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Intramedullary Clear Cell Ependymoma in the Thoracic Spinal Cord: A Case with Its Crush Smear and Ultrastructural Findings

Clear cell ependymoma was included in the World Health Organization classification of the nervous system in 1993, and all the reported cases, except for two in the spinal cord, were located in the brain, mainly in the supratentorial compartment. Astrocytomas outnumber ependymomas in the spinal cord, and the two entities partly share cytologic findings such as long, bipolar glial processes and oval to round nuclei resembling those seen in pilocytic astrocytoma. Here, we report the first Korean case of intramedullary clear cell ependymoma of the spinal cord, which is the third case situated in the spinal cord in the literature. The crush smear revealed round-to-oval nuclei with occasional nuclear eosinophilic inclusion and rare nuclear grooves. Cytoplasm had fluffy eosinophilic glial processes, and acellular fibrillary zone. On hematoxylin-eosin stain, oval to round tumor cells had large central nuclei with indistinct nucleoli and a moderate amount of clear cytoplasm, i.e. perinuclear halo, mimicking oligodendroglioma. Perivascular pseudorosettes and ependymal clefts were rarely found. In retrospect, perinuclear halo was absent on crush smears. Ultrastructurally, they had extensive surface microvilli and edematous cytoplasm filled with abundant glial filaments and microlumens with or without microvilli. Intercellular long cell junctions of the zipper-like zonula adherens type were found.

Key Words : *Ependymoma, Clear Cell; Spinal Cord; Crush Smear; Microscopy, Electron*

Na Rae Kim, Dong-Hae Chung, Sang Koo Lee*, Seung-Yeon Ha

Departments of Pathology and Neurosurgery*, Gachon University of Medicine and Science, Gil Medical Center, Incheon, Korea

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Address for correspondence
Seung-Yeon Ha, M.D.
Department of Pathology, Gachon University of Medicine and Science, Gil Medical Center, 1198 Guwol-dong, Namdong-gu, Incheon 450-760, Korea
Tel : +82.32-460-3078, Fax : +82.32-460-3073
E-mail : syha@gilhospital.com

INTRODUCTION

Clear cell ependymoma is a recently recognized rare variant of ependymoma that is largely composed of round clear tumor cells with few, if any, characteristic patterns of ependymomas such as true ependymal rosettes, or perivascular pseudorosettes, which may be misdiagnosed as oligodendroglioma, central neurocytoma or metastatic renal cell carcinoma of the clear cell type (1). Clear cell ependymoma is known to have a predilection for extraneural metastases and early recurrence. Therefore, resection followed by local radiotherapy is the treatment of choice as operation is possible (2). Only two cases of clear cell ependymomas that developed in the spinal cord have been reported in the literature (3, 4). Albeit being rare, distinguishing clear cell ependymoma from astrocytic tumor is important because the operation modality between the two entities is quite different (5).

Here, we emphasize the cytologic, histologic, immunohistochemical, and ultrastructural characteristics of clear cell ependymoma arising in the thoracic spinal cord.

CASE REPORT

Patient

A 73-yr-old woman was transferred to the Gil Medical Center due to low back pain that had begun two months previously. Spine magnetic resonance (MR) images revealed a 2.0 cm-sized intramedullary mass at the T12 level. The mass appeared as isosignal on T1-weighted images and high signal on T2-weighted images (Fig. 1). Intraoperative frozen examination and subtotal resection with laminoplasty was performed. After the operation, radiation therapy was initiated. During 33 months of follow-up, her condition did not deteriorate and she slightly regained a mobility of both limbs. Thirty-four months after, she experienced recurrence of the disease. Conservative treatment was done for pain control.

Frozen crush smears

On crush smears, oval to round tumor cells had abundant fibrillary cytoplasm and euchromatic round nuclei with occasional chromatin clumping (Fig. 2A). Acellular fibrillary zones

were also found (Fig. 2B). Vesicular nuclei, eosinophilic nuclear pseudoinclusions, and nuclear grooves were occasionally detected (Fig. 2C).

Histology

The resected tissue consisted of multiple fragments of grayish-white tissue. The tissue was fixed in 10% buffered formalin and then embedded in paraffin. Five micrometer-thick sections were stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS), masson-trichrome, and reticulin. Histologically, the mass was moderately cellular. Round to oval tumor cells had large central nuclei with indistinct nucleoli and moderate amounts of clear cytoplasm, i.e. perinuclear halo, giving a honeycomb appearance of the tumor cells (Fig. 3A). Chicken-wire patterns of blood vessels were absent. This clear cell appearance, however, was not identified in the frozen touch smear. Nuclear grooves and intranuclear eosinophilic inclusions that were seen on touch smears were rarely detected. Entrapped blood vessels showed perivascular hyalinization and pseudorosettes (Fig. 3B). The ependymal rosettes and clefts were rarely found. A few mitotic figures and occasional nuclear pleomorphism were found, but did not fulfil the criteria for anaplasia (Fig. 3C). Granular cytoplasm was stained positive for PAS.

Immunohistochemistry

Immunohistochemistry was done by the avidin-biotin-peroxidase complex method using antibodies against the antigens shown in Table 1. The tumor cells were positive for S-100 protein, glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), and vimentin, whereas they were negative for epithelial membrane antigen (EMA), desmin, neurofilament, synaptophysin, chromogranin, pancytokeratin, and p53 protein. The Ki-67 labeling index was 1% of the tumor cells.

Electron microscopy

For electron microscopy, fresh samples were randomly taken and fixed in 2.5% glutaraldehyde, followed by 1% osmium with propylene dioxide, and finally embedded in Epok 812 (Oken Shoji Ltd., Tokyo, Japan). The thin sections (1 μm) were stained with toluidine blue and Azure B solutions. They were examined with a transmission scanning electron microscope (H-7100, Hitachi High-Technologies Corporation, Tokyo, Japan) at an accelerating voltage of 75 kv. Electron microscopy revealed that round to oval tumor cells had edematous vacuolated cytoplasm with microluminas (Fig. 4A). The tumor cells showed abundant cytoplasmic long processes containing abundant glial filaments, microtubules, free ribosomes, rough endoplasmic reticulums, lipid vacuoles, and dilated mitochondria. The nuclei showed occasional cytoplasmic inclusions. Long, zipper-like cell junctions of zonula adherens type were frequently found at the inter-

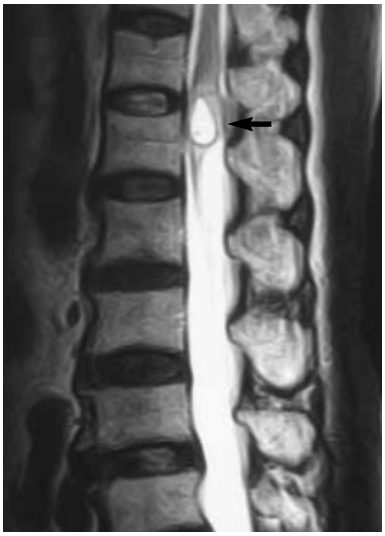

Fig. 1. MR images of the thoracic spinal cord. A T1-weighted image demonstrates that the tumor is isodense (arrow).

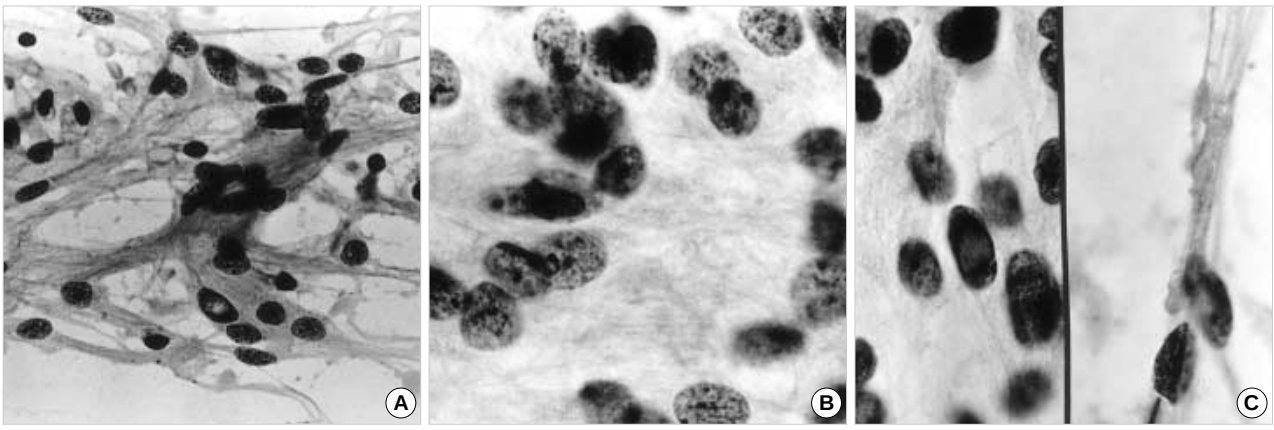

Fig. 2. Touch preparation on H&E stain. Smear preparation with long glial processes and oval to round tumor nuclei. (A) Tumor cells show fibrillary cytoplasm and euchromatic round to vesicular nuclei. (B) Acellular fibrillary zones. (C) Intranuclear pseudoinclusions (left) and nuclear grooves (right).

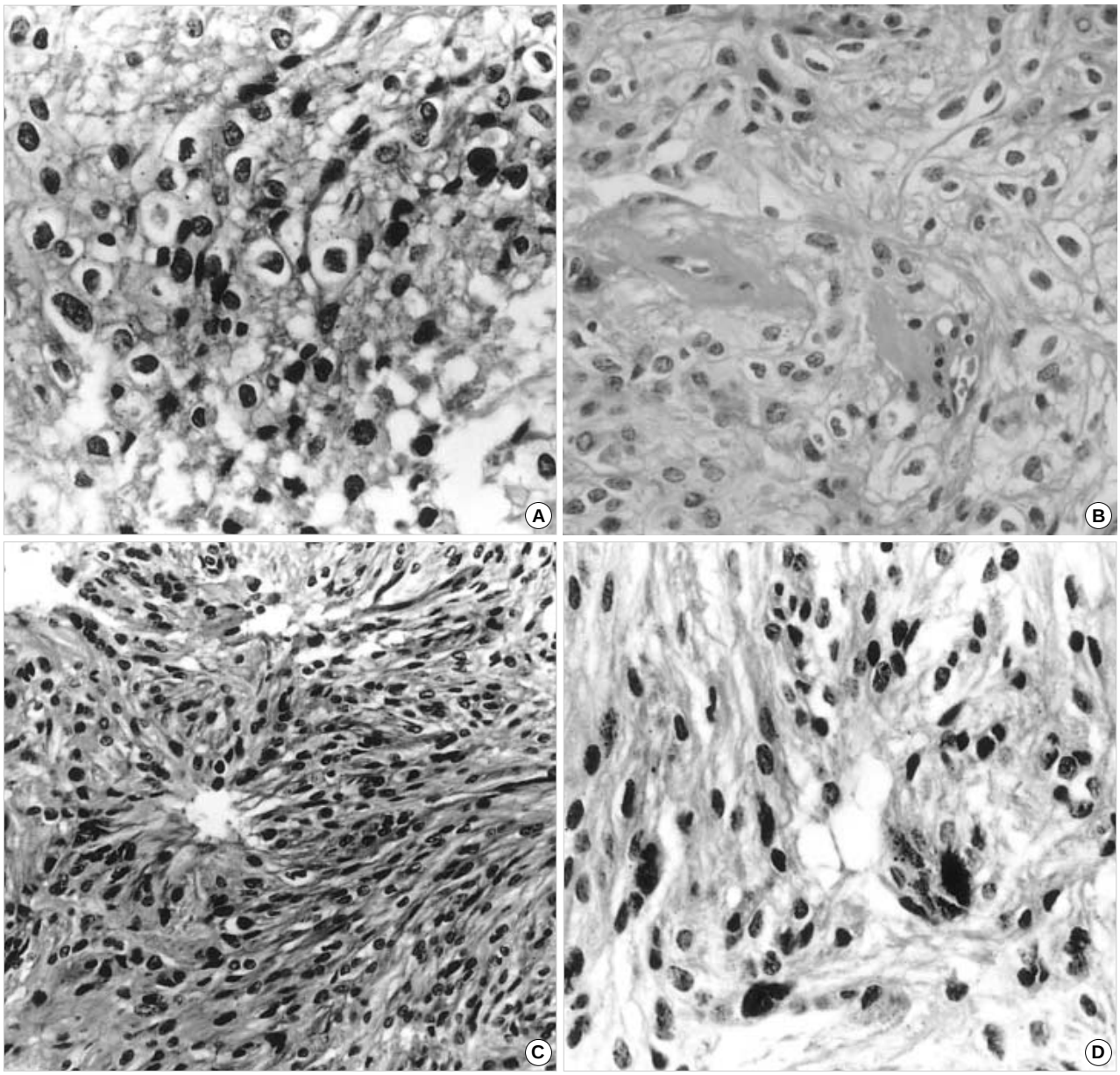

Fig. 3. H&E stain. (A, B) Clear cells with round nuclei with perinuclear halo and eosinophilic cytoplasm. (C) Ependymal rosettes. (D) Nuclear pleomorphism.

cellular spaces that were lined by abundant microvilli with rare cilia (Fig. 4B).

Based on the above findings, a diagnosis of clear cell ependymoma of spinal cord was made.

DISCUSSION

Because clear cell ependymoma of the spinal cord does not show typical histology of conventional ependymoma including perivascular pseudorosettes and ependymal rosettes or canal, it should be distinguished from other clear cell tumors of the central nervous system including astrocytoma, oligodendroglioma, central neurocytoma, hemangioblastoma, and even metastatic renal cell carcinoma on routine histologic examination with H&E stain. Previously cases of clear cell ependymomas, except for two, developed in the brain (1-4). Astrocytomas outnumber ependymomas in the spinal cord, and they share cytologic findings such as long, bipolar glial processes, and oval to round nuclei. However, with ultrastructural and immunohistochemical aids, most cases of clear cell ependymomas can be distinguished. Now, the clear cell ependymoma subtype previously classified as foramen Monro ependymoma is recognized as central neurocytoma by immu-

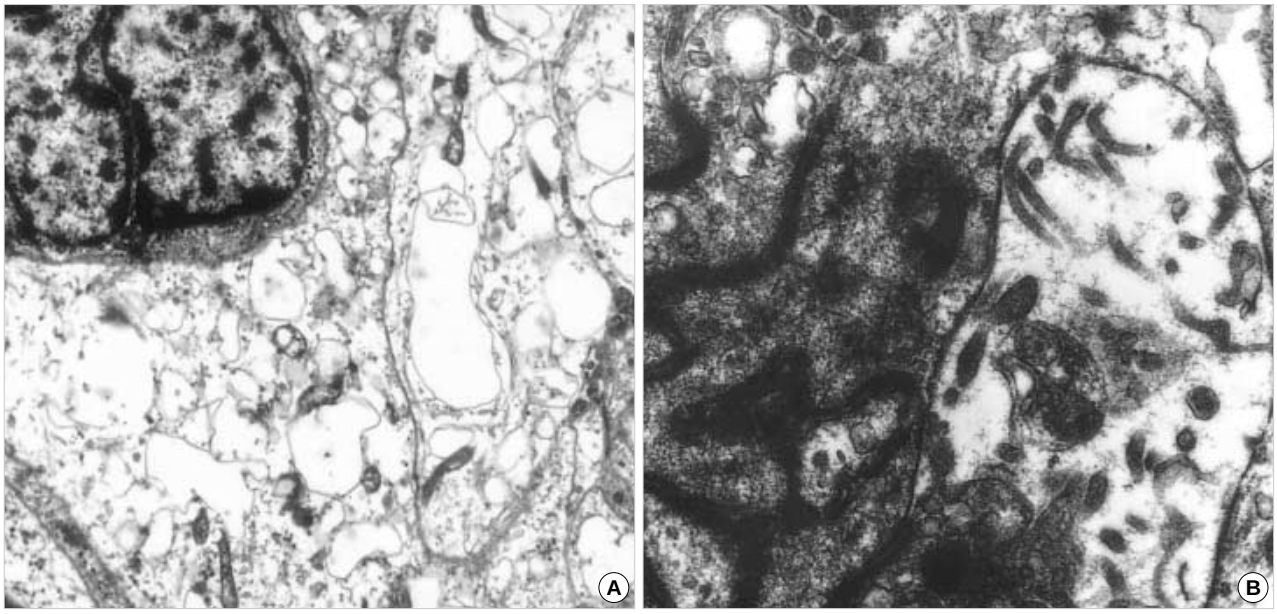

Fig. 4. (A) The tumor cells show abundant cytoplasmic long processes containing intermediate filaments, microtubules, free ribosomes, rough endoplasmic reticulums (×5,400). (B) Cell junctions of zonula adherens type and abundant microvilli with rare cilia are found (× 27,000).

Table 1. Antibodies used for immunohistochemical studies

Target protein	Clone	Dilution	Source
S-100 protein	Polyclonal	1:1,200	Zymed, San Francisco, CA, U.S.A.
GFAP	6F2	Prediluted	Dako, Glostrup, Denmark
Desmin	D33	Prediluted	Dako, Glostrup, Denmark
Pancytokeratin	AE1/AE3	Prediluted	Dako, Glostrup, Denmark
Synaptophysin	SY38	1:40 dilution	Dako, Glostrup, Denmark
Chromogranin	DAK-A3	1:2,000 dilution	Dako, Glostrup, Denmark
Phosphorylated neurofilament	2F11	Prediluted	Dako, Glostrup, Denmark
NSE	BBS	Prediluted	Dako, Glostrup, Denmark
Vimentin	VIM3B4	Prediluted	Dako, Glostrup, Denmark
EMA	E29	1:50	Dako, Glostrup, Denmark
p53 protein	DO-7	Prediluted	Dako, Glostrup, Denmark
Ki-67	Polyclonal	1:250	Dako, Glostrup, Denmark

GFAP, glial fibrillary acidic protein; NSE, Neuron specific enolase; EMA, epithelial membrane antigen.

nohistochemical and ultrastructural studies (6). Immunohistochemically, central neurocytoma shows eosinophilic fibrillary regions showing synaptophysin and chromogranin-reactivity. Under electron microscopy, central neurocytoma shows numerous cytoplasmic processes filled with microtubules and synaptic vesicles with dense core granules, and oligodendroglioma shows concentric onionskin-like lamination. Tumor cells of hemangioblastoma have abundant electron-lucent cytoplasm of lipid droplets, reminiscent of neuroendocrine granules. However, the diagnosis of clear cell ependymoma is quite difficult, especially in frozen slides because of the absence of clear cell morphology (perinuclear halo) in frozen slides. Min et al. (7) described that clear cell ependymomas take the same biologic behavior as that of other ependymomas, and a proliferation index is required for the evaluation of malignancy. Here, we carefully examined ultrastructural details to find different features of clear cell ependymoma from classic ependymoma. Intracytoplasmic lumen is one of the histological characteristics and ultrastructural feature of ependymoma that corresponded to microlumina with or without microvilli-lining under electron microscopy (8). Regardless of the type of ependymoma, the intracytoplasmic lumen is one of the common findings. One case frequently containing granulo-tubular materials has been reported, which were regarded as degraded microvilli of the tumor cells (8).

Kumar (9) compared the cytologic findings of classic ependymomas on crush smear with those of other tumors including meningiomas, schwannomas, astrocytomas, oligodendrogliomas, medulloblastomas, pituitary adenomas, choroid plexus papilloma, craniopharyngioma, and metastatic tumors. The most common findings out of 21 reviewed cases were ependymal rosettes (100%), nuclear grooves (71.4%), perivascular pseudorosettes (52.4%), and acinar structures (33.3 %). Papillary clusters (9.5%), calcification (9.5%), and intranuclear inclusions (9.5%) and myxomatous material (4.8%) can be seen in ependymoma. Intranuclear inclusions can be seen in meningiomas, and calcification is a feature shared by choroid plexus tumor, oligodendroglioma, and meningioma.

Among them, nuclear grooves are specific cytologic findings of ependymomas that could not found in other tumors.

As described in the previous articles, small foci of perivas-

cular hyalinizing pseudorosettes are rarely found on H&E stain of clear cell ependymomas, which were previously and frequently misdiagnosed as oligodendroglioma (7). In retrospect, perinuclear halo was absent on touch smears. Like oligodendroglioma, which show no clear cell change on frozen sections, the morphogenesis of clear cell morphology in clear cell ependymoma is not supposed to be different from those of oligodendroglioma, and this perinuclear halo does not appear in classic ependymoma, whatever the fixation artifact, thus supporting the clear cell ependymoma as a different entity from the classic ependymoma (10).

In summary, cytologic findings of nuclear grooves and eosinophilic nuclear inclusions are helpful findings in clear cell ependymoma as in classic ependymoma. Here we emphasize those findings on frozen examinations as well as acellular fibrillary zones, ependymal rosettes, and acinar structure because clear cell ependymoma does not show the typical morphology of classic ependymoma and it rarely occurs in spinal cord.

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