Dysembryoplastic Neuroepithelial Tumor (DNT): An Immunohistochemical and Ultrastructural Study

Takanori Hirose, Bernd W. Scheithauer, M. Beatriz S. Lopes, and Scott R. VandenBerg

Abstract. To assess the range of differentiation of the cells comprising dysembryoplastic neuroepithelial tumor (DNT), particularly the oligodendrocyte-like cells (OLC), 14 DNT were immunohistochemically studied with a spectrum of neuronal and glial markers. Eight tumors were also studied ultrastructurally. Neurofilament protein, class III β-tubulin, and synaptophysin preparations stained a few OLC in two, six and one lesion, respectively. In addition, many OLC within a single cortical nodule were reactive for class III β-tubulin. The vast majority of OLC were strongly S-100 protein positive. Glial fibrillary acidic protein labeled a fair number of OLC in two cases and one nodule consisted almost entirely of immunoreactive astrocytes. Ultrastructurally, many OLC resembled oligodendrocytes in exhibiting microtubules, prominent Golgi and short cell processes; pericellular lamination of cell processes, a characteristic of oligodendroglia, was noted in only one tumor. In two cases, OLC with astrocytic features were seen to contain small numbers of intermediate filaments. In four cases, a few OLC resembled immature neurons with scant dense-core granules or synapses. This study confirms the glioneuronal nature of DNT, a lesion composed of heterogeneous cells, many resembling oligodendrocytes and a few showing early astrocytic and neuronal differentiation. Although their relation to OLC is unclear, the presence and peculiar distribution of mature neurons is nonetheless an integral diagnostic feature of the lesion.

Key Words: Dysembryoplastic neuroepithelial tumor; Glioneuronal lesion; Immunohistochemistry; Neurons; Oligodendrogia; Ultrastructure.

INTRODUCTION

Only recently recognized, dysembryoplastic neuroepithelial tumor (DNT) is an uncommon neuroglial lesion of adolescents and young adults, one with a benign clinical course (1). Originally described cases were always associated with a long history of intractable partial complex seizures. Focal neurologic deficits were not noted. All lesions were supratentorial and intracortical, affecting primarily the frontal and temporal lobes, and were characterized by multinodularity as well as by a diffuse proliferation of what appear to be oligodendrocytes.

Histologically, DNT are stereotypic and exhibit a number of highly characteristic features. Both within and between nodules, the numerous, small, round oligodendrocyte-like cells (OLC) are associated with mucin accumulation. Astrocytes, often of pilocytic type, contribute significantly to some nodules. Mature, albeit sometimes abnormal neurons of different sizes may be seen to “float” within small mucin pools, particularly in internodular cortex. These essential features are often accompanied by dysplasia within surrounding cortex. Lack of recurrence and an excellent prognosis, even with subtotal resection or biopsy alone, suggest that DNT are hamartomatous or “dysembryoplastic” in nature (1).

Since publication of the original series (1), only a few additional cases of DNT have been published (2–4). Hasegawa et al (2) reported a lesion located in the right temporal lobe of a 16-year-old male with a 5-year history of intractable seizure. Pryson and Estes (3) described two patients, an 8- and a 19-year-old female, both of whom had partial complex seizures of long standing and temporal lobe involvement. Recently, a small DNT in a 75-year-old female with a 60-year history of seizures was reported by Gottschalk et al (4) who supported the concept that DNT is hamartomatous in nature. Although brief immunohistochemical and ultrastructural descriptions were provided, no large, comprehensive studies have been performed.

Unresolved issues remain, not only regarding the basic nature of DNT but also its constituent cells, particularly the OLC. Whether they represent true oligodendroglia, neuroglial precursors, or cells akin to neurocytes, a suggestion made by at least two authors (5, 6), remains to be established. In order to assess the range of differentiation of its component cells, 14 DNT were immunohistochemically studied with a spectrum of neuronal and glial markers. In addition, eight examples were systematically examined at the ultrastructural level.

MATERIALS AND METHODS

Fourteen cases of DNT, all of which exhibited typical clinicopathological features, were selected from the files of the Mayo Clinic Tissue Registry (Table 1). All but a single, recently diagnosed case were included in the original study of Daumas-Dupont et al (1). Nine patients were male and five were female, their ages at operation ranging from 4 to 29 years (mean, 15.3 years; median, 16 years). All tumors were associated with in-
TABLE 1
Clinical Data

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CTX = chemotherapy; XRT = radiation therapy; NED = no evidence of disease; AWD = alive with disease; * dead of radionecrosis; yr = years; mo = months.

tractable seizures and were supratentorial; cerebral lobes involved included the frontal (6), temporal (5), frontotemporal (1), frontoparietal (1) and temporoparietal (1). Follow-up ranged from 4 months to 17 years (mean, 8.2 years; median, 8 years). Neither clinical nor radiologic evidence of recurrence was noted in any case.

Tissues obtained at surgery were fixed in neutral, buffered formalin, routinely processed and paraffin embedded. All were stained by hematoxylin-eosin (H&E), Luxol fast blue-periodic acid Schiff (LFB-PAS), and the Bielschowsky silver method for axons. Immunohistochemical stains were performed using the avidin-biotin-peroxidase complex (ABC) method of Hsu et al (7). Primary antibodies utilized were directed toward the following: neurofilament protein (2F11, 1:75, DAKO, Carpinteria, CA), phosphorylation independent, high- and middle-molecular weight neurofilament protein (SMI-33, 1:1,000, Sternberger Inc., Baltimore, MD), phosphorylated epitope, high-molecular weight neurofilament protein (TnpNP-1A3, 1:100, donated by Dr. Collins [8], Ludwig Inst., Stockholm), class III β-tubulin (TUJ1, 1:500, donated by Dr. Frankfurter [9], University of Virginia, Charlottesville, VA), synaptophysin (SY58, 1:40, ICN, Costa Mesa, CA), S-100 protein (1:800, HSC, Toronto), glial fibrillary acidic protein (GFAP, 1:300, DAKO) and vimentin (V9, 1:10, DAKO).

For ultrastructural study, eight tumors were promptly fixed in Trunps' solution (phosphate buffered 4% formalin and 1% glutaraldehyde), postfixed in osmium tetroxide, en bloc stained with uranyl acetate, and embedded in Spurr's resin. Thin sections were stained with lead citrate and examined with a JEOL 1200 electron microscope. For the purpose of comparison, five ordinary oligodendrogliomas and two specimens of normal gray matter were also examined.

RESULTS

Light Microscopy

The lesions were largely limited to the cortex and were composed of (a) irregularly distributed zones of so-called "specific component," defined as "an extranodular cortical alteration characterized by diffuse oligodendroglial hypercellularity and mucin accumulation with floating neurons" (10), and (b) multiple nodules of variable size (0.5-3 mm). The "specific component" often exhibited microcystic change and accumulation of a mucinous matrix between cells, most of which were small OLC with uniform, hyperchromatic, round nuclei containing occasional indistinct nucleoli (Fig. 1). Their cytoplasm being scant, the cells often exhibited a perinuclear halo formation similar to that seen in ordinary oligodendroglioma. Furthermore, neurons of different sizes appeared to "float" within the mucin between the OLC; unlike in oligodendroglioma, no significant perineuronal satellitosis was observed. The large, conspicuous neurons had round, vesicular nuclei with a prominent, centrally placed nucleolus, abundant amphiphilic cytoplasm containing Nissl substance, and occasional processes. Smaller neurons were easily distinguished from OLC; although their cytoplasm was much less abundant than that of larger neurons, they exhibited clearly neuronal nuclear and cytoplasmic characteristics. No cells were observed with features transitional or intermediate between OLC and neurons. In addition to mucoid substance, a background of delicate, fibrillar neuropil was evident both within nodules and in the surrounding specific component. A delicate capillary network was often present in the specific component. In mucin-rich areas, OLC were prone to gather around capillaries.

The cellular components of the nodules were occasionally varied, even within a single lesion. Although cells of many nodules were similar to those of the specific component, namely OLC and neurons, some nodules were composed predominantly of OLC (Fig. 2A). Only a single, small cortical nodule in case 2 consisted of bipolar astrocytes (Fig. 2B), a few exhibiting mitotic activity. As a rule, mitoses were rarely encountered; only two tumors dem-
 demonstrated mitoses in small numbers. Some granular bodies were present in the specific component of three tumors. Delicate calcifications and minimal perineuronal satellitosis of OLC were identified in two and one lesion, respectively.

**Immunohistochemistry**

The results of immunochemical studies are summarized in Table 2. Each of the three neurofilament protein antibodies showed a characteristic pattern of staining. Reactivity for 2F11 was mainly localized to nerve fibers, the perikarya of the nerve cells being either nonstaining or only weakly and infrequently positive. On the other hand, SMI-33 showed primarily perikaryal reactivity. Both nerve fibers and perikarya were stained with the TpNFP-1A3 antibody. In summary, overall immunoreactivity for neurofilament proteins was as follows. Both within the specific component and the nodules of eight tumors, numerous, irregularly distributed immunopositive nerve fibers were present between neurons of different sizes and OLC (Fig. 3). In contrast, positive fibers were usually sparse or lacking within normal cortex. Perikaryal staining of some large and small neurons was also noted in ten tumors (Fig. 3), whereas neurons in extraleosional cortex were basically negative. Positive neurons of small size were easily distinguished from the nonstaining OLC by their larger, somewhat vesicular nuclei and prominence of nucleoli. Although OLC were devoid of neurofilament

![Fig. 1. "Specific component" characterized by diffuse OLC hypercellularity, a mucin-rich matrix and "floating neurons" of varying size. H&E (Case 1).](image)

![Fig. 2. Nodules composed of OLC (A) and astrocytes (B). H&E (Case 2).](image)
TABLE 2

Immuno-histochemical Features

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NF = neurofilament proteins; SYN = synaptophysin; TUB = Class III β-tubulin; S100 = S-100 protein; GFAP = glial fibrillary acidic protein; VIM = vimentin; NEU = neuron; OLC = oligodendrocyte-like cell; + = rare positive cells; ++ = scattered positive cells; +++ = numerous positive cells; = negative. *: This lesion contained a nodule composed of Class III β-tubulin-positive OLC. **: A nodule composed largely of astrocytic cells positive for GFAP, S-100 protein and vimentin.

In most cases, class III β-tubulin staining was localized to some obvious intralesional neurons of different sizes and to traversing nerve fibers. Although few in number, scattered, class III β-tubulin-immunoreactive OLC were also noted in six lesions (Fig. 5). In addition, a small cortical nodule in case 2 was mainly composed of class III β-tubulin-positive OLC (Fig. 6). This class III β-tubulin antibody occasionally showed weak staining of reactive astrocytes, but such cells were readily distinguished from OLC by their characteristic stellate configuration.

Synaptophysin staining was principally localized to neuropil-like, granular matrix between single and groups of cells. The amount of staining varied, not only between cases but within the same case. The neuropil often lay close to the cell membranes of OLC. Although in some instances it was difficult to determine whether immunoreactivity was localized to the membranes of adjacent OLC or to synapses of pre-existing nerve fibers, most OLC appeared immunonegative for synaptophysin. Only a few OLC in a single case (case 12) were equivocally positive.

In all tumors, the vast majority of OLC were strongly positive for S-100 protein. The reaction products were localized within both their cytoplasm and nuclei (Fig. 7). On the other hand, intra- and extralosomal neurons were generally negative or only faintly reactive. The tubulin-reactive OLC comprising the small cortical nodule of case 2 were also negative or only very weakly reactive for

Fig. 3. Intralosomal neurofilament protein immunoreactivity pattern. Reactive products are localized within the perikarya of some neurons and within numerous distorted neurites within the lesion. Neurofilament protein (2F11) immunostain (Case 5).
S-100 protein. Stellate, clearly reactive astrocytes were S-100 protein-positive.

Glial fibrillary acidic protein staining clearly demonstrated reactive astrocytes within all tumors. Whereas infrequent GFAP-positive OLC were recognized in all cases, in only two instances (cases 10, 11) were a fair number of positive OLC noted (Fig. 8). Furthermore, a cortical nodule in case 2 was seen to be composed of strongly GFAP-positive, bipolar astrocytes (Fig. 9). Aside from the localization of vimentin in endothelial cells, the results of vimentin staining were similar to those for GFAP. Vimentin-positive OLC were often seen in three lesions (Cases 10, 11, 13) and were infrequent in four other lesions.

**Electron Microscopy**

Ultrastructural observations were concentrated upon the loose-textured, mucin-rich areas of both nodules and the specific component. Mature neurons were observed in five specimens but OLC were well represented in all. Myelinated axons and nerve cell terminations accompanied by frequent, well-formed synapses were abundant in six of the specimens which appeared to contain neuropil in semithin sections.

The OLC were usually scattered singly but a small proportion lay closely apposed without intervening cell junctions (Fig. 10). Their round to ovoid nuclei often showed some indentations and contained heterochromatin beneath the nuclear membrane as well as single, small nucleoli. Cytoplasm was small to moderate in quantity and contained well-developed, albeit nonspecific organelles. In most OLC, these included a prominent Golgi apparatus, numerous free polyribosomes, mitochondria and a few microtubules (Fig. 10). Occasional cells contained a centriole, some coated vesicles, small lysosomes and a few segments of rough endoplasmic reticulum. Most OLC possessed a small number of short, slender processes, wherein some microtubules and a few ribosomes were present but synapses and dense-core granules were lacking. In one case (case 14), laminated, myelin sheath-like, membranous structures surrounded occasional OLC (Fig. 11). In two instances (cases 7, 11) some cells contained intermediate filaments in addition to microtubules, an appearance suggesting astrocytic differentiation (Fig. 12).
In four examples (cases 8–10, 12), minimal neuronal differentiation of OLC was evidenced by scant (50–160 nm) membrane-bound, dense-core granules within the cytoplasm of occasional cells (Fig. 13, lower inset). Furthermore, in three instances (cases 8, 10, 12) a few axonal terminations were seen to attach directly to the cell membranes of OLC. In areas of contact, the OLC membrane appeared dense and thick (Fig. 13). Clear presynaptic vesicles in small numbers were also noted within the apposed axonal terminations (Fig. 13, upper inset). The OLC engaged in synapse formation appeared to contain more numerous polyribosomes, although their ultrastructural features were otherwise similar to those of OLC without synapses. Between the OLC many well-differentiated nerve fibers were seen to engage in synapse formation, but most lacked observable connection to any cell bodies (Fig. 14).

Mature neurons were present in five specimens. Large and polygonal in shape, they were characterized by well-developed stacks of rough endoplasmic reticulum, numerous free polyribosomes and prominent Golgi complexes (Fig. 14). Some mitochondria, small lysosomes, lipofuscin granules, microtubules, and a few small, dense-core granules were also noted within their abundant cytoplasm. Neuronal nuclei showed a euchromatin pattern and exhibited a single, large, prominent nucleolus. Some neuronal cells possessed occasional long processes ter-

Fig. 7. Specific component demonstrating numerous S-100 protein-positive OLC in association with immunonegative, mature neurons. S-100 protein immunostain (Case 4).

Fig. 8. OLC exhibiting GFAP immunoreactivity are readily distinguished from reactive astrocytes. GFAP immunostain (Case 11).

Fig. 9. A cortical nodule composed of GFAP-positive astrocytes. GFAP immunostain (Case 2).
Fig. 10. OLC with short cell processes. Well-developed Golgi complexes, some mitochondria and microtubules are noted within the cytoplasm, although neither astrocytic nor neuronal differentiation is identified (×10,700). Inset: Many microtubules are also present within the cytoplasm (×26,900). (Case 7).

minating in synapses. Mature neurons, even small ones, could readily be distinguished from OLC associated with synapses. The neurons not only contained more numerous polyribosomes and well-organized rough endoplasmic reticulum but their chromatin pattern was different from that of OLC. No transitional cells with features intermediate between OLC and mature neurons were noted in any lesion.

Stellate, reactive astrocytes containing numerous intermediate filaments within their cytoplasm and processes were observed in four lesions (cases 1, 7, 13, 14). Tumor cells of the control oligodendrogliomas studied were basically similar to the OLC of DNT. The cytoplasm of neoplastic oligodendrocytes contained numerous mitochondria, free polyribosomes, prominent Golgi complexes, an occasional centriole and some microtubules. They also had relatively short, slender processes. Pre-existing myelinated axons and synapses were frequently observed between such tumor cells. Ultrastructural features of neurons in the normal gray matter were similar to those of mature neurons of DNT.

DISCUSSION

The unconfirmed nature of OLC contributes significantly to the nosologic problem surrounding DNT and its differential diagnosis. An oligodendrocytic character was suggested based on light microscopic similarities, but the precise derivation or differentiation of OLC remains uncertain (1). In that light microscopic similarities alone are insufficient proof that OLC are oligodendroglial in nature, it is no surprise that alternatives have been suggested. A possible neurocytic nature of OLC was recently proposed by Miller et al (5) and Nishio et al (6). Both groups reported synaptophysin immunoreactivity as well
as the ultrastructural presence of neurites with synapses and dense-core granules within multinodular, intracortical tumors composed of small, round tumor cells accompanied by lesser numbers of astrocytic and ganglion cells. Their lesions were also characterized by mucoid and microcystic changes. The authors considered these tumors, some of which we suspect represented DNT, to be nonclassical site (cerebral) neurocytomas. The characterization of the constituent cells of DNT, particularly of OLC, is therefore not only timely but of value in differential diagnosis.

In the present study, OLC showed complex and somewhat unexpected immunohistochemical features. Most OLC were strongly positive for S-100 protein, a glial-associated protein (11). Clearly, this protein is not specific to glial cells in that it is expressed by a variety of cells, including Schwann cells, melanocytes, sustentacular cells of the adrenal medulla and paranganglia, stellate cells of the anterior pituitary, etc. (11). Its localization in neurons is controversial. It has been stated that the α-subunit of S-100 protein is primarily located within neurons, whereas the β-subunit is present in glial and Schwann cells (12). The anti-S-100 protein antibody utilized in the present study reacts chiefly with the β-subunit; most neurons in our study, both intra- and extrasional, were immunonegative. Our observation of strong immunoreactivity of OLC for S-100 protein thus suggests that they are glial in nature. Indirect support for that contention is the observation that some OLC in a minority of tumors showed immunoreactivity for GFAP, a clearly glial marker. Immunostains demonstrated at least one intracortical nodule composed solely of GFAP-positive astrocytic cells. As had been stated in the original description of DNT (1), wide variation in cytology characterizes the nodular component.

Of particular interest was the expression of some neuronal markers by occasional OLC. A few cells were positive for neurofilament proteins, synaptophysin and class III β-tubulin in two, one and six tumors, respectively. These OLC could be readily distinguished from immuno-positive small neurons which exhibited large, vesicular nuclei and prominent nucleoli. One nodule, composed entirely of OLC, was diffusely immunopositive for class III β-tubulin. The latter is a neuron-specific isotype of β-tubulin that is among the first cytoskeletal proteins to appear in neuronal development (9, 13-15). In that this nodule was immunonegative for both neurofilament proteins and synaptophysin, it would appear to consist of immature neuronal cells. This may also be the case since class III β-tubulin is an earlier, more sensitive marker of neuronal differentiation than are neurofilament proteins or synaptophysin. In the present series, class III β-tubulin immunoreactivity was observed more often than were the two other markers. Our immunohistochemical study thus suggests that OLC consist of a heterogeneous pop-

Fig. 11. An OLC is surrounded by a myelin sheath-like, laminated, membranous structure (∗7,700). (Case 13).
the presence of occasional axo-somatic synapses provided definitive evidence of neuronal differentiation. In terms of other ultrastructural features, these OLC resembled those lacking specific features. Such primitive-appearing neuronal cells bore no resemblance to surrounding mature neurons, even small cortical neurons. Our ultrastructural study thus suggested the occurrence of divergent neuroglial differentiation in OLC. Although most cells resembled oligodendrocytes, a minority showed features of astrocytes or immature neurons.

Mature neurons, both large and small, are considered a basic constituent of DNT (1, 10). Anticipating some differences in immunophenotype and morphology between intra- and extralesional neurons, our studies showed some interesting immunochemical findings. The perikarya and axons of some neurons of DNT, both large and small, were immunoreactive for neurofilament proteins, whereas the perikarya of most neurons in surrounding normal cortex lacked reactivity. Numerous, irregularly distributed nerve fibers, mainly within the specific component, were also positive for neurofilament proteins. Daumas-Duport et al (1), in noting prominent neurite staining within cortical nodules, also suggested an inherent abnormality of intralesional neurons. Despite selective neuronal immunoreactivity, our ultrastructural study revealed no unusual ultrastructural features within the neurons of DNT. Dense-core granules were similar in size, distribution and number to those in normal cortical neurons. Not only were intralesional neurons readily distinguishable from OLC, no transitional cells were identified.

The question remains as to whether a histogenetic relationship exists between OLC and the mature neurons of DNT. Considering the occasional binucleation, irregular shape and loss of polarity of large neurons, as well as the maldistribution of nerve fibers within these lesions, many of the otherwise mature-appearing neurons of DNT may well be dysplastic. Otherwise normal neurons may become morphologically abnormal in some situations. For example, such secondary changes have been described in deafferentation and in irradiated tissue (22). Although similar mechanisms may underlie the neuronal abnormalities in DNT, they do not explain the often noted cortical dysplasia surrounding the lesion.

The present immunohistochemical and ultrastructural study confirmed the mixed glioneuronal nature of DNT (1). This complicated cellular constitution is the basis for possible misdiagnosis, particularly in small or fragmented

Fig. 12. An OLC with bundles of intermediate filaments within the cytoplasm shows astrocytic differentiation (×28,500). (Case 13).
Fig. 13. Primitive neuronal differentiation of an OLC is evidenced by the presence of a synapse (arrow) (×12,700). (Case 12)
Upper inset: The synapse at higher magnification. Note two presynaptic vesicles within the axonal termination (×67,300). (Case 12)
Lower inset: Dense-core granules within an OLC (×25,900). (Case 12).

specimens. In order to avoid unnecessary radiation and/or chemotherapy, the distinction of DNT from other lesions is of paramount importance. The prognosis of patients with DNT is excellent; no recurrences have been reported despite subtotal resection (1-4). The differential diagnosis includes oligodendroglioma, oligoastrocytoma, ganglioglioma, and neurocytoma (1, 5, 6, 10). Indeed, of the DNT in the present series, most had originally been diagnosed as mixed oligoastrocytoma, oligodendroglioma, and low-grade astrocytoma. A definitive diagnosis of DNT rests upon the identification of cortical nodules, most of which are OLC-rich, and the so-called “specific component.” Furthermore, although neuronal characteristics may be expressed by only a few OLC, the demonstration of such differentiation in OLC by immunohistochemical or ultrastructural methods may be of importance in distinguishing DNT from other glial tumors. Gangliogliomas consist of glial and neuronal elements but the former are nearly always fibrillary or pilocytic astrocytes; oligodendroglial cells are infrequently represented (10, 23). In addition, the neurons of DNT differ from those of ganglioglioma wherein dense-core granules are numerous, both within perikarya and in microtubule-rich cell processes (23, 24). The numerous synapses and neuritic processes demonstrated in “non-classical site (cerebral) neurocytomas” by Miller et al (5) and by Nishio et al (6) seem too well differentiated to be considered those of a neurocytoma. Indeed, synapse formation is a relatively infrequent feature in central neurocytoma (10, 25, 26). Similarly, the study of Hasegawa et al (2), as well as our own, demonstrated numerous synapses and nerve fibers within DNT. Most seemed to belong to mature, albeit abnormal neurons or to pre-existing cortical neurons, since almost no continuity was demonstrated between such nerve fibers and OLC.

The histogenesis of DNT remains unsettled. The OLC may be derived from progenitor cells capable of glioneuronal differentiation, ones originating in the secondary germinal layer of the developing central nervous system, particularly the subpial granular layer. Abnormalities in the proliferation of such cells and their migration to the cortex may be the basic abnormalities underlying DNT (1, 27). Whether a small proportion of OLC exhibiting early neuronal differentiation undergo further maturation to small or even large neurons, thus contributing to the pool of aberrant cortical neurons, remains unanswered.

In summary, our study supports the concept that DNT is a hamartomatous lesion, one consisting of both glial
Fig. 14. Synapse formation (arrows) among nerve fibers and upon a mature neuron containing well-developed rough endoplasmic reticulum, numerous polyribosomes and lipofuscin granules (×12,000). (Case 12).

and neuronal cells. The excellent prognosis associated with this uncommon lesion is in keeping with that interpretation.

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