

The Differential Diagnosis of Central Nervous System Tumors

A Critical Examination of Some Recent Immunohistochemical Applications

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• **Context.**—As we write, novel antibodies that may well alter the routine practice of surgical neuropathology are in development, characterization, and the early stages of clinical use. These will be used for purposes of tumor subclassification, as prognostic markers, as identifiers of potential therapeutic targets, and as predictors of treatment response.

Objective.—To provide for nonspecialists a critical assessment of the peer-reviewed literature (necessarily colored by our own experience) as it pertains to several immunohistochemical reagents that have been recently for-

warded as adjuncts to the histologic typing of central nervous system tumors.

Data Sources.—We address in these pages only antibodies that are commercially available, that have been the subjects of multiple published series, and that we have had occasion to use in the course of everyday problem solving.

Conclusions.—Discussion concentrates on the use of 4 antibodies: BAF47 in the diagnosis of atypical teratoid/rhabdoid tumor, OCT4 in intracranial germinoma, β -catenin in craniopharyngioma, and NeuN as a marker of neuronal differentiation in neuroepithelial neoplasms.

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EMBRYONAL NEOPLASMS: BAF47 AND THE IDENTIFICATION OF ATYPICAL TERATOID/RHABDOID TUMORS

The atypical teratoid/rhabdoid tumor (AT/RT) shares with rhabdoid tumors of the kidney and soft tissues a predilection for infants and young children, an aggressive clinical biology, and characteristic genetic abnormalities.^{1,2} The adjectival “rhabdoid,” of course, recognizes the presence within these neoplasms of cells having eccentrically positioned, large, and vesicular nuclei, prominent nucleoli, and densely eosinophilic cytoplasm often compacted in a globose, inclusion-like fashion (Figure 1). “Teratoid” components that can be identified in central nervous system (CNS) variants include poorly differentiated neuroepithelial elements of small cell type (these may be indistinguishable from medulloblastoma, pineoblastoma, or supratentorial primitive neuroectodermal tumor [sPNET]), spindled mesenchymal constituents, squamous nests, and glandular or tubulopapillary formations.^{1,3} In fact, only a minority (approximately 25%) of AT/RTs are composed solely of rhabdoid cells and these are obscured in a significant subset of cases by vastly predominant primitive neuroectodermal tumor (PNET)-like populations.^{1,3} There is now compelling genetic evidence (see later) that select neoplasms wholly devoid of rhabdoid forms and quali-

fying on histologic grounds as medulloblastomas or other embryonal tumors of central neuroepithelial type actually represent “lopsided” AT/RT variants (Figure 2). This differential diagnosis is hardly an academic matter, as AT/RTs are predictably unresponsive to standard regimens used effectively against medulloblastomas and the like but may be controlled in some cases by intensified adjuvant treatment strategies.^{4,5} That AT/RTs can occur in a setting of heritable genetic predisposition further mandates their identification by surgical pathologists.² The difficulties practitioners face in doing so are compounded by the rhabdoid cytologic features on display in some medulloblastomas and PNETs of large cell/anaplastic type, choroid plexus carcinomas, high-grade gliomas, meningiomas, melanomas, metastatic carcinomas, and sarcomas.⁶

A confident diagnosis of AT/RT is facilitated in the prototypical case by immunolabeling of rhabdoid elements for vimentin, epithelial membrane antigen, smooth muscle actin, and glial fibrillary acidic protein.^{1,3} Such cells may also exhibit reactivity for cytokeratins, neurofilament proteins, and synaptophysin. Not all cases are so obliging in the matter of antigen display, however, and, as mentioned, rhabdoid cells may not be much in evidence. A major breakthrough in segregating AT/RTs from potential mimickers came with the linkage of these neoplasms (as well as renal and other extrarenal rhabdoid tumors of the pediatric cohort) to inactivating abnormalities of *hSNF5/INI1/SMARCB1/BAF47*, a tumor suppressor gene on chromosome 22q11.2.² Commonly designated by the abbreviated *hSNF5/INI1* or *INI1* alone, this encodes a ubiquitously expressed protein active in chromatin remodeling. *INI1* inactivation in the rhabdoid tumor group conforms in pattern to the 2-hit mechanism operative in the disabling of

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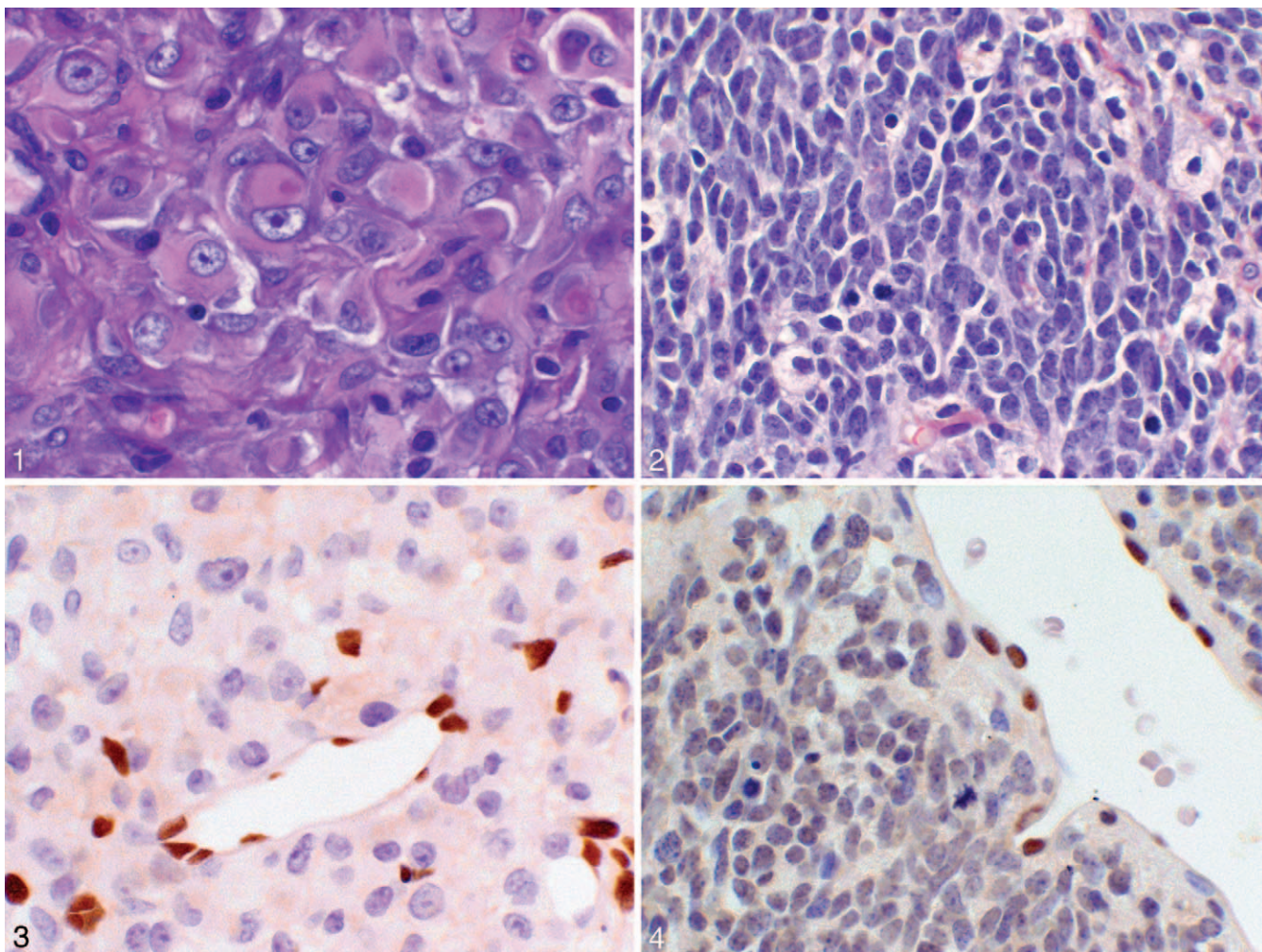


Figure 1. Atypical teratoid/rhabdoid tumor showing rhabdoid cytologic features with prominent nucleolus and eccentric eosinophilic inclusion-like cytoplasm (hematoxylin-eosin, original magnification $\times 400$).

Figure 2. Atypical teratoid/rhabdoid tumor with predominant small blue cell pattern simulating primitive neuroectodermal tumor (hematoxylin-eosin, original magnification $\times 200$).

Figure 3. Atypical teratoid/rhabdoid tumor showing lack of nuclear labeling for INI1; note positivity of endothelial and lymphohistiocytic cells as internal control (original magnification $\times 400$).

Figure 4. Atypical teratoid/rhabdoid tumor with small blue cell appearance shows no labeling for INI1; endothelial cells show nuclear staining (original magnification $\times 200$).

other tumor suppressing genes, loss of 1 copy resulting from partial deletion or monosomy 22 and the second copy suffering a nonsense or frameshift mutation that produces a novel stop codon. Deletions or mutations involving both *INI1* copies may also be encountered. Transmitted germline mutations of this gene underlie a familial rhabdoid tumor syndrome characterized by potentially multifocal, neural and extraneural primaries presenting in the first year of life.²

Genetic assessment may certainly facilitate the distinction of AT/RTs from other CNS neoplasms, as the investigations communicated to date indicate that *INI1* mutations are largely, if not entirely, restricted to the former.^{2,7,8} *INI1* mutant tumors morphologically acceptable as medulloblastomas or sPNETs have been reported,^{9–11} but such cases have generally exhibited the clinicobiologic features of AT/RTs and it has been reasonably proposed that these be classified and approached as such.¹² Choroid plexus tumors (mainly carcinomas) have also been described as

occasionally exhibiting *INI1* mutation,^{8,9,10,13} but, as presently discussed, there are grounds for questioning the pathologic diagnosis of the lesions in question. Undeniable, however, is that chromosome 22q deletions/monosomy have been identified in a variety of neoplasms, including choroid plexus carcinomas,¹⁴ that enter in this differential. Chromosome 22q11.2 deletions, in addition, are not detectable in AT/RT cases with uniparental disomy.¹⁵ Most problematic in regard to genetic diagnosis is the fact that only 70% to 75% of neoplasms exhibiting the morphologic and immunophenotypic attributes typical of the AT/RT demonstrate *INI1* deletion/mutation on karyotypic and molecular genomic appraisal.² Pertinent to the current discussion is the observation that a significant subset of ostensibly *INI1* intact cases nonetheless manifests decreased gene expression on reverse transcriptase–polymerase chain reaction analysis or undetectable INI1 protein product by Western blot.² Accordingly, an antibody to the INI1 protein would constitute a potentially more

sensitive adjunct to the diagnosis of AT/RT than current genetic assays. Such an antibody is now commercially available in monoclonal form for use on formalin-fixed, paraffinized material and is variously designated as BAF47/SNF5, anti-BAF47 or, simply, BAF47.

The BAF47 target protein is localized in the nuclei of normal cell types, including neurons, glia, and stromal and reactive elements. Thus, endothelium and lymphohistiocytic infiltrates serve as positive internal controls in the evaluation of tumor specimens even if adjoining neuroparenchyma is not represented in submitted surgical samples. A total failure of tumor cell nuclei to label with BAF47 has been consistently observed on immunohistochemical evaluation of bona fide AT/RTs, renal and extrarenal rhabdoid tumors.^{11,14,16–18} Loss of INI1 immunoreactivity has been a constant feature of cases exhibiting genetic evidence of inactivating *INI1* deletions/mutations (Figure 3) and of histologically and immunophenotypically classic examples without evidence of *INI1* abnormality, with decreased INI1 expression on RNA analysis, or simply not assessed for *INI1* status. We would emphasize the complete absence of BAF47 nuclear reactivity that must be demonstrated in using this antibody for purposes of diagnosis confirmation, as regional labeling loss can be encountered in non-AT/RTs and may very occasionally be extensive. In the absence of appropriate reactivity of included endothelial and lymphohistiocytic nuclei, results must be considered noninformative. Cytoplasmic labeling is irrelevant.

BAF47 is a reasonably discriminating reagent as applied to neurosurgical material. In the largest and most comprehensive survey of malignant pediatric brain tumors published to date (N = 289 cases), Haberler et al¹¹ found nuclear BAF47 labeling to be retained in glioblastomas (n = 41), anaplastic astrocytomas (n = 6), anaplastic ependymomas (n = 27), anaplastic oligodendroglioma (n = 1), choroid plexus carcinomas (n = 2), germ cell tumors (n = 8), and nodular/desmoplastic and large cell/anaplastic medulloblastomas (n = 17 and 20, respectively). Six of 158 classic medulloblastomas and 2 of 27 sPNETs failed to exhibit nuclear BAF47 reactivity. These INI1 nonexpressors generally presented in infants or very young children (≤ 3 years at diagnosis), responded poorly to conventional treatment regimens and coexpressed vimentin and epithelial antigens (epithelial membrane antigen, cytokeratins) despite their lack of rhabdoid constituents. Rhabdoid elements were ultimately identified in 2 of these cases at autopsy, and an additional 2 manifested evidence of homozygous 22q11.2 deletion on fluorescence in situ hybridization analysis. These apparent outliers, again, would seem to represent AT/RTs with domineering medulloblastoma/sPNET-like components (Figure 4). The smaller series of Judkins et al¹⁷ and Sigauke et al¹⁸ additionally attest to the general retention of nuclear BAF47 immunoreactivity in malignant childhood and adult-onset brain tumors of non-AT/RT type, as well as craniopharyngiomas (n = 2), pilocytic astrocytomas (n = 6), choroid plexus papillomas (n = 2), intramedullary ependymomas (n = 2), and gangliogliomas (n = 2). All 16 rhabdoid meningiomas assessed by Perry and colleagues⁶ proved BAF47 positive, as did 2 rhabdoid variants of glioblastoma included in their analysis.

Only an isolated CNS neoplasm seeming to fall outside the AT/RT group has been reported as entirely BAF47 silent, this being 1 of 2 pediatric oligodendrogliomas stud-

ied by Judkins et al.¹⁷ Fluorescence in situ hybridization probing did not disclose evidence of chromosome 22 deletion in this case. As mentioned, an anaplastic oligodendroglioma of childhood assessed by Haberler et al¹¹ retained nuclear BAF47 immunoreactivity and Sigauke et al¹⁸ found 4 adult oligodendrogliomas (3 of these anaplastic) to be similarly BAF47 positive. Judkins and colleagues¹⁷ also described regional loss of BAF47 reactivity in an anaplastic oligoastrocytoma and only faint nuclear labeling in a pituitary adenoma. By contrast, diffuse BAF47 expression characterized all 5 pituitary adenomas studied by Sigauke et al.¹⁸ Extraneural neoplasms reported as BAF47 negative include an isolated example of rhabdoid soft tissue leiomyosarcoma that proved chromosome 22q11.2 intact on fluorescence in situ hybridization analysis,⁶ a medullary carcinoma of the kidney,¹⁸ and a subset of epithelioid sarcomas.¹⁸ In regard to the latter tumor types, we would point out that chromosome 22 loss and abnormalities of 22q.11 have been reported in the setting of renal medullary carcinoma,^{19,20} that *INI1* deletions have been described in tumors classified as “proximal type” epithelioid sarcomas,²¹ and that a “distal type epithelioid sarcoma” devoid of INI1 protein expression is on record.²¹ Whether at least some of these *INI1* abnormal lesions actually represent rhabdoid tumors is open to argument. Other tumor types reported to be BAF47-expressing include Ewing sarcoma/peripheral PNET, clear cell sarcoma of soft tissues, desmoplastic small round cell tumor, Wilms tumor, renal clear cell sarcoma, congenital mesoblastic nephroma, Xp11.2 translocation-associated renal tumors, alveolar and embryonal rhabdomyosarcomas, malignant peripheral nerve sheath tumors, synovial sarcoma, angiosarcoma, chondrosarcomas (conventional, myxoid, and mesenchymal), adrenal neuroblastoma, rhabdoid malignant melanomas, and a variety of carcinomas including rhabdoid variants arising in the lungs, kidneys, liver, uterus, and gastrointestinal tract.^{6,16,18}

Given assertions that epithelial neoplasms of the choroid plexus, particularly carcinomas, may share with AT/RTs inactivating *INI1* mutations and occur in the setting of inherited, germline mutation of this gene,^{8,9,10,13} additional comment on the differential diagnosis of these entities seems warranted. The capacity of AT/RTs to present as intraventricular growths and to differentiate along epithelial lines potentially confounds this exercise, as does the presence in some choroid plexus carcinomas of anaplastic solid components that can include cells of suggestively rhabdoid appearance. Choroid plexus carcinomas, furthermore, may exhibit an AT/RT-like immunophenotype (with labeling for vimentin, epithelial membrane antigen, cytokeratins, and glial fibrillary acidic protein) and can manifest chromosome 22 deletions/monosomy (typically against a background of complex genetic aberration).¹⁴ In a targeted investigation of 28 tumors with a submitting diagnosis of choroid plexus carcinoma, BAF47 labeled all 20 cases in which the latter classification was upheld on “blinded” and independent review by 2 or more expert neuropathologists.¹⁴ Monosomy 22 was detected in 3 of 5 tumors in this subset, but all 7 subjected to mutation analysis evidenced normal *INI1* gene profiles. Complete loss of BAF47 nuclear reactivity characterized all 6 cases firmly placed in the AT/RT group on central review, these including examples with monosomy 22 previously published as choroid plexus carcinomas with “overlap,” AT/RT-like genetic features.²² Obviously, the

data support a role for BAF47 in distinguishing these tumor types and call into serious question the appropriate classification of lesions reported as *INI1* mutant neoplasms of choroid plexus derivation.

BAF47 immunoassessment is a simple, sensitive, and reasonably specific method for confirming or excluding the diagnosis of AT/RT in candidate cases and identifies a subset of medulloblastoma-, sPNET-, and pineoblastoma-like neoplasms that are appropriately regarded and approached as AT/RT variants. The recommendation¹¹ that CNS tumors of embryonal appearance be routinely screened for *INI1* expression using this reagent strikes us as prudent, similar considerations applying to poorly differentiated neoplasms for which a diagnosis of choroid plexus carcinoma is entertained.¹⁴ We would further agree that BAF47 assessment may not be indicated in the special circumstance of posterior fossa tumors with the appearance of conventional desmoplastic/nodular medulloblastoma, that is, manifesting the “pale islands” that define the latter entity.¹¹ This distinctive morphology has consistently correlated with retained BAF47 labeling and has never been evidenced, in our experience or to our knowledge, by a bona fide AT/RT.

CNS GERM CELL TUMORS: OCT4 AND THE IDENTIFICATION OF GERMINOMAS

The histopathologic evaluation of suspected CNS germ cell tumors can be a frustrating exercise given the scant and artefactually distorted neurosurgical specimens that are often submitted as biopsy material in this setting. The identification of germinomas, the most common subtype encountered in clinical practice, may be further complicated by obscuring lymphohistiocytic and granulomatous infiltrates that prompt considerations of neurosarcoidosis or other inflammatory/infectious processes. We refer the reader elsewhere for a comprehensive and fully annotated discussion of immunohistochemical profiling as applied to the problem of CNS germ cell tumor diagnosis and subclassification.²³ Here we discuss a relatively recent addition to the battery of reagents routinely used for these purposes.

OCT4 (also known as OCT3, OCT3/4, OTF3, and POU5F1) is an 18-kd POU-domain transcription factor, normally expressed in embryonic stem cells and primordial germ cells, that is integral to maintenance of the pluripotent state and requisite to primordial germ cell survival.^{24–27} Studies of gonadal germ cell tumors have demonstrated the consistent nuclear immunoreactivity of OCT4 by classic seminomas, dysgerminomas, embryonal carcinomas, the germ cell components of gonadoblastomas, and neoplastic intratubular germinal elements in the testis.^{28–32} By contrast, OCT4 reactivity has proved foreign to spermatocytic seminomas, yolk sac tumors, choriocarcinomas, and teratomas in these investigations. Our experience with CNS germ cell tumors to date suggests that nuclear labeling for OCT4 is a regular feature of (and phenomenon restricted to) germinomas (Figure 5) and embryonal carcinomas (Figure 6) in this setting.

Hattab et al³³ have assessed the comparative performance of commercially available antibodies to OCT4 and placental alkaline phosphatase (PLAP) against a series of 25 intracranial germinomas. Immunolabeling for PLAP, a primordial germ cell “marker,” is familiar, of course, to all pathologists as a finding used for years to support the diagnosis of germinoma but is neither a constant nor spe-

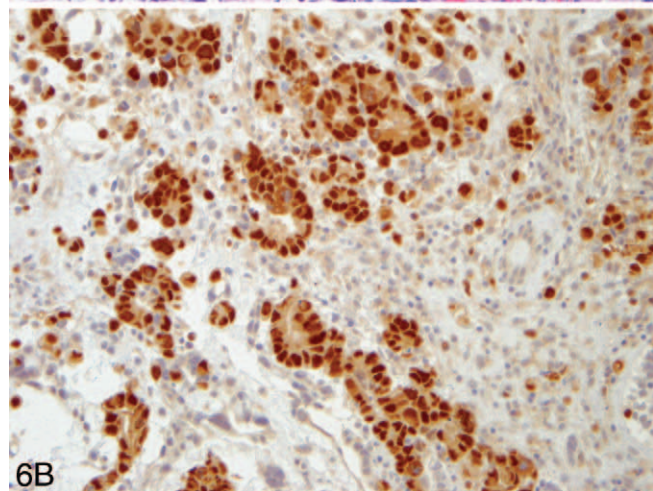
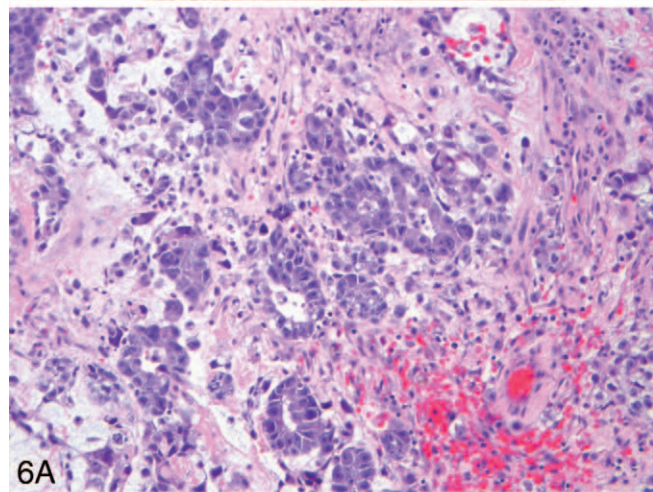
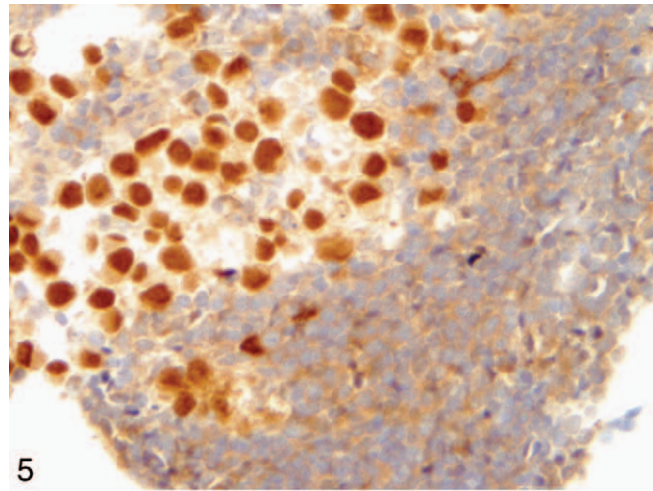


Figure 5. Germinoma with strong nuclear staining for OCT4; note absence of staining in lymphoid cells at lower right (original magnification $\times 200$).

Figure 6. A, Embryonal carcinoma with gland formation and typical cytologic features (hematoxylin-eosin, original magnification $\times 100$). B, Same case of embryonal carcinoma showing nuclear labeling for OCT4 (original magnification $\times 200$).

TUMORS AND CYSTS OF THE SELLAR REGION: NUCLEAR β -CATENIN IMMUNOLABELING AS A MARKER OF ADAMANTINOMATOUS CRANIOPHARYNGIOMA

cific attribute of the latter tumor type.²³ In the hands of Hattab and colleagues,³³ 23 of 25 germinomas were PLAP immunoreactive, but only 2 exhibited strong labeling and in 6 cases reactivity was described as weak. Furthermore, the authors acknowledged difficulties in distinguishing the membranous/cytoplasmic labeling of tumor cells for PLAP from nonspecific background staining in some instances. All 25 germinomas proved OCT4 positive, with diffuse nuclear labeling of strong intensity in 22 cases and moderate intensity in the remainder. As illustrated, tumoral nuclei retained OCT4 reactivity even in areas of severe crush artifact and necrosis. Nonspecific background labeling was not encountered and intense nuclear OCT4 staining permitted the ready identification of isolated germinoma cells in regions of heavy lymphocytic infiltration. In 1 case, OCT4-positive germinoma was admixed with entirely unlabeled elements of teratoma. All "control" cases were OCT4 negative, these including glioblastomas (n = 10), lymphomas (n = 4), pineoblastomas (n = 3), metastatic malignant melanomas (n = 4), capillary hemangioblastomas (n = 10), pituitary adenomas (n = 5), meningiomas (n = 4), schwannomas (n = 4), and metastatic carcinomas (2 lung, 2 colon, 1 breast).

Our experience with germinomas conforms in every respect to that of Hattab et al³³ and we think that PLAP assessment can be abandoned in the immunophenotypic subtyping of CNS germ cell tumors. Anti-OCT4 analysis is more sensitive and easily interpreted. The cited study, however, did not survey nongerminomatous germ cell tumor types and we cannot agree with the conclusion that OCT4 immunoreactivity is a specific germinoma marker. As depicted (Figure 6), the anti-OCT4 antibody used by Hattab and colleagues³³ strongly labels constituent nuclei in embryonal carcinomas of the CNS. The shared OCT4 expression of germinomas and embryonal carcinomas mandates additional immunophenotyping in cases that cannot be confidently subclassified on histologic grounds. Specifically, embryonal carcinomas can be recognized by their cytoplasmic labeling for CD30 (as well as their typically intense and diffuse expression of various cytokeratins), whereas germinomas depart from the latter in their membranous (and, in many cases, Golgi pattern) immunoreactivity for c-Kit (CD117)^{23,34,35} (Figures 7 and 8). In a companion study to the one detailed previously, Hattab et al³⁴ found that 23 of the 25 intracranial germinomas assessed were CD117 positive with 4+ staining intensity in 20 examples. Kamakura and colleagues³⁵ found all 13 cases of germinoma collected in their series to show membranous CD117. We, too, have found the great majority of germinomas to exhibit strong CD117 immunoreactivity. We have yet to encounter a CD117-reactive embryonal carcinoma, although we have seen a single "outlier" case of yolk sac tumor with membranous CD117 labeling and agree with Kamakura et al³⁵ that teratomatous epithelial and mesenchymal elements may display cytoplasmic reactivity for this antigen. As the differential diagnosis of CNS germ cell neoplasms includes AT/RT, we should mention having assessed several examples of the latter for OCT4 expression. None demonstrated nuclear immunolabeling. The reader is referred to our discussion of AT/RTs and BAF47 reactivity for additional comments relevant to this distinction.

The differential diagnosis of neurosurgical material deriving from the sellar region and suprasellar compartment may distill down to several entities that can share well-differentiated squamous components and, consequently, be difficult to distinguish (particularly in specimens of limited volume). These include craniopharyngiomas of both adamantinomatous and squamous papillary type as well as Rathke cleft cysts exhibiting the common phenomenon of squamous metaplasia. Nuclear immunoreactivity for β -catenin may be a property restricted, in this setting, to adamantinomatous craniopharyngioma.

The protein β -catenin normally functions in the maintenance of cellular adhesion through its complexing with cadherins at the level of the plasma membrane. A "free" cytosolic pool of β -catenin molecules constitutes a potential downstream component in the Wnt signaling cascade, a pathway involved in the developmental regulation of cellular morphology, motility, polarity, and proliferation.³⁶ In differentiated cells, this pool is kept to extremely low levels through a process of phosphorylation-dependent proteosomal degradation that is abrogated in a variety of human neoplasms by genetic mutations affecting the glycogen synthase kinase 3 β (GSK3 β) phosphorylation site of the β -catenin protein. Such mutations permit soluble β -catenin to accumulate and translocate to cell nuclei, where it may interact with transcription factors up-regulating the expression of target genes that include those encoding c-Myc and cyclin D1.^{37,38} Activating β -catenin mutations of this sort are shared by adamantinomatous craniopharyngiomas as a group but are foreign to craniopharyngiomas of squamous papillary type.³⁹⁻⁴³ In their initial report of these divergent genetic features, Sekine et al³⁹ noted corresponding differences in the immunohistochemical localization of β -catenin that have been confirmed in subsequent investigations.^{41,42,44}

Squamous papillary craniopharyngiomas exhibit exclusively cytoplasmic β -catenin immunoreactivity that is concentrated along tumor cell membranes in the manner characteristic of mature, nonneoplastic epithelia. Although this expression pattern is retained in portions of the adamantinomatous variant, the latter commonly manifests nuclear labeling for β -catenin as well (Figure 9). Usually correlating with mutations involving the GSK3 β binding domain but also occurring in some cases in which the latter are not demonstrable,⁴² this phenomenon is not encountered throughout the tumoral epithelium. Rather, nuclear reactivity for β -catenin is concentrated in areas of "morular," whorling cell aggregation and bordering foci of "wet keratinization" (ie, ghost/shadow cell change). Comparable immunoreactivity patterns have been documented in pilomatricomas, calcifying odontogenic cysts, and other neoplasms exhibiting morular formations or ghost/shadow cell keratinization and underlying mutations affecting the β -catenin GSK3 β phosphorylation site or Wnt-activating genetic lesions of other pathway members (APC) that result in increased β -catenin expression.⁴⁵⁻⁵⁰ Nuclear β -catenin reactivity may also be seen in palisaded epithelial cells at the interface of adamantinomatous craniopharyngiomas with stromal elements or neuroparenchyma.

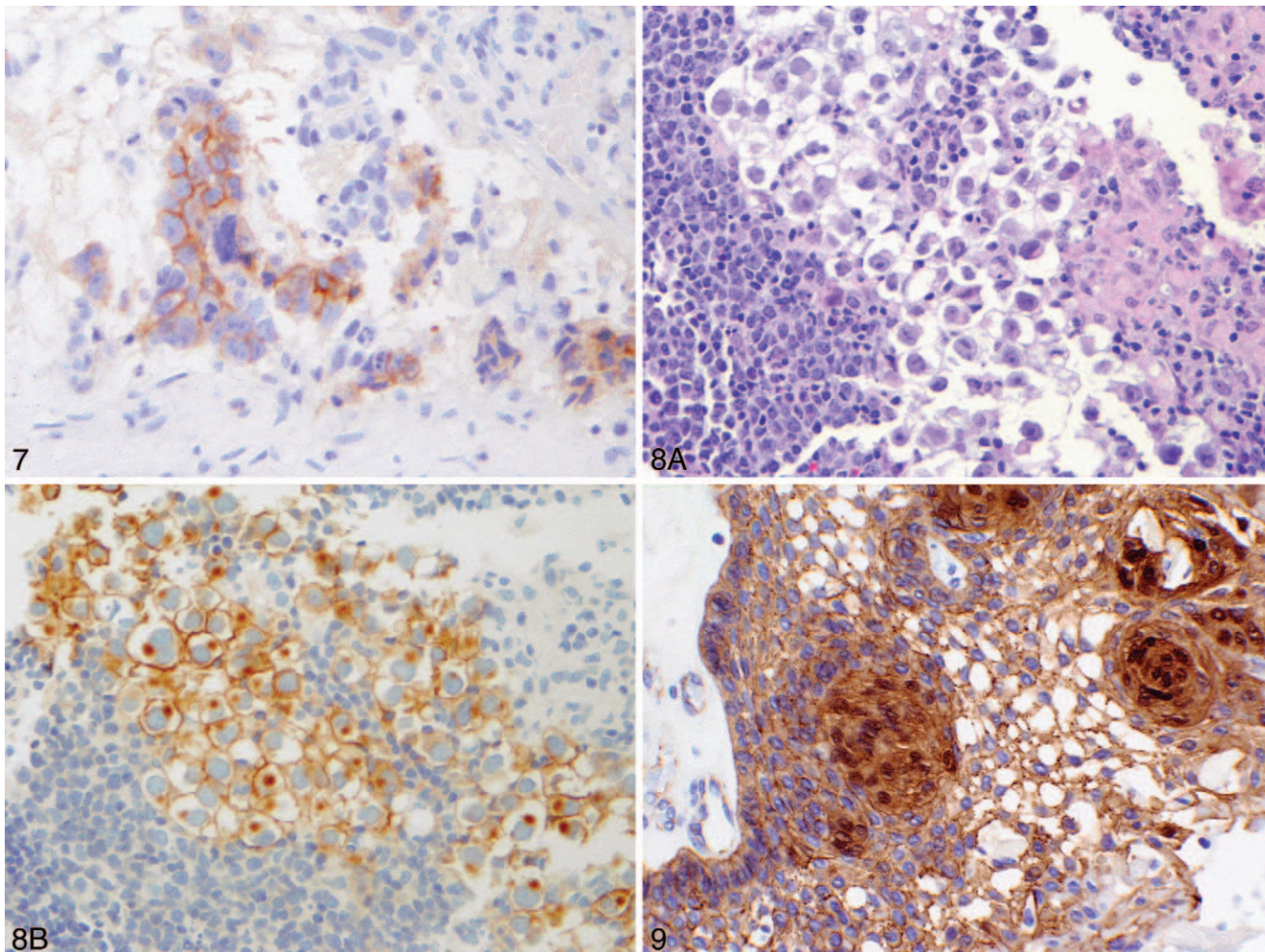


Figure 7. Embryonal carcinoma with membranous staining pattern for CD30 (original magnification $\times 200$).

Figure 8. A, Germinoma showing large cells with clear cytoplasm, prominent nucleoli and heavy associated lymphoid infiltrate (hematoxylin-eosin, original magnification $\times 200$). B, Germinoma from A showing membranous and Golgi labeling patterns in CD117 immunostain (original magnification $\times 200$).

Figure 9. Adamantinomatous craniopharyngioma with β -catenin immunostain showing membranous staining except in whorls where nuclear labeling is present (original magnification $\times 100$).

Buslei et al⁴² studied β -catenin immunolabeling patterns in a large comparative series including craniopharyngiomas of adamantinomatous ($n = 49$) and squamous papillary ($n = 8$) types, Rathke cleft cysts ($n = 10$), arachnoid cysts ($n = 8$), sellar xanthogranulomas ($n = 6$), and pituitary adenomas ($n = 58$). Regional shifts from cell membrane/cytoplasmic to nuclear reactivity were restricted to adamantinomatous craniopharyngiomas, occurring in 46 of 49 cases. Only small volumes of tumor were available for study in the 3 examples that failed to exhibit this phenomenon. Squamous metaplasia was not described as a feature of the Rathke cleft cysts assessed in this series. As noted by the authors, some workers⁵¹ have described frequent nuclear β -catenin labeling of pituitary adenomas despite evidence that underlying mutations of the β -catenin gene are either foreign to these tumors^{43,52} or exceptional in this setting.⁵¹ In an expansion of the series reported by Buslei and colleagues,⁴² Hofmann et al⁴⁴ found foci of nuclear β -catenin expression in 38 of 42 adamantinomatous craniopharyngiomas (4 outlying cases being restricted, again, to neurosurgical specimens of limited volume),

these including small examples of purely cystic type. Only cell membrane/cytoplasmic labeling was encountered in squamous papillary craniopharyngiomas ($n = 9$) and Rathke cleft cysts ($n = 30$, including 9 cases with squamous metaplasia).

These data support nuclear β -catenin immunoreactivity as a feature distancing craniopharyngiomas of classic adamantinomatous type from squamous papillary variants, as well as Rathke cleft cysts manifesting squamous metaplasia, and suggest that adamantinomatous craniopharyngiomas differ fundamentally from the latter lesions in their pathogenesis. The regional nature of this nuclear β -catenin accumulation, however, somewhat reduces the sensitivity of such assessment as applied to small specimens for differential diagnostic purposes. Craniopharyngiomas of both types have been reported in 1 relatively small series to differ from Rathke cleft cysts in their expression of cytokeratins 8 and 20.⁵³ Limited study also suggests that craniopharyngioma variants may evidence differing patterns of cytokeratin 7 and KL-1 (56-kd cytokeratin) expression,⁵⁴ the adamantinomatous subtype fur-

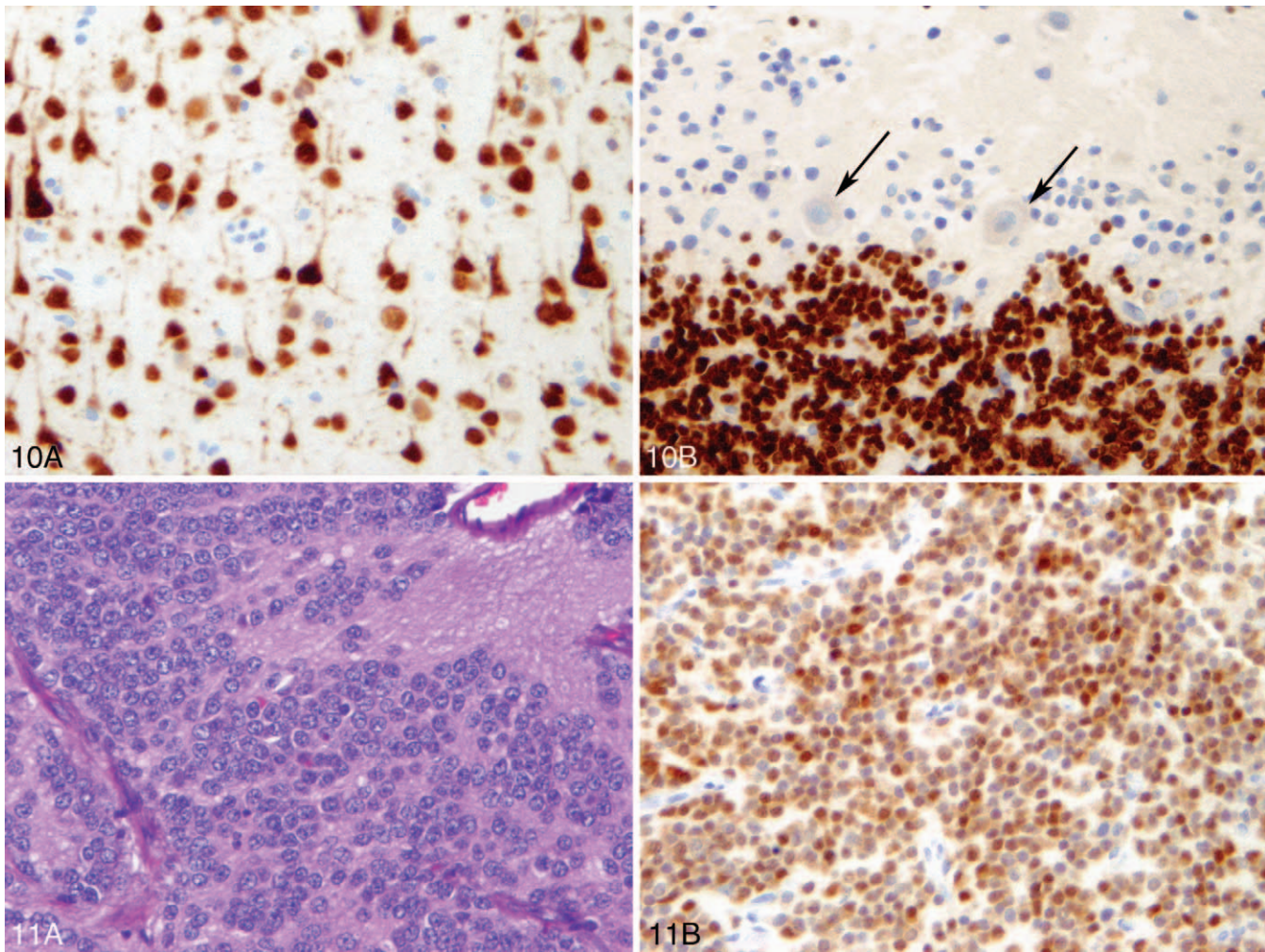


Figure 10. A, Cerebral cortex immunostained for NeuN showing nuclear and cytoplasmic labeling of neuronal cell bodies and absence of staining in glial cells (original magnification $\times 200$). B, Cerebellar cortex immunostained for NeuN shows strong nuclear reactivity in granular layer with unstained Purkinje cells at arrows (original magnification $\times 200$).

Figure 11. A, Typical histologic appearance of central neurocytoma with monotonous round cells and perivascular neuropil (hematoxylin-eosin, original magnification $\times 200$). B, Central neurocytoma with strong nuclear labeling for NeuN (original magnification $\times 200$).

ther proving in 1 analysis⁵⁵ to immunolabel for various enamel proteins and an odontogenesis-associated transcription factor (LEF1) not expressed by squamous papillary examples.

THE IDENTIFICATION OF NEOPLASTIC NEURONAL ELEMENTS: NeuN IMMUNOEXPRESSION AS A DIAGNOSTIC "MARKER"

A variety of immunohistochemical reagents may be used in the assessing of formalin-fixed, paraffin-embedded tumor samples for neuronal components. The A60 monoclonal antibody differs from the great majority of these, which identify cytoplasmic or cell membrane-associated antigens, in that its target is a DNA-binding nuclear protein—designated NeuN—that is developmentally neuron restricted and expressed in association with terminal neuronal differentiation.⁵⁶ A60 labels the nuclei and perikarya of postmitotic neurons throughout the central and peripheral nervous systems of vertebrates, including sensory ganglion cells and gastrointestinal neurons of the submucosal and myenteric plexi, although Cajal-Retzius neurons in layer 1 of the cerebral cortex, Purkinje cells,

inferior olivary and dentate nuclear neurons, retinal photoreceptor cells, mitral cells of the olfactory tracts, and ganglion cells of the sympathetic chain have been found nonreactive^{56,57} (Figure 10). Glia of all types, pineocytes, adenohipophyseal cells, and satellite and Schwann cells are normally NeuN negative, as are other nonneuronal tissue constituents.^{56,57}

Use of the A60 monoclonal antibody as a neuronal tumor "marker" spread widely among neuropathologists on the strength of only limited characterizations of its performance against neoplastic surgical material.⁵⁸⁻⁶⁰ The cited references collectively found nuclear NeuN expression foreign to gliomas of all varieties, as well as carcinomas metastatic to the brain, and a property restricted to the patently neuronal components of gangliogliomas, ganglioneuromas, and Lhermitte-Duclos disease (dysplastic cerebellar gangliocytoma), the cellular constituents of central neurocytomas, well-differentiated neurons and rare oligodendrocyte-like cells within some dysembryoplastic neuroepithelial tumors, and limited cellular subsets of the occasional medulloblastoma. Parker and colleagues,⁶¹ however, reported focal nuclear reactivity for NeuN in 11

of 17 intracranial ependymomas of childhood (with anaplastic lesions overrepresented in the NeuN-positive group) and Koperek et al⁶² noted, in a study of potential "look-alikes," that 2 of 10 clear cell ependymomas and 1 of 10 oligodendrogliomas harbored NeuN-reactive subpopulations (the 10 central neurocytomas assessed in this series all manifesting diffuse and strong nuclear A60 labeling; Figure 11).

Only recently has a truly broad investigation of NeuN immunoreactivity by CNS tumors (n > 600) been published.⁶³ In this analysis, Preusser and colleagues found at least focal nuclear NeuN labeling (with or without cytoplasmic reactivity) in examples of all major glioma variants assessed save for pilocytic astrocytomas (n = 100). NeuN-positive neoplasms in this study included select cases of diffuse astrocytoma (2/106), glioblastoma (4/115), low-grade and anaplastic oligodendroglioma with demonstrated chromosome 1p deletion (4/41), and both clear cell and conventional ependymomas of varying grade (9/107). Nuclear reactivity for NeuN was a feature of all central neurocytomas (n = 10) and of many medulloblastomas (63/86 classic, 13/14 desmoplastic, 11/13 large cell/anaplastic, 1/1 extensively nodular, and 1/1 medulloblastic) but was surprisingly manifested by only 4 of 14 tumors in the ganglioglioma/gangliocytoma group. Ostensibly glial neoplasms exhibiting widespread NeuN immunoreactivity (defined as $\geq 60\%$ of tumor tissue reactive) included 1 oligodendroglioma, 2 clear cell ependymomas, and 3 glioblastomas (2 conventional, 1 giant cell type). Among clear cell brain tumors, widespread nuclear labeling was displayed by all central neurocytomas, 2 of 11 clear cell ependymomas, and only 1 of 59 tumors classified as oligodendroglioma or oligoastrocytoma.

Our experience with the A60 anti-NeuN reagent conforms in nearly all respects to that of Preusser et al.⁶³ We, too, have noted that the neuronal components of ganglion cell tumors frequently fail to express NeuN even when highly differentiated in appearance (Figure 12) and think that this attribute is potentially useful in identifying mature-looking neurons as neoplastic so long as internal controls label appropriately. We further agree that central neurocytomas regularly exhibit nuclear NeuN reactivity (and that such reactivity is reasonably strong supporting evidence of a neurocytic neoplasm when diffusely apparent) but have found only focal labeling in some bona fide cases. Most importantly, we concur in the view that tumors otherwise qualifying as astrocytomas, oligodendrogliomas, and ependymomas may exceptionally manifest nuclear NeuN immunoreactivity. Although some might argue that the latter phenomena reveal A60 labeling to lack absolute specificity as an indicator of neuronal differentiation in the neoplastic context, neuroscientists in growing numbers would invoke such seemingly aberrant immunoprofiling data as just additional evidence that CNS tumors long regarded as glial in lineage and maturational commitment in fact derive from pluripotent precursors and retain some developmental plasticity. To take the matter of oligodendroglioma by way of example, we refer interested readers to investigations of normal neurocytogenesis documenting the isolation from the rat cerebral cortex of progenitors specifically programmed to spawn both oligodendrocytes and neurons⁶⁴ as well as studies in various species demonstrating that *OLIG* gene expression by common precursors is requisite to the de-

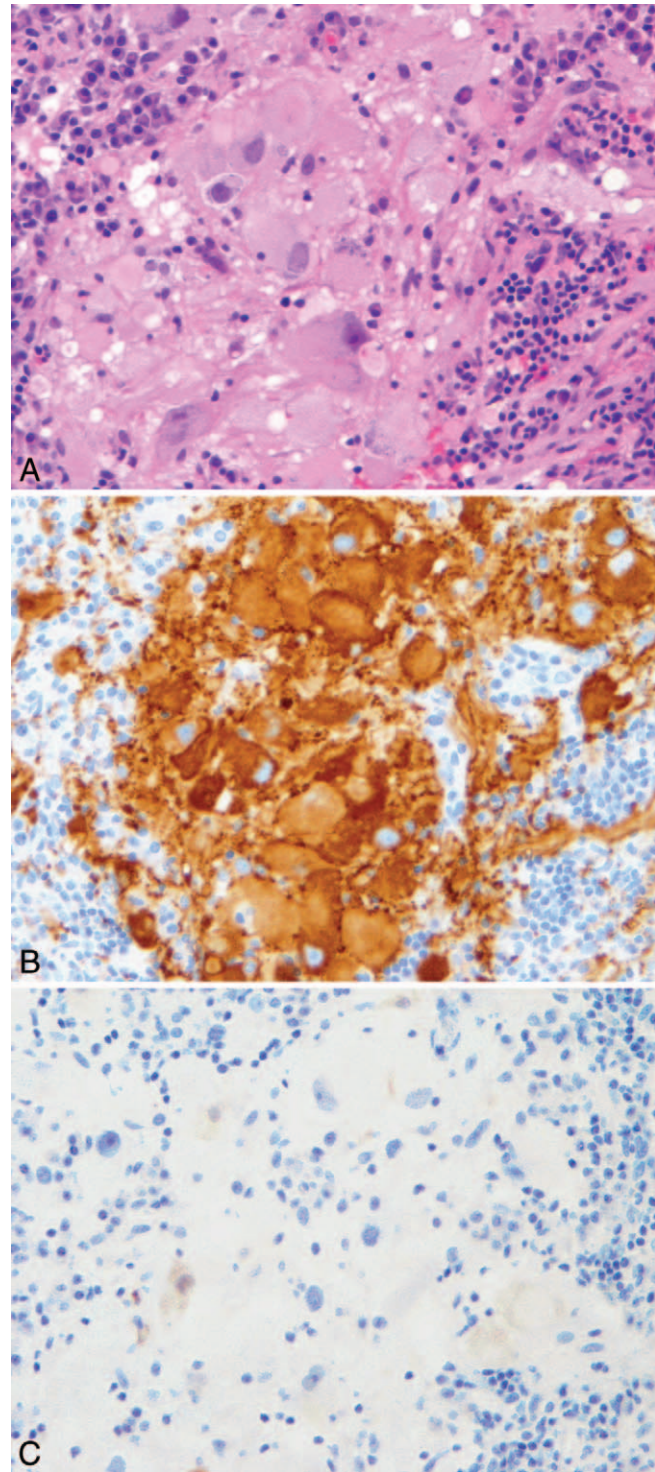


Figure 12. A, This ganglioglioma shows large, dysmorphic ganglion cells with abnormally distributed Nissl substance (hematoxylin-eosin, original magnification $\times 200$). B, Synaptophysin immunostain showing the area illustrated in A demonstrates cytoplasmic and perikaryal labeling (original magnification $\times 200$). C, Some ganglion cells from the area illustrated in A fail to stain for NeuN (original magnification $\times 200$).

velopment of oligodendroglia and select neuronal populations.^{65,66} The potentially neuronal attributes of neoplastic "oligodendrocytes" have been documented, furthermore, in physiologic,⁶⁷ ultrastructural,⁶⁸ immunohisto-

chemical,^{59,68–70} and gene expression⁷¹ analyses. Overt neurocytic differentiation with formation of synaptophysin-rich rosettes has been encountered in otherwise conventional oligodendrogliomas,⁷² including lesions manifesting the chromosome 1p/19q codeletions characteristic of this tumor group, and a similar phenomenon has been documented in association with neoplasms of diffuse astrocytic^{73,74} and ependymal⁷⁵ appearance.

On a utilitarian note, we do not practice or advocate the routine immunoscreening of neurosurgical tumor specimens for NeuN expression. To date, evidence has not been forwarded that would attach any special biologic import to nuclear NeuN labeling per se when displayed by neoplasms exhibiting those morphologic features that characterize the various glioma and embryonal tumor subtypes.

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