Molecular Genetic Aspects of Oligodendrogliomas Including Analysis by Comparative Genomic Hybridization

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Oligodendrogliomas are a subgroup of gliomas with distinctive morphological characteristics. In the present study we have evaluated a series of these tumors to define their molecular profiles and to determine whether there is a relationship between molecular genetic parameters and histological pattern in this tumor type. Loss of heterozygosity (LOH) for 1p and 19q was seen in 17/23 (74%) well-differentiated oligodendrogliomas, in 18/23 (83%) anaplastic oligodendrogliomas, and in 3/8 (38%) oligoastrocytomas grades II and III. LOH for 17p and/or mutations of the TP53 gene occurred in 14 of these 55 tumors. Only one of the 14 cases with 17p LOH/TP53 gene mutation also had LOH for 1p and 19q, and significant astrocytic elements were seen histologically in the majority of these 14 tumors. LOH for 9p and/or deletion of the CDKN2A gene occurred in 15 of these 55 tumors, and 11 of these cases were among the 24 (42%) anaplastic oligodendrogliomas. Comparative genomic hybridization (CGH) identified the majority of cases with 1p and 19q loss and, in addition, showed frequent loss of chromosomes 4, 14, 15, and 18. These findings demonstrate that oligodendroglial neoplasms usually have loss of 1p and 19q whereas astrocytomas of the progressive type frequently contain mutations of the TP53 gene, and that 9p loss and CDKN2A deletions are associated with progression from well-differentiated to anaplastic oligodendrogliomas. 

Oligodendroglial neoplasms are characterized by a high incidence of loss of chromosomes 1p and 19q, as determined by loss of heterozygosity (LOH) analyses.5–9 Mutations of the TP53 gene are uncommon in these tumors, but few studies have addressed other genetic alterations in this group of neoplasms. Although a few oligodendrogliomas have been included in comparative genomic hybridization (CGH) studies of gliomas,10 no CGH analysis of a large series of oligodendroglial neoplasms has been reported to date. Here we examine a series of 61 oligodendroglial tumors from 56 patients for LOH for 19q and 1p and profile by CGH. We also evaluate genetic alterations commonly seen in astrocytic neoplasms, including alterations of the PTEN, CDKN2A, and TP53 genes and their loci, and amplification of the EGFR, PDGFRα, MYC, Cyclin D1, CDK4, and GLI genes. Based on these findings, we describe how this group of oligodendroglial neoplasms resembles and differs from astrocytic tumors.

Materials and Methods

Consecutive gliomas, which by routine diagnostic evaluation were considered to be composed partially or completely of neoplastic oligodendroglia and for which frozen tissue and normal peripheral lymphocytes were available, were included. Tissue samples collected fresh at the time of surgery were cut into blocks, snap frozen in liquid nitrogen, and stored at −135°C. Individual blocks were examined histologically, and those blocks composed of more than 70% neoplastic cells and representative of the oligodendroglial component were selected for DNA isolation. DNA from tumor tissue and corresponding lymphocytes were obtained by standard procedures.

Histological Classification

All tumors were subjected to consensus review by three neuropathologists. Only cases on which there was agreement between at least two of the three observers were considered to be suitable for analysis. Oligodendroglial neoplasms are characterized by a high incidence of loss of chromosomes 1p and 19q, as determined by loss of heterozygosity (LOH) analyses.5–9 Mutations of the TP53 gene are uncommon in these tumors, but few studies have addressed other genetic alterations in this group of neoplasms. Although a few oligodendrogliomas have been included in comparative genomic hybridization (CGH) studies of gliomas,10 no CGH analysis of a large series of oligodendroglial neoplasms has been reported to date. Here we examine a series of 61 oligodendroglial tumors from 56 patients for LOH for 19q and 1p and profile by CGH. We also evaluate genetic alterations commonly seen in astrocytic neoplasms, including alterations of the PTEN, CDKN2A, and TP53 genes and their loci, and amplification of the EGFR, PDGFRα, MYC, Cyclin D1, CDK4, and GLI genes. Based on these findings, we describe how this group of oligodendroglial neoplasms resembles and differs from astrocytic tumors.

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DNA (5 m) Aaronson for the pT11C1B1 (a gift from Drs. Steven Tronick and Stuart was scored as LOH. Tumor lane compared to the corresponding blood lane for another set of primers and run on an ABI 310 fluorescent analyzer. A reduction in intensity of more than 50% in the absence of dimethyl sulfoxide, and the annealing temperature was 58°C. Fragmented and analyzed on 2% agarose gels and stained with ethidium bromide. The PCR reactions were carried out for 26 cycles at 95°C denaturation for 50 seconds, annealing was allowed to proceed for 30 seconds, and extension was allowed to proceed at 72°C for 50 seconds. For exon 1, 5% dimethyl sulfoxide was included in the PCR buffer, and the touch down PCR profile included annealing at 68°C for four cycles, 64°C for four cycles, 62°C for four cycles, and 60°C for 10 cycles. Exon 3 was amplified in the absence of dimethyl sulfoxide, and the annealing temperature was 58°C for all 26 cycles. A reduction in intensity of more than 80% was considered to be due to a homozygous deletion of the exon. All PCR reactions were repeated once to confirm the results.

**Gene Amplification**

To assess gene amplification, an EcoRI digest of tumor DNA (5 μg) was Southern blotted and hybridized with the following probes: 1.6-kb EcoRI fragment of pEP7 for the EGFR gene; 1.6-kb StsI fragment of pHSR-1 for MYC, 1.6-kb PstI insert of pKk36P1 (a gift from Dr. Bert Vogelstein) for the GLI gene; 1.8-kb BamH1 fragment of pT11C1B1 (a gift from Drs. Steven Tronick and Stuart Aaronson) for the PDGFR α gene; 0.9-kb XhoI fragment of c14-2 (a gift from Dr. Bert Vogelstein) for the MDM2 gene; and PCR-amplified fragments of CDK4 and the CCND1 gene. Amplification was defined as a signal more than five times the reference signal.

**SSCP Analysis**

Exons 5, 6, and 7 of the TP53 gene were PCR-amplified individually, and exons 8 and 9 were amplified together, from tumor DNA, and subjected to SSCP analysis. After completion of PCR, an equal volume of sequencing stop solution containing 20 mmol/L NaOH was added, heated at 95°C, and cooled on ice, and 1–1.5 μl was loaded on a 6% acrylamide–0.5× TBE (45 mmol/L Tris-Borate, pH 8.3, 2 mmol/L EDTA) gel containing 5% glycerol. Electrophoresis was carried out at room temperature (30 W for 3–7 hours) with a cooling fan. Gels were dried and autoradiographed for 1–3 days.

**CDKN2A Analysis**

Exons 1 (primers 5’TGGCGCTCGGCGCTGCGAGA-3’ and 5’TCCCCTGCTCCGCTCGGAGA-3’) and 3 (primers 5’TTCCTTTCTGGCCTGGAGA-3’ and 5’TGAAGTCCGACCTTCCG-3’) of the CDKN2A gene were PCR-amplified and analyzed on 2% agarose gels and stained with ethidium bromide. The PCR reactions were carried out for 26 cycles at 95°C denaturation for 50 seconds, annealing was allowed to proceed for 30 seconds, and extension was allowed to proceed at 72°C for 50 seconds. For exon 1, 5% dimethyl sulfoxide was included in the PCR buffer, and the touch down PCR profile included annealing at 68°C for four cycles, 64°C for four cycles, 62°C for four cycles, and 60°C for 10 cycles. Exon 3 was amplified in the absence of dimethyl sulfoxide, and the annealing temperature was 58°C for all 26 cycles. A reduction in intensity of more than 80% was considered to be due to a homozygous deletion of the exon. All PCR reactions were repeated once to confirm the results.

**Comparative Genomic Hybridization**

Identification of DNA sequence copy number changes was accomplished by comparative genomic hybridization (CGH). Genomic DNA was extracted from the tumor biopsies. The reference DNA was isolated from normal female (GM10959) and male (GM01247B) lymphoblastoid cell lines (Coriell Institute Laboratories, Camden, NJ). Approximately 2 μg of tumor and normal genomic DNA was labeled with Rhodamine 110 (Applied Biosystems, Foster City, CA) and Spectrum Orange (Vysis, Downers Grove, IL) directly conjugated nucleotides, respectively, by a modified nick translation protocol. A
The final DNase I concentration of 0.00043 U/μl and 0.8 U/μl of DNA polymerase I was added to each reaction. Approximately 200–400 ng of the R110-labeled tumor (green) and Spectrum Orange labeled normal reference (red) DNA was ethanol-precipitated along with 15 mg of Cot-1 blocking DNA (Gibco BRL), resuspended in 10 ml of Master Mix 1.0 (50% formamide; 10% 20× SSC (pH 6.3); 100 mg/ml dextran sulfate), and hybridized to a normal denatured metaphase spread for 3 days, followed by stringency washes adapted from a standard fluorescence in situ hybridization (FISH) protocol. For each hybridization, at least six metaphases were captured and analyzed with the Quips XL image analysis computer program (Vysis). Interpretation of the profiles was made according to published guidelines.17 Specifically, a ratio of 1.0 depicts neither a gain nor a loss of tumor DNA sequences, and a ratio of 1.20 or 0.85 indicates a gain or loss, respectively, of tumor loci compared to normal loci.

Results

Well-Differentiated Oligodendroglioma, Oligoastrocytoma, and Well-Differentiated Astrocytoma (WHO II)

The majority of well-differentiated oligodendrogliomas had LOH for 19q (17/23, 74%), 1p (18/23, 78%), or both (17/23, 74%) (Table 1). One tumor in this group had LOH for 10q25, three cases had LOH for 10p, and one case had LOH for 10p, 10q23, and 10q25, but none of these tumors had PTEN gene mutations. Three of 21 (14%) informative cases had 9p loss, but homozygous deletion of the CDKN2A locus was not seen in any of the grade II tumors. Five of the 23 oligodendrogliomas had LOH for 17p and/or mutation of the TP53 gene. None of the grade II tumors in this group had gene amplification. The single OA had LOH for both 1p and 19q, but no other alterations. By CGH, 16 oligodendroglioma cases had loss of 1p and 19q, either alone, in six cases, or with additional gains or losses (Table 1, Figures 1 and 2). These 16 cases were among the 17 cases in which 1p and 19q loss was seen by LOH analysis. The most frequent additional abnormalities in this group were loss of all or part of chromosome 4, 14q, and the Y chromosome (Figure 2). Five of the seven cases that lacked 1p and 19q loss showed a variety of gains and losses with no consistent pattern. The single OA had loss of 1p and 19q, with no other alterations.

Anaplastic Oligodendroglioma (WHO III)

These tumors had many molecular features in common with the well-differentiated oligodendrogliomas (Table 2). Specifically, a high proportion of cases had LOH for 19q...
Figure 1. CGH profile of well-differentiated oligodendroglioma 969A, which shows only loss of 1p and 19q.

Figure 2. Composite of CGH findings for the 23 well-differentiated oligodendrogliomas. In addition to the frequent losses of 1p and 19q, losses of all or part of chromosomes 4, 14, and Y are noted.
One tumor had LOH for 10q23, one had LOH for 10q25, three cases had LOH for 10p, and one case had LOH for 10q23, 10q25, and 10p, but none of these cases had PTEN gene mutations. Aberrations of the TP53 gene and its locus were uncommon, occurring in 4/24 (17%) of cases. Deletion of 9p, the CDKN2A locus, or both was seen in 11/24 (42%) cases, and no tumors in this group contained gene amplification.

By CGH, 20 cases in this group had loss of 1p and 19q, either alone in one tumor or combined with additional abnormalities (Figures 3 and 4, Table 2). These 20 cases included the 18 cases with loss of 1p and 19q by LOH studies. The most common additional deviations were loss of chromosomes 4, 9p, 15, and 18 (Figure 4). The four cases that lacked 1p and 19q loss had a variety of abnormalities. Three of these four cases were among the four cases in this group with loss of 17p by CGH. Loss of 9p was demonstrable by CGH in seven of the 11 cases, with loss of 9p by LOH analysis.

### Anaplastic Oligoastrocytoma (WHO III)

Three of seven (43%) cases had 19q LOH, 2/7 (29%) had 1p LOH, and 2/7 (29%) cases had LOH for both 19q and 1p (Table 3). Three of seven (43%) cases had LOH for all chromosome 10q loci, and one tumor had LOH for 10p, but the cases in this group lacked PTEN gene mutations. One of seven (14%) tumors had a deletion of the CDKN2A locus. Five of seven (71%) tumors had 17p deletion and/or TP53 gene mutation, and 3/7 cases had gene amplification.

By CGH two cases had loss of 1p and 19q, as had been shown by LOH studies. Partial or complete chromosome 10 losses were seen in four tumors by CGH. Loss of 17p was not seen by CGH, and amplification at the EGFR locus was demonstrated in one case.

### Glioblastoma Multiforme (WHO IV)

One case originally classified as a glioblastoma had loss of 1p and 19q by both LOH and CGH studies (Figure 5).
and would have been classified as an anaplastic oligodendroglioma by some observers (see section on histology). Among the remaining four glioblastomas, three cases were included in this series because they contained oligodendroglial areas, and one tumor was the recurrence of a well-differentiated oligodendroglioma. One of these four cases had LOH for 19q, and one case had LOH for 1p (Table 3). Three of four (75%) cases had LOH for all or part of chromosome 10, but none of those three tumors had a PTEN gene mutation. Three of four (75%) cases had LOH for 9p and 2/4 (50%) of cases had 17p LOH, or TP53 gene mutation. One case had amplification of the EGFR gene, one case had CDK4 gene amplification, and one case had amplification of both the CDK4 and MYC genes.

By CGH these four tumors had changes associated with glioblastomas, including gain of chromosome 7 (three cases), amplification at the EGFR locus on 7p (one case), and loss of chromosome 10 (three cases) (Figure 6).

**Recurrent Tumors**

Both primarily resected tumors and recurrences were studied in five patients (Table 4). Additional changes in the two cases with only 1p and 19q losses, initially, included loss of chromosomes 4 and 9 by CGH. In one of these tumors the 9p loss was also documented by LOH studies, whereas in the other it was not. The other three cases had significant astrocytic elements, contained TPS3 gene mutations in both the initial resection and the recurrence, and did not show the 1p, 19q loss pattern. Additional changes in these cases included loss of 9p, 10q and a CDKN2A deletion (one case), and MYC and CDK4 gene amplification (one case).

**Histology**

Histological slides were reviewed in view of the molecular genetic and CGH findings to determine whether there were morphological differences between cases with loss of complete copies of 1p and 19q and the cases lacking this abnormality. Among the 23 tumors classified as oligodendroglioma, the 16 cases with 1p and 19q loss consisted of 17 tumors composed entirely or predominantly of oligodendroglial elements (Figure 7, C and D), and three tumors with astrocytic components. In contrast, all of the four cases that lacked the 1p 19q loss pattern contained significant amounts of astrocytic elements and might be considered oligoastrocytomas by some observers.

Among the 24 cases classified as anaplastic oligodendroglioma, the 20 cases with complete 1p and 19q loss consisted of 17 tumors composed entirely or predominantly of oligodendroglial elements (Figure 7, C and D) and three tumors with astrocytic components. In contrast, all of the four cases that lacked the 1p 19q loss pattern contained significant amounts of high-grade astrocytoma.

Among the seven cases classified as anaplastic mixed gliomas, the single case with complete loss of 1p and
19q showed a predominantly oligodendroglial component. The remaining six cases contained significant regions of anaplastic astrocytoma, combined with areas of glioblastoma or well-differentiated astrocytoma.

The single case classified as glioblastoma that had complete loss of 1p and 19q was composed exclusively of neoplastic oligodendroglia with focal areas of anaplasia, necrosis, and endothelial proliferation (Figure 8, A and B) and would be classified as an anaplastic oligodendroglioma by some observers. The other four cases showed the typical morphology of glioblastoma, although there were hypercellular regions with a fine vascular pattern, reminiscent of oligodendroglial neoplasms in three of these four cases (Figure 8, C and D).

**Discussion**

An association between LOH for regions on 19q and glial neoplasms, including tumors containing an oligodendroglial component, was reported by Ransom et al.\(^1\) and von Diemling et al.\(^1\) Bello et al.\(^7\) and Reifenberger et al.\(^8\) noted that oligodendroglial tumors also contain a high incidence of 1p deletions. Interestingly, further analysis of chromosomes 1 and 19 has revealed that although tumors of astrocytic and oligodendroglial derivation share LOH for regions on 19q, these categories of neoplasms also show distinctive genetic patterns.\(^5,9\) Specifically, oligodendroglial tumors contain loss of the entire 1p and 19q chromosomal arms, with retention of 1q and 19p. Astrocytic tumors, in contrast, show a more varied pattern of chromosome 19 abnormalities, ranging from loss of the whole chromosome to retention of most of the chromosome with an interstitial deletion of the 19q13.3 region.

In the present study, using a combination of molecular approaches, including CGH, we have confirmed that the majority of well-differentiated oligodendrogliomas (WHO grade II) show loss of chromosomes 1p and 19q with retention of 1q and 19p, with only rare occurrences of LOH for chromosome 10, 9p, 17p, and TP53 gene mutations and no cases of PTEN gene mutations, homozygous deletion of the CDKN2A gene, or gene amplification.

The anaplastic oligodendroglioma (WHO grade III) is a neoplasm with oligodendroglial morphology that, in addition, possesses features such as cytological anaplasia and hypercellularity, necrosis, endothelial proliferation, and a high mitotic rate. Patients with these tumors typically have shorter survival times than those with low-grade oligodendrogliomas, but the anaplastic oligoden-
droglioma responds to chemotherapy, such as PCV and Temazolamide. Anaplastic oligodendrogliomas share with well-differentiated tumors a high incidence of 19q and 1p loss. An allelotyping study of oligodendroglial tumors published by Reifenberger et al. showed that anaplastic tumors are more likely than well-differentiated oligodendrogliomas to have loss of additional chromosomal regions, where 9p is the chromosomal area with the highest incidence of loss. Cairncross et al. have demonstrated that anaplastic oligodendrogliomas with CDKN2A deletion respond poorly to therapy with PCV. Loss of 17p has also been reported in a few oligodendroglial tumors.

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*Loss of heterozygosity.
†No loss of heterozygosity.
‡Reduced amplification of CDKN2A (p16) gene exons.
§Copy number changes of partial chromosome arms.
¶Amplified region.
 Amplified oncogene.
Prior **surgery, ††radiation therapy.

Glioblastoma

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*Loss of heterozygosity.
†No loss of heterozygosity.
‡Reduced amplification of CDKN2A (p16) gene exons.
§Copy number changes of partial chromosome arms.
¶Amplified region.
 Amplified oncogene.
Prior **surgery, ††radiation therapy.

Figure 5. CGH profile of case 1072, which was classified as a glioblastoma based on the presence of necrosis with pseudopalisading (see Figure 8, A and B). Loss of 1p and 19q, seen here, along with amplification of 17q, suggests that this lesion should be considered an anaplastic oligodendroglioma.

Table 3. Molecular and CGH Profile of Anaplastic Oligoastrocytomas and Glioblastomas
droglial tumors, although it is not associated with TP53 gene rearrangements.8

In the series reported here we found that the majority of anaplastic oligodendrogliomas had loss of 1p and 19q. In contrast to the well-differentiated tumors, nearly half of the cases had 9p LOH, homozygous deletion of the CDKN2A gene, or both. Although a few tumors had loss of all or part of chromosome 10, none of these cases had mutations of the PTEN gene, gene amplification, or the \( ^{17}, ^{21}0 \) pattern characteristic of glioblastoma by CGH. Previous studies have reported PTEN mutations in 0/1420 and 2/22 oligodendrogliomas.21 The actual incidence of PTEN mutations in oligodendrogliomas may be higher because in the present study only tumors with chromosome 10 loss were evaluated for the presence of PTEN mutations. As with the well-differentiated tumors, most of the cases with TP53 gene mutations and/or 17p loss did not show complete loss of 1p and 19q by CGH and LOH and, in retrospect, contained significant regions of astrocytoma of various grades.

Table 4. Molecular and CGH Profile of Recurrent Tumors

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*Loss of heterozygosity.
†No loss of heterozygosity.
‡Reduced amplification of CDKN2A (p16) gene.
§Copy number changes of partial chromosome arms.
¶Amplified oncogene.
**Recurring CGH alterations in bold type.
***Not informative.
††All cases except H no. 500 are also presented in Tables 1–3.

Figure 6. CGH profile of case 905, which is a glioblastoma with oligodendrogliial features (see Figure 8, C and D). The gain of chromosome 7, amplification at the EGFR locus, and loss of 9p and chromosome 10 support classification of this tumor as a glioblastoma.
The WHO classification scheme recognizes mixed gliomas containing both astrocytic and oligodendroglial elements (oligoastrocytomas). Malignant progression can occur in either component, producing an anaplastic mixed glioma. Molecular analysis of this category of neoplasm has shown that most cases show either the 1p,19q loss pattern, suggesting an oligodendroglial origin, or TP53 gene mutations, suggesting an astrocytic origin, and that these changes were inversely related. Review of the histology in these cases confirmed that the molecular pattern frequently predicted whether the oligodendroglial or astrocytic component predominated. In the present study, for anaplastic mixed gliomas and lesions classified as glioblastoma with oligodendroglial features, complete loss of 1p and 19q was infrequently found in tumors with TP53 mutations. Cases with 1p and 19q loss had predominantly oligodendroglial morphology, and lacked gene amplification, whereas tumors with TP53 gene mutations showed mainly astrocytic features and included cases with gene amplification and the +7, −10 pattern of glioblastoma.

The evidence to date suggests that oligodendroglial neoplasms have a distinctive molecular and cytogenetic profile, characterized by complete loss of 1p and 19q, with only rare examples of TP53 gene mutations. Progressive changes include losses of chromosomes 3p, 4, 6 and 9p, 11, 14q, 15q, 18, Y. Although anaplastic oligodendrogliomas tend to contain more chromosomal deviations than well-differentiated lesions, the only change consistently associated with histological progression is loss of 9p, particularly when associated with homozygous deletion of the CDKN2A gene. Although some mixed oligoastrocytomas with predominantly oligodendroglial components by histological evaluation have the profile described above, a subset has features in common with astrocytomas of the progressive or secondary type. These alterations include loss of 17p and/or TP53 gene mutations, amplification of the CDK4 gene, and loss of all or part of chromosome 10 without PTEN gene mutations. Furthermore, Kraus et al have shown that histologically separable regions of three oligoastrocytomas showed the same 1p19q loss pattern within oligodendroglial and astrocytic areas. Analysis of microdissected portions of histologically mixed oligoastrocytomas or FISH studies may well demonstrate that regions with differing molecular and cytogenetic profiles exist within the same tumor. The findings presented here, as well as those reported by others to date, suggest, however, that in at least the majority of oligoastrocytomas, one component dominates.

In view of the subjectivity of the histological classification of gliomas and the clinical importance of distinguishing oligodendroglial from astrocytic neoplasms, the findings presented here, along with reports from other investigators, suggest that molecular profiles might be useful in the categorization of these tumors. Loss of 1p accompanied by loss of 19q identifies most oligodendroglial tumors. Furthermore, for oligodendrogliomas, loss of
9p and/or deletion of the CDKN2A gene is associated with anaplastic features. For astrocytic tumors, loss of all or part of chromosome 10 and amplification of the CDK4 gene reflect progression to high-grade lesions. De novo glioblastomas, in contrast, are frequently characterized by gains of chromosome 7, losses of 9p and chromosome 10, deletions of the CDKN2A gene, and EGFR gene amplification but lack the other characteristics seen in oligodendrogial tumors and progressive astrocytomas.

Our findings also identify methods that are useful for demonstrating these molecular characterizations. Although LOH analysis is regarded as the gold standard for detecting loss of 1p and 19q in oligodendroglial neoplasms, we demonstrate an excellent correlation between loss of these chromosomal arms as detected by CGH and LOH, undoubtedly because the regions of loss are large. For 9p loss, the abnormalities were not reliably demonstrated by CGH, and 17p loss was seldom encountered in the CGH studies shown here, either because the lesions were too small to be seen by this method or because the mechanism for LOH may have been mitotic recombination. In view of these observations, the application of CGH to detect complete loss of 1p and 19q, along with LOH analysis to detect 9p and 17p loss, and SSCP with sequencing to demonstrate mutations of the TP53 gene and PCR studies to identify homozygous deletion of the CDKN2A gene might be useful in providing a reproducible means of categorizing these tumors. Furthermore, CGH has shown frequent losses of chromosomes 4, 14, 15, 18, and Y in subsets of tumors in this series. With long-term follow-up, it will be possible to determine whether these patterns of genetic progression are useful in predicting survival and responsiveness to therapy.

References

5. von Deimling A, Nage J, Bender B, Lenartz D, Schramm J, Louis DN,


