Gangliogliomas: An Intriguing Tumor Entity Associated With Focal Epilepsies

INGMAR BLÜMCKE, MD AND OTMAR D. WIESTLER, MD

Abstract. Gangliogliomas represent the most frequent tumor entity in young patients suffering from chronic focal epilepsies. In a series of 326 gangliogliomas collected from the University of Bonn Epilepsy Surgery Program and other departments of neuropathology in Germany, Austria, and Switzerland, epidemiological findings and histopathological hallmarks of gangliogliomas are systematically reviewed. The majority of these tumors occur within the temporal lobe and reveal a biphasic histological architecture characterized by a combination of dysplastic neurons and neoplastic glial cell elements. However, gangliogliomas exhibit a considerable variability in their histopathological appearance. Immunohistochemical studies are an important tool to discriminate these neoplasms from other tumor entities. Almost 80% of gangliogliomas reveal immunoreactivity for CD34, a stem cell epitope not expressed in normal brain. Immunohistochemical reactions for MAP2 or NeuN can be employed to characterize the dysplastic nature of neurons in those areas difficult to discriminate from pre-existing brain parenchyma. Less than 50% of the cases display binucleated neurons. With the frequent finding of “satellite” tumor clusters in adjacent brain regions, gangliogliomas are macroscopically less circumscribed than previously assumed. The distinction from diffusely infiltrating gliomas is of considerable importance since tumor recurrence or malignant progression are rare events in gangliogliomas. Only little is known about the molecular pathogenesis of these glioneuronal tumors. Our findings support a dysontogenic origin from a glioneuronal precursor lesion with neoplastic, clonal proliferation of the glial cell population. Candidate genes appear to associate with neurodevelopmental signaling cascades rather than cell cycle control or DNA repair mechanisms. The reelin signaling and tuberin/insulin growth receptor pathways have recently been implicated in ganglioglioma development. Powerful new molecular genetic and biological tools can now be employed to unravel the pathogenesis of these intriguing lesions.

Key Words: Brain tumor; CD34; Epilepsy; Malformation; MAP2; Stem cell.

INTRODUCTION

Gangliogliomas are rare neoplasms, with an incidence of only 1.3% in large brain tumor series (1–4). However, they represent the most common tumor entity in young patients suffering from chronic, intractable focal epilepsy (5–9). The following overview summarizes our experience with a series of 326 gangliogliomas obtained from the University of Bonn Epilepsy Surgery Program during the period of 1991–2001, as well as of additional cases referred from colleagues in Germany, Austria, and Switzerland.

Particular emphasis will be paid to the histopathological variability of gangliogliomas, which requires their distinction from several other tumor entities, such as diffuse astrocytomas, oligodendrogliomas, or dysmyeloplastic neuroepithelial tumors (DNT). This is of major importance, since clinico-pathological follow-ups consistently identify only a low risk for tumor recurrence and malignant progression in patients suffering from focal epilepsies and gangliogliomas (6, 7, 10).

Epidemiological and Clinical Findings

Gangliogliomas predominantly occur in young patients. In our series of 279 WHO grade I tumors, the mean age was 22.1 ± 11.2 yr (range: 8 months to 67 yr). The majority of patients present with a history of earlier symptoms, such as focal epilepsies. There is a prevalence of male compared to female patients (Table 1). The vast majority of tumors occurred in the temporal lobe (71.3%). Other areas included the frontal lobe (8.2%), occipital and parietal lobes (5% and 4%, respectively), the cerebellum (5%), spinal cord (3%), pituitary gland, pineal gland, hypothalamus, and brainstem (percentages are given since detailed clinical records including tumor location were available only in 195 cases).

Gangliogliomas with atypical features, such as increased cellularity and proliferation activity, correspond to WHO grade II (see below). Age and sex distribution is similar to grade I tumors, as well as their location in the temporal lobe and association with focal epilepsies. In rare instances, anaplastic gangliogliomas have been encountered. There is a trend for these tumors to occur in older patients with a mean age of 35 ± 14.5 yr (range: 10 to 88 yr). In our series of 17 cases, tumor location was more evenly distributed with involvement of the temporal lobes (33%), parietal lobes (33%), frontal lobes (11%), as well as the spinal cord (22%).

The postoperative course in gangliogliomas is favorable. A clinical follow-up of 7 yr was available from 86 patients included in the present series (10). During this period, tumor recurrence of gangliogliomas (WHO
TABLE 1
Series of Gangliogliomas Presented in this Study

<table>
<thead>
<tr>
<th>WHO Grade</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Sex (f/m)</th>
<th>Temporal location (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO I</td>
<td>279</td>
<td>22.1</td>
<td>1:1.3</td>
<td>71.3</td>
</tr>
<tr>
<td>WHO II</td>
<td>30</td>
<td>24</td>
<td>1:1.7</td>
<td>79.7</td>
</tr>
<tr>
<td>WHO III</td>
<td>17</td>
<td>35</td>
<td>1:1.4</td>
<td>45.5</td>
</tr>
</tbody>
</table>

Tumor location is given in percentages from those cases in which detailed clinical information was available (n = 195). Abbreviations: f, female; m, male.

Fig. 1. Age and sex distribution (years) of gangliogliomas based on 338 cases (GG I, GG II, and GG III according to WHO grading scale). Abbreviations: GG I, gangliogliomas WHO grade I; GG II, atypical gangliogliomas WHO grade II; GG III, anaplastic gangliogliomas WHO grade III.

grades I and II) occurred in only 1 patient. In this unusual case, a glioblastoma (WHO grade IV) has been detected in the same location 1 yr after the diagnosis of a temporal lobe ganglioglioma. Histopathologically, we found no evidence for a malignant cell component in the primary tumor specimen. Another unusual case encountered in our series involved a glioblastoma (WHO IV), which displayed an additional component corresponding to a highly differentiated, slowly proliferating ganglioglioma. Similar cases have been previously described (11, 12).

Eighty-eight percent of our patients in which the diagnosis of a ganglioglioma could be verified became seizure-free, with a follow-up period of 7 yr (10). The remaining 12% of patients still benefit from epilepsy surgery with significant seizure reduction of 75% or 50% (Engel class II and III, respectively). However, seizure relief may not directly correlate with tumor resection. It appears, rather, to rely on a careful examination of the entire epileptogenic area. This applies particularly to temporal lobe gangliogliomas, which frequently affect limbic structures such as amygdala or hippocampus. In all epilepsy patients, therefore, a systematic neurophysiological and psychological work-up has to precede the tailored resection in order to achieve seizure control and to avoid post-surgical deficits in mnemonic and/or emotional functions (13).

Histopathological Findings

The histopathological hallmark of gangliogliomas is a combination of neuronal and glial cell elements (14). Both cell populations exhibit marked heterogeneity. Their variable microscopic appearance can pose a real challenge to the surgical neuropathologist, and it is not unusual for these neoplasms to be misdiagnosed as low-grade gliomas or other tumor entity.

The morphological spectrum of gangliogliomas varies from a predominantly neuronal phenotype (cortical dysplasias, gangliocytomas) towards variants with a prominent glial population (diffuse astrocytomas) (Figs. 2, 3).
However, these tumors may also display a clear cell morphology, which raises the differential diagnosis of oligodendroglioma or DNT. The specific immunohistochemical profile of gangliogliomas will usually allow a greater distinction (see below).

According to radiological findings and surgical features, gangliogliomas appear as circumscribed lesions well demarcated from the adjacent brain parenchyma (15). Histopathological examination of the resection specimens reveals, however, that tumor growth often permeates the pre-existing cytoarchitecture, such as neocortex, hippocampus, and amygdala. Here, the distinction between neoplastic and normal brain parenchyma can be very difficult and requires specific immunohistochemical reactions. In addition, malformative lesions have been consistently observed adjacent to gangliogliomas (14, 16, 17). The stem cell epitope CD34, which cannot be observed in normal brain tissue, has evolved as a helpful diagnostic tool. It is consistently expressed in gangliogliomas. This observation may point towards a pathogenic relationship with malformative precursor lesions and identifies a previously not well-recognized feature of gangliogliomas (18). Many small CD34-immunoreactive tumor satellite clusters that may escape routine histopathological inspection can be observed in the adjacent normal brain. Most tumors can be readily identified by their dysplastic neuronal component (Fig. 3). Dysplastic neurons should be characterized by (i) loss of cytoarchitectural organization, (ii) abnormal (subcortical) localization, (iii) clustered appearance, (iv) cytomegaly, or (v) perimembranous aggregated Nissl substance. In our series, however, only 40% of the specimens contain a distinct population of bi- or multinucleated neurons.

Glia l cell elements in gangliogliomas show substantial variability (Fig. 3). As a rule, the glial component constitutes the proliferative cell population of the tumor and, therefore, defines its biological nature/behavior (19). The spectrum of glial cells in gangliogliomas is broad and includes cell types resembling fibrillary astrocytoma, oligodendroglioma, or pilocytic astrocytoma. Indeed, the presence of Rosenthal fibers and protein droplets can be observed in a significant number (10%–20%). A glial fiber matrix is usually prominent and may contain microcystic cavities and/or mucous substance. A distinct reticular fiber network can be developed apart from the vasculature. Papillary architectures or small lobules of tumor cells may also account for the variable tumor architecture and can be very prominent. Recently, a new variant designated as papillary glioneuronal tumor has been established (20, 21). Gangliogliomas featuring a prominent glial component and only inconspicuous neuronal elements can be easily misinterpreted and classified as diffuse astrocytoma. We suggest, therefore, to carefully exclude the diagnosis of ganglioglioma in tumor specimens obtained from the temporal lobe of young patients whether information on clinical history (e.g. chronic epilepsy) is available or not.

Additional histopathological features frequently identified in gangliogliomas are (i) calcifications, either excessive or as neuronal/capillary incrustation, (ii) extensive lymphoid infiltrates along perivascular spaces or within the tumor/brain parenchyma, and (iii) a prominent capillary network. In few cases, however, the latter manifests as malformative angiomatous component.

**Immunohistochemical Characterization**

A systematic analysis of our series of epilepsy-associated tumors identified a distinct immunohistochemical profile that allows for a reliable distinction of gangliogliomas and related tumor entities (Fig. 2; Table 2). The immunohistochemical reaction panel not only includes well-characterized glial marker proteins, such as glial fibrillary acidic protein (GFAP), S-100, and neuronal epitopes (i.e. NeuN, neurofilaments, synaptophysin or MAP2). Most characteristically, the stem cell epitope...
CD34 can be detected in the vast majority of gangliogi-
liomas (18). The assessment of tumor cell proliferation
and nuclear accumulation of p53-protein are additional
markers to be considered.

The Stem Cell Epitope CD34 is Consistently Expressed
in Gangliogliomas

In our series, 80% of gangliogliomas showed immu-
noreactivity for the stem cell epitope CD34 (Fig. 4).
There appeared to be a slight association between CD34
expression and tumor location. Whereas most CD34-pos-
tive tumors are found in the temporal lobe, 45.5% of
CD34-negative gangliogliomas can be found in other
brain regions, i.e. frontal and parietal lobes, cerebellum,
or hypothalamus. The striking association between CD34
immunoreactivity and temporal location can be observed
in WHO grades I and II gangliogliomas. In anaplastic
variants (WHO grade III), the temporal lobe appears less
frequently affected (57%). Whether this finding also point
towards different pathogenic mechanisms remains to be
shown. It is tempting to speculate, however, that gan-
gliogliomas located outside the temporal lobe may arise
from a different precursor cell population and may ex-
hibit a slightly higher risk for neoplastic progression. On
the other hand, the group of CD34-negative gangliogi-
liomas comprises those tumors predominated by dysplas-
tic neurons. These tumors lack virtually any proliferation
activity and may rather be classified as gangliocytoma or
cortical dysplasia (Figs. 2–4).

The glycoprotein CD34 is abundantly expressed on en-
dothelial cells of the developing and adult brain, whereas
neuroepithelial cell elements never reveal CD34 immu-
noreactivity in mammals (18) with the exception of
eye precursors during neural tube formation (22). In
gangliogliomas, dysplastic/neoplastic cellular elements
consistently demonstrate CD34 immunoreactivity of cel-
lar surface membranes (Figs. 4, 5). The staining covers
not only the perikarya but also densely ramifying pro-
cesses. A detailed characterization of CD34-immunore-
active neuroectodermal cells failed to identify a specific
cellular lineage. Confocal laser scanning microscopy re-
vealed no co-localization between CD34 and GFAP la-
beled astrocytes. CD34-positive cells consistently co-ex-
pressed the S100 protein (18). Lack of double staining
with anti-CD68 or anti-HLA-DR excluded a microglial
nature. Some CD34-immunoreactive profiles resembled
neurons. This observation has been confirmed by double
labeling of CD34 and the neuronal nuclear protein NeuN
(Fig. 4). CD34-immunoreactive neurons were also la-
beled with MAP2 and neurofilament antibodies (Fig. 4).
A significant proliferation capacity of CD34-immunore-
active cells was not detected with the Ki-67 antigen.
CD34-immunoreactive cells can be observed in the center
of tumor, either as homogeneous staining including the
matrix, as cell clusters, or in a band-like fashion spreading
along adjacent normal brain tissue (Fig. 5A). In all
specimens examined, additional “CD34-immunoreactive tumor satellites” have been detected in brain tissue that appeared normal in routine histopathological stains (Fig. 5).

The function of CD34 has yet to be clearly identified, although this epitope is commonly used for the characterization and isolation of hematopoietic stem cells (23). This relationship suggests that CD34 expression in gangliogliomas may reflect an immature phenotype of the tumor cells and/or an origin from a malformative dysontogenic precursor lesion (16). Whether CD34 itself is involved in tumorigenesis remains to be studied since few other tumor entities of the brain, peripheral nervous system, or stomach also express this epitope (24).

Additional Immunohistochemical Findings in Gangliogliomas

Neuronal marker proteins such as MAP2, NeuN, neurofilaments, and synaptophysin are useful tools to demonstrate the neuronal component in gangliogliomas. There is still no specific marker available to differentiate dysplastic/neoplastic neurons from normal counterparts. CD34 immunoreactivity appears helpful in this respect because it is not present in adult brain and co-localization with neuronal profiles points towards their abnormal nature.

Particular attention should be paid to the staining pattern of antibodies directed against MAP2 epitopes in the differential diagnosis of gangliogliomas and other low-grade neuroepithelial brain tumors. In a series of 400 diffusely infiltrating gliomas, all tumors of oligodendrogial and most tumors of astrocytic origin were strongly immunoreactive for low and high molecular weight MAP2 isoforms (25). The morphological and molecular similarity between MAP2-immunoreactive glial precursor cells of the developing human brain (including the exon 13 splice variant of the MAP2 gene) and diffuse gliomas would be compatible with an origin of these tumors from persisting glial precursor cells. The pattern of MAP2 immunoreactivity can facilitate the differential diagnosis of low-grade gliomas; that is, labeled tumor cells in oligodendrogliomas are characterized by round-shaped cell bodies with only few sparse processes. In contrast, astrocytic neoplasms display prominent, ramifying processes. Neuronal profiles can also be identified using MAP2 immunohistochemical reactions. In gangliogliomas, however, the glial component is not immunoreactive for MAP2, except in very rare instances. On the other hand, MAP2-labeled dysplastic neurons of various morphologies can be identified in the tumor, i.e. small granular neurons that are difficult to recognize in routine histological preparations.

Semi-quantitative estimation of the Ki67-labeling index can be used to characterize the biological behavior of the tumor (14, 17). In our series, the characteristic ganglioglioma showed a very low proliferation index with only occasional cells labeled per high power field (~1%). Since Ki67-labeled reactive cellular infiltrates such as microglia, lymphocytes, and macrophages are often present in the tumor tissue, the tumor-associated proliferation index in gangliogliomas may be even lower. Nuclear p53 accumulation has been only occasionally observed in gangliogliomas and no mutations of the TP53 gene were described (26).

The glial component of gangliogliomas should be immunoreactive for antibodies directed against GFAP or S-100 protein. Substantial heterogeneity in the expression of these antigens with a more consistent fraction of S-100-immunoreactive glial elements may be observed. However, these ubiquitous markers do not help to differentiate gangliogliomas from other glial neoplasms.

Atypical and Anaplastic Variants

In the vast majority of patients the biological behavior of gangliogliomas corresponds to WHO grade I (1). This is in agreement with a recent clinical study including 86 patients of our present series and a postsurgical follow-up period of 7 yr (10). In only 1 patient of this cohort was tumor recurrence observed (see above). According to the WHO consortium on the classification of brain tumors, histopathological features for atypia or anaplasia manifest in the glial component. In our series, 30 tumors were classified as atypical gangliogliomas (WHO grade II) characterized by increased cellularity and proliferation activity (the latter being identified by Ki67 labeling in ~5% of tumor cells). Seventeen tumors were classified as anaplastic gangliogliomas (WHO grade III) with substantial mitotic activity/proliferation activity in 10% or more of the tumor cell population (27). The detection of microvascular proliferates and necrosis are additional criteria indicating an anaplastic transformation of the tumor. Anaplastic features were never observed in the neuronal component.

Incomplete tumor resection/local tumor recurrence was established in 12 of 151 WHO grade I gangliogliomas, in which the clinical history of the patient was available for analysis. In most of these patients, surgical resection of residual tumor tissue was performed to achieve seizure control rather than to eliminate local tumor growth. Three of 12 atypical gangliogliomas (WHO grade II) and 3 of 8 anaplastic gangliogliomas (WHO grade III) showed local recurrence and/or malignant progression.

The immunohistochemical profile for atypical gangliogliomas is very similar to grade I tumors with most cases expressing the stem cell epitope CD34. However, anaplastic tumors did not follow this scheme, with significantly fewer cases immunoreactive for CD34. This observation may indicate differences in histogenesis or changes that occur during tumor progression.
The number of cases and long-term clinical follow-up is, however, still too small to establish a reliable histopathological grading scale suitable for the differentiation of atypical or anaplastic gangliogliomas.

The Differential Diagnosis of Gangliogliomas

Considering the histomorphological spectrum of gangliogliomas, these neoplasms have to be distinguished from various other glial or glioneuronal tumor entities. Although rare, gangliogliomas represent the most common tumor in young patients with chronic pharmaco-resistant seizures. We propose to carefully exclude the following panel of differential diagnoses when examining surgical specimens obtained from such patients.

Pilocytic Astrocytoma (WHO Grade I): Pilocytic astrocytomas predominantly occur in young patients and may affect any brain region. In addition, pilocytic astrocytomas of the temporal lobe are frequently associated with chronic epilepsy (28). These tumors may share many features with the glial component in gangliogliomas. The discrimination between pre-existing brain parenchyma and dysplastic neuronal elements can be of critical importance and should rely on an immunohistochemical reaction panel. Lack of CD34 immunoreactivity and presence of MAP2-labeled neoplastic glial cells are consistent findings in pilocytic astrocytomas. Proliferation activity is usually higher than in gangliogliomas with a range of 2%–3%.

Astrocytoma (WHO Grade II): Gangliogliomas with a diffuse growth pattern and predominant glial differentiation may be difficult to differentiate from diffuse astrocytomas. These tumors usually occur in adults, but have also frequently been encountered in epilepsy patients (29–31). Diffuse astrocytomas have a considerable potential for recurrence and malignant progression (10). They correspond to WHO grade II. Discrimination from gangliogliomas is therefore beyond academic purpose. The critical question of whether intermingled neurons represent pre-existing brain parenchyma and dysplastic neuronal elements can be of critical importance and should rely on an immunohistochemical reaction panel. Lack of CD34 staining corroborate this diagnosis. Proliferation activity in diffuse astrocytomas is approximately 5% and significantly higher than in gangliogliomas.

Oligodendroglioma (WHO Grade II): The honeycomb-like clear cell architecture is a histopathological hallmark of oligodendrogliomas. However, clear cell elements can also be encountered in gangliogliomas. In addition, both tumor entities can be associated with chronic epilepsies. Since oligodendrogliomas harbor the risk of tumor recurrence and malignant progression (WHO grade II), they must be carefully distinguished from gangliogliomas. The recently reported pattern of MAP2 immunoreactivity in oligodendrogliomas (25) can be helpful in this respect.

Similar to diffuse astrocytomas, proliferation activity is significantly higher compared to gangliogliomas.

Dysembryoplastic Neuroepithelial Tumor (DNT) (WHO Grade I): DNT, another tumor frequently associated with chronic focal epilepsies in young patients, was described in 1988 (32). DNTs are characterized by their specific glioneuronal element: a small and clear cell glial population embedded in a mucoid matrix and harboring mature “floating” neurons. Their multifocal nodular appearance within cortical brain regions is another characteristic finding of this tumor entity. The diagnosis of DNTs can pose considerable problems in cases with small, fragmented biopsy specimens or in the complex DNT variant with a prominent glioma component. DNTs lack, however, CD34 immunoreactivity.

Pleomorphic Xanthoastrocytoma (WHO Grade II): Pleomorphic xanthoastrocytomas arise from the astrocytic lineage and are predominantly localized in superficial cortical regions with involvement of the subarachnoid space. Pleomorphic, often multinucleated tumor cells are a histological hallmark, as well as the presence of foamy cells. The latter contribute to the xanthochrome macroscopical appearance of the tumor tissue. Significant numbers of reticulin fibers can be observed. Immunohistochemically, the tumor cells display a strong GFAP reaction and frequently show CD34 expression (unpublished observation). Lack of a neuronal component, expression of MAP2-immunoreactive neoplastic glia, and increased proliferation activity differentiate this glial neoplasm from gangliogliomas. There are few reports indicating, however, that gangliogliomas coexist in the setting of PXA (33).

Cortical Dysplasia: Cortical dysplasias (CD) may be difficult to discriminate from those gangliogliomas with a prominent dysplastic neuronal component. CDs appear to develop on the basis of dysontogenic abnormalities of neuronal migration and differentiation (34). In contrast to their terminology, cortical dysplasias do not carry an increased risk for neoplastic transformation, and the classification scheme for these lesions is still a matter of ongoing debate. A recent proposal by Palmini and Lüders suggests assigning most variants into 2 classes: mild forms of cortical malformation/heterotopia or focal cortical dysplasias (with or without dysmorphic neurons) (35). Among the latter category, focal cortical dysplasia of Taylor’s balloon cell type can be characterized as distinct clinico-pathological entity with frequent alterations of the TSC1 locus on chromosome 9q13.3 (36). These molecular-genetical studies may help to achieve a classification system that is based on a combination of both pathogenetic concepts and histomorphological features. Significant CD34 expression has not been observed in cortical dysplasia with the exception of occasional balloon cells in Taylor’s dysplasia (Fig. 5F) (37).
Gangliocytoma (WHO Grade I): In our series, we have not identified a single case of a differentiated, purely neuronal neoplasm with proliferation activity in the neuronal tumor component (except for Lhermitte-Duclos tumors of the cerebellum). Whether gangliocytomas really constitute a distinct tumor entity thus awaits further studies. They may well represent dysplastic or malformative lesions.

Gangliogliomas and Epileptogenesis

Pathogenic mechanisms underlying focal hyperexcitability in patients with gangliogliomas have not yet been delineated. Two major hypotheses are to be considered. The neuronal component of the tumor itself may contribute to epileptic activity. Immunohistochemical studies demonstrated that neuroactive molecules such as glutamatergic neurotransmitter receptors are expressed by dysplastic neurons (14, 38). Whether these neurons functionally integrate and excite neuronal pathways remains to be determined. Recent studies, however, support this notion. Intracerebral recordings from electrodes implanted to allocate seizure onset identified early ictogenic discharges from a ganglioglioma (Kirschstein et al, personal communication). These findings would support a hyperexcitable neuronal tumor component functionally integrated into excitatory circuits.

An alternative mechanism involves tumor-associated epileptogenic changes in the adjacent brain (39). Altered expression patterns of neuroactive molecules within the perilesional brain parenchyma, as well as the clinical observation that the epileptogenic area may be substantially larger than the tumor mass itself, would support this hypothesis. In many patients, seizure relief cannot be achieved if only the lesion is resected (13, 40). Neuronal hyperexcitability within perifocal brain areas may be evoked by kindling mechanisms in limbic structures such as hippocampus and amygdala. Similar phenomena can be observed in experimental animal models (41).

Molecular Pathogenesis of Gangliogliomas

The focal nature of gangliogliomas, the differentiated glioneuronal phenotype, and the benign clinical course suggest an origin from a developmentally compromised or dysplastic precursor lesion (14, 16). Subsequent neoplastic transformation would be confined to the glial component. This pathogenic model is supported by 2 recent observations. Expression of the stem cell epitope CD34 points towards a malformative nature of gangliogliomas. In addition, laser microdissection of individual tumor cell populations revealed a distinct mutation of the TSC2 gene present only in the glial component of a ganglioglioma (42).

With the recent progress in stem cell neurobiology, pluripotent precursor cells have been identified also in the mature human brain, i.e. the hippocampus (43, 44). These neural stem cells have the potential to generate a variety of glial and neuronal cell lineages. Focal alterations in signaling pathways regulating migration and differentiation of such progenitors may represent an alternative mechanism contributing to the development of gangliogliomas.

Molecular genetic studies on genes and genetic loci affected in human gliomas (including TP53, PTEN and EGF receptor and various chromosomal loci) have consistently yielded negative results in gangliogliomas (26, 45). Such observations strongly indicate that other signaling pathways play a role in these intriguing neoplasms. Considering the dysontogenic nature of gangliogliomas, genes associated with glioneuronal differentiation and migration may represent interesting candidates. Recent studies from our laboratory have spotted 2 developmentally regulated signaling cascades that may be involved in the pathogenesis of gangliogliomas.

Tuberous Sclerosis Complex (TSC)

The observation of an anaplastic ganglioglioma in the Eker rat, a strain carrying a TSC2 germline mutation, provided an initial clue (46). In addition, histomorphological similarities with TSC-associated pathologies point towards TSC1 (hamartin) and TSC2 (tuberin) as potential candidate genes in gangliogliomas. TSC-associated brain lesions (i.e. cortical tubers and subependymal nodules) are both composed of dysplastic neuronal and glial cell elements (47). Subependymal giant cell astrocytomas also represent characteristic brain lesions in TSC patients. Recent reports have allowed insights into the function of the TSC1 and TSC2 gene products. A functional interaction of tuberin and hamartin has been reported in Dro sophila. It appears to play a role in proliferation and regulation of cell cycle control via the insulin signaling pathway (48). The incidental finding of enlarged, binucleated neurons in gangliogliomas and of gigantic, multinucleated balloon cells in cortical tubers would support an involvement of TSC1 and TSC2 in cell cycle control. In addition, tuberin and hamartin interact with CDK1 and cyclin B1 (49, 50). Alterations in the TSC1 gene compromise the binding of hamartin to ezrin/radixin/moesin (ERM) proteins and affect the regulation of Rho GTPases. These changes interfere with cell adhesion (51).

In our comprehensive mutation and mRNA and protein expression analysis we were able to identify 7 polymorphisms in the TSC1 and 28 polymorphisms, as well as a single mutation in the TSC2 gene in a cohort of 55 patients with gangliogliomas (42, 52). The frequency of polymorphisms in the ganglioglioma group was significantly increased in intron 4 and exon 41 of the TSC2 gene compared to controls. However, mutations in the coding region of the TSC1 or TSC2 genes have not been identified (42, 52, 53). Abundance and clustering of
TSC2 polymorphisms, which manifest either in the vicinity of splice sites or accumulate within certain intronic regions (i.e. close to the GAP-related domain or within the coiled-coil hamartin interaction domain of TSC2) suggest a functional significance of these alterations and lend further support to a role of such TSC alleles in the development of sporadic gangglioglias.

With the identification of a somatic TSC2 mutation in intron 32, we were able to address the intriguing question of whether glial and neuronal cells simultaneously contribute to neoplastic transformation in gangglioglias. Laser microdissection and harvesting of individual tumor cell components allowed the assignment of the mutation to the glial portion but not the dysplastic neuronal elements of the tumor. This finding strongly suggests genetic heterogeneity between individual tumor components and is in line with the hypothesis of the glioma representing the neoplastic element in gangglioglias. Another observation in favor of this model is the immunohistochemical detection of increased Ki67 proliferation activity only in glial compared to neuronal tumor cells as recently reported (14).

Although we did not identify mutations that inactivate the TSC2 gene, our quantitative mRNA analysis points towards a significant reduction of mRNA levels in gangglioglias compared to normal brain tissue (unpublished observation). Immunohistochemical reactions using tuberin-specific antibodies confirmed reduced staining within the tumor parenchyma. Reduced tuberin expression was also reported in subependymal giant-cell tumors in patients with TSC (54). These findings appear to emphasize a potential function of TSC2 in the development of sporadic glioneuronal tumors (53). Since it appears that mutations are not responsible for decreased TSC2 expression, promoter inactivation, altered protein stability, or compromised protein-protein interactions may operate as pathogenic mechanisms.

Reelin Signaling Pathway

The reelin signal transduction cascade plays a major role in neuronal development, remodeling of the cytoskeleton, and cellular migration processes (55, 56). Key effector components of the reelin pathway are doublecortin (DCX; Xq22.3-Xq23) and cyclin dependent kinase 5 (CDK5; 7q36). DCX is expressed at high levels in fetal CNS. An altered DCX function in vitro results in interruption of microtubules (57, 58). The double cortex syndrome represents a neuronal migration disorder caused by (inactivating) mutations of the DCX gene (59), and is clinically characterized by seizures, cognitive dysfunctions, and neurological deficits.

The CDK5 gene is specifically expressed in postmitotic neurons and muscle cells (60, 61). CDK5 functions during the transition from G- to S-phase of the cell cycle and interacts with cyclin D (D1, D2, D3) (62). CDK5 and its complex partner p25 hyperphosphorylate the microtubule-associated protein Tau. Phosphorylation reduces the potential of Tau to interact with microtubules (63). CDK5 ablation in mice results in severe migratory defects of the CNS (64–66). CDK5 has also been implicated in apoptotic processes where it functions as a tumor suppressor gene (67, 68).

CDK5 and DCX thus represent interesting effector genes of the reelin signaling cascade. In a recent study, we performed a systematic mutational and expression analysis of both genes in 23 patients suffering from intractable chronic epilepsy and gangglioglias (69). Significantly decreased mRNA transcript levels were detected for both genes in the tumor group compared to brain tissue from gender-matched controls. Neither mutations in the DCX gene nor CpG islands in the DCX promoter region have been identified. Therefore, downregulation of DCX mRNA may result from a negative feedback mechanism operating upstream of the reelin signaling pathway. “In silico” analysis of the CDK5 gene revealed 2 CpG islands within the promoter region adjacent to exon1 suitable for methylation as a possible mechanism for CDK5 transcript downregulation.

Whether loss of proteins associated with the reelin signaling pathway plays a role for the development of neuronal dysplasia in gangglioglias requires further investigation. Our findings are compatible with developmentally regulated signaling cascades involved in the pathogenesis of gangglioglias. However, neoplastic transformation of the glial component may be driven by other neurodevelopmental tumor suppressor genes yet to be identified (69).

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