Immunohistochemical staining for ganglioside GD1b as a diagnostic and prognostic marker for primary human brain tumors¹

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Immunohistochemical staining intensity for ganglioside GD1b was determined for 108 human neuroectodermal tumors. Most of the tissue elements that immunostained were tumor cells; only a few axons and occasional neurons reacted in some specimens. All pilocytic astrocytomas stained very positively, whereas none of the ependymomas and only 11% of primitive neuroectodermal tumors, 20% of glioblastomas, and 28% of anaplastic astrocytomas showed more than faint staining. A similar association between grade and immunostaining was seen in tumors containing an oligodendrogliomatous component, but reactivity was not as strong as in astrocytic tumors or primitive neuroectodermal tumors. Results of Cox regression showed significant associations between immunostaining intensity and survival for all cases taken together ($P = 0.007$); for the group consisting of astrocytomas, oligoastrocytomas, and oligodendrogliomas ($P = 0.002$); and for astrocytomas alone ($P = 0.04$). Results were also significant using a proportional hazards model controlling for patient age (all cases $P =$

0.005; astrocytomas only $P = 0.02$), but not when controlling for tumor grade. Our results indicate that immunohistochemical staining for GD1b is correlated with tumor grade and that it may be of prognostic utility in some primary human brain tumors, especially astrocytomas. *Neuro-Oncology* 1, 261–267, 1999 (Posted to *Neuro-Oncology* [serial online], Doc. 98-27, September 9, 1999. URL <neuro-oncology.mc.duke.edu>)

Systems of classification and grading of human gliomas are currently based on their histologic features. Within each category and grade, gliomas with classic features are easily recognized. Within all categories and grades, however, there are ranges of histologic appearance, the aspects of which may merge imperceptibly, being sometimes placed in one category or grade, and sometimes in another. Thus, a diagnosis often rests upon the subjective interpretation of clusters of histologic features about which even experts can disagree. For this reason, considerable effort has been expended to identify diagnostic and prognostic molecular markers of greater objectivity in glioma diagnosis.

Gangliosides are sialic acid-containing complex glycosphingolipids present in all mammalian tissues studied to date and are potentially useful in tumor diagnosis. Gangliosides are classified according to the sequence and linkages of neutral sugars, amino sugars, and sialic acids in the oligosaccharide portion of the molecule (Ledeen and Yu, 1982). The oligosaccharide is synthesized in the Golgi complex, with several metabolic pathways leading to different families of gangliosides that are named (Chester, 1998; Svennerholm, 1980) on the basis of their oligosac-

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³Abbreviation used is as follows: TBS, Tris-buffered saline.

Table 1. Ganglioside structure and most commonly used systems of nomenclature

Svennerholm ^a	IUPAC-IUB ^b	Schematic structure
GM3	II ³ NeuAc-LacCer	NeuAcα2→3Galβ1→4Glcβ1→1Cer
GM2	II ³ NeuAc-GgOse ₃ Cer	GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GM1, GM1a	II ³ NeuAc-GgOse ₄ Cer	Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GM1b	IV ³ NeuAc-GgOse ₄ Cer	NeuAcα2→3Galβ1→3GalNAcβ1→4Galβ1→4Glcβ1→1Cer
GD3	II ³ (NeuAc) ₂ -LacCer	NeuAcα2→8NeuAcα2→3Galβ1→4Glcβ1→1Cer
GD1a	IV ³ NeuAc, II ³ NeuAc-GgOse ₄ Cer	NeuAcα2→3Galβ1→3GalNAcβ1→4(NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GD2	II ³ (NeuAc) ₂ -GgOse ₃ Cer	GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GD1b	II ³ (NeuAc) ₂ -GgOse ₄ Cer	Galβ1→3GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GT1b	IV ³ NeuAc, II ³ (NeuAc) ₂ -GgOse ₄ Cer	NeuAcα2→3Galβ1→3GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glcβ1→1Cer
GQ1b	IV ³ (NeuAc) ₂ , II ³ (NeuAc) ₂ -GgOse ₄ Cer	NeuAcα2→8NeuAcα2→3Galβ1→3GalNAcβ1→4(NeuAcα2→8NeuAcα2→3)Galβ1→4Glcβ1→1Cer

^aSvennerholm, 1980.

^bChester, 1998.

charide sequences (Table 1). One of the major families of gangliosides in normal adult brain, the “1b” family, is derived from a parent compound called GD1b. The latter accounts for most of the 1b family gangliosides in normal human brain (Vanier et al., 1971). The other major 1b gangliosides in human brain include GT1b, which has one sialic acid attached to the GD1b structure, and GQ1b, which has two additional sialic acids (Wiegandt, 1982). A consistent finding in studies of gangliosides in human astrocytomas is a shift in the pattern of gangliosides, resulting in a relative loss of those with complex oligosaccharides and an increase in those that have simpler oligosaccharides (Berra et al., 1985; Fredman et al., 1988; Jennemann et al., 1990; Kostic and Bucheit, 1970; Sung et al., 1993; Traylor and Hogan, 1980). In previous studies, we found that loss of the 1b gangliosides was associated with higher histologic grade and poorer survival (Sung et al., 1993; 1994). There were two possible interpretations of this finding. One was that, within the lower grade gliomas, the 1b gangliosides are located within tumor cells, but those gangliosides are not expressed in the higher grade tumors. The other interpretation was that the loss of 1b gangliosides from the higher grade tumors is simply due to destruction by invading tumor cells of nonneoplastic cellular elements normally containing 1b gangliosides. The present study was designed both to resolve this issue and to determine whether an immunohistochemical assay for the major 1b ganglioside in brain (GD1b) could help in the classification, grading, and prognosis of human gliomas.

Methods

Tumor Tissues

Tumor samples were obtained from patients within 30 min of surgical removal, examined grossly, and divided into pieces for diagnostic and research purposes. Those samples intended for immunohistochemical staining were snap frozen by placing the tissue in a cryomold (Tissue-Tek, Miles Inc., Elkhart, Ind.) filled with OCT (Shandon, Pittsburgh, Penn.) and immersing it into isopentane

(2-methyl butane, Fisher Scientific, N.J.) in the vapor phase of liquid nitrogen. The histologic diagnoses and grades assigned represented the majority opinions of three neuropathologists (B.W.S., P.C.B., and A.J.Y.) who independently reviewed hematoxylin and eosin-stained sections that had been formalin fixed, routinely processed, and paraffin embedded.

Immunohistochemical Procedure

Tissues were sectioned on a cryostat (−20°C) at 5-μm thickness and placed on Superfrost Plus slides (Fisher Scientific, Pittsburgh, Penn.). For each sample three sections were cut: one for the assay, another as a negative control, and the last for a hematoxylin and eosin stain as a control for tumor content. After the slides were air dried, they were fixed in cold acetone at −20°C for 3–4 min, allowed to dry for 2 h, and rehydrated with 0.05 M Tris-HCl (pH = 7.6, TBS³) for 4 min at room temperature. Nonspecific binding was blocked with 10% normal horse serum (Vector Labs, Burlingame, Calif.) for 30 min at room temperature. Primary antibody GGR12 (Ozawa et al., 1992) was added undiluted, and the sections were incubated in a humid chamber overnight at 4°C (average 16–18 h). As a negative control, a similar isotype antibody (mouse IgG2b, Dako, Carpinteria, Calif.) was used at a 3-μg/ml concentration. Primary and control antibodies were removed and the slides washed in TBS for 4 min. The secondary antibody (horse antimouse biotin conjugated) was then applied at a dilution of 1:200 (2–4 μg/ml) in a 2% normal horse serum in TBS for 30 min at room temperature in a moist chamber before being washed in TBS for 4 min. Endogenous peroxidase activity was blocked with a 2.25% peroxide in methanol solution for 30 min at room temperature and washed; avidin:biotin complexed horseradish peroxidase (Vector Elite Kit, Burlingame, Calif.) was added for 30 min at room temperature. The samples were then washed in TBS and placed into a 0.02 M acetate buffer for 5 min before transfer into 3-amino-9-ethylcarbazole. The reaction was started by adding 20 ml of a 3% hydrogen peroxide solution to the 3-amino-9-ethylcarbazole substrate,

Table 2. Distribution of staining intensities among diagnostic categories

Staining intensity ^a	Astrocytoma	Oligo	Anaplastic oligo	Ependymoma	PNET	Total
0	35 (63.6) (52.2)	0 (0.00) (0.00)	5 (9.09) (33.3)	7 (12.7) (100)	8 (14.5) (88.9)	55 (100) (50.9)
1	9 (64.3) (13.4)	1 (7.14) (10.0)	4 (28.6) (26.7)	0 (0.00) (0.00)	0 (0.00) (0.00)	14 (100) (13.0)
2	14 (50.0) (20.9)	9 (32.1) (90.0)	4 (14.3) (26.7)	0 (0.00) (0.00)	1 (3.57) (11.1)	28 (100) (25.9)
3	9 (81.8) (13.4)	0 (0.00) (0.00)	2 (18.2) (13.3)	0 (0.00) (0.00)	0 (0.00) (0.00)	11 (100) (10.2)
Total	67 (62.0) (100)	10 (9.26) (100)	15 (13.9) (100)	7 (6.48) (100)	9 (8.33) (100)	108 (100) (100)

Abbreviations: Oligo, oligodendroglioma; PNET, primitive neuroectodermal tumor.

Numbers in **bold** are the number of cases. Numbers in the top parentheses are percentage of tumors expressing a particular staining intensity for GD1b (percent of row). Numbers in the bottom row of parentheses are the percentage of specific tumor types expressing a particular staining intensity for GD1b (percent of column).

^aA staining intensity of 0 is equivalent to negative control. A staining intensity of 3 is equivalent to positive control (rat cerebral cortex).

and coloration was allowed to develop for 16 min at room temperature. The slides were then washed briefly with acetate buffer, placed under warm tap water for 5 min, counterstained with Gill's #1 solution (Fisher Scientific, N.J.) for 1 min, washed with tap water, fixed briefly with 0.01 M Tris (pH = 9.5), washed with double distilled water, and coverslipped with warmed glycerin jelly.

Staining Intensity

Two investigators (T.C.C. and A.J.Y.) independently examined the slides and expressed the staining intensity on a 4-tiered scale. Staining intensity of the tumor samples was related to two controls run with each assay. Staining was 3+ when it was equivalent to the intensity of the staining observed in rat cerebral cortex. Tissue that was similar to the negative controls was considered to be 0 staining. Intermediate staining was considered to be either 1+ (mild) or 2+ (moderate). Interobserver agreement was over 90%; disagreements were resolved by subsequent simultaneous viewing through a double-headed microscope.

Ganglioside Analyses

Tissues were extracted first with chloroform/methanol/water (1:2:20%) and then with chloroform/methanol/water (1:1:5%). The supernatants were pooled and reconstituted in chloroform/methanol/water (30:60:8), after which acidic glycolipids were isolated using a DEAE-Sephadex column (Ledeen et al., 1973). Gangliosides were purified, separated using thin-layer chromatography, and quantitated using the methods described by Sung et al. (1994).

Results

A total of 108 tumors were examined immunohistochemically for GD1b. The number of tumors in each

major diagnostic category is shown in Table 2. Pilocytic astrocytomas stained quite positively in both a cytoplasmic and surface pattern. In the few cases of glioblastoma multiforme that were positive, there was staining of both the scanty cytoplasm and the surface membrane of tumor cells. Typical examples of positive staining in a pilocytic astrocytoma and a negative reaction in a glioblastoma multiforme are shown in Fig. 1. The distribution of staining intensities among the major diagnostic categories is shown in Table 2 and among the four grades of astrocytomas in Table 3. All of 11 pilocytic (grade 1) astrocytomas exhibited reactivities of either 2+ or 3+. Five of 7 anaplastic (grade 3) astrocytomas had either no staining or only 1+ staining of the tumor cells; as a rule most anaplastic regions were nonstaining. Of the 47 glioblastomas multiforme, 32 (68%) were negative and none had 3+ staining. Whereas 9 of 10 oligodendrogliomas and oligoastrocytomas (90%) stained 2+, fully 9 of 15 anaplastic oligodendrogliomas and anaplastic oligoastrocytomas (60%) stained either 1+ or were negative. Only 1 of 9 primitive neuroectodermal tumors and none of 7 ependymomas contained any immunopositive tumor cells. In all cases studied, positive immunostaining occurred mainly in the tumor cells; at most, only scant, infiltrated normal neural elements (axons and neurons) in the tumor tissue stained positively. This reflects the care that was taken at the time of tissue dissection to select portions of solid tumor.

To determine the validity of the immunohistostaining intensities, we plotted these semiquantitative values against the percentage of GD1b contained in the total amount of ganglioside, measured chemically, in fresh tissues of the same cases. These measurements will form the basis of a separate report. Fig. 2 shows that, for all tumors, regardless of diagnosis, there was a good correlation between these two estimates of GD1b content. Correlation was even better for the 52 astrocytomas studied ($P < 0.005$). However, in no group did tumors

Fig. 1. Immunohistochemical staining for GD1b in a pilocytic astrocytoma (A,B) and a glioblastoma multiforme (C,D). A and C are positive staining with anti-GD1b; B and D are negative controls using the same isotype (IgG2b) antibody directed against a nonrelevant antigen. X50.

with intensities 1+ and 2+ separate well. Thus for this data set, no information would be lost in using an alternative 3-tiered system that combines 1+ and 2+ into a single intermediate group. An analysis of variance (ANOVA) comparing the percentages of GD1b in a 3-tiered system showed that the staining intensity levels were all significantly separated using the least significant difference post hoc test (low to intermediate, $P = 0.005$; intermediate to high, $P = 0.06$; low to high, $P = 0.0004$).

Taking all cases together, we found a significant ($P = 0.007$) association between intensity of immunostaining for GD1b and survival of patients in a univariate analysis using the Logrank test (Fig. 3). Similar results were obtained when analyzing data inclusive of astrocytomas, oligoastrocytomas, and oligodendrogliomas ($P = 0.001$) (Fig. 4), as well as data limited to astrocytomas ($P = 0.04$) (Fig. 5). Results were also significant in the multivariate analyses controlling for patient age with the Cox proportional hazards regression method (all cases, $P = 0.004$; astrocytoma plus oligoastrocytoma plus oligodendroglioma cases, $P = 0.02$; astrocytomas only, $P = 0.02$). Using Cox regression, however, we found no significant difference when tumor grade was also added into the model.

Table 3. Frequency of staining intensities for all grades of astrocytoma

Staining intensity ^a	Histologic grade				Total
	1	2	3	4	
0	0 (0.00) (0.00)	1 (2.86) (50.0)	2 (5.71) (28.6)	32 (91.4) (68.1)	35 (100) (52.2)
1	0 (0.00) (0.00)	0 (0.00) (0.00)	3 (33.3) (42.9)	6 (66.7) (12.8)	9 (100) (13.4)
2	4 (28.6) (36.4)	0 (0.00) (0.00)	1 (7.14) (14.3)	9 (64.3) (19.1)	14 (100) (20.9)
3	7 (77.8) (63.6)	1 (11.1) (50.0)	1 (11.1) (14.3)	0 (0.00) (0.00)	9 (100) (13.4)
Total	11 (16.4) (100)	2 (2.99) (100)	7 (10.4) (100)	47 (70.1) (100)	67 (100) (100)

Abbreviations: Oligo, oligodendroglioma; PNET, primitive neuroectodermal tumor.

Numbers in **bold** are the number of cases. Numbers in the top parentheses are percentage of tumors expressing a particular staining intensity for GD1b (percent of row). Numbers in the bottom row of parentheses are the percentage of specific tumor types expressing a particular staining intensity for GD1b (percent of column).

^aA staining intensity of 0 is equivalent to negative control. A staining intensity of 3 is equivalent to positive control (rat cerebral cortex).

Fig. 2. Correlation between chemical and immunohistochemical estimates of GD1b in tumors. The box plot shows GD1b as a percent of total gangliosides determined chemically versus the intensity of immunohistochemical staining for GD1b. The box frames the upper and lower quartiles. The whiskers show the range of the contiguous data. Outliers are displayed individually. The shaded regions give a 95% confidence interval for the median.

Discussion

Histologic grading of astrocytomas and oligodendrogliomas is based on the expression of several of the features of anaplasia. Early methods included those of Kernohan et al. (1949) and Ringertz (1949), who described four- and three-tiered systems, respectively, for grading astrocytomas. These were subjective in nature and were based on finding a composite of several of these features. Daumas-Duport proposed a more objective system for grading astrocytomas in which the presence or absence of four histologic features (nuclear atypia, mitotic figures, endothelial proliferation, and necrosis) were determined; the number of positive features were totaled to generate a grade (Daumas-Duport, 1992; Daumas-Duport et al., 1988). Referred to as the St. Anne-Mayo method, this system correlates quite well with the survival of patients with diffuse or fibrillary astrocytomas. In the World Health Organization's classification of CNS tumors, pilocytic astrocytoma is considered to be grade I on the basis of its biological behavior (Kleihues et al., 1993, p. 112). However, pilocytic astrocytomas may show histologic features, such as occasional mitotic figures, vascular proliferation, and necrosis, which in fibrillary astrocytomas would increase the grade to III or even IV. This accounts for the frequency with which some low-grade tumors are misdiagnosed as high-grade (Finlay et al., 1995). Therefore, it is critically important to identify pilocytic astrocytomas so the St. Anne-Mayo or World Health Organization grading system is not inappropriately applied. The availability of a marker that would aid the identification of pilocytic astrocytomas would be extremely useful, especially to pathologists less familiar with this entity. In our study, all 14 pilocytic

Fig. 3. Relationship between staining intensity and survival for all cases studied. Survival data were available for 8 of 11 patients whose tumors stained at level 3 intensity (1 of 8 patients died); for 25 of 28 patients whose tumors stained at level 2 intensity (7 of 25 died); for 13 of 14 patients whose tumors stained at level 1 intensity (5 of 13 died); and for 52 of 55 patients whose tumors stained at level 0 intensity (38 of 52 died).

astrocytomas immunostained either 2+ or 3+ for GD1b. In contrast, no glioblastomas multiforme stained 3+, and fully 84% showed only faint reactivity or no reactivity at all. Unfortunately, staining of anaplastic astrocytomas was distributed across all levels of intensity.

There are changes in the ganglioside composition of normal human brain with development and aging. On the basis of ganglioside sialic acid, the proportion of total ganglioside accounted for by GD1b progressively increases from approximately 10% at birth to 30% in adults (Segler-Stahl et al., 1983; Vanier et al., 1971). This is the inverse of what we found for the relation between

Fig. 4. Relationship between staining intensity and survival for astrocytomas, oligoastrocytomas, and oligodendrogliomas. Survival data were available for 8 of 11 patients whose tumors stained at level 3 intensity (1 of 8 patients died); for 24 of 27 patients whose tumors stained at level 2 intensity (7 of 24 died); for 13 of 14 patients whose tumors stained at level 1 intensity (5 of 13 died); and for 37 of 40 patients whose tumors stained at level 0 intensity (33 of 37 died).

Fig. 5. Relationship between staining intensity and survival for astrocytomas. Survival data were available for 7 of 9 patients whose tumors stained with level 3 intensity (1 of 7 patients died); for 11 of 14 patients whose tumors stained for level 4 intensity (4 of 11 died); for 8 of 9 patients whose tumors stained at level 1 intensity (4 of 8 died); and for 32 of 35 whose tumors stained at level 0 intensity (30 of 32 died).

intensity of immunostaining for GD1b in tumors and the age of the patients harboring them. The median age for tumors with no immunostaining for GD1b was 52 years and for those with 3+ staining was 13 years; those with intermediate intensities of staining were 34 and 39 years. Therefore, the differences in immunostaining intensities are not due to age-related differences in ganglioside composition of residual brain.

No universally accepted system has emerged for grading oligodendrogliomas, although several have been proposed. Based on the overall degree of anaplasia, the World Health Organization system divides oligodendrogliomas into two groups: oligodendrogliomas per se and a group with malignant features termed anaplastic oligodendrogliomas (Kleihues et al., 1993, p. 112). Oligoastrocytomas are graded in a similar two-tiered fashion, but the distinction between these two tumors is highly subjective. Eight of the nine oligodendrogliomas we studied stained strongly (2+ or 3+), whereas six of the eight anaplastic oligodendrogliomas showed either no staining or only faint staining. Therefore, immunostaining for GD1b could be of use in grading these gliomas. Although all three oligoastrocytomas stained either 2+ or 3+, no distinction was seen when comparing them with anaplastic oligoastrocytomas. There were simply too few tumors in either category, however, to draw a firm conclusion as to the usefulness of GD1b immunostaining in grading.

The diagnosis of ependymoma in adequately sized specimens is usually made readily, but this can be problematic in very small specimens. Furthermore, histologic grading of ependymomas has been found to be a poor indicator of patient survival (Ross and Rubinstein, 1989). In none of the seven ependymomatous tumors we studied, however, was there any immunostaining for GD1b. Although this immunostain is of little value in identifying or grading ependymomas, it could be useful to rule out this diagnosis if immunostaining is strongly positive.

Eight of the nine primitive neuroectodermal tumors were unreactive with the GD1b antibody, which confirms the general correlation between low GD1b immunostaining and high-grade CNS neoplasia. Although making the correct diagnosis of primitive neuroectodermal tumor is usually not a problem, positive immunostaining for GD1b in what appears to be a poorly differentiated primary tumor of the CNS should caution one against this diagnosis.

One interesting finding of our study is that GD1b was detected within tumor cells. Indeed, in the tumor tissues we examined, the vast majority of immunostaining for GD1b was within tumor cells. Although occasionally there was some immunopositivity in elongate structures that might have been axons, this could account for only a small amount of the total GD1b within the tissues. The animal studies of Seyfried and colleagues were interpreted to indicate that a significant proportion of the glycolipid in glioma tissues they studied was present in lymphocytes and macrophages infiltrating the tumor (El-Abbadi and Seyfried, 1994; Seyfried et al., 1996). However, GD1b is not a major component of lymphoid or myeloid cells, so the finding of glycolipids of this type could not be attributed to infiltration by such cells.

In conclusion, our results demonstrate that immunostaining for GD1b gives a reasonably accurate estimate of the GD1b content of gliomas. In pilocytic astrocytomas, tumors known for their relatively solid, noninfiltrative nature, most of the GD1b is in the tumor cells rather than in residual neuronal elements. Because fibrillary astrocytomas and oligodendrogliomas of higher grade exhibit little or no staining for GD1b, immunoreactivity for this glycolipid may be useful in establishing their histologic grade.

References

- Berra, B., Gaini, S.M., and Riboni, L. (1985) Correlation between ganglioside distribution and histological grading of human astrocytomas. *Int. J. Cancer* **36**, 363–366.
- Chester, M.A. (1998) IUPAC-IUB Joint Commission on Biochemical Nomenclature (JCBN). Nomenclature of glycolipids. Recommendations 1997. *Eur. J. Biochem.* **257**, 293–298.
- Daumas-Duport, C. (1992) Histological grading of gliomas. *Current Opin. Neurol. Neurosurg.* **5**, 924–931.
- Daumas-Duport, C., Scheithauer, B., O'Fallon, J., and Kelly, P. (1988) Grading of astrocytomas: A simple and reproducible method. *Cancer* **62**, 2152–2165.
- El-Abbadi, M., and Seyfried, T.N. (1994) Influence of growth environment on the ganglioside composition of an experimental mouse brain tumor. *Mol. Chem. Neuropathol.* **21**, 273–285.
- Finlay, J.L., Boyett, J.M., Yates, A.J., Wisoff, J.H., Milstein, J.M., Geyer, J.R., Bertolone, S.J., McGuire, P., Cherlow, J.M., Tefft, M., Turski, P.A., Wara, W.M., Edwards, M., Sutton, L.N., Berger, M.S., Epstein, F., Ayers, G., Allen, J.C., Packer, R.J. (1995) Randomized phase III trial in childhood high-grade astrocytoma comparing vincristine, lomustine, and prednisone with the eight-drugs-in-1-day regimen. *J. Clin. Oncol.* **13**, 112–123.
- Fredman, P., von Holst, H., Collins, V.P., Granholm, L., and Svennerholm, L. (1988) Sialyllactotetraosylceramide, a ganglioside marker for human malignant gliomas. *J. Neurochem.* **50**, 912–919.
- Jennemann, R., Rodden, A., Bauer, B.L., Mennel, H.D., and Wiegandt, H.

- (1990) Glycosphingolipids of human gliomas. *Cancer Res.* **50**, 7444–7449.
- Kernohan, J.W., Mabon, R.F., Svien, H.J., Adson, A.W. (1949) A simplified classification of the gliomas. *Mayo Clinic Proc.* **24**, 71–74.
- Kleihues, P., Burger, P.C., and Scheithauer, B.W. (1993) *Histological Typing of Tumours of the Central Nervous System*. International Histological Classification of Tumours, World Health Organization. Second edition. New York: Springer-Verlag.
- Kostic, D., and Bucheit, F. (1970) Gangliosides in human brain tumors. *Life Sci.* (Pt. 2) **9**, 589–596.
- Ledeen, R.W., and Yu, R.K. (1982) Gangliosides: Structure, isolation, and analysis. *Methods Enzymol.* **83**, 139–191.
- Ledeen, R.W., Yu, R.K., and Eng, L.F. (1973) Gangliosides of human myelin: Sialosylgalactosylceramide(G7) as a major component. *J. Neurochem.* **21**, 829–839.
- Ozawa, H., Kotani, M., Kawashima, I., and Tai, T. (1992) Generation of one set of monoclonal antibodies specific for b-pathway ganglio-series gangliosides. *Biochim. Biophys. Acta* **1123**, 184–190.
- Ringertz, N. (1949) Grading of gliomas. *Acta Pathol. Microbiol. Scand.* **27**, 51–64.
- Ross, G.W., and Rubinstein, L.J. (1989) Lack of histopathological correlation of malignantependymomas with postoperative survival. *J. Neurosurg.* **70**, 31–36.
- Segler-Stahl, K., Webster, J.C., and Brunngraber, E.G. (1983) Changes in the concentration and composition of human brain gangliosides with aging. *Gerontology* **29**, 161–168.
- Seyfried, T.N., El-Abbadi, M., Ecsedy, J.A., Bai, H.W., and Yohe, H.C. (1996) Influence of host cell infiltration on the glycolipid content of mouse brain tumors. *J. Neurochem.* **66**, 2026–2033.
- Sung, C.C., Pearl, D.K., and Yates, A.J. (1993) Ganglioside composition of human brain tumors. *J. Neurochem.* **61**, S232.
- Sung, C.C., Pearl, D.K., Coons, S.W., Scheithauer, B.W., Johnson, P.C., and Yates, A.J. (1994) Gangliosides as diagnostic markers of human astrocytomas and primitive neuroectodermal tumors. *Cancer* **74**, 3010–3022.
- Svennerholm, L. (1980) Ganglioside designation. *Adv. Exp. Med. Biol.* **125**, 11.
- Traylor, T.D., and Hogan, E.L. (1980) Gangliosides of human cerebral astrocytomas. *J. Neurochem.* **34**, 126–131.
- Vanier, M.T., Holm, M., Ohman, R., and Svennerholm, L. (1971) Developmental profiles of gangliosides in human and rat brain. *J. Neurochem.* **18**, 581–592.
- Wiegandt, H. (1982) The gangliosides. In Agranoff, B.W., and Aprison, M.H. (Eds.), *Advances in Neurochemistry*, Vol. 4. New York: Plenum Press. pp. 149–223.