High-Grade Neuroepithelial Tumor with *BCL6* Corepressor-Alteration Presenting Pathological and Radiological Calcification: Case Report

Running Head: HGNET-BCOR with Calcification

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Abbreviations

BCOR, *BCL6* corepressor; CNS, central nervous system; EMA, epithelial membrane antigen; GFAP, glial fibrillary protein; HGNET-*BCOR*, high-grade neuroepithelial tumor with *BCOR* alteration; CCSK, clear cell sarcoma of the

kidney; IHC, immunohistochemistry; ITD, internal tandem duplication; MRI, magnetic resonance imaging; NOS, not other specified; PCR, polymerase chain reaction; PMMTI, primitive myxoid mesenchymal tumor of infancy, PNET, primitive neuroectodermal tumor; URCS, undifferentiated round cell sarcoma; WI, weighted imaging

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Abstract

A 5 year-old girl presented with headache and vomiting. Head computed tomography and magnetic resonance imaging showed a right frontal lobe tumor with marked calcification. The patient underwent resection surgery with suspicion of anaplastic ependymoma, and the tumor was gross totally removed. Pathological examination revealed areas of dense tumor cells with a high nucleocytoplasmic ratio and myxoid areas consisting of tumor cells with a round-shaped nucleus and eosinophilic cytoplasm. Perivascular pseudorosette, necrosis, circumscribed growth, and microcalcification were also observed. Immunohistochemistry demonstrated negative staining for glial fibrillary protein and epithelial membrane antigen. Diagnosis of a high-grade neuroepithelial tumor (HGNET) with *BCL6* corepressor (*BCOR*) alteration was made based on pathological findings and internal tandem duplication in the exon 15 of *BCOR*. Although calcification on radiological and pathological examination is not typical, it would be essential to recognize that calcification could appear in HGNET-*BCOR*.

Keywords: BCOR, high-grade neuroepithelial tumor, pediatric, PNET

INTRODUCTION

Pediatric brain tumor harboring internal tandem duplication (ITD) in the exon 15 of *BCL6 corepressor* (*BCOR*) is classified as high-grade neuroepithelial tumor with *BCOR* alteration (HGNET-*BCOR*).[1] This is a novel entity of pediatric malignant brain tumor identified through reclassification of primitive neuroectodermal tumor (PNET) in the central nervous system (CNS) by deoxyribonucleic acid (DNA) methylation profiling.[1] ITD in *BCOR* is frequently observed in several pediatric soft tissue tumors, including clear cell sarcoma of the kidney (CCSK), undifferentiated round cell sarcoma (URCS), and primitive myxoid mesenchymal tumor of infancy (PMMTI), as well as CNS tumors.[2-5] A frequently duplicated region of approximately 30 amino acids exists in the exon 15 of *BCOR*, which is common in HGNET and other soft tissue tumors with ITD in *BCOR*.[3, 6, 7]

Pathological features of HGNET-*BCOR* include findings that resemble gliomas or ependymomas in morphology.[1, 6, 7] Therefore, a subset of HGNET-*BCOR* has initially been diagnosed as gliomas or ependymomas as histopathological diagnosis.[1] In spite of morphological resemblance to gliomas or ependymomas, on immunohistochemistry (IHC), these tumors present negative staining for glial fibrillary protein (GFAP) and epithelial membrane antigen (EMA), whereas positive staining for BCOR is usually observed.[6, 7]

Previously reported radiological features of HGNET-*BCOR* are a well-demarcated mass in the cerebrum or cerebellum with high signal intensity on T2-weighted magnetic resonance imaging (MRI).[6, 7] An intratumoral high signal intensity lesion suggesting hemorrhage is also frequently observed.[6, 7]

Although previously reported cases of HGNET-*BCOR* with pathological and radiological findings have been limited, calcification has been rarely detected in tissue sections or images.[1, 6, 7] In this report, the authors present a case of a supratentorial calcified tumor that was diagnosed as HGNET-*BCOR*. The radiological findings of this case mimicked an anaplastic ependymoma due to intratumoral calcification. Because some HGENT-*BCOR*s have been pathologically diagnosed as ependymomas previously,[1] we should recognize this novel entity and consider further molecular genetic analysis when atypical pathological findings including calcification are observed.

CLINICAL SUMMARY

A 5 year-old girl suffered from headache for 1 month before she was referred to our institution. Neurological examination revealed no apparent deficit except for headache. Head computed tomography showed a mass lesion with marked calcification in her right frontal lobe (Fig. 1a). The tumor presented isointensity on T1-weighted imaging (WI), mixed high intensity on T2WI, and heterogeneously enhanced on gadolinium-enhanced T1WI (Fig. 1, b–d). An intra-axial high-grade brain tumor such as an anaplastic ependymoma was strongly suspected based on these radiological examinations. She underwent emergent surgery, and the tumor was gross totally resected. Postoperative MRI presented no evidence of residual tumor (Fig. 1e). After pathological examination, she did not undergo postoperative adjuvant treatments because her parents did not accept chemoradiotherapy considering adverse events and late complications. She was discharged to the hospital without any neurological deficit and was observed. MRI 3 months after the initial surgery showed local recurrence (Fig. 1f).

PATHOLOGICAL FINDINGS

Hematoxylin and eosin staining showed areas of dense tumor cells with a high nucleocytoplasmic ratio (Fig. 2a, b) and myxoid areas consisting of tumor cells with a round nucleus (Fig. 2c, d). Foci of necrosis were evident (Fig. 3a). The tumor was almost well-circumscribed, but focally invaded adjacent brain parenchyma (Fig. 3b). Microcalcification was also prominently observed (Fig. 3c). Perivascular pseudorosettes were frequently observed (Fig. 3d). On immunohistochemistry (IHC), the tumor was negative for EMA and GFAP (Fig. 4a, b). Furthermore, the tumor was positive for BCOR, Olig2, NeuN, INI1, and CD56 (Fig. 4c, d) and negative for synaptophysin, neurofilament, S-100, Chromogranin A, and IDH-R132H (Table 1). The Ki-67 labeling index was 38% at the highest area. Although anaplastic ependymoma was initially considered based on the presence of perivascular pseudorosettes, the result of IHC was not typical and genetic analysis was conducted with the suspicion of HGNET-*BCOR*.

Genetic Analysis

DNA was extracted from frozen tumor tissue, and polymerase chain reaction (PCR) and Sanger sequencing were performed as described previously.[8] A hot spot region of ITD in exon 15 of *BCOR* was amplified by PCR using the following oligonucleotide primers: forward (5

DISCUSSION

Radiological Findings of HGNET-*BCOR*

In most previously reported cases, a well-circumscribed mass with mixed high intensity on T2WI has been the characteristic of HGNET-*BCOR*.[6, 7, 9-12] Central necrosis or hemorrhage has frequently been identified.[7, 11] Restricted diffusion on diffusion WI is also a hallmark of HGNET-*BCOR*.[6, 7, 11] Among 24 reported cases of HGNET-*BCOR*, calcification was suspected only in 1 case based on imaging findings,[6, 7, 9-14] and all other cases had no description about calcification.

Because calcification has been observed in 21% to 75% of supratentorial ependymomas on preoperative radiological examinations,[15, 16] an ependymoma was strongly suspected based on preoperative radiological examinations with calcification. Although the frequency of radiological calcification in HGNET-*BCOR* is uncertain, this case would suggest that calcification in radiological examination is not a definite finding that dismisses a diagnosis of HGNET-*BCOR*.

Pathological Findings of HGNET-*BCOR*

Previous research has reported several pathological features commonly observed in HGNET-*BCOR*, including a solid or compact growth pattern,[6, 7] ependymoma-like perivascular pseudorosettes,[1, 6, 7] glioma-like fibrillarity,[1, 7] necrosis,[6, 7] high vascularity with branching vessels demonstrating a chicken-wire appearance,[6, 7] or solid growth of spindle-shaped cells in bundles with the same vascular pattern.[6] Furthermore, various pathological findings, including myxoid and microcystic background,[6, 7] stromal and perivascular hyalinization,[7] Homer Wright-like rosettes,[7] rosette-like formation,[6] hemangiopericytoma-like stag-horn vascular pattern,[6] and infiltration of tumor cells[6, 7] have been observed in a subset of HGNET-*BCOR*. On the other hand, calcification has been rarely described in a series of HGNET. Only Ferris et al. reported that microcalcification was seen in a minority of HGNET-*BCOR*.[7] The presence of calcification in pathological examination like this case would be not common, but it is important to recognize that calcification is part of histopathological findings in HGNET-*BCOR*.

As HGNET-*BCOR* has been confirmed through genomic classification of CNS-PNETs, a subset of HGNET-*BCOR* has been diagnosed as ependymomas or gliomas based on pathological findings.[1] Similar to radiological examinations, calcification is common as pathological findings of ependymomas[17, 18] in contrast to HGNET-*BCOR*. In this case,

although an ependymoma-like component and calcification were observed on pathological examination that mimicked an ependymoma, other pathological findings including negative staining for EMA and genetic analysis of *BCOR* could provide a correct diagnosis of HGNET-*BCOR*.

Because perivascular pseudorosette is frequently observed in HGNET-*BCOR*[6, 7], ependymoma is the most commonly considered differential diagnosis in such cases. Additionally, glioblastoma, medulloblastoma, or CNS embryonal tumor (not other specified [NOS]) also have been considered as differential diagnoses of HGNET-*BCOR*[6, 7]. Morphologically, Yoshida et al. reported that the characteristic features of monotonous round to oval shape nuclei and fine chromatin are the most helpful findings for distinguishing HGNET-*BCOR* from other brain tumors[6]. In this case, although morphological nuclei findings were consistent with these previous reports, the presence of distinct areas of different cell density, as shown in Figure 2, were considered atypical findings. We consider that these findings would be similar to the case presented by Ferris et al.[7] that included an anaplastic component with chromosomal gains and losses.

Nuclear findings of HGNET-BCOR are also characteristic findings for CCSK, URCS, and PMMTI with ITD of *BCOR*[3, 6]. Moreover, majority of these soft tissue sarcomas pathologically present with an arborizing capillary network and myxoid areas[3, 5]. In the present case, although the nuclear findings and presence of myxoid areas were reminiscent of sarcomas with ITD of *BCOR*, there was no evidence of arborizing vessels.

Treatment Outcomes of HGNET-*BCOR*

Because HGNET-*BCOR* is a novel entity that has been proposed recently, optimal treatment has not been standardized. Among previously reported cases, two patients who underwent partial resection died after surgery.[6] Therefore, surgical resection should be achieved as gross total resection. Systemic chemotherapy would also be necessary, as shown in five patients who underwent solely observation or irradiation and have experienced recurrence within a short term.[6, 7, 12] Notably, two patients with subcutaneous recurrence after observation or local irradiation have been reported.[6, 12] Chemoradiotherapy, including temozolomide, has been used for four reported cases with 2 to 37 months of observation and reported between 12 months and 4.5 years of overall survival without death.[7, 9] Platinum-based multi-agent chemotherapy with or without autologous stem cell transplantation has been performed for nine reported cases with between 2 months and 14.2 years of observation.[6, 7, 10, 11] Because of the lack of sufficient period after treatment, the efficacy of multi-agent chemotherapy and

autologous stem cell transplantation for HGNET-*BCOR* is still unclear. Among these cases, three cases have demonstrated long-term survival of more than 5 years.[1, 7, 11]

In CCSKs with ITD of BCOR, preoperative chemotherapy (with actinomycin and vincristine) and postoperative chemotherapy (with etoposide, carboplatin, and ifosfamide alternated with cyclophosphamide and doxorubicin) are recommended[19]. Abdominal irradiation is recommended for stage 2 or higher cases[19]. Treatment outcomes for CCSK have improved, with a 5-year overall survival rate of 75%–90% [20], which is higher than that of HGNET-*BCOR*. Considering equal genetic alteration as ITD of *BCOR*, knowledge on CCSK may be applied to the treatment strategy for HGNET-*BCOR*.

Finally in this report, the authors shared a case of HGNET-*BCOR* with marked calcification on radiological and pathological examinations that resembled an ependymoma. Although it would not be typical, HGNET-*BCOR* should not be ruled out in radiological and pathological diagnosis based on the presence of calcification.

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Disclosure Statement

The authors declare that they have no conflict of interest.

Author Contributions

YI contributed to the study's conception, design, genetic analysis, and draft writing. AS, SN, and ST contributed to the pathological diagnosis and draft revisions. ET performed the pathological analysis. MS contributed to clinical data acquisition and draft revision. MO, HM, SH, YC, AI, and AM also contributed to clinical data acquisition. SY supervised the study, contributed to data acquisition, and revised the draft. All authors approved the final manuscript.

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Figure Legends

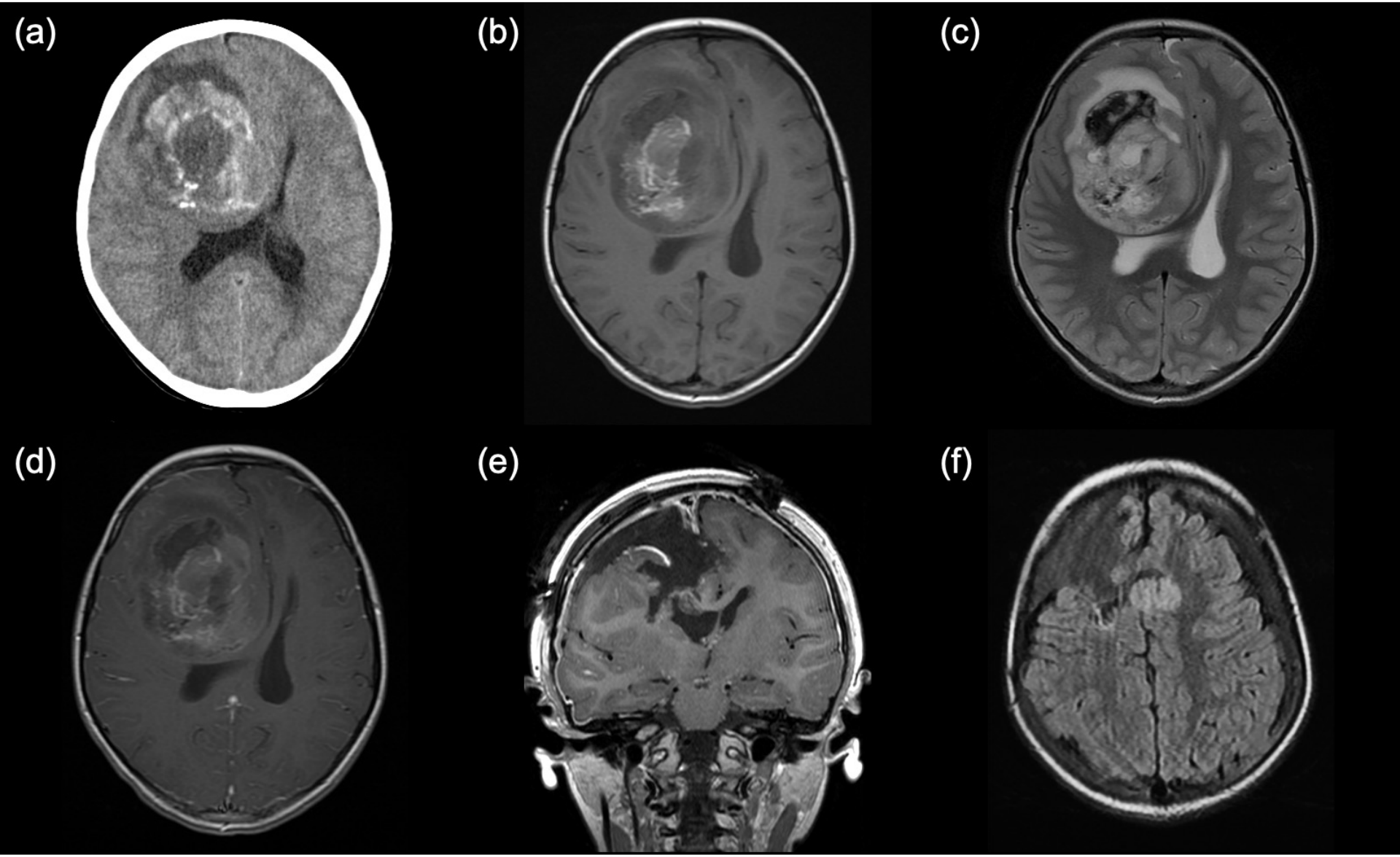
Figure 1. Radiological images. (a) Head computed tomography showing a right frontal lobe tumor with a high density region suggesting intratumoral calcification. (b) T1-weighted magnetic resonance imaging (MRI) showing a low-intensity tumor. The presence of subacute hemorrhage was also suggested by an intratumoral high intensity region. (c) T2-weighted MRI showing a mixed high intensity tumor. (d) Gadolinium-enhanced T1-weighted imaging showing enhancement of the tumor. (e) Gadolinium-enhanced T1-weighted imaging taken on the day after surgery showing no evidence of the tumor. (f) Fluid attenuated inversion recovery imaging 3 months after surgery showing local recurrence of the tumor.

Figure 2. Basic morphological features of HGNET-*BCOR*. (a) Hematoxylin and eosin staining showing areas with dense tumor cells and myxoid areas (original magnification, ×4). (b) Tumor cells with a high nucleocytoplasmic ratio in dense areas (original magnification, ×40). (c) Tumor cells with a round-shaped nucleus and eosinophilic cytoplasm in myxoid areas (original magnification, ×40).

Figure 3. Characteristic morphological features observed in the present case. (a) Massive necrosis. (b) Some parts of the tumor border presented a circumscribed growth pattern. (c) Microcalcification was frequently observed, especially in areas with sparse tumor cells. (d) Perivascular pseudorosettes were observed frequently.

Figure 4. Immunohistochemistry and molecular analysis of ITD of HGNET-*BCOR*. (a)

The tumor was negative for glial fibrillary acidic protein. (b) The tumor was negative for epithelial membrane antigen. (c) The tumor was positive for BCOR. (d) The tumor was positive for NeuN. (e) Electrophoresis of the polymerase chain reaction product of *BCOR* presenting two distinct bands suggesting the presence of ITD. Control deoxyribonucleic acid (DNA) was extracted from an adult with a high-grade glioma. (f) Sanger sequencing presenting insertion of 1 bp (T) and duplication of 89 bps in exon 15 of *BCOR*. The yellow-shaded region indicates duplicated sequence, and the blue-shaded region indicates its parental sequence.


(a)
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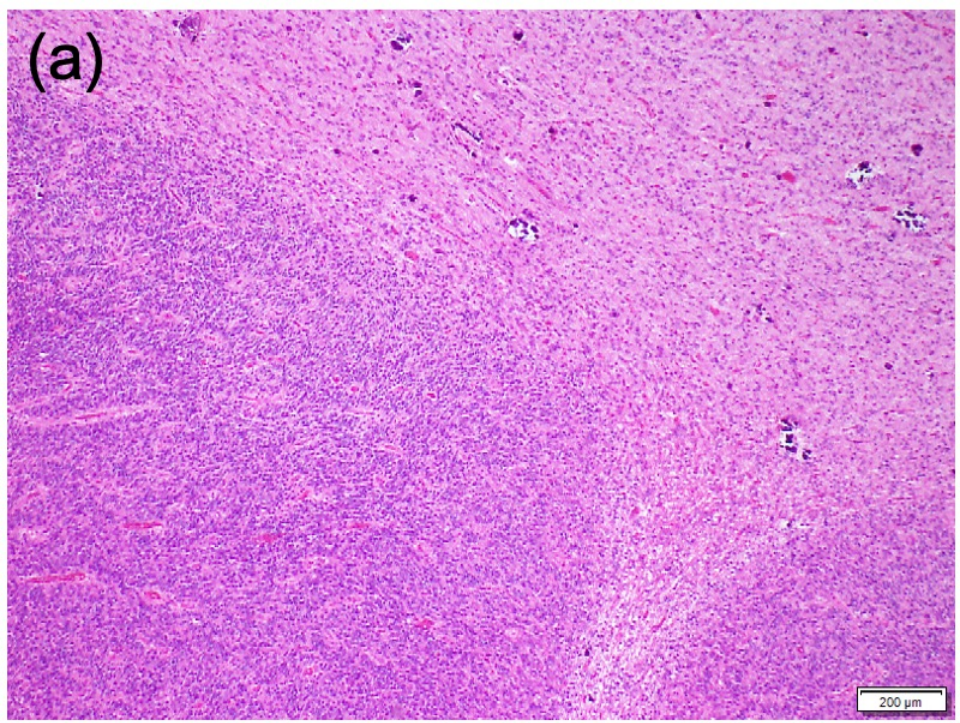
(a)
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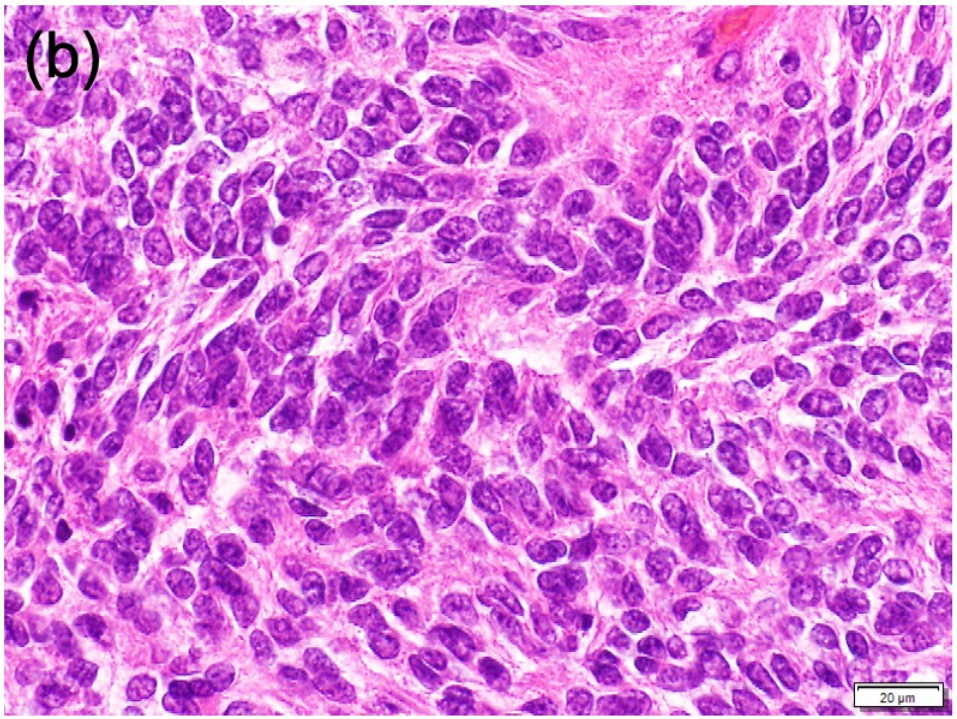
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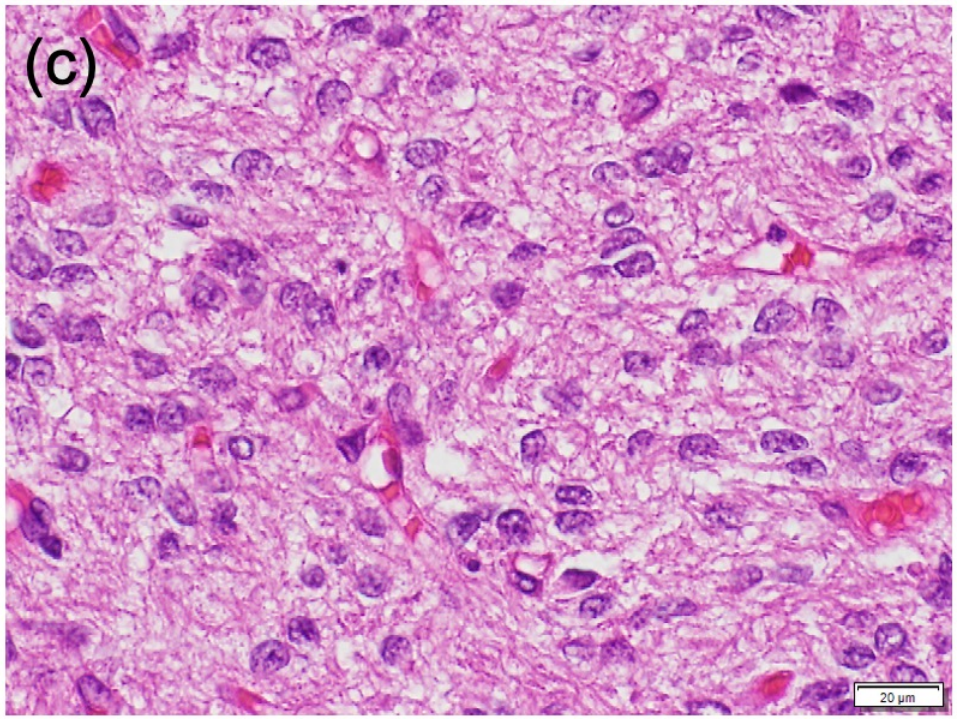
(c)
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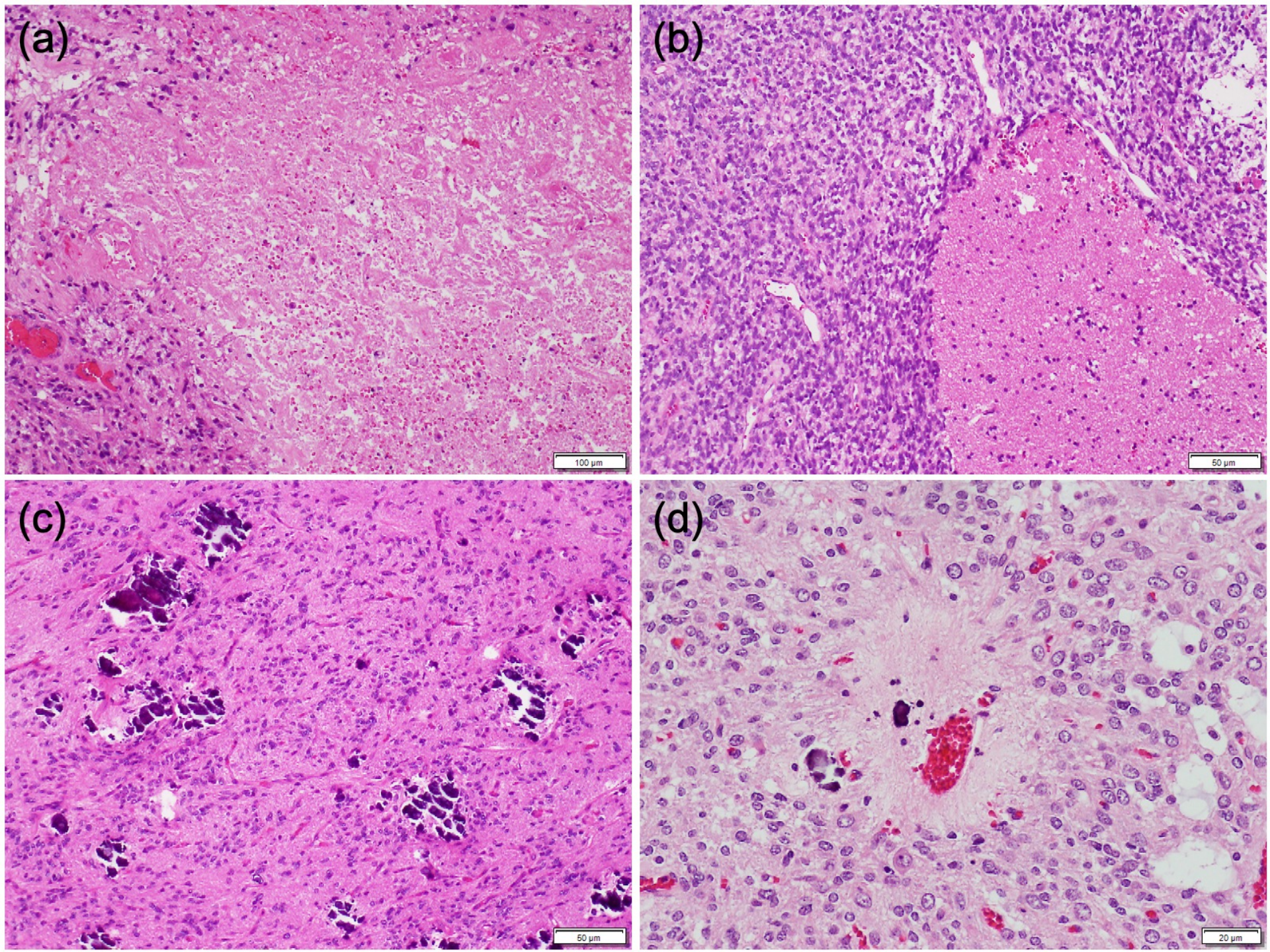
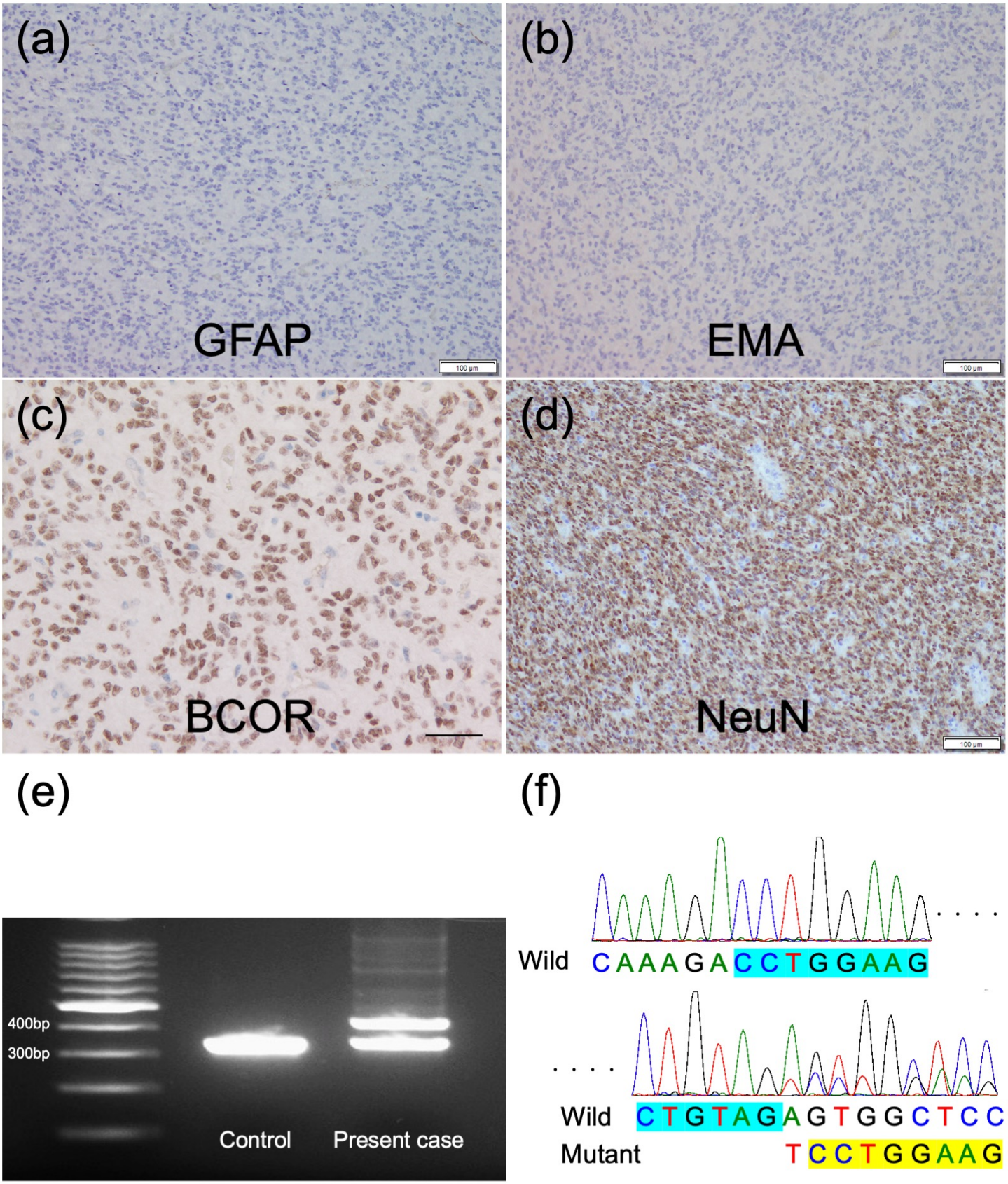

(a)
100 µm
(b)
50 µm
(c)
50 µm
(d)
20 µm



c.5224_5225ins90 [insertion of 1 bp (T) upstream of duplicated region and c.5136_5224dup]

Table 1. Results of immunohistochemistry.

Antibody	Present case	Previous reports
GFAP	(-)	(-)
EMA	(-)	(-)
BCOR	(+)	(+)
Olig2	(+)	(-)—(+)
NeuN	(+)	(-)—(+)
Synaptophysin	(-)	(-)
Neurofilament	(-)	(-)—(+)
S-100	(-)	(-)
INI1	(+)	(+)
CD56	(+)	(+)
Chromogranin A	(-)	ND
IDH1-R132H	(-)	(-)

ND; not described