.

Recently, Allen et al. (12) developed a methylation-based clonality assay that takes advantage of a highly polymorphic trinucleotide repeat (CAG) in the coding region of the first exon of the human androgen receptor gene on the X chromosome (HUMARA). This CAG repeat is closely linked to four methylation sites that have served as the basis for studying patterns of X chromosome inactivation in female carriers. The advantages of this clonality assay include high frequency of heterozygosity (90%), reliable methylation patterns, and that it is PCR-based so that a small amount of tumor specimen from OCT or paraffin can be successfully analyzed (13). By using this technique, monoclonality in Langerhans cell histiocytosis has been documented, and the results were confirmed with the PGK and M27ß assays (14).

However, there has been concern that differential methylation at these loci is not a reliable marker for the state of activation of the X chromosome or that variable methylation may occur in association with malignancy (15–19). This is well exemplified at the DXS255 locus analyzed with the M27ß probe, which is hypermethylated in a significant proportion of acute myelogenous leukemia blast populations (8, 9). Inconsistent data have been reported by groups using the methylation and expression assays. For example, parathyroid adenomas were determined as multicellular in origin by an expression-based clonality assay (20), whereas the same type of tumor was shown to be monoclonal by methylation-based clonality techniques (21, 22). This discrepancy may be due to several causes, including the complexity of tumor, normal tissue contamination, abnormal methylation, or variable expression of the G6PD gene between tumor and normal tissues. Therefore, it is very important to conduct comparative clonality determinations in the same tumor using both expression- and methylation-based clonality assays. Until recently, no single locus has had a coding polymorphism coupled with differential methylation sites that
would allow comparison of both the expression- and methylation-based assays at the same locus. Such a combination would allow validation of methylation-based assays in comparison with expression analysis at the same locus.

A transcription assay was recently developed at the HUMARA locus using a RT-PCR strategy that allows clonality determination without relying on differential methylation of the X chromosomes (23). It permits direct comparison of methylation-based clonality assay with transcription-based assay at the same locus. However, the technique is limited by the level of transcription of the androgen receptor gene in the tissue of investigation (24). In order to validate this new assay and to compare the results of transcription- and methylation-based clonality assays, we decided to investigate the origin of clonality in a large number of solid tumors with both methylation- and transcription-based clonality techniques.

Meningiomas are one of the most common primary intracranial tumors and are thought to arise from the arachnoid cells of the meninges. They occur frequently in middle to late years of life as sporadic solitary tumors with slow growth and rare infiltration of surrounding tissues. They also occur as heritable tumors associated with bilateral acoustic neurofibromatosis (NF2) (25, 26). Jacoby et al. (27) have shown that meningiomas are monoclonal in origin by methylation-based method with PGK and HPRT probes. Cytogenetic and molecular genetic analyses have demonstrated that loss of part or a copy of chromosome 22 (monosomy) is a predominant genetic abnormality in meningiomas (28, 29). Recently, it has been shown that the NF2 tumor suppressor gene, on chromosome 22 is inactivated in 60% of sporadic meningiomas (30, 31).

There is some evidence that meningiomas may be stimulated by hormonal components. It is known that the incidence of meningioma is twice as high in women than men and the tumors enlarge during pregnancy (25, 26). There is also association between meningioma and breast cancer (32, 33). Many studies have shown an increased level of expression and nuclear localization of steroid hormone receptors, including progesterone receptor and androgen receptor, in meningiomas (34, 35).

Based on the results that the androgen receptor is expressed in most meningiomas (35) and the availability of a large number of tumor specimens from female patients, we decided to directly compare the two clonality assays through analysis of meningiomas. We examined 19 informative sporadic meningiomas from female patients using both methylation- and transcription-based clonality assays at the human androgen receptor gene locus (HUMARA). Our data demonstrate that clonality results obtained with the two techniques are concordant in most cases and confirm that most meningiomas are monoclonal, although some samples are polyclonal.

**MATERIALS AND METHODS**

**Collection of Tissue and Blood Samples, Pathological Evaluation of Tissue, and OCT Tumor Specimens.** Meningioma specimens were obtained from patients who had undergone surgery for the removal of meningioma. Tissue samples were obtained from the surgical specimen and stored at —70°C. The tissue samples were then processed for methylation analysis using DNA extracted from fresh frozen tissue.

**Isolation of Blood DNA, Tumor DNA and RNA from Frozen Tissues.** Tumor DNA and RNA were extracted from FFPE tissue sections using the QIAamp DNA/RNA Mini Kit (Qiagen). DNA was isolated from peripheral blood leukocytes by SDS/Protease K digestion followed by phenol and chloroform extraction (36). Tumor DNA and RNA were extracted simultaneously from the same fresh frozen tissue by a modified procedure as described previously (37, 38). Briefly, one 6-μm and 4–5 10-μm sections were cut serially at ~20°C in a cryostat. The 6-μm section was stained with hematoxylin and eosin for pathological examination, and the adjacent sections were subjected to DNA isolation with proteinase K digestion followed by phenol/chloroform extractions (39).

**Methylation-based Clonality Assay at the Androgen Receptor Locus.** The PCR-based clonality analysis at the human androgen receptor gene (HUMARA) was performed as described previously with the following modifications (Fig. 1A; Ref. 12). In a 20-μl volume, 200 ng of genomic DNA were pretreated with or without 12 units HpaII (International Biotechnologies, Inc., New Haven, CT) in 10× PCR buffer (0.5 M KCl, 0.2 M Tris (pH 8.6), 1 μg/μl BSA, and 15 mM MgCl₂). After digestion, 5 μl was taken and added to a PCR mixture of 10 μl final volume containing [32P]dATP (3000 Ci/mmol; NEN, DuPont) end-labeled primer, and the PCR reaction was then performed according to conditions described (12, 38).

**Transcription-based Clonality Assay at the Androgen Receptor Locus.** The transcription-based clonality analysis was conducted as reported (Fig. 1B; Ref. 23) with the following changes. The RNA samples (1 μg) were first extracted from peripheral blood leukocytes by SDS/Protease K digestion followed by phenol/chloroform extractions (36). The PCR products were mixed with an equal volume of formamide loading buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol), denatured at 95°C for 5 min, and cooled on ice. About 2 μl of each sample were loaded onto a 6% denaturing polyacrylamide gel containing 8 M urea, and the gel was run at 100 W for 4 h. Subsequently, the gel was fixed, dried, and exposed to XAR-5 (Kodak) film with an intensifying screen at ~80°C for 1–3 days.

**Table 1 Clonality results and clinicopathological features of meningiomas**

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a NB, normal brain; N. Ara, normal arachnoid; NE, no detectable expression of the androgen receptor gene; NI, not informative; Mono, monoclonal; Poly, polyclonal; ND, not done.
b Corrected ratio for normal tissue only.
c CR (see "Materials and Methods").
d Ratio of two alleles in RT+ sample.

**Table 1** Clonality results and clinicopathological features of meningiomas.
CLONALITY ANALYSIS OF MENINGIOMAS

A

Genomic DNA

Restriction digestion with Hpa II or Hha I

Digested DNA

(CAG)n

Primers

PCR with HUMARA primers

Polyacrylamide gel

Cut and dispersed by collagenase. Cell culture conditions were the same as described previously (40). DNA was extracted from second and third passages of the cultured cells using the same protocol as described above for tumor DNA isolation. LOH analysis of DNAs from tissue and primary cultured cells was performed using a PCR-based microsatellite marker at F3BVWFP, a tetranucleotide repeat on chromosome 22 (41).

Quantitation and Determination of CR. To obtain a precise determination of the ratio between the two X-linked alleles generated by the HUMARA clonality assay, the same gel after film autoradiography was exposed to a Phosphorimager screen (Molecular Dynamics), and the relative amount of PCR products of each allele was measured with ImageQuant software. The clonality of each tumor was determined by the value of the CR by the following procedure. The corrected tumor ratio (ctr) was calculated by the formula: ctr = (a/b)/(c/d), where a/b is the band intensity ratio of upper band over lower band with HpaII precut tumor sample, and c/d is the band intensity ratio of upper band over lower band of the control (nonprecut) tumor sample. This corrected ratio compensates for any potential preferential amplification of one of the two alleles in the PCR reaction. We analyzed noninvolved tissue (DNA from blood leukocytes) for each patient to control for Lyonization (42), since skewing towards one allele to an extent that meets criteria for clonal derivation may be as high as 25% in normal females (8, 43). The Lyonization ratio was calculated from corrected blood ratio (cbr) with the same formula as ctr. Finally, the CR was obtained by the following formula: CR = ctr/cbr, or cbr/ctr, whichever is greater than 1. This CR corresponds to the deviation from the original Lyonization ratio of the individual female caused by clonal cells contained in the tumor sample. With these stringent criteria for the clonality assay, a polyclonal sample will have a CR close to 1.0, and a sample containing an appreciable number of clonal cells (>50%) will have a CR greater than 3.0. For the transcription-based clonality assay, the clonality ratio equals the band intensity ratio of the two alleles in the presence of reverse transcriptase (+). Quantitation of each allele in LOH of chromosome 22 analysis was carried out by exposing the same gel after film autoradiography to a Phosphorimager screen. The intensity of each band was determined using the ImageQuant software.

RESULTS

Analysis of Frozen Tissues and OCT Tumor Specimens by Methylation-based Clonality Assay at HUMARA. Among 23 female patients, 19 were informative at the HUMARA locus (83%) after screening for heterozygosity in lymphocyte DNA. Samples of normal arachnoid and two normal brain tissues from patients heterozygous at the HUMARA locus were used as controls. Two bands of closely matched intensities in the HpaII-digested normal samples were evident as shown in Figs. 2A and 4A with CRs of 2.6, 1.6, and 1.3, respectively, which demonstrate that normal tissues are polyclonal (Table 1). In four patients (M16, M17, M18, and M19), the pattern of X chromosome inactivation was extremely skewed towards one allele (unequal Lyonization) in lymphocyte DNA, similar to the skewing pattern in tumor DNA, which precluded accurate clonality determination in tumors (Fig. 2A, M19; Ref. 13). The PCR-based methylation-dependent clonality analysis of the remaining 15 blood and tumor pairs showed that 9 samples were monoclonal in origin with CRs > 3 and 6; samples were polyclonal with CRs < 3 (Fig. 2; Table 1). To assess the possibility that normal tissues mixed with the tumor specimens were amplified by PCR, resulting in polyclonal patterns in the six tumors, we repeated the clonality analysis on DNAs extracted from OCT samples where the presence of tumor cells had been confirmed by histopathological examination (Fig. 3). The histological examination of adjacent serial section confirmed that the tissue samples came from the leseional tissue. Although nonneoplastic elements are present in all tumors, these elements appeared to represent a minority of these regions chosen under investigation (Fig. 3). The methylation-based clonality analysis demonstrated that all six samples from OCT are still polyclonal in origin (Fig. 2B), consistent with clonality data obtained from frozen tumor tissues (Fig. 2). The CRs of

Fig. 1. Schematic diagram comparing experimental procedures of the methylation- (A) and transcription-based (B) clonality assays at the HUMARA locus. The two assays are based on different principles for identifying the pattern of X chromosome inactivation in female mammals. In the methylation assay, methylation-sensitive restriction enzymes, such as HpaII (filled vertical bars) and HhaI (open vertical bars), are used to distinguish the inactive X chromosome, which is often methylated and resistant to HpaII digestion, from the active X chromosome, which is mostly unmethylated and sensitive to HpaII cleavage (A). In contrast, the transcription clonality assay is based on the fact that most genes, including the androgen receptor gene, are expressed from the active, but not the inactive, X chromosome (B). Therefore, HpaII digestion and RT are used for methylation and transcription assays, respectively. Subsequent reactions to amplify the products after restriction cleavage or RT are the same for the two assays.

B

Total RNA

AAAA 3'

Reverse transcription

cDNA

Polyacrylamide gel

(cDNA)

Polycrional

Monoclonal

Monoclonal

Polycrional

Monoclonal

Monoclonal

POLYCLONALITY ANALYSIS OF MENINGIOMAS

Treated with DNase I according to the manufacturers instructions (Life Technologies, Gaithersburg, MD), and the enzyme was subsequently inactivated by heating at 95°C for 15 min. The total mixture was then subjected to RT using SuperScript (Life Technologies, Gaithersburg, MD) with 20 pmol of oligo dT (Pharmacia Biotech, Inc., Piscataway, NJ) at 37°C for 60 min. The subsequent conditions for PCR reaction, gel separation, and autoradiography are the same as described above.

Methylation-based Clonality Assay at the PGK Locus. Screening for heterozygosity at the PGK locus was carried out as described (10). To determine clonality, 100 ng of DNA were digested with or without 12 units HpaII (International Biotechnologies, Inc., New Haven, CT) and then amplified with conditions as described previously (10), except one primer was end-labeled with [γ-32P]ATP (3000 Ci/mmol; NEN, DuPont). The BstXI-digested PCR products were separated on a 1.5% agarose gel, and the gel was Southern transferred to a nylon filter and subjected to autoradiography at −80°C overnight.

Cell Culture, DNA Extraction, and LOH Analysis of Chromosome 22. Primary cultured cells were obtained from one tumor tissue (M12). After examination by a neuropathologist, a portion of the fresh tumor specimen was

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Fig. 2. Methylation-based clonality analysis of fresh frozen samples (A) and frozen tissue and OCT specimens (B) at the HUMARA locus on the X chromosome. The marker is a trinucleotide tandem repeat (CAG), and its PCR products are around 280 bp. A, parallel samples for blood (B) and tumor (T) were predigested with (+) or without (−) HpaII restriction enzyme. The normal arachnoid was served as a control for polyclonality with a corrected ratio of 2.6. The CRs for M5, M1, M2, M3, and M13 are 6.0, 3.4, 19.6, 6.2 and 1.1, respectively. M19 was precluded from clonality determination due to extreme skewing towards one allele in the blood (Lyonization). B, both frozen and OCT samples were from the same tumor specimens. M2 was identified as monoclonal in origin (A) and was served as a positive control for complete HpaII digestion of DNA extracted from OCT. The CRs for both frozen tissues and OCTs are listed in Table 1. R, blood; T, frozen tissue; OCT, tumor slices cut out of OCT compound; −, no HpaII; +, with HpaII.

both frozen tissues and OCT tumors are shown in Table 1. It is unlikely that the polyclonal patterns observed here resulted from incomplete HpaII digestion, since one master mix was prepared for all HpaII digestions and a positive control included in all experiments was always monoclonal (Fig. 2, M2). In addition, each experiment was repeated at least two times, and the results were the same as shown in Fig. 2.

Analysis of Tumor Tissues by Transcription-based Clonality Assay. Compared to the methylation-based assay, the transcription-based clonality analysis uses RT of RNA to cDNA, which obviates the need to use methylation status at a given locus as a surrogate for X chromosome status (Fig. 1). That is, androgen receptor transcripts will only be expressed from an active X chromosome. Total RNA used for this analysis was isolated from the same tumor tissue where DNA was obtained for methylation clonality assay using a modified procedure to extract DNA and RNA simultaneously (38). One kidney and two normal brain samples (NB1 and NB2) expressing detectable levels of androgen receptor message were included as polyclonal controls. Methylation-based clonality analysis of DNA from these normal tissues was performed for comparison with transcription assay results.

Fig. 3. Representative pathological examination of tumor specimens from OCT sections. Sectioning of OCT blocks was carried out in a cryostat at −20°C. One 6-μm section was cut and stained with hematoxylin and eosin. A, meningiothelial meningioma from M2; B, transitional meningioma from M12; C, transitional meningioma from M15; D, secretory meningioma from M14.
and for the positions of the two alleles. For each RT, two identical reactions with (+) and without (−) RT were carried out, where RT− reactions served as controls for possible DNA contamination, which would produce two bands. In each of the three normal tissues, two bands corresponding to the ones amplified from DNA were observed in RT+ lanes, while no band was detected in RT− lanes (Fig. 4A). The CRs for the kidney and two normal brains were 1.5, 1.5, and 1.0, respectively, indicating that they are polyclonal in origin by the transcription assay (Fig. 4A; Table 1). No expression of the androgen receptor gene was detected in two meningiomas (Fig. 4B, M2 and M9; Table 1). The absence of RT-PCR products from M2 and M9 likely results from a limited or lack of gene expression from the androgen receptor rather than degradation of the RNA during DNase I treatment, since a master solution was used for all the samples and RT-PCR amplifications were observed in most specimens (Table 1; Fig. 4). In addition, RT-PCR products were observed for other genes, such as dopamine 1 and 2 receptors, from the M2 RNA and other meningiomas using the same batch of DNase I (data not shown).

Application of this assay to the remaining 13 meningiomas that were shown to be polyclonal by methylation assay, we further studied clonality in tumors, including M10 and M12, which had discordant results in the previous assays. Representative results are shown in Fig. 6. The intensity ratio of the 530-bp band to the 433-bp band was approximately 3:1 for polyclonal samples. This is due to the formation of heteroduplex (50%) after PCR amplification, which resists the BstXI digestion. Therefore, only the predicted 25% PCR products that have the BstXI site on each strand of a homoduplex would be digested (10). Based on this PGK assay, M4 and M7 are monoclonal, M10 (data not shown) and M12 are polyclonal, and M18 is precluded from clonality determination due to extreme Lyonization in the blood (Fig. 6; Table 1).

LOH Analysis of Chromosome 22 and Clonality Analysis of Primary Cultured Cells by the Methylation Assay. To further study the biology of one tumor with discordant results, we took advantage of the primary cultured cells available from one tumor tissue (M12) prepared previously for other experiments. A portion of the original tumor specimen (M12) was excised and subjected to cell culture upon receipt of the tissue from the operating room. After two and three passages, the cells were harvested, and DNAs were isolated. LOH analysis of tumor DNA and DNA from cultured cells was performed with a polymorphic marker, F8VWFP, on chromosome 22 (41). Partial loss (incomplete loss) of chromosome 22 was detected in frozen tissue of all four informative samples (M12, M13, M10, and M15) of the six tumors, which are polyclonal in origin by methylation assay (Fig. 7A). However, complete loss of chromosome 22 was observed in DNA from second and third passages of primary cultured cells (Fig. 7A, M12). Methylation-based clonality analysis (HUMARA) of DNA from cultured cells demonstrated that these cells are monoclonal in origin with a CR of 6.5 (Fig. 7B), although polyclonality was detected in the parallel tumor tissue (Fig. 7B) and in the OCT sample from the same patient (Fig. 2B).
Table 1). Seven of the nine tumors were identified as monoclonal in band intensities between the two alleles in both methylation (Fig. 2B, Therefore, opposite skewings of the alleles between HpaI-treated only the allele transcribed from the active X chromosome is amplified. generated by the two techniques are concordant in nine cases (69%; clonal origin of these two tumors was demonstrated by expression of the androgen receptor gene was not detected in two cases (Fig. 45. M2 lymphocyte DNA (Fig. 2A. M19; Table 1). The frequency (4 of 19) of differential methylation of X chromosomes (23). We have compared the results of a transcription assay through analysis of RNA (23) with that of a methylation assay of DNA (12) from the same tumor at the same locus in meningiomas.

Twenty-three sporadic meningioma specimens from female patients were collected, of which 19 were heterozygous at the HUMARA locus (83%). Four cases (21%) were excluded from clonality determination due to severe skewing towards one allele in HpaII-digested lymphocyte DNA (Fig. 2A, M19; Table 1). The frequency (4 of 19) of extreme Lyonization observed in our experiments (21%) is consistent with observations in other clonality studies (8, 43). Expression of the androgen receptor gene was not detected in two cases (M14 and M15), polyclonality was demonstrated by methylation-based clonality assay only (Fig. 2A; Table 1). Clonality androgen receptor probe demonstrated transcription of such cells with androgen receptor probe demonstrated transcription of androgen receptor between clonal tumor and other populations of clonal cells. This will bias the assay in the favor of the small population of clonal tumor cells expresses a significant higher level of the androgen receptor in comparison to reactive or another population of clonal cells. This will bias the assay in favor of this small population of clonally derived cells. We, therefore, view the assay as complementary, with the DNA methylation-based assay giving an estimation of the number of clonal cells in the sample and the transcription assay in this setting confirming the presence of a clonal population of cells.

Results obtained with LOH analysis of chromosome 22 support this explanation. Incomplete LOH of chromosome 22 in all four informative cases of the six tumors provides evidence that there are two or more different population of cells within the tumor mass (Fig. 7A). Complete LOH detected in primary cultured cells derived from the same tumor suggests that the clonal population of cells has a proliferative advantage during cell culture, as would be expected for neoplastic cells (Fig. 7A, M12). In support of this hypothesis, cultured meningioma cells derived from polyclonal tissue were monoclonal by the methylation assay (Fig. 7B). Northern blot analysis of RNA from such cells with androgen receptor probe demonstrated transcription of androgen receptor at similar levels as detected in tumor tissue (M12;
data not shown). Based on these data, we speculate that these six tumors may be heterogeneous in composition containing clonal tumor cells and another polyclonal component, which most likely represents normal tissue due to hormonal stimulation, since it has been shown that many hormone receptors, including progesterone receptor and androgen receptor, are expressed in meningiomas (34, 35).

Jacoby et al. (27) have shown that meningiomas are monoclonal in 9 informative patients by an HPRT methylation assay. However, in four of the nine tumors (44%), the CRs of the four tumors were less than 3.0, including two cases at the border line between monoclonal and polyclonal, which is consistent with our results (27). Recently, Rutledge et al. (46) reported that three tumor fragments randomly separated from the same tumor mass (case 119) with no difference from one another pathologically were subjected to LOH analysis of chromosome 22. They found LOH in one of the fragments but not the other two fragments. This suggests that the tumor is not homogeneous at the DNA level, although they are indistinguishable by pathological means. Similar observations have been reported in thyroid nodules, where inconsistent data were obtained between molecular analysis and histomorphological examination of 39 thyroid nodules (47). Genetic heterogeneity in colorectal adenomas, but not in adenocarcinomas, has been reported by Shibata et al. (48). They found c-K-ras gene mutations only at discrete portions in four of seven adenomas, whereas c-K-ras mutations were detected throughout the tumor specimens in three of seven adenomas and all seven adenocarcinomas.

Interestingly, a similar frequency of polyclonality in meningiomas has been observed recently by Wu et al.5 They found that 6 of 17 (35%) of informative meningioma tumors are polyclonal by the M27β probe, a methylation-based clonality assay at the DXS255 locus.5 In 1976, Nowell (49) proposed a model of clonal evolution of tumor cell populations from precancerous cells that acquired one or more genetic changes and exhibited growth advantage over adjacent cells. This model suggests that in the early stages of tumorigenesis, such as benign tumors, the cell mass may be heterogeneous. Tumor heterogeneity within a single neoplasm is well recognized (50, 51) and has been described in many types of tumors, including human gliomas (52), where 3 to 21 subpopulations were observed karyotypically in each of 8 glioma tissue specimens studied. Our clonality results demonstrate that some meningiomas contain mostly clonally expanded tumor cells that are monoclonal, whereas others may be polyclonal, consisting of reactive cells by hormonal stimulation or several distinct clonal populations of tumor cells.

In summary, we have shown that methylation- and transcription-based clonality assays are often but not always concordant. Our data also demonstrate that most meningiomas are monoclonal, but some are apparently mixed or contain polyclonal cells, suggesting that heterogeneity or reactive cells due to hormonal stimulations may be present in a high proportion of tumors, which may represent early stages of clonal evolution during tumorigenesis.

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Analysis of Meningiomas by Methylation- and Transcription-based Clonality Assays

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