CLINICAL–PATIENT STUDIES

Oligodendroglioma with neurocytic differentiation versus atypical extraventricular neurocytoma: a case report of unusual pathologic findings of a spinal cord tumor

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Abstract Differentiating oligodendroglioma from extraventricular neurocytoma by conventional light microscopy alone can present a diagnostic challenge. We report pathologic findings of an unusual spinal cord tumor from a 33-year-old male patient which showed hybrid features of oligodendroglioma and extraventricular neurocytoma. Magnetic resonance imaging (MRI) showed an enhancing intramedullary mass in the cervicothoracic region (C7 through T6). Histologic examination revealed a clear cell neoplasm containing ganglion-like cells and calcifications, prompting the differential diagnosis of oligodendroglioma and extraventricular neurocytoma. The immunohistochemical analysis disclosed neural differentiation of the neoplastic cells with strong synaptophysin and neurofilament staining consistent with extraventricular neurocytoma, as well as strong S-100 and glial fibrillary acidic protein (GFAP) expression. Molecular studies with fluorescent in situ hybridization (FISH) revealed chromosome 1p/(partial) 19q deletions, a finding commonly observed in oligodendroglioma. The proliferation index (using antibody MIB1) of the tumor was ~30%. The morphologic findings and these results strengthen the hypothesis that these tumors may share a common progenitor cell, which has also been observed by others. Because there are differences in patient management and long-term prognosis, it is important to attempt to distinguish between oligodendroglioma and neurocytoma. This unusual case and similar rare reported cases support the need to reclassify tumors showing pathologic features common to both neurocytoma and oligodendroglioma as a unique entity, while the effort continues to identify the cell of origin.

Keywords Oligodenroglioma · Extraventricular neurocytoma · Spinal cord

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Introduction

Neoplasms composed of oligodendrocyte-like cells often pose a diagnostic challenge. In the intramedullary spinal cord compartment, the principal differential diagnosis includes oligodendroglioma and extraventricular neurocytoma. Although immunohistochemical and ultrastructural investigation can be helpful in such cases, overlapping features may prove difficult to distinguish between the two entities. More recently, the application of molecular diagnostic techniques to certain types of primary central nervous system (CNS) tumors has increased the diagnostic accuracy over conventional microscopy, but at the same time raises important nosological questions.

Central neurocytomas are rare, generally circumscribed, intraventricular CNS tumors first recognized by Hassoun et al. [1]. Clinically, they often present as

calcified supratentorial masses in young adults with symptoms of increased intracranial pressure. Microscopically, the neoplastic cells are monotonous with clear cytoplasm and small round nuclei, and often with a surrounding halo that resembles oligodendroglioma. In some cases, there are rosette-like structures consisting of irregular acellular zones with a fine, fibrillary matrix. Immunohistochemistry demonstrates evidence of neuronal differentiation with consistent synaptophysin positivity and variable staining for neurofilament proteins, especially prominent with the fibrillary matrix [2–4]. These findings are substantiated by ultrastructural features consisting of microtubules, neurofilaments, neurotic processes, secretory granules, and synaptic junctions [3, 5, 6]. Neurocytomas are thought to arise from mature neuronal cells but in vitro culture studies have shown that the progenitor cells are likely to be glioneural with the potential to differentiate into both glial and neuronal cells [7, 8]. Glial fibrillary acidic protein (GFAP) and S-100 expression is typically not observed in the tumor cells but in the surrounding reactive astrocytes [2]. More recently, extraventricular neurocytomas (EVN) have been reported as brain and spinal cord masses [9–19].

Oligodendrioglioma was first described by Bailey and Bucy in 1929 based on morphologic similarity of the neoplastic cells to non-neoplastic oligodendrogliocytes [20]. It is the third most common type of gliomas, comprising 4–7% of primary brain tumors [21]. The classic microscopic appearance is that of a neoplasm composed of clear cells surrounded by perinuclear halos and embedded in a scaffolding of "chicken wire" vessels. The tumor lacks expression of markers of mature oligodendrocytes; however, there may be focal expression of GFAP, especially in gliofibrillary oligodendrocytes and minigemistocytes [22]. Although the cell of origin of either oligodendroglioma or neurocytoma has not been identified, several investigators have suggested that these two tumors may have a common cell of origin [23, 24].

We report the pathologic findings of a spinal cord tumor from a 33-year-old male, which showed unusual combination of morphology, immunohistochemistry, and fluorescent in situ hybridization (FISH) results, underscoring the challenge of discriminating between oligodendroglioma and neurocytoma and suggesting a possible common origin.

Case history

A 33-year-old male patient with long standing history of neck pain, right hand numbness, and left ankle weakness presented to a tertiary care provider. On admission, there were no abnormal findings on general physical examination. Neurologic examination failed to reveal extremity weakness or sensory loss.

Magnetic resonance imaging (MRI) studies at the time showed abnormal T2 signal with an intramedullary mass in the cervico-thoracic spinal cord extending from C7 to T7. The mass lesion showed heterogeneous signal intensity and enhancement throughout the lesion (Fig. 1). A radiologic diagnosis of astrocytoma was considered. The patient underwent complete resection of the tumor, which was submitted to surgical pathology in its entirety.

The patient tolerated the procedure well. His postoperative hospital course was complicated by weakness of right iliopsoas and quadriceps dorsiflexion for which he received physical and occupational therapy. After 2 weeks of post-surgical hospital stay he was transferred to a rehabilitation center with a follow up visit in 1 month.

Material and methods

Light microscopy

A portion of the specimen was received for frozen section diagnosis. The remaining specimen was submitted

Fig. 1 T2 weighted sagittal MRI shows intramedullary mass in the thoracic spinal cord (C7–T7) with heterogenous signal intensity and enhancement throughout the lesion

entirely and processed routinely. Histological sections were prepared from 10% formalin-fixed, paraffin-embedded tissue, and stained with hematoxylin-eosin for routine light-microscopic examination.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections using an avidin-biotin-horseradish peroxidase system after the tissue was deparaffinized and rehydrated according to standard protocol. The following antibodies were used: polyclonal antibodies against S-100, chromogranin and synaptophysin (Dako, Carpinteria, CA, USA), and monoclonal antibodies against epithelial membrane antigen (EMA), neurofilament, neuron specific enolase (NSE), MIB-1, GFAP, vimentin (Dako), CD99 (O-13) (Signet; Dedham, MA, USA), and keratin (a cocktail of AE1/AE3, CAM 5.2, and 35βH11) (Ventana Medical Systems, Tucson, AZ, USA). In every case, formalin-fixed tissue was subjected to heat-induced antigen retrieval.

Fluorescence in situ hybridization (FISH) analysis

Chromosomal analysis was performed on deparaffinized formalin-fixed, paraffin-embedded tissue sections using commercially available Vysis dual-color probe sets (LSI 1p36/LSI 1q25 and LSI 19q13/LSI 19p13) as described in the manufacturer's catalog (Vysis Inc., Downers Grove, IL, USA).

Results

H&E stained sections showed solid areas of uniform, polygonal cells with small round nuclei and delicate chromatin with clear perinuclear halos, clear cytoplasm accompanied by a capillary vascular arcade that compartmentalized the tumor cells, as well as foci of small or large cells with round nuclei and eosinophilic cytoplasm suggesting gangliocytic differentiation (Fig. 2a–c). Mitotic figures were rare and necrosis was not observed.

Table 1 summarizes the immunohistochemical results. The tumor cells including with clear cytoplasm stained strongly for NSE, neurofilament, synaptophysin (Fig. 3a), GFAP (Fig. 3b), and S-100 (Fig. 3c). The cells resembling ganglion cells stained for chromogranin (Fig. 3d), NSE, S-100, neurofilament, and synaptophysin and were negative for GFAP. The clear cells were negative for vimentin, whereas the cells with eosinophilic cytoplasm and ganglion-like cells were positive. The proliferative activity of the tumor was estimated to be 30% using MIB1 antibody in the clear cells, whereas it was negative or very low in the remainder of the tumor. The neoplastic cells did not express keratin, CD99, or EMA.

Fluorescent in situ hybridization analysis revealed fewer than two orange signals for 1p36 indicating deletion of 1p36 region on chromosome 1 in ~80% of the tumor cells (Fig. 4a), whereas no deletion of 1q25 region was present (green signal, Fig. 4b). Also, in ~10–20% of the tumor cells fewer than two orange signals for chromosome 19q13 were also observed indicating deletion of chromosome 19q13 region, but not 19p13 region (data not shown).

Discussion

This case highlights the limitations of the present diagnostic criteria for classifying primary clear cell neoplasms of the CNS. Since current nomenclature of brain tumors is based on morphology, ultrastructure, and immunohistochemistry rather than molecular genetic criteria, it may be considered incongruent to classify our case as an oligodendroglioma. At the same time, the prognostic role of chromosome 1p/19q deletion in oligodendrogliomas has generated great interest in targeted therapy which may be associated with chemosensitivity and higher survival [25–27]. In addition, our findings lend further support to a proposed common progenitor cell for these two tumors [23, 24].

According to in vitro animal studies, the O-2A oligodendrocyte precursor cell has the ability to generate oligodendrocytes or type 2 astrocytes in rats [28]. Similarly, human gliomas contain glial precursor cells that can give rise to cells of astrocyte or oligodendrocyte lineage [29]. Williams et al. [23] demonstrated the existence of a bi-potential precursor cell with the ability to differentiate to either oligodendrocytes or neurons in rats. Using immunohistochemical markers in an in vitro model, the authors identified mixed clones with neurons and oligodendrocytes. Similar findings were reported by Price and Thurlow [30], who demonstrated a progenitor cell in the rat cerebral cortex that could differentiate into both neurons and white matter glial cells. These findings are not surprising since cultured rat spinal cord neuroepithelial cells, which are distinct from neural crest stem cells, can differentiate into neurons, oligodendrocytes, and astrocytes [31]. O-2A cells have been shown to express platelet derived growth factor α receptor (PDGF α R) and NG2 chondroitin sulfate proteoglycan in vitro, which can be detected in tissue sections. Based on immunohistochemical analysis of human gliomas,

Fig. 2 Representative morphologic features from formalin fixed paraffin embedded sections of the tumor. *Lower magnification* shows monomorphic cells with clear cytoplasm as well as areas rich in ganglion-like cells (**a**) (hematoxylin and eosin, ×20). Higher magnification of the clear cells (**b**) and the ganglion-like cells (**c**) (hematoxylin and eosin ×100, each)
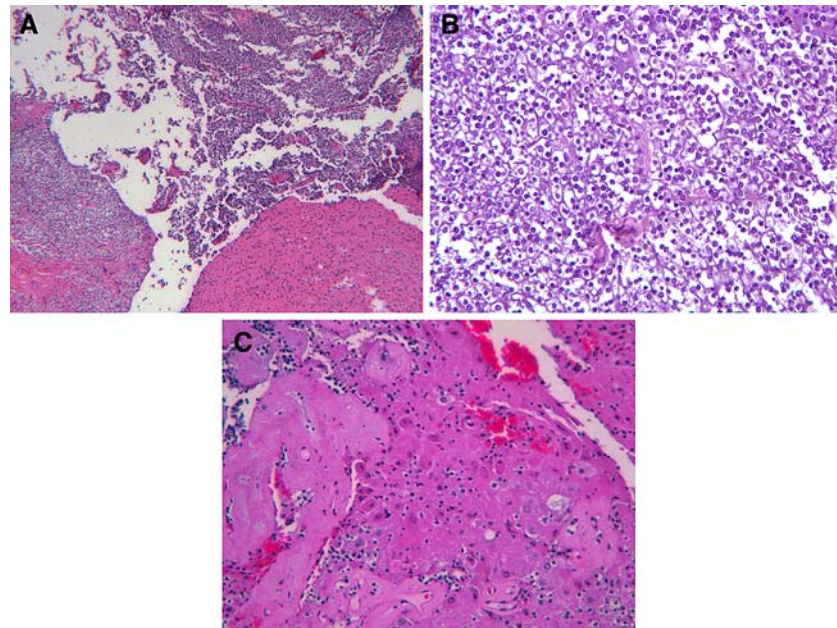
Shoshan et al. [32] suggested that oligodendrogliomas might arise from NG2+/PDGF α R + progenitor cells. As cell division is important in tumorigenesis, proliferating progenitor cells are more susceptible to oncogenesis than post-mitotic cells supporting the hypothesis that NG2+/PDGF α R + cells could be the precursor of oligodendrogliomas. It is not clear if similar cells are also progenitors of neurocytomas, but because oligodendrocytes can be induced from adult human olfactory epithelial-derived precursors [33], it is possible that human oligodendriogliomas and neurocytomas are derived from a common precursor cell.

Table 1 Results of immunohistochemical staining by cell type

Antibody used	Cells with clear cytoplasm	Smaller cells with eosinophilic cytoplasm	Ganglion cells
S-100	Positive (2+)	Positive	Positive
Chromogranin	Negative	Negative	Positive
NSE[a]	Positive (3+)	Positive	Positive
Neurofilament	Positive (2+)	Positive	Positive
Synaptophysin	Positive (3+)	Negative	Positive
GFAP[b]	Positive (2+)	Negative	Negative
Vimentin	Negative	Positive	Positive
Pancytokeratin	Negative	Negative	Negative
EMA[c]	Negative	Negative	Negative
CD99	Negative	Negative	Negative
MIB-1 (Ki 67)	Positive in 30%	Positive in less than 5%	Negative

[a] Neuron specific enolase; [b] Glial fibrillary acidic protein; [c] Epithelial membrane antigen; 3+, staining in more than 70% of the clear cells; 2+, staining in 30–70% of the clear cells

Few cases of neurocytoma have been reported in extraventricular sites including the spinal cord [9–19]. As in the present case, most spinal neurocytomas occur as intramedullary cervical or cervico-thoracic tumors. EVN are histologically similar to their centrally located counterparts, but often exhibit glial and ganglionic differentiation [11]. Accordingly, the typical immunohistochemical profile consists of synaptophysin and neurofilaments positivity with variable GFAP expression. Similar to our case, ganglion cells in EVN stain for chromogranin, which is not observed in central neurocytomas.

In general, central and extraventricular neurocytomas have a good prognosis [2, 11]. Complete resection of neurocytomas is the treatment of choice. The diagnosis of atypical neurocytoma has been assigned to those tumors showing necrosis, vascular proliferation, increased mitotic activity, and MIB1 labeling index of more that 2%. The MIB1 index has been shown to correlate better with survival and recurrence than cellular atypia alone [34]. Our case lacked cellular atypia and necrosis; however, the MIB1 proliferation index approached 30%, which is higher than reported cases of atypical neurocytoma [17, 18, 34].

Ho-Keung et al. [35] were the first to report that oligodendrogliomas exhibited staining for the neuronal markers synaptophysin and NSE. Subsequently, Wharton et al. [36] reported synaptophysin and neurofilament expression and neuron-like electrophysiologic properties in cultured oligodendroglioma cells. Although the neoplastic clear cells in our case showed inconsistent staining for neurofilaments and synaptophysin, this can

Fig. 3 By immunohistochemistry, the clear cells are positive for synaptophysin (**a**), GFAP (**b**), and S-100 (**c**), (×100, each). The ganglion-like cells are positive for chromogranin (**d**) (×400)
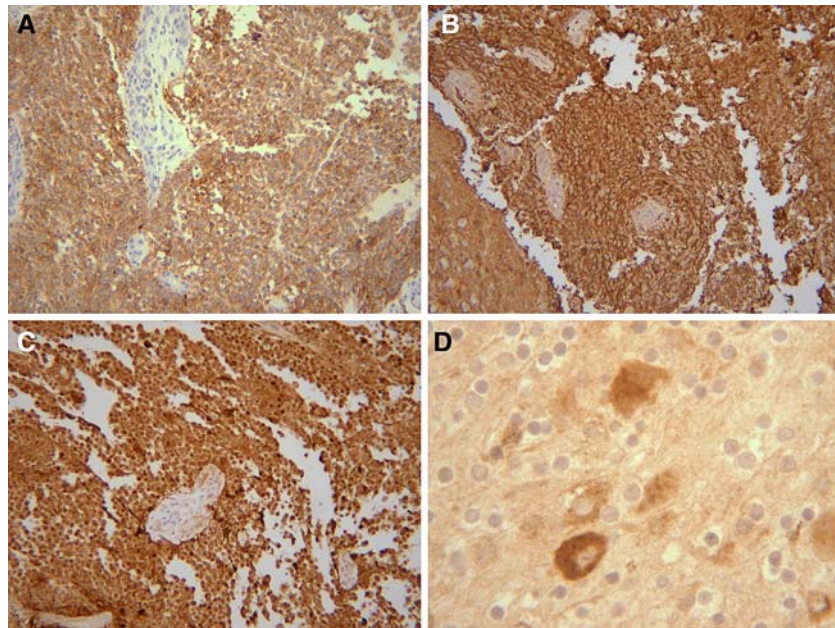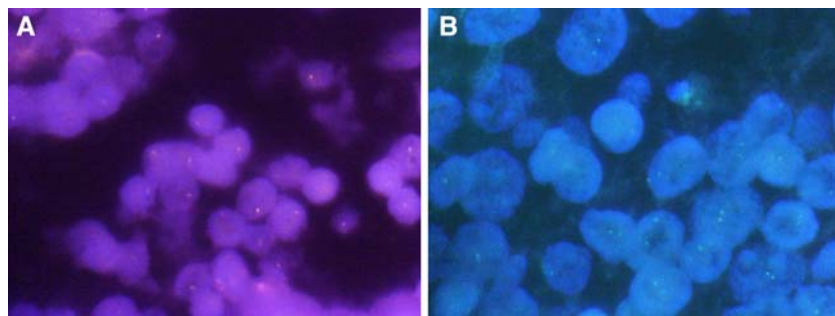

Fig. 4 FISH analysis demonstrates 1p36 deletion in the majority of the tumor cells (**a**) (×1,000), whereas no deletion of 1q25 was observed (**b**)

be explained by loss of immunoreactivity with formalin fixation and embedding or true oligodenroglial differentiation. Morphologically, the identification of focal ganglion cell differentiation in our case confirmed by chromogranin immunostain and synaptophysin and neurofilament expression in clear cells supports the diagnosis of EVN rather than oligodendroglioma. However, the presence of S-100 and GFAP staining and lack of vimentin in clear cells is more in favor of oligodendroglioma. In in vitro experiments with neurocytomas, a shift from synaptophysin to GFAP expression has been observed with successive cell passages [7]. Also in rats transplacental ethyl-nirosourea administration induces oligodendrioglioma-like tumors with both neuronal and glial markers [37]. These observations favor a common bi-potential progenitor cell of origin for neurocytomas and oligodendrogliomas.

Given the lack of specific immunohistochemical, histological or ultrastructural markers for oligodendrogliomas, greater emphasis has been placed on molecular genetic criteria, that is, allelic deletions on chromosome 1p and 19q to make the diagnosis [25, 26]. Studies have shown that chromosome 1p loss occurs in 40–92% of oligodendrogliomas, whereas 19q loss occurs in 50–80% of cases [26]. Loss of chromosome 1p/19q is not commonly present in neurocytic tumors [38]; however, this has been observed in EVN [15, 24]. Some investigators found 1p/19q deletions in tumors showing immunohistochemical and ultrastructural features of neuronal differentiation similar to our case, which prompted reclassification of the tumors as oligodendroglioma [38].

In summary, this unusual case illustrates the need to reclassify tumors showing pathologic features of both neurocytoma and oligodendroglioma as a unique entity, while the effort to identify the cell of origin continues. Further study of larger numbers of cases of the biologic behavior and the possible expression of cell surface molecules such as NG2 and PDGF α R by tumors showing differentiation of both oligodendroglioma and neurocytoma may help in directing therapy and improving prognosis.

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