


Long-term epilepsy-associated tumor in the amygdala of a 16-year-old boy: report of a rare case having intranuclear filaments

Yoon Jin Cha¹ · Dong-Seok Kim² · Seung-Koo Lee³ · Hoon-Chul Kang⁴ ·
Se Hoon Kim⁵ 

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Abstract The term “long-term epilepsy-associated tumor (LEAT)” encompasses brain lesions associated with drug-resistant epilepsy over a long duration (≥ 2 years). Notably, some LEATs do not fit into any of the classifications of the World Health Organization (WHO). Herein, we report a LEAT that occurred in the left amygdala of a 16-year-old patient with intractable epilepsy. Histological examination of the resected amygdala revealed diffusely infiltrating tumor cells in the cortex. Perineuronal satellitosis and perivascular aggregation of tumor cells were apparent, along with mild nuclear enlargement and cytologic atypia. Tumor cells were positive for oligodendrocyte transcription factor 2 and neuronal markers including NeuN, neurofilaments, and synaptophysin, but were negative for CD34 and nestin. The most intriguing finding was intranuclear filaments, which appeared as rod- or needle-like shapes under high-power view. Ancillary ultrastructural analysis revealed thin filamentous intranuclear structures in tumor cells. Based on the glioneuronal nature of

these cells as well as the infiltrative growth pattern, a diagnosis of LEAT was rendered that was deemed WHO grade I to II; however, the clinicopathological implications of the intranuclear inclusions remain unknown. The patient is currently alive and well without seizures.

Keywords Brain · Epilepsy · Temporal lobe · Neoplasm

Introduction

“Long-term epilepsy-associated tumors (LEATs)”, introduced by Luyken et al., refer to brain lesions that cause drug-resistant epilepsy for 2 years or more [1, 2]. While any brain lesion can provoke a seizure, LEATs are primary brain tumors that mostly affect the temporal lobes of young patients; they exhibit slow growth and are associated with intractable seizures. Generally, LEATs have benign biologic behaviors, but recurrence and malignant transformation have also been reported [2, 3]. While such tumors are classified according to current World Health Organization (WHO) classification, some LEATs have mixed features of two different tumor types (so-called mixed LEATs) or are of the diffuse cortical infiltrative type (so-called diffuse LEATs); these do not fit under current WHO classifications. Herein, we report a LEAT in the amygdala of a 16-year-old boy that showed diffuse infiltrative growth and unique intranuclear filamentous inclusions.

Clinical summary

A 16-year-old boy with intractable epilepsy was referred to our institution for further observation and surgical treatment. He had taken antiepileptic drugs for generalized

✉ Se Hoon Kim
paxco@yuhs.ac

¹ Department of Pathology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea

² Department of Pediatric Neurosurgery, Severance Children’s Hospital, Yonsei University College of Medicine, Seoul, South Korea

³ Department of Radiology, Severance Hospital, Yonsei University College of Medicine, Seoul, South Korea

⁴ Division of Pediatric Neurology, Department of Pediatrics, Epilepsy Research Institute, Severance Children’s Hospital, Yonsei University College of Medicine, Seoul, South Korea

⁵ Department of Pathology, Severance Hospital, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, South Korea

tonic–clonic (GTC) seizures for 4 years, after which the GTC seizures disappeared, but medically intractable complex partial seizures persisted and increased in frequency (up to 1–3 times/day with durations of 30 s to 1 min).

On admission, he had complex partial seizures of eye opening (during sleep) or aura (on awake state), as well as sitting with tonic posturing followed by early unforced head turning, eye blinking, agitation, and glancing around; he progressed to ipsilateral hand automatism with contralateral late version and oral automatism.

Video electroencephalography (EEG) monitoring revealed inconsistent asymmetric slowing in the right fronto-temporal areas and occasional sharp wave discharges from the right sphenoidal electrode (right temporal area) that were associated with suppression of background rhythm. These were followed by the rhythmic evolution of ictal discharges from the right mesial temporal areas with postictal slowing on the right temporal areas lasting for 80–120 s. These findings suggested underlying encephalopathy in the right temporal area, with partial seizures originating from the right mesial temporal areas.

Brain magnetic resonance imaging showed possible cortical dysplasia in the right uncus and amygdala and a relatively preserved hippocampus (Fig. 1). The patient underwent electrocorticography (ECoG)-guided right hippocampal-sparing temporal lobectomy; intraoperative findings revealed thickened gray matter of the right anteromedial area of the temporal lobe and the amygdala, with an intact hippocampus. After anterior temporal resection, ECoG monitoring revealed a spike-free zone. Although minimal aura has persisted, the patient has since

become seizure-free following 14 months of medication, and his EEG is normal.

Pathologic findings

During gross examination, specimens were adjusted according to gyral patterns and cut perpendicularly to the cortical surface (3–5 mm thick). Along with routine hematoxylin and eosin staining, immunohistochemical staining for oligodendrocyte transcription factor 2 (OLIG2) (clone H-68; Santa Cruz Biotechnology, Santa Cruz, USA; dilution 1:200), glial fibrillary acidic protein (GFAP) (clone 6F2; Dako, Carpinteria, CA, USA; dilution 1:100), isocitrate dehydrogenase 1 (IDH1)-R132H (clone H09; Dianova, Hamburg, Germany; dilution 1:80), CD34 (clone QBEnd 10; Dako; dilution 1:50), NeuN (clone A60; Millipore, Billerica, USA; dilution 1:3000), synaptophysin (clone DAK-SYNAP; Dako; dilution 1:450), CD99 (clone 12E7; Dako; dilution 1:200), S-100 protein (clone bBS/NC/VI-H14; Dako; dilution 1:2000), microtubule-associated protein 2 (MAP2) (clone HM-2; Sigma-Aldrich, St. Louis, MO, USA; dilution 1:200), neurofilament (clone 2F11; Dako; dilution 1:100), and Ki67 (clone MIB-1; Dako; dilution 1:150) were performed using a Ventana Discovery XT automated staining system (Ventana Medical System, Tucson, AZ, USA).

Microscopically, the left temporal lobe had well-preserved gray matter ribbon (Fig. 2a). No cortical dyslamination was found in the left temporal lobe; however, there were few heterotopic neurons as well as perineuronal

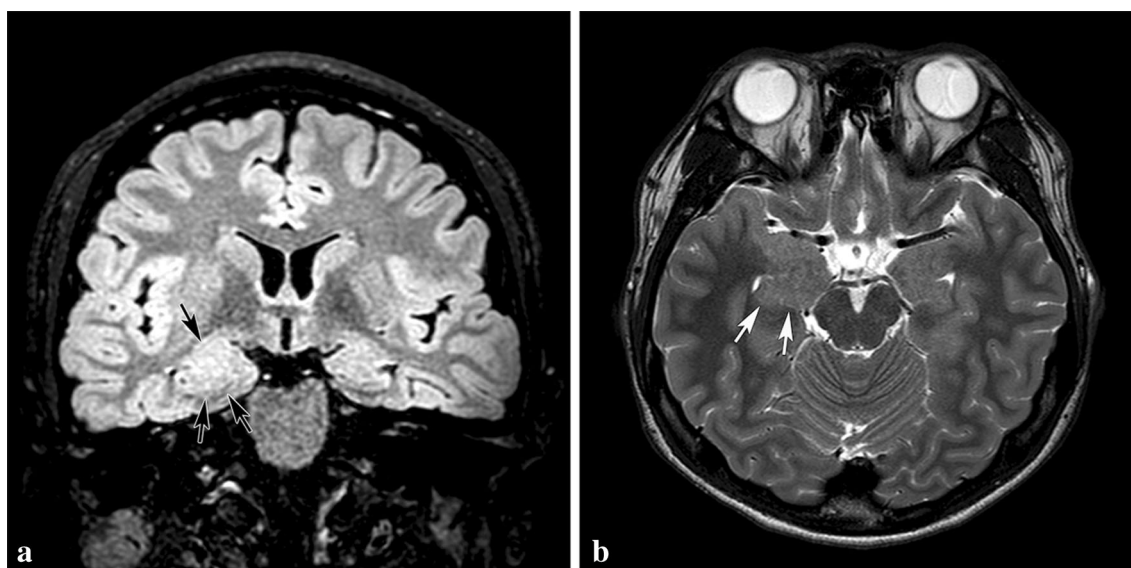


Fig. 1 **a** Coronal T1 image shows focal thickening of the right uncus (*arrows*) with a relatively preserved hippocampus. **b** Axial T2 image shows a slightly enlarged right amygdala (*arrows*) owing to polymicrogyria, suspicious finding of cortical malformation

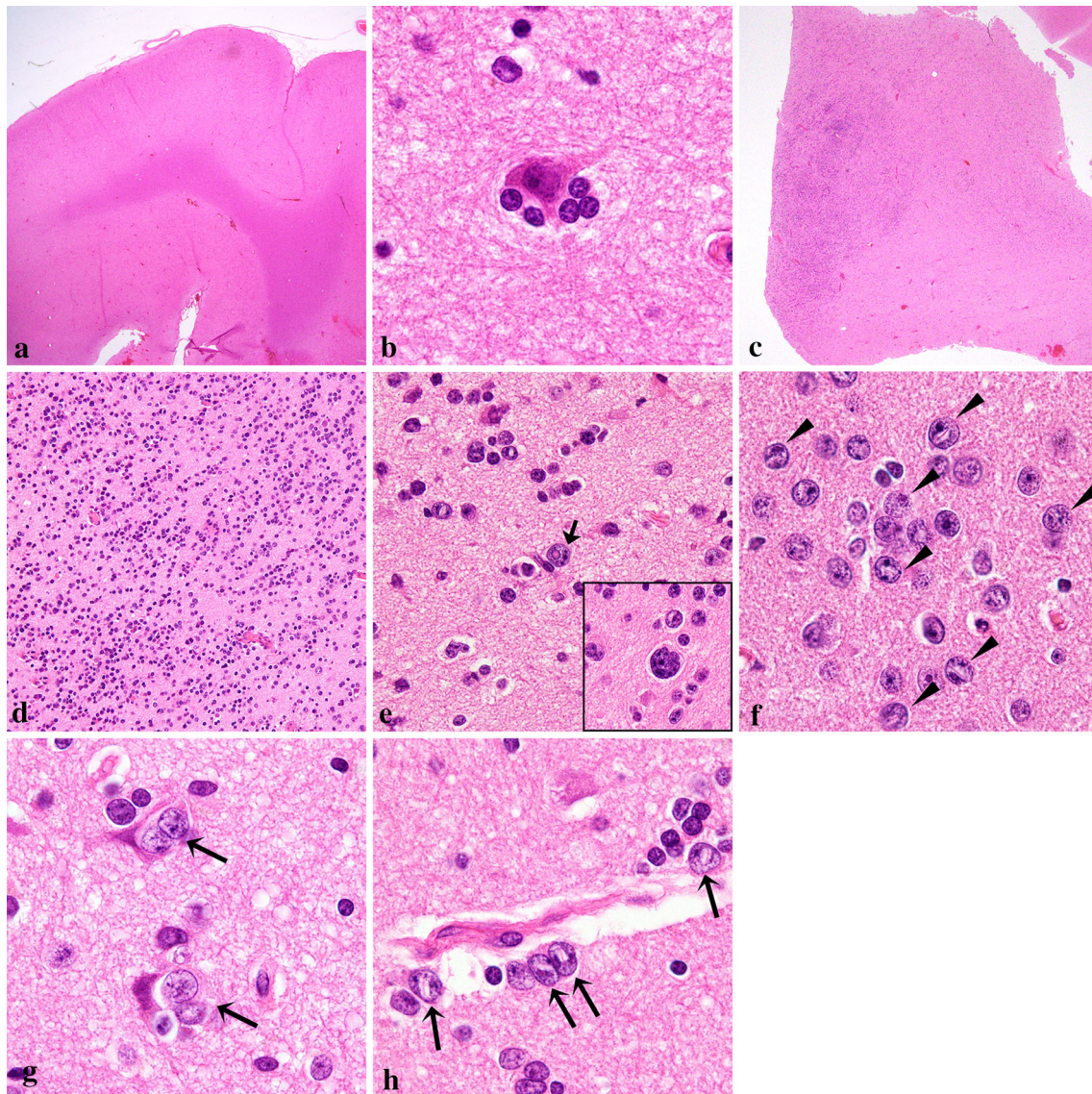


Fig. 2 Histology of temporal lobe (**a**, **b**) and the tumor of the amygdala (**c**–**h**). **a** Low power view of the resected temporal lobe. There is no blurring in the corticomedullary junction. **b** In the temporal lobe, perineuronal satellitosis is frequently observed, a feature of reactive gliosis. **c** The resected amygdala with a notable hypercellular zone on the *left side*. **d** Small-to-medium-sized, hyperchromatic cells with uneven distribution. **e** Tumor cells showing a high nuclear-to-cytoplasmic ratio and slight nuclear membrane

irregularity with chromatic clumping (*inset*). Note the nuclear pseudoinclusion in the tumor cell (*arrow*). **f** Characteristic intranuclear rod-like or needle-like inclusions in the tumor cells (*arrowheads*). **g** Perineuronal satellitosis of tumor cells (*arrows*). Medium-sized tumor cells show chromatic clumping. Compare those perineuronal tumor cells with the reactive glial cells in temporal lobe (**b**). **h** Tumor cells also show perivascular aggregation (*arrows*). Note the clumped chromatin and nuclear inclusion in the tumor cells

satellitosis of small round glial cells that were considered reactive gliosis (Fig. 2b).

Sections from the amygdala revealed an ill-defined localized hypercellular zone (Fig. 2c). Round to ovoid cells that were predominantly small-to-medium in size were diffusely proliferating in the cortex. Given its cellularity and nuclear atypia, a tumor was highly suspected. Some cells were arranged in microcolumns, while others were diffusely and unevenly distributed in a non-specific pattern (Fig. 2d). Infiltrating cells had monotonous nuclei

with coarse chromatin, inconspicuous nucleoli, and sparse cytoplasm. Most tumor cells had mild nuclear membrane irregularity with occasional large, hyperchromatic cells. Tumor cells had scant cytoplasm; since no specific differentiation could be identified, they were considered to have primitive features. Occasional nuclear pseudoinclusions were observed that reflected nuclear membrane wrinkling (Fig. 2e). Most intriguingly, intranuclear rod-like or needle-like inclusion bodies were present in these tumor cells (Fig. 2f). In the peripheral area of the

hypercellular region, tumor cells were aggregated around neurons or capillaries, and corresponded to perineuronal satellitosis and perivascular aggregation, an infiltrating pattern of glioma (Fig. 2g, h).

Since primitive round cells were proliferating in a non-specific pattern, differential diagnoses included malignant small round cell tumors (such as primitive neuroectodermal tumors and embryonal tumors like medulloblastoma), as well as LEAT. Although no specifically matched tumor was listed in current WHO classification, an intra-axial tumor, particularly LEAT, was most strongly suspected considering the low-grade nuclear atypia, lack of mitosis, and involvement of secondary structures of Scherer. On immunohistochemistry, tumor cells were diffusely and strongly positive for OLIG2 (Fig. 3a) but negative for IDH1-R132H. Perineuronal satellitosis and perivascular aggregation were also highlighted by OLIG2 staining (Fig. 3a, inset). Reactive astrocytes within atypical cells were clearly delineated by S-100 protein and GFAP, whereas tumor cells were diffusely but weakly positive for S-100 protein (Fig. 3b) and negative for GFAP (Fig. 3c). NeuN expression was weak in tumor cells, but strong in intervening neurons (Fig. 3d). Neurofilaments (Fig. 3e) and synaptophysin (Fig. 3f) were diffusely positive in the tumor cells, while CD34 (Fig. 3g) was negative in tumor cells. Nestin was also negative in tumor cells, and no balloon cells were recognized (Fig. 3h). Ki67 revealed approximately 5% positivity in the hypercellular area of atypical cells (Fig. 3i).

One of the interesting findings of this tumor was intranuclear inclusion bodies of tumor cells. They appeared to be coffee bean-like nuclear grooves in the tumor cells. With higher magnification, nuclear grooves were clear-spaced within the nuclei, and rod-like or needle-like eosinophilic inclusion bodies were present (Fig. 4a). Additionally, we performed ultrastructural examination to delineate the intranuclear inclusions. Electron microscopic examination revealed 1–2 thin filamentous structures measuring approximately 4–5 μm long and 50–100 nm wide (Fig. 4b), but their diagnostic implications were unclear.

Since tumor cells were positive for OLIG2, and some co-expressed neuronal markers including NeuN, MAP2, neurofilaments, and synaptophysin, a glioneuronal tumor was the most likely diagnosis. We concluded this tumor is most consistent with LEAT, given its clinical presentation and the glioneuronal nature of tumor cells. Although this tumor was not classifiable by current WHO classification, hypercellularity and involvement of secondary structures of Scherer appeared to reflect its infiltrative nature; hence, we considered that biologic behavior of this tumor might correspond to WHO grade I to II, although most features fit to WHO grade I.

Discussion

Although any brain tumor can cause seizures, LEATs encompass tumors in patients with long durations (two or more years) of drug-resistant seizures [2]. Low-grade glial and glioneuronal tumors of the supratentorial cortex are generally described as epilepsy-associated tumors. According to current WHO classifications, glioneuronal tumors, representatively dysembryoplastic neuroepithelial tumor and ganglioglioma and glial tumors such as pilocytic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, oligodendroglioma, and angiocentric glioma are considered LEATs, and most correspond to WHO grade I or II [1, 4]. On the histologic spectrum of epilepsy-associated brain lesions, focal cortical dysplasias (FCDs) are present, and were first described by Taylor et al. within resected brain tissues of patients with drug-resistant epilepsy that exhibited histological cortical abnormalities [5]. After evaluating a few neuropathologic grading systems [6, 7], a consensus classification of FCD by the International League Against Epilepsy task force was adopted [8]. Although FCD type IIIb denotes dysplasias with associated tumors, no FCD-like area was found in our patient. The resected temporal lobe showed sharp cortical ribbons without blurring or dyslamination of the cortex. However, relatively frequent perineuronal satellitosis of small glial cells was found in the temporal lobe; this was more likely indicative of reactive gliosis associated with long-term intractable epilepsy; as such findings were previously reported in patients with temporal lobe epilepsy [9].

Based primarily on histological features, the present case was more likely to be a low-grade tumor rather than a reactive condition or small round-cell malignancy because of its localized hypercellularity, cellular monotony, and mild nuclear atypia without brisk mitosis. Coexpression of OLIG2 and neuronal markers on immunohistochemistry confirmed the tumor's glioneuronal nature, and the patient's clinical history of intractable epilepsy was consistent with LEAT. With respect to epileptiform activity, a larger neuronal component appears to be associated with highly epileptiform discharge patterns in glioneuronal tumors or FCDs [10]. Abrupt tissue damage resulting from progressive high-grade gliomas can also produce seizures [11]. Furthermore, a previous *ex vivo* study showed that perineuronal invasion (perineuronal satellitosis) of glioma can alter the discharge level of the preexisting neuron, which may contribute to the bursting neurons in the seizure-initiating zones [12]. Intractable epilepsy in the present case appeared to be associated with neuronal differentiation and localized hypercellularity of the tumor cell area, as well as peritumoral perineuronal satellitosis of the tumor cells.

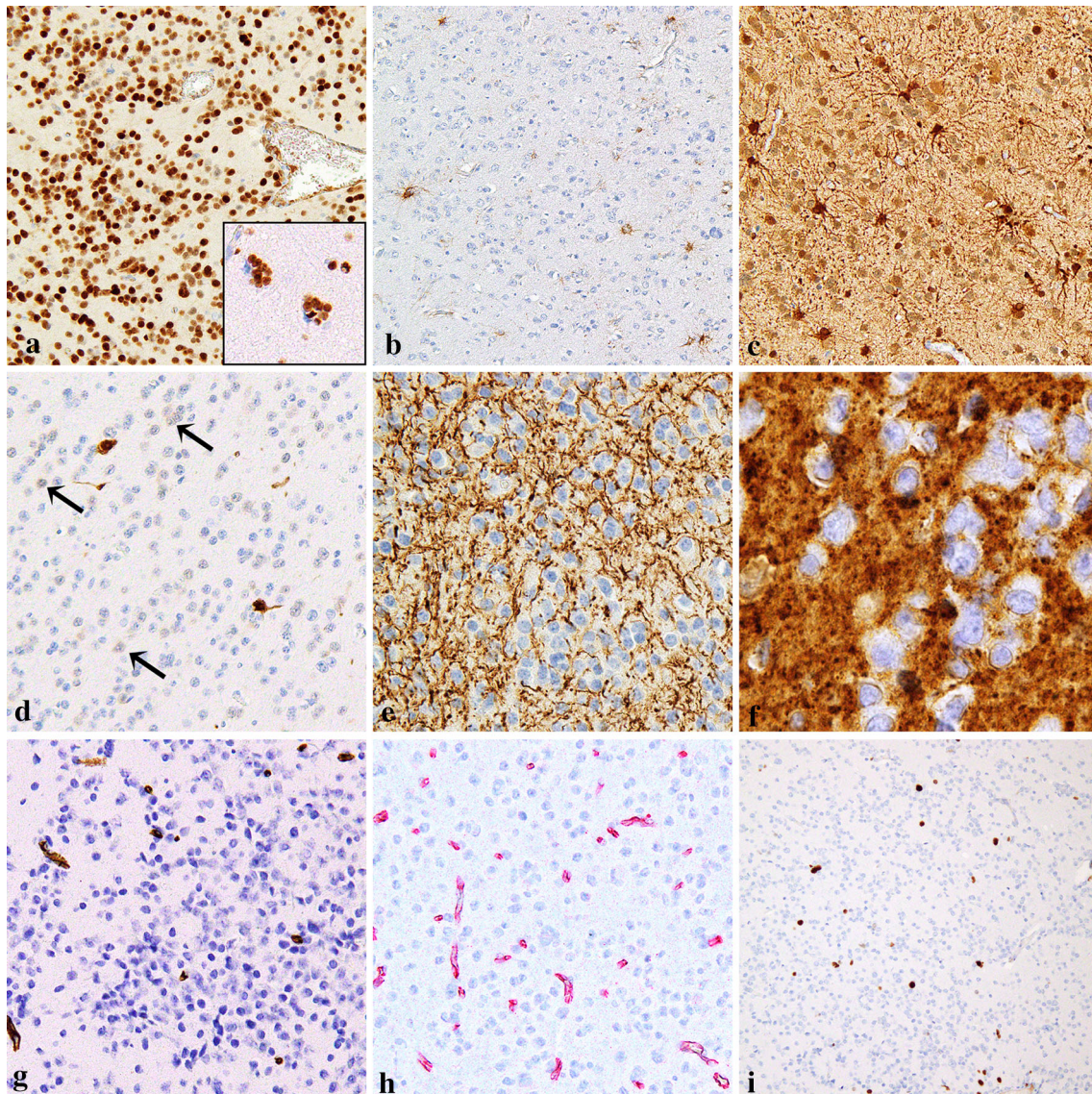


Fig. 3 Immunohistochemical staining and ultrastructural examination. **a** Tumor cells are diffusely positive for oligodendrocyte transcription factor 2 (OLIG2). **b** Perineuronal satellitosis of the tumor cells in the periphery is highlighted by OLIG2 (*inset*). **c** Tumor cells are negative for glial fibrillary acidic protein (GFAP) but positive for S-100 protein. Note the scattered reactive astrocytes expressing **b** GFAP and **c** S-100 protein. **d** Among neuronal markers,

NeuN is focally weakly positive in tumor cells (*arrows*) whereas diffuse positivity is recognized with **e** neurofilaments and **f** synaptophysin. The tumor cells are negative for **g** CD34 and **h** nestin. Note that only endothelial cells are positive for CD34 (**g**) and nestin (**h**). **i** Ki67 staining reveals approximately 5% positivity among the tumor cells

Another interesting histologic finding that was evidence of tumorigenicity was that nearly every tumor cell had intranuclear rod-like inclusion bodies, which were better visualized under high-power light microscopy because of the peri-inclusion clear space. We also confirmed the intranuclear filamentous structures via electron microscopy. Intranuclear inclusions in neurons have been sporadically reported in patients with multiple sclerosis [13], as well as in the neurons of normal brains [14]. In a previous study, intranuclear inclusions were immunoreactive for cytoskeletal protein class III β tubulin, and were found

in gangliogliomas and posterior fossa ependymomas in children, as well as adult ependymomas of the lateral ventricle, spine, and posterior fossa in varying proportions [15]. Although tubulin staining was not available in the present study, the histologic findings, shapes, and sizes of intranuclear inclusions as observed under an electron microscope were consistent with those of the previous study. Our case illustrated the glioneuronal nature of the tumor cells, almost all of which had intranuclear inclusions; this was also evidence of clonal proliferation indicative of tumorigenicity, although the implications

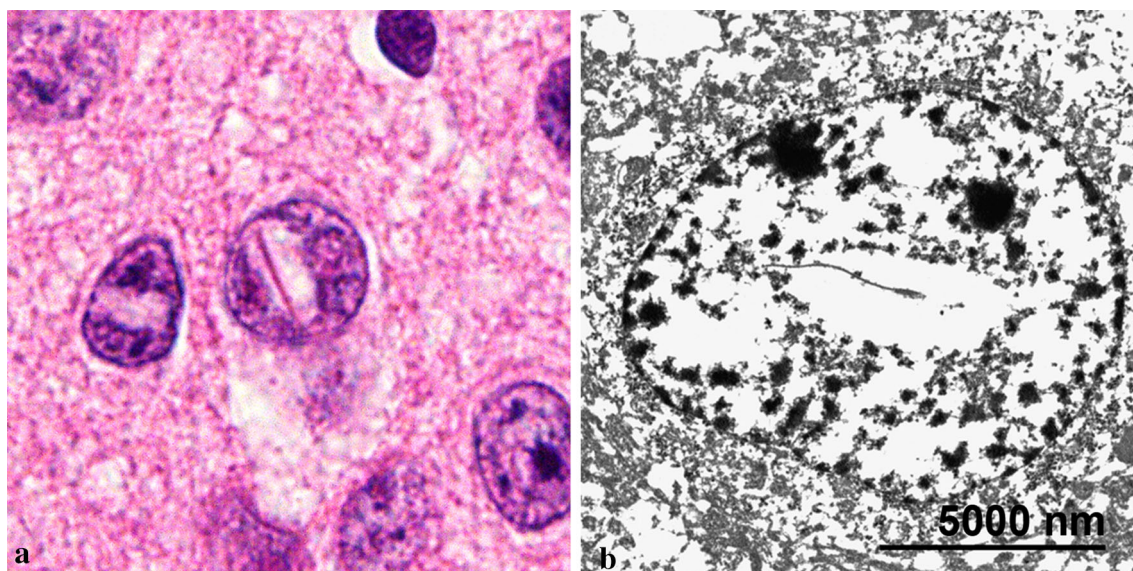


Fig. 4 **a** Tumor cells have intranuclear rod- or needle-like eosinophilic inclusion. **b** Ultrastructural examination reveals intranuclear filamentous material in the tumor cells

were unclear. To date, intranuclear inclusions have not been reported in LEATs that are of a known specific tumor in WHO classification. Whether intranuclear inclusion is associated with a specific tumor type or clinical presentation, or is a non-specific feature, remains to be determined.

Although we mentioned that our patient's tumor appeared to correspond to WHO grade I to II, he was not treated with adjuvant postoperative chemotherapy or radiotherapy. He remains alive and well with no tumor recurrence or residual seizures. Taken together, the LEAT that we reported, with its uncommon histologic findings and intranuclear inclusions with an infiltrative growth pattern resembling low-grade glioma, appears to be a unique finding.

Compliance with ethical standards

Conflict of interest None declared.

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