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Differentiation between reactive gliosis and diffuse astrocytoma by in situ hybridization

Article abstract—The authors examined the use of chromosomal analysis by in situ hybridization to differentiate between nonneoplastic reactive gliosis and astrocytomas in cases in which routine histology was inconclusive. Numerical chromosomal aberrations were found in 80% of low-grade astrocytoma specimens and in none of the reactive gliosis specimens. Aneusomic tumor cells were detected in four of 13 stereotactic samples with an initially inconclusive tissue diagnosis, three of which were later diagnosed as astrocytoma. The in situ hybridization procedure may have additional value in the differential diagnosis of reactive gliosis versus low-grade astrocytoma.

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Stereotactic biopsy specimens from patients with low-grade diffuse astrocytomas may result in inconclusive diagnoses because of small sample size and histopathologic heterogeneity.¹ Attempts to differentiate between a nonneoplastic and a neoplastic cause of astrocytic proliferation have used a range of methods, such as the intra-operative cytologic wet smear technique and additional immunohistochemical assays for p53 and Ki-67 antigen.

Conventional cytogenetic procedures can also be of additional value in the differentiation between reactive gliosis and astrocytoma. However, most of these techniques only detect sporadic chromosomal aberrations in low-grade astrocytoma, besides loss on chromosome arm 17p and mutations of the p53 gene.² The in situ hybridization (ISH) technique allows a more frequent detection of chromosomal aberrations in astrocytomas.^{3,4}

The purpose of the current study was to determine whether or not the ISH technique offers additional diagnostic value by detecting tumor cells in areas of low-grade astrocytomas that are not typical enough

to allow a morphologic diagnosis. The ISH results were compared with the tumor cell proliferation (Ki-67) index, which is considered to have additional value in the differentiation between nonneoplastic and neoplastic astrocytic proliferation.⁵

Materials and methods. *Tissue material.* The formalin-fixed, paraffin-embedded archival material consisted of three groups of tissue samples: reactive gliosis from patients who died from a nonneoplastic neurologic cause (n = 10), low-grade diffuse astrocytomas (grade 2) (n = 20), and stereotactic biopsy specimens with an inconclusive tissue diagnosis, i.e., no differentiation between nonneoplastic reactive gliosis and low-grade astrocytoma (n = 13).

Methods. In situ hybridization was performed following a recently optimized protocol.⁶ The probes were specific for the heterochromatin (sub)centromere region of chromosome 1 (1q12, pUC 1.77), the alphoid region of chromosome 7 (p7t1), and the centromere region of chromosome 10 (D10Z1). After overnight hybridization, the biotin labeled DNA probes were indirectly detected by a peroxidase precipitation reaction. Diaminobenzidine was applied for visualization of the peroxidase activity. Nuclear counterstaining was done with hematoxylin or alternatively with 4,6-diaminophenylindol (DAPI). Monosomy was defined as >25% of nuclei with no or only one signal per nucleus. Nuclei were classified trisomic if >5% of nuclei contained three or more signals. Nuclei containing four or more signals were defined as tetrasomic and polysomic.

The proliferation status was assessed as the Ki-67 immunostaining index, using the mouse monoclonal MIB-1 antibody (Immunotech S.A., Marseille, France) after antigen retrieval by treatment with citrate buffer in a domestic microwave oven for 10 minutes at 700 W.

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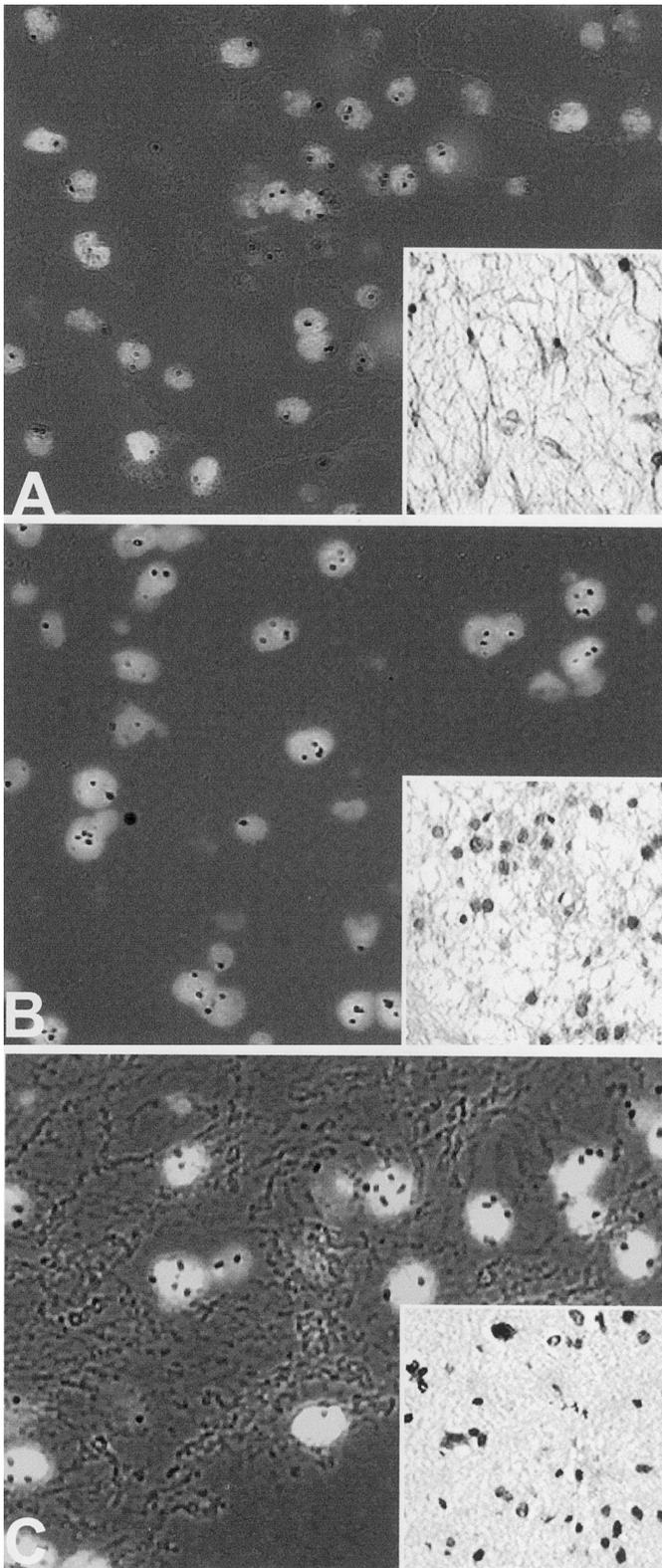


Figure. Routine microscopy and in situ hybridization (ISH) with a probe for chromosome 7 in samples from the investigated groups. No numerical chromosomal aberrations are present in the sample with nonneoplastic reactive gliosis (A), whereas in both the low-grade astrocytoma (B) and the sample with an inconclusive tissue diagnosis (C) frequent nuclei with three (trisomy), four (tetrasomy), and more than four signals (polysomy) are present. Microscopy before reduction: $\times 200$; ISH: (A, B) $\times 800$, (C) $\times 1,000$.

Results. *Reactive gliosis.* All evaluable cases (87%) of reactive gliosis revealed disomy for all three chromosomes (figure, A). Very sporadically, nuclei with three signals were seen, but none of the samples met the criteria for trisomy. In one sample with reactive gliosis the Ki-67 labeling index was 3.5%, whereas in the other samples it did not exceed 1% (table 1).

Low-grade astrocytomas (grade 2). The mean age of the patients with low-grade astrocytomas was 39.9 years (range: 24 to 68 years) and the median interval to tumor progression was 35 months. Numerical chromosomal aberrations for one or more of the chromosomes investigated were present in 80% of the low-grade astrocytomas: in 60% for chromosome 1, in 65% for chromosome 7, and in 50% for chromosome 10 (see table 1). Only one sample showed monosomy for chromosomes 1 and 7. All other aberrations comprised trisomy, tetrasomy, and polysomy (see the figure, B). The mean Ki-67 labeling index in the astrocytomas was 2.1% (range <1 to 9%).

Cases with inconclusive diagnosis. All samples with inconclusive tissue diagnosis were stereotactic biopsy specimens in which it was not possible to distinguish between reactive gliosis and diffuse astrocytoma on the basis of routine histology. Revision of one sample, derived from a patient with a suspected recurrent astrocytoma after radiotherapy, revealed endothelial cell proliferation and necrosis, which we considered to be nonneoplastic radiation damage. In all other samples neuropathologic revision agreed with the initial inconclusive diagnosis. On the basis of follow-up data, the samples could be divided into three groups, for which ISH results and Ki-67 labeling indices are shown in table 2.

1. Samples in which astrocytoma was diagnosed at a later stage (Cases 1 to 7): In three of these samples (Cases 1 to 3) the primary biopsy already showed numerical chromosomal aberrations (figure, C). Ki-67 labeling indices of these samples were 1, 10, and 5%. In Case 7 no confirmation of malignancy was obtained but higher-grade astrocytoma was diagnosed by combining histopathology and clinical and radiologic progression of the space-occupying lesion.

2. Samples in which the follow-up period was too short and in which a second biopsy or resection was deferred (Cases 8 to 11): In Case 8, the primary biopsy showed numerical chromosomal aberrations for chromosomes 1 and 7. The Ki-67 labeling index of this sample was less than 1%.

3. Samples in which a nonneoplastic diagnosis was made at a later stage (Cases 12 to 13): Case 12 showed no numerical chromosomal aberrations, and Case 13 was not evaluable by ISH due to cell lysis.

Discussion. The current study showed that in some cases with inconclusive histologic diagnoses the ISH technique allows differentiation between reactive gliosis and diffuse astrocytoma. Because the distinction between these two histomorphologic entities is often difficult, this finding has potential clinical relevance.

None of the samples with reactive gliosis showed chromosomal aberrations, whereas 80% of the samples containing evident low-grade astrocytomas were classified as genetically aberrant for chromosomes 1,

Table 1 Summarized results of numerical chromosomal aberrations and Ki-67 labeling index (LI) for the three investigated patient groups

Diagnosis	N	Number of samples with numerical chromosomal aberrations (%)			Aberrant for one or more chromosomes (%)	Ki-67 LI, % cells (range)
		Chr. 1	Chr. 7	Chr. 10	Chr. 1, 7, and/or 10	
Gliosis	10	0	0	0	0	<1 (<1-3)
Astrocytoma	20	12 (60)	13 (65)	10 (50)	16 (80)	2.1 (<1-9)
Inconclusive	13	3 (23)	4 (31)	1 (8)	4 (31)	2 (<1-10)

Chr. = chromosome.

7, and/or 10. The absence of numerical chromosomal aberrations in the nonneoplastic reactive gliosis corroborates the findings of the only previous ISH study in gliotic brain tissue.⁷

The ISH technique is more efficient in finding chromosomal aberrations in astrocytomas than classic karyotyping or loss of heterozygosity studies because all interphase cells can be evaluated. Earlier ISH studies on small series of low-grade astrocytomas have already revealed hyperdiploid cells, gain of chromosome 7, loss of chromosome 10, and aberrations of chromosomes 1 and 17.^{3,4,8,9} Using three chromosome probes with single-target ISH analysis allowed us to detect high percentages of aneusomic cells in most low-grade astrocytomas.

ISH detected aneusomies in four samples with an initial inconclusive tissue diagnosis. Of these four cases, three showed rapid progression to high-grade astrocytoma. However, this may also suggest that

these patients, whose differential tissue diagnosis was a choice between reactive gliosis and low-grade astrocytomas, most probably had higher-grade astrocytomas, which were not detected at first diagnosis because of sampling errors. In the fourth case, no progression was seen after 4 months of follow-up. Because of the high frequency of genetic aberrations in this case, the patient is now being clinically monitored on a regular basis.

Our findings indicate that the detectability of genotypic alterations extends well beyond the histomorphologic border of astrocytomas.

Two of the four inconclusive cases had high Ki-67 labeling indices, which supports the highly malignant character of these lesions. However, the two other lesions had low Ki-67 levels, suggesting that this marker has a lower sensitivity for the detection of lesions with a malignant clinical course than the ISH technique.

Table 2 *In situ* hybridization results and Ki-67 labeling index in samples with uncertain tissue diagnosis

Case No.	Numerical chromosome aberrations (%)			Ki-67 LI, % cells	Follow-up, mo.
	Chromosome 1	Chromosome 7	Chromosome 10		
1	Tri (10%)	Tri (10%)	Tri (5%)	5	Malignant progression Postmortem: GBM, 10
2	Tri (21%)	Tri (19%), Tetra (11%), Poly (1%)	D	1	Resection: AA, 5
3	D	Tri (25%)	D	10	Second biopsy: GBM, 2
4	D	D	D	<1	Resection: AA, 4
5	D	D	D	<1	Second biopsy: GBM, 1
6	D	D	NE	<1	Second biopsy: GBM, 40
7	D	D	D	4	MRI, 35
8	Tri (10%)	Tri (32%), Tetra (9%)	NE	<1	No progression in time followed 4
9	D	D	NE	<1	20
10	D	D	D	<1	6
11	D	D	D	<1	22
12	D	D	D	<1	Nonmalignant diagnosis Multiple sclerosis
13	NE	NE	NE	NE	Radiation necrosis

D = disomy; Tri = trisomy; Tetra = tetrasomy; Poly = polysomy; NE = not evaluable; AA = anaplastic astrocytoma; GBM = glioblastoma; MRI = progression on MRI; Ki-67 LI = Ki-67 labeling index.

Because the numbers in the investigated groups were relatively small, the role that chromosomal abnormalities can play in the diagnosis of astrocytomas needs confirmation in a larger series of cases.

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Guillain-Barré syndrome associated with IgG monospecific to ganglioside GD1b

Article abstract—The authors examined serum antiglycolipid antibodies in 445 patients with Guillain-Barré syndrome (GBS). Among them, nine had anti-GD1b IgG antibodies with no reactivity to other glycolipids tested. All those patients had sensory disturbance, and none had the primary axonal form. Anti-GD1b IgG antibodies may bind to primary sensory neurons and paranodal myelin, where GD1b is localized, and be involved in the pathogenesis of sensory disturbance and demyelination. However, more study is needed to substantiate the roles of anti-GD1b IgG antibodies.

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Guillain-Barré syndrome (GBS) is an acute motor-dominant inflammatory neuropathy. A significant increase in the titers of various kinds of antiganglioside antibodies in the acute phase sera from patients with this syndrome has been reported. The relationship between presence of antiganglioside antibodies and a certain clinical feature has been discussed.

Ganglioside GD1b is localized in dorsal root ganglia (DRG) neurons and in the paranodal myelin of human peripheral nerve.¹ Three patients with GBS, exhibiting an increase in the level of the monospecific anti-GD1b IgG antibody, have been reported to

show clinical characteristics of acute cerebellar or sensory ataxia.²⁻⁴ One patient with postinfection sensory neuropathy and IgG anti-GD1b antibody also has been reported.⁵ However, no report on clinical features of GBS based on a large population of GBS patients with the monospecific anti-GD1b IgG antibody has been published.

Methods. *Samples.* Serum samples from patients fulfilling the criteria for GBS proposed by Asbury and Cornblath⁶ were obtained from several hospitals throughout Japan between December 1992 and June 1999. Serum samples from cases showing progression of GBS beyond 4 weeks, marked laterality of limb muscle weakness, or high CSF cellular count (more than 50) were excluded from the study. Antibody immunoreactivities against gangliosides (GM1, GM2, GM3, GD1a, GD1b, GalNAc-GD1a, GM1b, GD3, GT1b, GQ1b), GA1, and galactocerebroside were assayed in our laboratory.⁷

Patient group. We investigated clinical features such as clinical course, symptoms, and electrophysiologic findings of patients with GBS whose sera had anti-GD1b IgG

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