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CASE REPORT

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Cerebrospinal fluid cytology in a case of epithelioid glioblastoma

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KEYWORDS

atypical teratoid/rhabdoid tumour, brain tumour, cerebrospinal fluid cytology, epithelioid glioblastoma

1 | INTRODUCTION

Epithelioid glioblastoma (eGB) is an aggressive histological subtype of isocitrate dehydrogenase (IDH)-wildtype glioblastoma characterised by the monotonous proliferation of loosely cohesive large round-to-oval neoplastic cells with abundant eosinophilic cytoplasm and eccentric nuclei.[1,2] This GB subtype affects children and young adults and exhibits a poor prognosis.[2,3] Genetically, *BRAF* V600E mutations, telomerase reverse transcriptase (*TERT*) promoter mutations and cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) homozygous deletions are characteristic genetic alterations in eGB.[2] Recently, three molecular subtypes of eGB have been reported: (i) favourable prognosis eGB in children and young adults grouped into the methylation profile of canonical pleomorphic xanthoastrocytoma (PXA), (ii) poor prognosis eGB in older adults grouped into the methylation profile of adult IDH-wildtype GB and (iii) intermediate prognosis eGB grouped into the methylation profile of receptor tyrosine kinase 1 (RTK1)-type paediatric high-grade glioma.[4] In eGB treatment, Kanemaru et al. reported the efficacy of the BRAF and mitogen-activated protein kinase (MEK) inhibitor combination therapy using an eGB cell line with *BRAF V600E* mutation, *TERT* promoter mutation and *CDKN2A/2B* homozygous deletion.[5]

Therefore, the recognition of eGB is important for improving patient treatment and prognosis. Here, we describe the cytopathological, histopathological and immunohistochemical features of eGBs in a young adult.

2 | CASE HISTORY

A 23-year-old man without a family history presented with a headache on his forehead. Seven days after the onset, brain magnetic resonance imaging at the outpatient clinic of a local hospital revealed a brain mass with peripheral oedema in the left medial temporal lobe, hypointense signals on T1-weighted images, slightly hyperintense signals on T2-weight images, and slightly heterogeneous enhancement on T1-weighted gadolinium-enhanced images with superficial dural attachment. Tumour dissemination was also observed in the spinal cord. Fifteen days after the onset, the patient was admitted to our hospital with vomiting, headache and poor food intake. Cerebrospinal fluid (CSF) cytology was performed after confirming the absence of hydrocephalus using magnetic resonance imaging (Figure 1A,B). One month after the onset, the patient experienced worsening intracranial hypertension. Therefore, tumour resection was performed using intraoperative cytology (Figure 1C–E) and intraoperative frozen-section pathology (Figure 2A). Subsequently, the final pathological diagnosis was made using a formalin-fixed and paraffin-embedded tumour specimen of the patient was used for the final diagnosis (Figure 2B–H).

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After the surgery, the patient experienced brain herniation and was subsequently in a state of brain death. Finally, the patient died four months after disease onset.

3 | RESULTS

3.1 | Cytopathological findings

Cerebrospinal fluid specimens were obtained via lumbar puncture and stained using the May–Grunwald Giemsa method. The specimen exhibited a rich neutrophil background with scattered, poorly cohesive, large round neoplastic cells (Figure 1A). Neoplastic cells have eccentric nuclei with high nuclear-to-cytoplasmic ratios. In addition, slightly reddish-purple intracytoplasmic globular inclusions were often detected (Figure 1B, black arrows) along with scattered mitotic figures. CSF cytology was diagnosed as "round cell neoplasm with rhabdoid features", and several differential diagnoses were made, including eGB and atypical teratoid/rhabdoid tumour (AT/RT).

Intraoperative imprint and smear cytology specimens were stained using the standard Papanicolaou (Pap) and haematoxylin and eosin methods. The specimen showed poor cohesion and highly cellular clusters with a myxoid background (Figure 1C). Many round neoplastic cells (Figure 1C,D) with round-to-oval eccentric nuclei, rough chromatin and prominent nucleoli (Figure 1E) were also observed. In addition, greenish-dense intracytoplasmic globular inclusions were detected (Figure 1E, black arrows). As shown in Figure 1E (arrowheads), mitotic figures were also observed.

3.2 | Histopathological findings

Highly cellular neoplasms with necrosis were observed in both the intraoperative frozen and permanent brain tumour sections (Figure 2A,B). The specimen consisted of round neoplastic cells with eccentric round-to-oval nuclei and rich eosinophilic cytoplasm, showing a poorly cohesive or sheet-like pattern (Figure 2A–C). The neoplastic cells often contained intracytoplasmic eosinophilic globular inclusions (Figure 2C). In addition, a lower-grade glial component

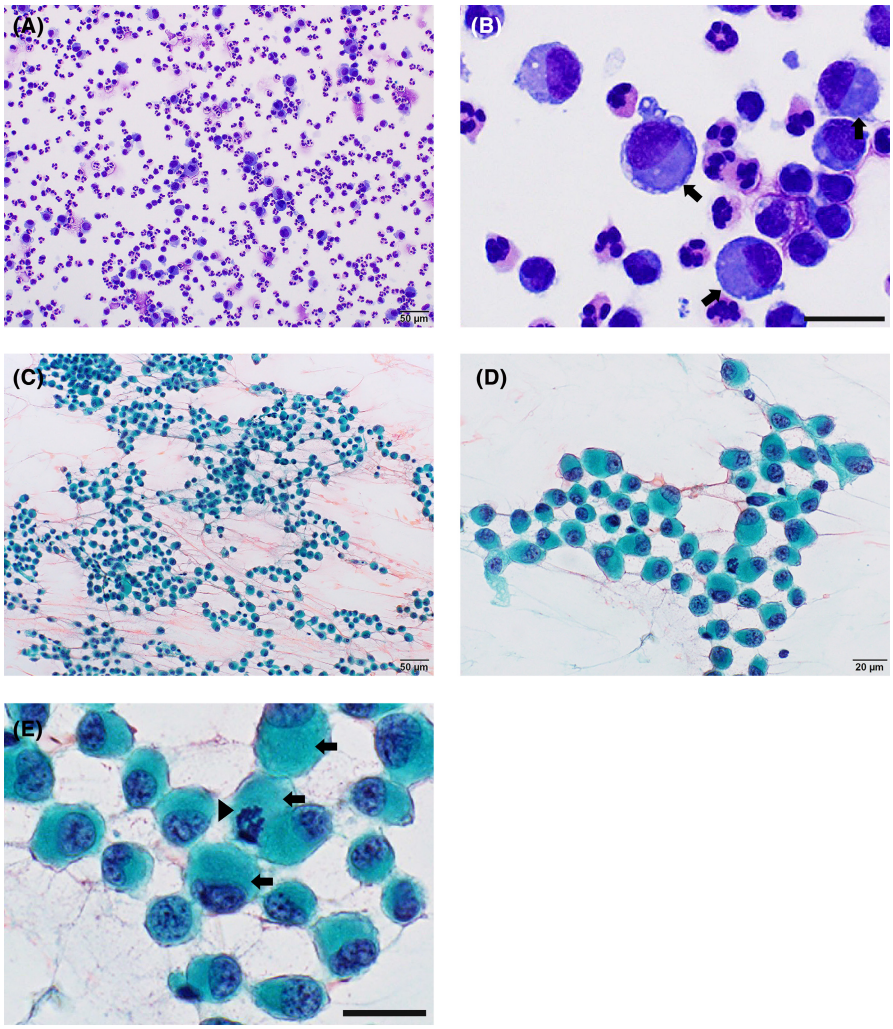

FIGURE 1 Cytopathological features of epithelioid glioblastoma. (A) Scattered, poorly cohesive, large and round neoplastic cells were observed in the rich neutrophil background (cerebrospinal fluid; magnification: 200×; May–Grunwald Giemsa stain). (B) Neoplastic cells contained light reddish-purple globular inclusions and eccentric nuclei in their cytoplasm (cerebrospinal fluid; magnification: 600×, scale bars: 20 µm; May–Grunwald Giemsa stain). (C) Poorly cohesive large round neoplastic cells in the slight myxoid background (intraoperative squash tumour cytology; magnification 200×; Papanicolaou [Pap] stain). (D) Poorly cohesive large round neoplastic cells with intracytoplasmic globular inclusion and eccentric nuclei (intraoperative squash tumour cytology; magnification: 600×; Pap stain). (E) Neoplastic cells with dense intracytoplasmic globular inclusions (black arrow) and frequently detected mitotic figures (black arrow head; intraoperative squash tumour cytology; magnification: 600×, scale bars: 20 µm; Pap stain).

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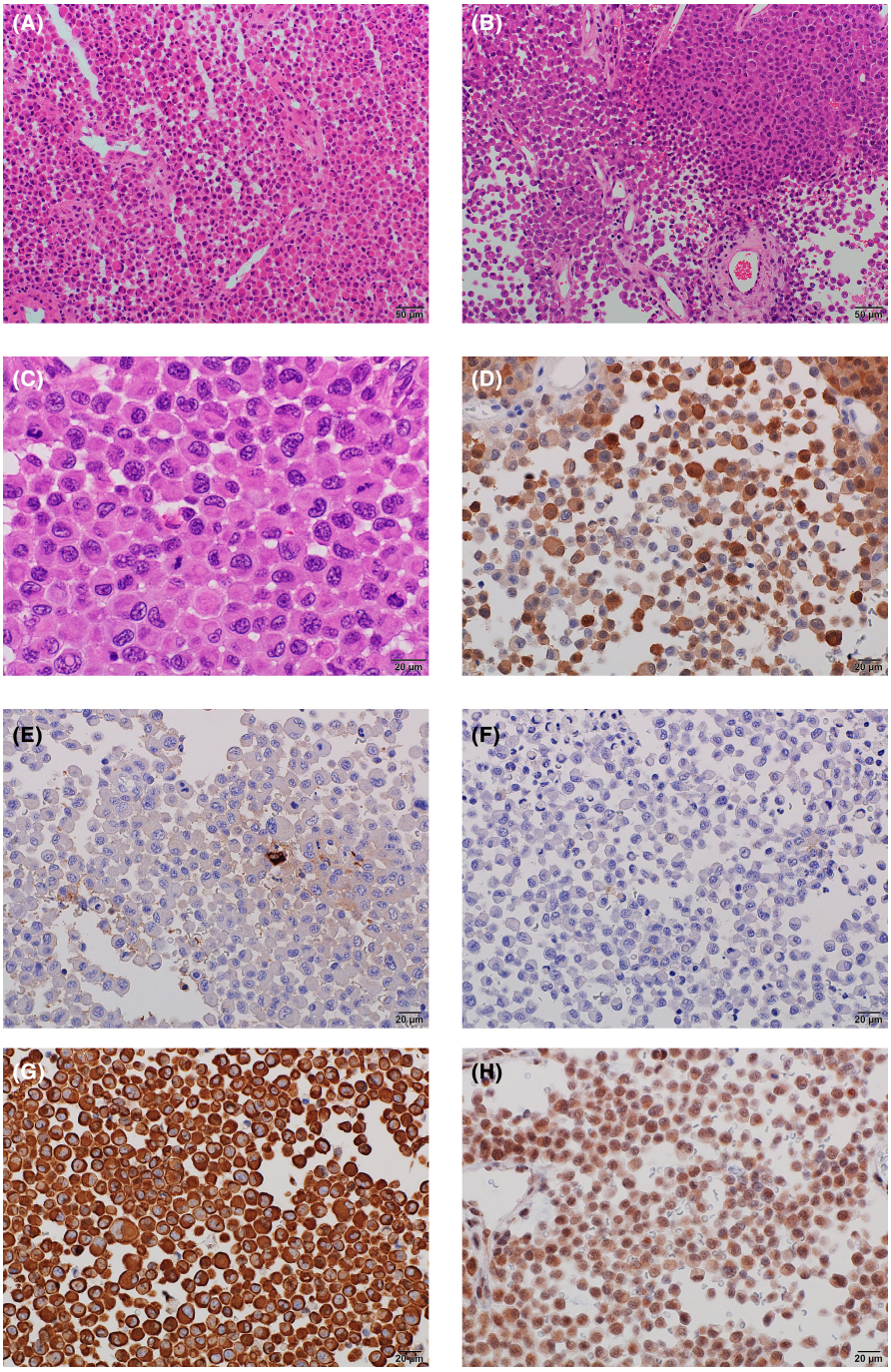

FIGURE 2 Histopathological and immunohistochemical features of epithelioid glioblastoma. (A,B) Highly cellular neoplasm with no characteristic features (intraoperative frozen section [A] and paraffin-embedded section [B]; magnification: 200×; haematoxylin and eosin [H&E] staining). (C) Poorly cohesive round neoplastic cells with eccentric nuclei and dense globular inclusions in their cytoplasm (magnification: 600×; H&E). (D–H) Many neoplastic cells showed immunopositivity for S100 protein (D; magnification: 400×) and a few for glial fibrillary acidic protein (E; magnification: 400×), but none were positive for the epithelial membranous antigen (F; magnification: 400×). Intracytoplasmic globular inclusions exhibited viemtin immunoreactivity (G; magnification: 400×). Neoplastic cells exhibited nuclear expression of INI-1 (H; magnification: 400×).

with fibrillary morphology was observed; however, no PXA-like features were detected. Mitotic figures were commonly observed in the former component (5 mitoses/10 high-power fields of 0.345 mm^2 in area).

Immunohistochemically, the neoplastic cells stained positive for S100 protein (Figure 2D) and nestin. Although rare, they also stained positive for glial fibrillary acidic protein (GFAP; Figure 2E). Neoplastic cells were immunonegative for oligodendrocyte lineage transcription factor 2 (Olig2), epithelial membrane antigen (Figure 2F), cytokeratin AE1/AE3 and the mutated IDH1-R132H antibody. Less than 5% of neoplastic cells expressed the P53 protein. The neoplastic cells did not exhibit MDM2 or CDK4 immunoreactivity. However, nuclear expression of alpha-thalassemia/mental retardation X-linked protein and INI-1 (Figure 2H) was also detected. The intracytoplasmic globular inclusions were positive for vimentin (Figure 2G), and the Ki67 labelling index was 16.8%.

Genetically, *BRAF* V600E and C228T mutations in *TERT* were identified via pyrosequencing. No mutations were detected in *IDH1/2*, *H3F3A*, *HIST1* or *FGFR1*.

The brain tumour was finally diagnosed as a central nervous system (CNS) grade 4 eGB according to the World Health Organization (WHO) Classification of CNS Tumours, 5th edition.[1]

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4 | DISCUSSION

The eGM is a rare subtype of GB.[6] This GB subtype had been previously described as an epithelioid/rhabdoid glioblastoma. However, it was recommended to avoid the term "rhabdoid glioblastoma" on the basis of loss of SMARCB1 (INI-1) nuclear expression.[7] In contrast, eGB has been listed as the subtypes of IDH-wildtype glioblastoma since the 2016 WHO classification of tumours of the CNS.[7] The eGB predominantly affects males (male: female ratio = 2.37–3.3:1).[4,6] At the time of eGM diagnosis, the patient age is approximately 25 years (range: 3–67 years) and the cerebral hemisphere is mainly affected.[4,6] The median overall survival is 23 months (range: 5–72 months).[4] Neuroradiologically, eGM presents as a large mass with complex cystic and solid areas on T1-weighted gadolinium-enhanced images.[6] Superficial dural attachment and CSF dissemination are also observed in many eGB cases.[6] In this study, the patient with eGB was a young adult male with a short survival of 4 months. Neuroradiological features included slightly heterogeneous enhancement on T1-weighted gadolinium-enhanced images with superficial dural attachment. Prior to pathological examination, neuroradiology suggested CSF dissemination in the spinal cord. These clinical and neuroradiological features were similar to those reported in previous studies.[4,6]

The histopathology of eGB is characterised by small to medium poorly cohesive neoplastic cells showing patternless sheet proliferation.[6] Although necrosis is observed in this GB subtype,[6] in contrast to typical IDH-wildtype GB, palisading type necrosis is not common. The neoplastic cells had a rounded rich cytoplasm and eccentric large nuclei with prominent nucleoli.[6] Globular intracytoplasmic inclusions were also frequently detected[6]; therefore, AT/RT should be considered as a differential diagnosis.[8–12] The clinicopathological and genetic features of eGB and AT/RT are summarised in Table 1. As shown in Table 1, both neoplasms were often disseminated into the CSF. However, in terms of clinical features, eGBs affect young adults, whereas AT/RTs mainly affect infants and children. Furthermore, ill-cohesive neoplastic cells were monotonously detectable in both the cytology and

TABLE 1 Clinical, cytological, histopathological and molecular differences between epithelioid glioblastoma and atypical teratoid/rhabdoid tumours.

	Epithelioid glioblastoma[1,4,6]	Atypical teratoid/rhabdoid tumour[8–12]
Clinical features		
Sex (male: female)	2.3–3:1	1.2–2:1
Age	25–26 years	18–24 months
Tumour localisation		
ST	87.5%–96.9%	50%–62%
IT	3.1%–12.5%	38%–47%
CSF involvement	12.5%	20%
Survival	23 months (range 5–72 months)	16.75 months (range, 1–96 months)
Cytopathological features		
Cell morphology	Monotonous medium-sized epithelioid or rhabdoid cells	Rhabdoid cell, undifferentiated small cell
Nuclei	Eccentric nuclei	Eccentric nuclei
Nucleoli	Distinct nucleoli	Distinct nucleoli
Intracytoplasmic inclusion	Rounded dense cytoplasmic inclusion	Indistinct round inclusion
Histopathological features		
Growth pattern	Patternless sheets of poorly cohesive neoplastic cells	Various patterns:embryonal tumour-like, epithelial-like, mesenchymal-like etc.
Necrosis	+	+
Immunohistochemical features		
	Immunophenotype of glial lineage *positive for GFAP (patchy), OLIG2, EMA (focal) and S100 protein	Polyimmunophenotype *Positive for GFAP, NFP, synaptophysin, EMA and alpha-SMA in various degree
Vimentin	+ (intracytoplasmic inclusion)	+ (intracytoplasmic inclusion)
BRAF p.V600E-mutant protein	+	–
INI1	Preserved nuclear expression	Loss of nuclear expression
Ki67 labelling index	42%–48%	20%–50%
Molecular alteration		
	BRAF p.V600E mutation	*SMARCB1(INI1)* or SMARCA4 alteration

Abbreviations: EMA, epithelial membrane antigen; GFAP, glial fibrillary acidic protein; IHC, immunohistochemistry; IT, infratentorial; OLIG2, oligodendrocyte transcription factor 2; SMA, smooth muscle actin; ST, supratentorial.

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histopathology of the eGB. In contrast, rhabdoid neoplastic cells and undifferentiated small cells were observed in the AT/RT. In terms of immunohistochemical features, eGB showed immunoreactivity for glial markers such as GFAP, S100 protein and OLIG2, whereas AT/RT showed a polyimmunophenotype. Importantly, in contrast to AT/RT, eGB exhibits INI-1 nuclear expression.[6]

Only a few studies have described the cytopathological features of eGB.[6,13] Its key cytopathological features include the presence of poorly cohesive medium-to large neoplastic cells with distinct nucleoli, eccentric nuclei and round cytoplasm.[6,13] Yamamoto et al. also described the dense condensation of the cytoplasm via squash cytology and revealed a large polygonal pale cytoplasmic inclusion via touch imprint cytology.[13] Furthermore, in their case, classic GB-like cells with cytoplasmic processes can be detected.[8] Although no GB-like neoplastic cells were observed, the cytopathological features of eGB in our case are the same as those previously reported. Therefore, intraoperative and CSF cytology are useful for evaluating tumour histology and diagnosing eGB.

Recently, Korshunov et al.[4] reported the molecular classification of eGB. According to the methylation patterns, copy numbers, mutational alterations and clinical features, eGB can be classified into the following three molecular subtypes: (i) favourable prognosis eGB of children and young adults grouped into the methylation profile of canonical PXA, (ii) poor prognosis eGB of older adults grouped into the methylation profile of adult IDH-wildtype GB and (iii) intermediate prognosis eGB grouped into the methylation profile of RTK1-type paediatric high-grade glioma.[4] However, we could not perform detailed molecular analyses, including methylation analyses, in this study. Our patient exhibited a poor prognosis; however, immunohistochemistry and genetic alterations were similar to those observed in the PXA eGB molecular subtype.

Arbour et al.[14] reported the efficacy of BRAF/MEK inhibitors for high-grade gliomas with *BRAF V600E* mutation and revealed that more than 80% of tumours, including glioblastoma, anaplastic PXA, anaplastic astrocytoma and anaplastic ganglioglioma, show complete or partial response to these inhibitors. Kanemaru et al.[5] also reported the favourable response to combined BRAF and MEK inhibitor therapy in an eGB case with *BRAF V600E* mutation. Therefore, keeping eGB in mind and making distinct pathological diagnosis of eGB, with genetic alteration of *BRAF* gene, is necessary for choice of therapies and patients' prognosis.

In conclusion, this report presents the cytopathological, histopathological and immunohistochemical features of eGBs in young adults. Although this glioblastoma subtype is rare, eGB exhibits characteristic cellular morphology and genetic alterations and predominantly affects young adults. A recent study demonstrated the efficacy of BRAF and MEK-1/2 inhibitors in eGB treatment.[5] Overall, our findings highlight the importance of considering this glioblastoma subtype to enhance patient care and prognosis.

AUTHOR CONTRIBUTIONS

Conceptualisation: Taku Homma. Patient care: Tomonari Suzuki, Mitsuaki Shirahata and Kazuhiko Mishima. Cytological investigation: Taku Homma, Tomomi Kato. Pathological investigation: Taku Homma. Genetic data: Tomonari Suzuki, Mitsuaki Shirahata and Kazuhiko Mishima. Writing – original draft: Taku Homma. Writing – review and editing: Taku Homma.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

All data related to this case are included in this case report.

INFORMED CONSENT

Written informed consent for medical research and presentation was obtained from patient.

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