Chromosome 1p Allelic Loss by Fluorescence In Situ Hybridization Is Not Observed in Dysembryoplastic Neuroepithelial Tumors

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Key Words: Dysembryoplastic neuroepithelial tumor; Oligodendroglioma; Chromosome 1; Chromosome marker

Abstract

Differentiation of dysembryoplastic neuroepithelial tumor (DNT) from cystic low-grade oligodendroglioma, particularly in a limited biopsy or fragmented specimen, may be impossible. Research has shown that allelic loss of chromosome 1p is a relatively common finding in oligodendrogliomas. Little is known about chromosome 1p status in DNT. We retrospectively evaluated 14 DNTs for loss of heterozygosity (LOH) on chromosome 1p by fluorescence in situ hybridization (FISH) and compared the results with 1p FISH analysis in 57 low-grade oligodendrogliomas (World Health Organization grade II). The 14 DNTs arose in 8 females and 6 males (mean age, 20.9 years at the time of surgery). All 14 DNTs were 1p intact by FISH analysis. The 57 low-grade oligodendrogliomas arose in 31 males and 26 females (mean age, 43.2 years). LOH on chromosome 1p was present in 31 (54%) of 57 tumors; the remaining 26 tumors were 1p intact. LOH on chromosome 1p may be a useful differential diagnostic feature (favoring oligodendroglioma) in a subset of cases in which specimen fragmentation or size raises the differential diagnosis of DNT vs oligodendroglioma.

In 1988, Daumas-Duport et al described a pathologically unique lesion characterized by a predominant intracortical location, multinodular architectural pattern, and heterogeneity of cellular composition, which arose predominantly in young patients with intractable epilepsy. They coined the term dysembryoplastic neuroepithelial tumor (DNT) for the lesion. The importance of recognizing the lesion lies in its relatively benign clinical course (World Health Organization [WHO] grade I tumor). The lesion generally is amenable to surgical resection, with resolution of seizures. If completely excised, there is little risk for recurrence or spread. Microscopically, the tumor is marked by a predominance of oligodendroglial-like cells, typically arranged against a microcystic background. In the proper clinical setting with resection of an intact tumor, the diagnosis is relatively straightforward. Occasionally, however, small biopsy or fragmented specimens are received in pathology, and distinguishing the DNT from a microcystic, low-grade oligodendroglioma (WHO grade II) is problematic and may be virtually impossible to do with any great degree of certainty. The clinical ramifications of the distinction are important, given the more aggressive clinical behavior of oligodendrogliomas and the potential for malignant degeneration.

During the last few years, recognition of the association of certain genetic alterations with oligodendrogliomas has been elucidated and well documented in the literature. This most notably involves allelic loss on chromosomes 1p and 19q, which are present in the majority of oligodendrogliomas, and has been correlated with chemotherapeutic response and survival, particularly in anaplastic oligodendrogliomas (WHO grade III). The status of chromosome 1p in DNTs has not been examined. Theoretically, in a subset of cases, in which owing to limited histologic material the
definite distinction between oligodendroglioma and DNT cannot be made, evaluation of the biopsy specimen for allelic loss on chromosome 1p can be performed. If a loss is demonstrated, a diagnosis of oligodendroglioma may be favored, providing that the DNT does not demonstrate loss of heterozygosity (LOH) on chromosome 1p. We evaluated a series of DNTs with regard to allelic loss on chromosome 1p and compared the results with those for a control group of low-grade oligodendrogliomas (WHO grade II), which were similarly evaluated with respect to allelic loss on chromosome 1p, using fluorescence in situ hybridization (FISH).

**Materials and Methods**

The surgical pathology files were searched for cases in which a diagnosis of DNT or low-grade oligodendroglioma was made. All of the available pathologic material was reviewed in these cases to confirm the diagnosis. Cases in which material was limited and the diagnosis of DNT was questioned were excluded from the study. A total of 14 DNTs and 57 low-grade oligodendrogliomas were included in the evaluation.

For each tumor, a representative block of tumor was selected, and 4-µm-thick sections were cut on positively charged slides. One section was stained with H&E. On the H&E-stained section, an area of the slide containing tumor was identified for FISH analysis. The unstained slide was deparaffinized in xylene and then rehydrated in graded alcohols and distilled water. Cell conditioning was performed in a target retrieval solution (DAKO, Carpinteria, CA) at 90°C in a water bath for 40 minutes, followed by cooling at ambient temperature for 20 minutes, then Proteinase K (Roche, Indianapolis, IN) for 6 minutes at 25°C. Probes specific to the centromeric and telomeric portions of chromosome 1 were applied to the section, and then the DNA target was denatured at 90°C for 6 minutes on a heat block. A centromeric probe (spectrum orange–labeled CEP1 probe, Vysis, Downers Grove, IL) was applied at a dilution of 1:10 in hybridization buffer and allowed to hybridize to the pericentromeric region of chromosome 1 (1p12). A digoxigenin-labeled telomeric probe mapping to chromosome 1p36 (p58 clk-1 DNA probe, Ventana, Tucson, AZ) that identifies the cell division cycle 2-like 1 (CDC2L1 locus) gene also was applied. Both probes were hybridized at 37°C for 16 to 18 hours. The coverslip was removed by washing in 2× standard saline citrate (SSC), and excess probe was removed with 0.5× SSC stringency washes, followed by graded SSC stringency washes. The digoxigenin-labeled probe was visualized using fluorescein isothiocyanate (FITC)-antidigoxigenin (Roche). Nucleated cells were identified using 4',6-diamidino-2-phenylindole (DAPI) counterstain.

The area of interest on each slide after staining was analyzed, using an epifluorescent microscope equipped with DAPI/FITC/Texas red band-path filters (Axioplan 2, Zeiss, Gottingen, Germany). Forty cells containing a minimum of 2 centromeric signals were counted, and a ratio of telomeric to centromeric signals was determined. A telomeric/centromeric signal ratio of less than or equal to 0.7 was interpreted as representing loss of chromosome 1p36, ie, allelic loss on chromosome 1p. A telomeric/centromeric signal ratio of greater than or equal to 0.8 was interpreted as representing no evidence of allelic loss on chromosome 1p, ie, 1p intact. Results between the DNT group and the oligodendroglioma tumor group were compared.

**Results**

A total of 14 DNTs were evaluated by FISH. The tumors were present in 8 females and 6 males. At the time of surgery, the patients ranged in age from 8 to 52 years (mean, 20.9 years; median, 16 years). Eight tumors were located on the right side, and 5 were on the left. In 1 tumor, the laterality was not known. Eight tumors were situated in the temporal lobe, 4 in the occipital lobe, and 2 in the parietal lobe. All tumors demonstrated the typical histologic features of DNT: multinodular architectural pattern Image 2, predominant intracortical location, focally microcystic pattern Image 3, and a predominance of oligodendrogial-like cells with smaller numbers of intermixed neurons and astrocytic cells. There was minimal cytologic atypia in any of the cellular components of the tumor. Focally, a prominent arcuate, capillary vascular pattern was noted in most tumors, There was no evidence of vascular (endothelial) proliferative changes or necrosis. Rare mitotic figures were observed in 2 tumors. None of the 14 tumors demonstrated an allelic loss on chromosome 1p by FISH Image 4.

A comparison group of 57 low-grade oligodendrogliomas (WHO grade II) likewise were evaluated by FISH for LOH on chromosome 1p. The patients in this group included 31 males and 26 females ranging in age from 17 to 72 years at the time of surgery (mean, 43.2 years; median, 43 years). Twenty-eight tumors were situated on the right side, and 25 were on the left. In 4 tumors, the laterality was not known. Thirty-five tumors were located in the frontal lobe and 8 each in the parietal and temporal lobes. Two tumors arose in the occipital lobe, 1 in the thalamus, and 1 in the frontotemporal lobes. In 2 tumors, the site of origin was not known. Histologically, all tumors fulfilled criteria for a WHO grade II oligodendroglioma. Thirty-one tumors (54%) demonstrated an allelic loss on chromosome 1p by FISH analysis Image 5. The remaining 26 (46%) low-grade oligodendrogliomas were chromosome 1p intact.
Discussion

Distinction of the DNT from low-grade oligodendroglioma, which it most closely mimics, is important from prognostic and therapeutic standpoints. DNTs potentially are curable by surgery and have minimal risk of spread or metastasis, provided rigid diagnostic criteria are adhered to in making the initial diagnosis. There is no role for adjuvant radiation therapy or chemotherapy.1,2,11-17 In its classic presentation, distinction from the low-grade oligodendroglioma is relatively straightforward. The typical DNT arises in pediatric-age patients, who often have a long history of medically intractable epilepsy. The vast majority of tumors arise in the temporal lobes; however, extratemporal DNTs are well-described, albeit less frequent, occurrences. In contrast, low-grade oligodendrogliomas...
typically arise in adults and are distinctly uncommon in the pediatric-age population. Oligodendrogliomas traditionally are considered to arise from white matter oligodendrocytes, and their distribution correlates roughly with the amount of white matter in various regions of the brain; therefore, the frontal lobe with the most white matter is the most common site of origin. Histologically, the typical DNT has a multinodular architectural pattern, with the bulk of the tumor located in the cortical region, in contrast with the uninnodular, predominantly white matter–based oligodendroglioma. Classically, DNTs are marked by microcystic change and consist of cells of multiple lineages, ie, oligodendroglial-like cells, astrocytes, and neuronally differentiated cells.18-21 Another distinctive feature of the DNT lesion is the coexistence of cortical dysplasia, marked by a malformative disorganization of the cortical architecture adjacent to the tumor.14,15,17,22 The coexistence of cortical dysplasia and a DNT has prompted some to suggest that the DNT lesion may have a malformative basis to its origin.

Despite the aforementioned salient characteristics of the DNT lesion, its distinction from low-grade oligodendroglioma is not always easy. A number of overlapping histologic features, under certain conditions, may make distinguishing one tumor from the other difficult, particularly when dealing with limited pathologic material. Small needle biopsy specimens or markedly fragmented specimens may make it impossible to assess whether the tumor is predominantly cortically based or multinodular. A subset of oligodendrogliomas will demonstrate microcystic change, similar to the DNT. By nature, oligodendrogliomas are infiltrative, and extension into the overlying cortex is not unusual. Micocalcifications and an arcuate or vascular pattern, which are features present in the vast majority of oligodendrogliomas, may be evident in DNT lesions. Both lesions are marked by low levels of cell proliferation, although cell proliferation indices tend to run slightly higher among low-grade oligodendrogliomas than among DNTs. Vascular proliferative changes and necrosis are not typical features of either low-grade oligodendroglioma or DNT. Minigemistocytic cells (signet-ring cell oligodendrocytes) may be a prominent component in a subset of oligodendrogliomas. Small gemistocytic-type cells also may be present focally in DNTs.

Besides a morphologic basis, there have been no significant inroads made in terms of immunohistochemical analysis or ultrastructural examination for distinguishing DNTs from low-grade oligodendrogliomas. Work examining the molecular genetics of oligodendrogial neoplasms has noted that a substantial number of oligodendrogliomas demonstrate LOH on chromosomes 1p and 19q. 1p loss has been described in variable numbers of oligodendrogliomas, ranging from 40% to 92%; likewise, 19q loss has been described to occur in 50% to 80% of oligodendrogliomas.23-30 Smith et al4 recently evaluated LOH on chromosome 1p, as determined by FISH, was observed in 6 tumors (38%). In the present study, we restricted 1p analysis by FISH to 57 low-grade oligodendrogliomas, since anaplastic oligodendrogliomas generally are not a differential diagnostic consideration with DNTs owing to their marked hypercellularity, nuclear atypia, and increased mitotic activity. LOH on

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<th>Feature</th>
<th>Dysembryoplastic Neuroepithelial Tumor</th>
<th>Low-Grade Oligodendroglioma</th>
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<td>Age</td>
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<tr>
<td>Location</td>
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<td>Arcuate vascular pattern</td>
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<tr>
<td>Mitoses</td>
<td>Rare</td>
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<td>Malignant progression</td>
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<td>Most (54% [31/57] in the present series)</td>
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chromosome 1p was noted in 54% (31/57) of the low-grade oligodendrogliomas in the present study, a figure similar to that recorded by Smith et al.4

To our knowledge, evaluation of LOH on chromosome 1p has not been reported in DNTs. None of the 14 DNTs evaluated for LOH on 1p in the present study showed evidence of deletion. Consequently, it seems that DNTs are different cytogenetically from oligodendrogliomas with respect to allelic loss on chromosome 1p. From a practical viewpoint, this information may be useful in evaluating a subset of cases in which the differential diagnosis of low-grade, microcystic oligodendroglioma vs DNT comes into question. In such lesions, in which many of the characteristic features of DNT are not readily identifiable and a diagnosis of low-grade oligodendroglioma also is being considered, evaluation for LOH on chromosome 1p may be helpful, particularly if the tumor shows evidence of allelic loss on 1p. In cases in which there is no evidence of LOH on chromosome 1p, the 1p analysis is not informative, in that a subset of oligodendrogliomas, like DNT, are 1p intact. In such cases, a description of the lesion with indication of the differential diagnosis is appropriate.

References


