Cortical Architectural Abnormalities and MIB1 Immunoreactivity in Gangliogliomas: A Study of 60 Patients with Intracranial Tumors

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Abstract. Gangliogliomas are generally low grade neoplasms composed of mixtures of neoplastic glial and neuronal elements whose origin and exact nature are still controversial. We studied a series of 60 intracranial gangliogliomas looking for coexistent cortical architectural abnormalities (cortical dysplasia, microdysgenesis) and to determine if tumor behavior correlates with MIB1 (marker of cellular proliferation) labeling. The patients included 34 males and 26 females who ranged in age from 6 months to 55 years (mean 20 years). Thirty-eight tumors (63%) were located in the temporal lobe and 6 (10%) in the frontal lobe. Fifty-four patients (90%) presented with seizures (most with intractable epilepsy) and the duration of seizures ranged from 1 to 38 years (mean 14 years). In all cases, the predominant glioma component resembled a low grade fibrillary astrocytoma. In 14 tumors (23%), an oligodendroglial component was present. In one case, the glial component resembled an anaplastic astrocytoma. The tumors were characterized variously by perivascular chronic inflammation (N = 45, 75%), vascular proliferation (N = 36, 60%), granular bodies (N = 54, 90%), binucleated neurons (N = 36, 60%), calcification (N = 28, 47%), and cystic degeneration (N = 26, 43%). Meningeal involvement by tumor was observed in five (8%) cases. In 38 patients, sufficient tissue was resected to evaluate for the presence of coexistent cortical architectural abnormalities. Cortical architectural abnormalities were identified in 10 but clearly separate from the tumor in 19 (30%) patients. Only four patients including the anaplastic tumor died with tumor progression. MIB1 indices (positive tumor cells/1,000 tumor cells counted) in 54 cases ranged from 0 to 10.8 (mean 1.1 ± 1.0). Mortality did not reliably correlate with the MIB1 index; however, the highest index was observed in the anaplastic tumor. The high incidence of associated architectural abnormalities suggests that gangliogliomas may arise on a maldevelopmental basis. Although generally benign and slow-growing tumors, as indicated by the generally low MIB1 indices, gangliogliomas can rarely behave in a malignant fashion. It is not possible to reliably predict prognosis on the basis of histologic features of MIB1 immunoreactivity.

Key Words: Cortical dysplasia; Ganglioglioma; Ki-67 antibody; MIB1 antibody; Migration disorders; Seizures; Tumor-associated epilepsy.

INTRODUCTION

According to the World Health Organization Histologic Typing of Tumours of the Central Nervous System, gangliogliomas are defined as benign tumors composed of neoplastic astrocytes and ganglion cells (1). Since the first description of these tumors in the late 1920s and early 1930s (2, 3), several cases and series of these tumors have been published in the literature (3–25). Although most gangliogliomas appear to have a relatively benign clinical course and are amenable to surgical resection alone, there is a small subset of these neoplasms which appears to behave in a somewhat more aggressive fashion (26–31). To date, predicting tumor behavior based solely on the histologic appearance of these tumors is unreliable.

In recent years, tumor cell kinetics and studies centering on the cell cycle have been employed in an attempt to more accurately determine the growth rate of a tumor, thereby helping to better characterize its malignant potential as well as prognosis. A variety of approaches in this arena have been employed including measurements of DNA content, silver-staining nucleolar organizer region-associated proteins (AgNORs), bromodeoxyuridine incorporation, proliferating cell nuclear antigen (PCNA), tritiated thymidine uptake, and Ki-67 antigen (32–47). Ki-67 monoclonal antibody was initially developed as a specific antibody against Reed-Sternberg cell nuclei and reacts with nuclear antigen expressed in active phases in the cell cycle (G1, G2, S and M phases). A major disadvantage of Ki-67 antibody is that it requires fresh or frozen material. Recently, the MIB1 antibody, a Ki-67 antibody which is able to be utilized in formalin-fixed, paraffin-embedded tissue, has been developed and appears to be an effective marker of cell proliferation (48–50). The Ki-67 antibody and MIB1 antibody have been shown to recognize slightly different epitopes; therefore, immunoreactivity with the two antibodies may vary slightly. The origin of gangliogliomas is still a matter of considerable controversy. Their slow evolution, long duration of symptoms prior to diagnosis, and the histologic appearance support a hamartomatous or maldevelopmental nature for these tumors (13, 31, 51, 52). Studies based primarily on immunohistochemical staining patterns of ganglioglioma have suggested that they arise from ectop-
ic neuronal cell rests derived from peripheral autonomic nervous tissue (22, 53–55). Others have hypothesized that gangliogliomas arise from a single stem cell which has the ability to differentiate along both glial and neuronal cell lines (20, 56). Recently, it has been observed that at least a subset of gangliogliomas appears to be associated with disorganized cortical architecture (cortical dysplasia) (25, 57, 58), suggesting that gangliogliomas may arise on a maldevelopmental basis, possibly from foci of cortical dysplasia.

In an attempt to examine the role cortical dysplasia might play in the origin, development, and surgical management of gangliogliomas, we studied a series of 60 intracranial gangliogliomas. The clinical features and histopathology are described. Immunohistochemical staining of a subset of these tumors was performed in order to determine whether there is a relationship between MIB1 labeling and the behavior of these tumors.

**MATERIALS AND METHODS**

The surgical pathology and autopsy files at the Cleveland Clinic Foundation were searched between 1974 and February 1994 for patients in whom a diagnosis of ganglioglioma was made. A total of 76 such patients were identified. All available microscopic slides were reviewed in each of these cases and a definite diagnosis of ganglioglioma was confirmed in 60 cases. Of the 16 cases that were excluded from study, most were reclassified as either a low grade fibrillary astrocytoma or a dysplastic neuroepithelial tumor (DNT). Anywhere from two to 33 microscopic slides (mean 13 slides) were available for examination in each case. In cases in which a biopsy or tumor resection alone was performed, all the tissue submitted to pathology was examined histologically. In the cases in which a partial lobectomy with gross total resection of the tumor was performed, at least half of the tissue submitted to pathology was examined histologically. Four micron thick hematoxylin and eosin-stained sections were routinely generated from formalin-fixed, paraffin-embedded material. Ancillary stains were not routinely performed in each case and were used to confirm the diagnosis in selected cases.

Histopathologic features tabulated included the following: classification (i.e. astrocytoma, oligodendroglioma) and grade (i.e. low grade, anaplastic) of the glial component of the neoplasm, presence or absence of binucleate ganglion cells, perivascular inflammation, vascular proliferation, cystic degeneration, calcification, necrosis, mitoses, and granular body formation. In addition, coexistent pathology including neuronal heterotopia, mesial temporal sclerosis, and cortical architectural abnormalities such as cortical dysplasia or microgyrnesses were noted. For the purposes of this study, a cortical architectural abnormality is defined as a malformative disorganization of the cytoarchitecture of the cortex relative to neurons (58, 59). Such foci were separate from but near areas of tumor. Neuronal heterotopia were defined as the presence of neurons within the white matter. Neurons located within the white matter immediately subjacent to the cortex were not considered heterotopia. Mesial temporal sclerosis consisted of hippocampal atrophy associated with neuronal loss and gliosis, particularly involving CA1, CA4, and dentate gyrus.

Immunohistochemical staining of tissue from 54 patients was performed with MIB1 antibody (1:10 dilution, AMAC Inc., Westbrook, ME) utilizing a microwave processing procedure and an avidin-biotinylated immunoperoxidase methodology as previously described (60, 61). Appropriate negative and positive controls were performed in each case. On each immunostained slide, 1,000 tumor cell nuclei were counted using a highpower field of 400× (A.O. Scientific Instrument binocular microscope, calculated area = 0.196 mm²). Only nuclear staining was interpreted as positive. In each case, a labeling index (MIB1 positive nuclei/1,000 tumor cell counted) was calculated.

Pathology and clinical records were reviewed for pertinent clinical information including the age and sex of the patient, tumor location, presentation, duration of symptoms, type and frequency of seizures, surgical procedure and extent of tumor resection, seizure outcome, evidence of tumor recurrence, adjuvant chemotherapy or radiation therapy, and status at the most recent follow-up.

**RESULTS**

A total of 60 patients with histologically confirmed intracranial gangliogliomas comprise the study group. Patients ranged in age from 6 months to 55 years (mean 20 years). The study group consisted of 34 males and 26 females. Fifty-four patients (90%) presented with seizures ranging from 1 to 38 years duration (mean 14 years). Thirteen patients (29%) had seizures for greater than or equal to 20 years duration prior to surgery. Seizures were characterized as complex partial with or without generalized tonic clonic seizures in 46/54 patients (85%). In 45/60 patients (75%), the seizures were deemed medically intractable, defined as repetitive and disabling seizures of greater than 2 years duration despite optimal anticonvulsant therapy. Six patients (10%) presented with symptomatology other than seizures including headaches, vomiting, decreased memory, ataxia, neck stiffness, ptoisis, and head tilt. Patients with intractable epilepsy had a mean duration of preoperative symptoms of 14 years as compared with those without epilepsy who had symptoms for a mean of 4.7 months preoperatively.

**Surgical Procedures**

Twenty-eight tumors were situated on the right side and 27 tumors on the left. In the remaining five tumors, laterality was not indicated or the tumor was midline in location. The most common site of involvement was the temporal lobe in 38 patients (63%). Other tumors were situated in the frontal lobe (N = 6, 10%), parietal lobe (N = 4, 7%), temporal/parietal/occipital area (N = 3, 5%), and cerebellum (N = 3, 5%). The remaining tumors were situated in the suprasellar region, left cingulate gyrus, midbrain, thalamus and the right atrium of the lateral ventricle.

Fifty-one patients underwent a single surgical proce-
Fig. 1. Ganglioglioma characterized by a mixture of neuronal cells and spindled glial cells resembling a low grade fibrillary astrocytoma. Hematoxylin and eosin.

dure for their tumor, including gross total resection of the tumor in 37 (73%) patients, subtotal resection in 12 (23%) patients and biopsy alone in two (4%) patients. Nine patients underwent more than one surgical procedure related to the tumor. In three patients who had an initial biopsy alone, one subsequently underwent a gross total resection, one underwent a subtotal resection, and one was rebiopsied. Of the two patients who had an initial subtotal resection, both had gross total resections at a later date for tumor recurrence. Of the four patients who had a gross total resection initially, all four were treated with a repeat gross total resection 6 to 19 years following the initial surgery and one was treated with a subsequent subtotal resection 5 years after the second surgery for recurrent tumor.

Histopathology

All 60 cases demonstrated histologic features consistent with ganglioglioma (Fig. 1). The abnormal neuronal component was characterized by an increased number of pleomorphic neuronal or ganglion cells with vesicular nuclei and prominent nucleoli. Binucleate neurons were observed in 36 (60%) cases. The predominant pattern of the accompanying glioma was that of a low grade fibrillary astrocytoma, according to the Ringertz classification schema, in 59 cases (98%). In one tumor, the glial component was characterized by increased nuclear pleomorphism, scattered mitotic figures, and prominent vascular proliferation and was felt to be histologically consistent with an anaplastic ganglioglioma. In 14 tumors (23%), focal areas resembling an oligodendroglioma were present. Eosinophilic granular bodies were observed focally in 54 tumors (90%). Areas of perivascular chronic inflammation consisting primarily of lymphocytes were focally present in 45 tumors (75%). A mild degree of vascular endothelial hyperplasia was observed in 36 tumors (60%). Focal microcystic change was seen in 26 tumors (43%) and calcification in 28 tumors (47%). In two cases, foci of necrosis which were thought to be related to infarction were seen. Mitotic figures were observed only in the tumor with anaplastic features. Focal Rosenthal fiber formation was seen at the periphery of two tumors (3%). In five patients (8%), the tumor focally extended to involve the overlying meninges (Fig. 2). Small areas of contusion most probably related to previous subdural electrode grid usage were seen in eight patients (13%). Focal areas of chronic meningeal inflammation overlying the tumor were observed in 12 cases (20%) and foci of subpial gliosis were present in 46 (77%).

Cortical architectural abnormalities including cortical dysplasia and microdysgenesis were observed near to but separate from the tumor in 19 of 38 patients (50%) in whom there was sufficient tissue adjacent to the tumor for adequate evaluation (Figs. 3, 4). In 11 patients, cortical dysplasia was characterized by disruption of the normal cortical architecture due to a malpositioning or malalignment of neuronal elements. In one of these patients, polymicrogyria as well as gray matter heterotopia were present. In ten patients, the cortical dysplasia consisted
of discrete clusters of neuronal and glial elements situated within the cortex. In three patients, an increased number of molecular layer neurons were observed. In four patients, two of the three above-mentioned patterns of cortical architectural disorganization were seen. In one patient, all three patterns were present.

In one patient, mesial temporal sclerosis was present. In many of the hippocampus sections, however, fragmentation and improper orientation made evaluation for mesial temporal sclerosis difficult or impossible. Heterotopic neurons were observed in 37 of 38 patients (97%) in whom adequate involved white matter uninvolved by tumor was available for histologic examination.

**MIB1**

MIB1 immunohistochemical staining of tumors in 54 patients was performed. The MIB1 index ranged from 0 to 10.2 (mean 1.1 ± 1.0). MIB1 positivity was observed only in the glial component of the tumors. No MIB1 immunoreactivity was noted in seven patients. The highest MIB1 reactivity (index = 10.2) was observed in the patient with an anaplastic astrocytoma glial component. Of the four patients who died or were presumed dead of tumor progression, the MIB1 indices were performed in three patients and were 0.2, 0.4 and 10.2. Of the five patients in whom the MIB1 index was determined on serial resections of the ganglioglioma, the second resection showed higher indices in all five cases. Of the 15 patients with indices greater than 1.0 (range 1.2–10.2, mean 3.1) one patient died with disease, three patients are alive with evidence of tumor, and the remaining patients are alive with no evidence of tumor with a mean follow-up interval of 5 years. There was no obvious relationship between the MIB1 labeling index and the presence or absence of cortical dysplasia, age and sex of patient, location of tumor, presenting symptomatology, and duration of symptoms prior to surgery.

**Follow-up**

Follow-up in all patients since the initial operation ranged from 6 months to 284 months (mean 63 months). Of the 54 patients with seizures, 33 are seizure free, 11 had a greater than 90% reduction in seizure frequency and 10 had persistent seizures at last known follow-up. Of the 39 patients with seizures who were treated with a gross total resection at the most recent surgery, none have had recurrent tumor on follow-up neuroimaging.

Four patients (follow-up range 12–284 months, mean 136 months) are known to have died with tumor. Two patients died directly related to tumor progression, including the one anaplastic ganglioglioma. Of the two remaining patients who died with an apparently histologically benign tumor, one died of postoperative pneumonia and one died of a presumed shunt malfunction associated with a seizure and choking episode. None of the 45 patients who presented with intractable epilepsy died. Of the four patients who were treated with adjuvant chemotherapy and radiation therapy, two died with tumor 12 months and 75 months after surgery and two are alive with tumor 54 months and 107 months after surgery. Of the three patients who were treated with radiation therapy alone, one patient died with tumor 284 months postoperatively, one patient is alive with evidence of tumor 68 months postoperatively, and one patient is alive with no evidence of tumor 10 months postoperatively.

**DISCUSSION**

Even though gangliogliomas are a relatively rare occurrence in the central nervous system, they have been the source of much literature. The exact etiology of this lesion, however, is still a matter of debate. In contrast to
the established association of cortical dysplasia with DNT (1, 62–66), a similar association has not been well documented in the literature for ganglioglioma. To our knowledge, only a few studies have suggested such a relationship (25, 57, 58). In 1993, Jay et al (57) described four cases of childhood temporal lobe ganglioglioma; two of these cases had associated cortical disorganization. They noted a transition from the ganglioglioma to areas of dysplastic cortex. In a recent study of 61 gangliogliomas, Wolf et al (25) found evidence of “glioneuronal hamartias” in 13% of cases. In 1993, the Cleveland Clinic Foundation reported a series of 13 tumors in patients with chronic epilepsy and associated cortical dysplasia (58). Of the 13 tumors, eight were gangliogliomas, four were dysembryoplastic neuroepithelial tumors, and two were low grade astrocytomas. This study prompted us to specifically examine all of our gangliogliomas, looking for evidence of coexistent cortical architectural abnormalities. Of the 38 tumors in which there was adequate tissue adjacent to the neoplasm for evaluation, cortical architectural abnormalities were identified in 50%. Because of limitations in tissue sampling and the reliance primarily on hematoxylin and eosin-stained sections, the actual incidence of coexistent pathology may actually be somewhat higher.

The presence of coexistent pathology raises interesting issues regarding the pathogenesis of ganglioglioma. The histologic similarity along with the apparent slow growth of most gangliogliomas and the incidence of coexistent dysplasia are suggestive of a causal relationship between the two lesions, i.e., gangliogliomas may represent a tumor form of cortical dysplasia or neoplastic transformation of a dysplastic focus. Interestingly, a number of similarities exist between the so-called DNT (dysplasia-associated tumor) and ganglioglioma including early age of presentation, history of chronic epilepsy, temporal lobe predilection, associated cortical dysplasia and generally excellent prognosis (62–66). Although in the most recent WHO classification of central nervous system tumors, ganglioglioma and DNT are separated as distinct entities based on histologic grounds, this distinction on etiologic grounds may not be clearcut and the two lesions may be more related than different.

Malignant degeneration of low grade gliomas into higher grade neoplasms has been well described in all glial cell lines. Clearly, there is a small percentage of gangliogliomas which behave in an aggressive fashion and even metastasize (26–31). Unfortunately, there are no histologic features that are absolutely predictive of such behavior. In only one patient out of our series were the histologic features worrisome enough to warrant the designation of anaplastic ganglioglioma. This patient died secondary to tumor progression. Only one of the three histologically benign-appearing tumors caused patient death due to tumor progression. Since the ability to predict tumor behavior based on histologic appearance is imperfect, as it currently stands, we examined the possible utility of a cell proliferation marker (MIB1) in order to determine whether there was a correlation between tumor behavior and labeling index. Of the three tumors which resulted in patient death and in which MIB1 indices were obtainable, only the anaplastic tumor had a significantly elevated index. In the other two tumors, the MIB1 indices were low and similar to the other benign more typically behaving tumors. Conversely, rare apparently low grade gangliogliomas had elevated MIB1 indices over 4.0. These tumors may eventually behave in a more aggressive fashion with continued follow-up. Perhaps if enough anaplastic gangliogliomas were examined, a rough distinction from ordinary low grade gangliogliomas could be made with MIB1 similar to other glial neoplasms. However, clearly MIB1 by itself is not absolutely predictive of tumor behavior. Tumors with increased mitotic activity or correspondingly high MIB1 indices and other worrisome histologic features such as nuclear pleomorphism or prominent vascular endothelial proliferation should, however, be watched more closely. Interestingly, clinical presentation is the only feature which seemed to be somewhat indicative of tumor behavior. None of the 45 patients who presented with intractable epilepsy died, while four of 15 (27%) of patients presenting with other symptoms died. Of those who died, the mean duration of symptoms prior to diagnosis was relatively short, i.e. 5 months.

MIB1 studies also support the notion that gangliogliomas are in general slow-growing tumors since the mean index was only slightly more than 1.0. Our findings were similar to those of Wolf et al (25) who observed a Ki-67 labeling index of less than 1% in 45/61 (74%) of tumors. In reported studies in which Ki-67 and MIB1 immunoreactivity were examined in tumors of the central nervous system, differences in methodologies of staining and cell counting and in the epitope recognition between Ki-67 and MIB1 index create some difficulty in making direct comparisons. In general, however, it appears that gangliogliomas have lower cell proliferation indices than other low grade malignancies such as meningioma, low grade astrocytoma, and subependymoma (39, 49). These findings are consistent with the prolonged clinical course and apparent slow growth of these tumors.

Diagnosis of ganglioglioma relies primarily on recognition of the mixture of the glial and neuronal components which comprise this tumor. In the vast majority of cases, the glial component resembled a low grade fibrillary astrocytoma. However, foci of areas in which the glial component of the tumor resembled an oligodendroglioma were seen, a finding previously observed by others (22, 31). A biopsy sampled from such an area could certainly cause diagnostic confusion with oligodendroglioma or even with DNT. Other histologic
features which were present in a majority of cases which are generally useful in helping identify these tumors include the presence of eosinophilic granular bodies, focal perivascular chronic inflammation and a mild degree of vascular hyperplasia. In general, the degree of vascular hyperplasia does not approach that seen in a typical anaplastic astrocytoma. Frequently, focal microcystic degeneration and calcification can also be observed. Although the presence of binucleated neuronal elements is useful in recognizing neuronal atypia, it is not an invariable feature of ganglioglioma and was observed in only 60% of cases. Extension of ganglioglioma to involve the overlying meninges has been previously described (67) and in general does not seem to be associated in itself with a bad outcome. Coexistent mesial temporal sclerosis, observed in one patient in this series, most probably represents coincidental pathology.

If there truly is an association between cortical dysplasia and ganglioglioma, this has certain ramifications with regards to the surgical approach to these tumors. There is much controversy regarding the most appropriate surgical treatment for patients with chronic epilepsy associated with gangliogliomas. While some feel that complete excision of the tumor is sufficient (7, 8, 10, 11, 12, 68), others argue that resection of the epileptogenic zones may provide added seizure control (69-71). Cortical dysplasia in and of itself is a well-recognized cause of chronic epilepsy (59, 72-75). Hypothetically, if one were to just excise the tumor and leave behind an epileptogenic focus of cortical dysplasia, one might cure the patient of the neoplasm but leave the patient with seizures. In one of the patients in our series, such a situation occurred. A ganglioglioma was initially resected with a slight decrease in seizure activity. In the ensuing year, the patient's seizure activity markedly increased. Repeated imaging studies failed to show evidence of tumor recurrence. Subsequent resection showed no evidence of tumor, however, cortical dysplasia was identified. The patient is seizure-free 9 months postoperatively. In a small percentage of patients, gross total resection of the tumor does not appear to result in relief of seizure activity. It is possible that remaining foci of cortical dysplasia may be causing continued seizures. Therefore, it would seem reasonable that for the best surgical result, tumor resection be continued until normal pial margins are reached so that areas of cortical dysplasia located near the tumor are incorporated in the resection. Currently, there appears to be no role for radiation therapy or adjuvant chemotherapy in treating routine intracranial gangliogliomas. The role of such adjuvant therapeutic modalities may be more justifiable in the rare anaplastic gangliogliomas.

The high incidence of coexistent cortical dysplasia and ganglioglioma in this study supports the notion that gangliogliomas may represent a maldevelopmental lesion which may either arise from cortical dysplasia or may even represent one end of a spectrum of changes of cortical dysplasia (tumoral dysplasia). This notion is further supported by the fact that most patients with ganglioglioma generally present earlier in life with symptoms of long duration and that complete tumor excision appears to be curative in the majority of cases. In addition, the mixed neuronal-glial elements of the ganglioglioma are somewhat reminiscent of certain forms of cortical dysplasia and of the dysplasia-associated DNT.

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