Ependymoma-like tumor with mesenchymal differentiation harboring \textit{C11orf95-NCOA1/2} or \textit{-RELA} fusion: A hitherto unclassified tumor related to ependymoma

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Abstract

Recurrent fusion genes involving \textit{C11orf95}, \textit{C11orf95-RELA}, have been identified only in supratentorial ependymomas among primary CNS tumors. Here, we report hitherto histopathologically unclassifiable high-grade tumors, under the tentative label of “ependymoma-like tumors with mesenchymal differentiation (ELTMDs),” harboring \textit{C11orf95-NCOA1/2} or \textit{-RELA} fusion. We examined the clinicopathological and molecular features in five cases of ELTMDs. Except for one adult case (50 years old), all cases were in children ranging from 1 to 2.5 years old. All patients presented with a mass lesion in the cerebral hemisphere. Histologically, all cases demonstrated a similar histology with a mixture of components. The major components were embryonal-appearing components forming well-delineated tumor cell nests composed of small uniform cells with high proliferative activity, and spindle-cell mesenchymal components with a low- to high-grade sarcoma-like appearance. The embryonal-appearing components exhibited minimal ependymal differentiation including a characteristic EMA positivity and tubular structures, but histologically did not fit with ependymoma because they lacked perivascular pseudorosettes, a histological hallmark of ependymoma, formed well-delineated nests, and had diffuse and strong staining for CAM5.2. Molecular analysis identified \textit{C11orf95-NCOA1}, \textit{-NCOA2}, and \textit{-RELA} in two, one, and two cases, respectively.
INTRODUCTION

Ependymomas develop anywhere throughout the central nervous system (CNS) and in all age groups, but are most commonly infratentorial in children and young adults, accounting for approximately 10% of pediatric intracranial brain tumors (1). Although ependymomas from different anatomical locations or from different age groups are hardly histopathologically distinguishable and the World Health Organization (WHO) grading is sometimes challenging because of not well defined criteria, recent genomic studies subdivided supratentorial (ST), posterior fossa, and spinal ependymomas into clinically meaningful and molecularly distinct subgroups, including ST ependymomas with \(C11orf95\)-\(RELA\) (2–7).

In the revised 4th edition of the WHO classification, ST ependymomas with \(C11orf95\)-\(RELA\) are defined as a separate entity (8). In a large cohort study, ST ependymomas with \(C11orf95\)-\(RELA\) accounted for 70% of all ST ependymomas, mostly in children; however, a significant portion (24%) was found in adults (3). Parker et al reported that the fusion genes resulted from clustered genomic rearrangements occurring in localized genomic regions, known as chromothripsis, at chromosome...
1lq12.1–1lq13.3, and that the fusion proteins led to NF-κB pathway activation with nuclear accumulation of p65/RelA (4). In addition, LICAM, which was originally identified as a neural adhesion molecule essential for axonogenesis (9), was reported to be overexpressed in ST ependymomas with C11orf95-RELA (4,10,11), suggesting that LICAM is a target of aberrant signaling of the fusion proteins (4). Overexpression of both p65/RelA and LICAM is identifiable by immunohistochemistry (4,10,11). Histologically, ST ependymomas with C11orf95-RELA often exhibit clear cell morphology and branching vessels (8).

In ST ependymomas, C11orf95 is also the fusion partner of other rare fusions with genes encoding transcription factors, such as NCOAI, YAP1, and MAML2, each with only one or two cases reported (4,5,12). The case of ependymoma with C11orf95-NCOAI presented clear cell morphology and was diagnosed as anaplastic ependymoma (5); however, histology was not detailed for the other cases (4). Other than in ST ependymomas, as recurrent fusion genes involving C11orf95, only C11orf95-MKL2 were identified in chondroid lipomas, benign lipogenic tumors developing mainly in the extremities and limb girdles of adults (13,14). Although it has been suggested that the zinc finger domains of C11orf95 may be essential oncogenic elements of these fusions involving C11orf95, the physiological function of C11orf95 is unknown (4). The breakpoints in C11orf95 for C11orf95-RELA in ST ependymomas are mostly between exons 2 and 3, whereas those for C11orf95-MKL2 in chondroid lipomas are within exon 5 (4,5,13,14).

In this study, we report five cases of hitherto histopathologically unclassifiable high-grade tumors with fusion genes involving C11orf95, with NCOAI, NCOA2, or RELA as fusion partners. These tumors, herein, referred to as “ependymoma-like tumors with mesenchymal differentiation (ELTMDs),” demonstrated a similar histology, including small round blue cell components with minimal ependymal differentiation, but they cannot be regarded as embryonal tumors or ependymoma as a whole.

2 | MATERIALS AND METHODS

2.1 | Tumor samples

We searched the consultation archives of two authors (S. Nobusawa and J.H.), comprising approximately 2500 cases of brain tumors, for cases demonstrating a similar histology described below (for details see “RESULTS”), and found five such cases (Table 1). Sections for histological and genetic analyses were prepared from formalin-fixed paraffin-embedded (FFPE) tissue specimens. This study was conducted in accordance with the ethical committees of Gunma University and the National Cancer Center.

2.2 | Conventional histological analysis

Three-micrometer-thick tissue sections were cut and stained with hematoxylin-eosin or periodic acid–methenamine silver (PAM). Immunohistochemical staining was performed on FFPE tissue sections. Primary antibodies against the following antigens were applied: vimentin (V9; 1:200; Dako, Glostrup, Denmark), glial fibrillary acidic protein (GFAP) (1:5000) (15), Olig2 (1:5000) (16), cytokeratin (CAM5.2; 1:5; BD Bioscience, San Jose, CA, USA), α-smooth muscle actin (aSMA) (1A4; 1:320; BioMakor, Rehovot, Israel), epithelial membrane antigen (EMA) (E29; 1:100; Dako), synaptophysin (27G12; 1:200; Novocastra, Newcastle upon Tyne, UK), NeuN (A60; 1:1000; Chemicon, Temecula, CA, USA), podoplanin (D2-40; prediluted; Nichirei, Tokyo, Japan), CD99 (12E7; 1:50; Dako), LICAM (UJ127; 1:100; Novus Biologicals, Littleton, CO, USA), p65/RelA (D14E12; 1:400; Cell Signaling Technology, Danvers, MA, USA), BAF47/IN1I (BAF47; 1:100; BD Bioscience, San Jose, CA, USA), BRG1 (polyclonal; 1:1000; Millipore, Temecula, CA, USA), and Ki-67 (MIB-1; 1:100; Dako). For coloration, a commercially available biotin-streptavidin immunopersidoxidase kit (Histofine, Nichirei) and diaminobenzidine were employed.

2.3 | RNA sequencing and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from FFPE (cases 2 and 5) or frozen (case 3) samples. RNA sequencing and RT-PCR were performed as described in Supporting Information.

2.4 | Fluorescence in situ hybridization (FISH) analysis

Dual-probe hybridization using an intermittent microbeam irradiation method was employed using 4-μm-thick FFPE tissue sections, as described previously (17). Probes for C11orf95, RELA, NCOAI, and NCOA2 were prepared from bacterial artificial chromosome (BAC) clones, as described previously (Table S1) (18,19). The BAC clones were labeled with either ENZO Orange-dUTP or ENZO Green-dUTP (Abbott Molecular Inc., Des Plaines, IL, USA), and metaphase FISH to verify clone mapping positions was performed using the peripheral blood cell cultures of a healthy donor.

2.5 | Whole exome sequencing (WES)

WES was performed on DNA isolated from FFPE tissue of cases 2–5 with sufficient quality and quantity using a NextSeq 500 DNA sequencer as described in Supporting Information.
| Case | Age/Sex | Initial symptoms | Location (size) | Neuroimaging | Surgery | Radiation | Chemotherapy | Outcome | Molecular status |
|------|---------|------------------|-----------------|--------------|---------|-----------|--------------|---------|------------------|
| 1    | 50/F    | Headache, hemiplegia | Cerebrum (right frontal lobe) (4.5 cm) | Cystic/solid, enhanced, calcification | GTR | Local 46 Gy | Temozolomide | RD (4 months) | C11orf95-RELA<sup>a</sup> |
| 2    | 2.5/F   | Ataxia, claudication | Cerebrum (right lateral ventricle) (5.5 cm) | Cystic/solid, enhanced | PR | No | Multiagent*1 (1st and 2nd courses) | SD (3 months) | C11orf95 (exon 5, partial)-NCOA1 (exon 15)<sup>a,b,c</sup> |
| 2 Rec | –       | –                | –               | Cystic/solid, enhanced | GTR | Local 27 Gy + CSI 23.4 Gy | Multiagent*1, (3rd and 4th courses), PBSCT | RD (2.8 years) → DOD (3.5 years) | – |
| 3    | 1/M     | Vomit, seizure    | Cerebrum (left parietal lobe) (8 cm) | Cystic/solid, enhanced, meningeal dissemination | No | Carboplatin, etoposide (at recurrence) | RD (1 years) → DOD (2.2 years) | C11orf95 (exon 5, partial)-NCOA2 (exon 14)<sup>a,b,c</sup> | No matching methylation classes with calibrated score ≥ 0.9<sup>d</sup> Methylation class ependymoma, RELA fusion with a low calibrated score (0.65)<sup>d</sup> |
| 4    | 2/F     | Seizure           | Cerebrum (right frontal lobe) (3 cm) | Solid, enhanced, well-circumscribed, calcification | GTR | No | Multiagent*2 (BBSFOP) | NED (4.5 years) | C11orf95-RELA<sup>a</sup> |
| 5    | 1.5/F   | Seizure, strabismus | Cerebrum (left frontal lobe) (6 cm) | Cystic/solid, enhanced | GTR | Local (proton) 50.4 Gy/30 Fr | Multiagent*3, PBSCT | RD → NED (3 years) | C11orf95 (exon 5, partial)-NCOA1 (exon 14)<sup>a,b,c</sup> No matching methylation classes with calibrated score ≥ 0.3<sup>d</sup> |

**Note:** *1: etoposide, cyclophosphamide, cisplatin, vincristine, and intrathecal methotrexate and doxorubicin, followed by thiopeta and melphalan; *2: carboplatin, procarbazine, etoposide, cisplatin, vincristine, and cyclophosphamide; *3: vincristine, triple-intrathecal (Methotrexate/Hydrocortisone/Cytarabine), followed by ifosfamide, cisplatin, and etoposide, and HIT 2000 protocol comprising cyclophosphamide, vincristine, methotrexate, carboplatin, and etoposide, and concomitant intrathecal methotrexate.

Abbreviations: CSI, craniospinal irradiation; DOD, died of disease; F, female; GTR, gross total resection; M, male; NED, no evidence of disease; PBSCT, peripheral blood stem cell transplantation; PR, partial resection; RD, recurrence of disease; SD, stable disease.

<sup>a</sup>Identified by FISH analysis.<br>
<sup>b</sup>Identified by RNA sequencing.<br>
<sup>c</sup>Identified by WES.<br>
<sup>d</sup>Analyzed by the DKFZ methylation classifier.
Information. Selected variants observed in more than two cases were categorized as follows: COSMIC database (https://cancer.sanger.ac.uk/cosmic)-registered variants, truncation mutation (not registered in COSMIC database), or variants of unknown significance (VUS) (Table S2).

2.6 | Genome-wide DNA methylation analysis

DNA of sufficient quality and quantity was extracted from cases 3 (frozen sample) and 5 (FFPE sample), and bisulfite modification of DNA was performed using an EZ Methylation DNA Kit (Zymo Research, CA, USA). Methylation profiling was performed as in Supporting Information.

2.7 | Array comparative genomic hybridization (CGH)

DNA from cases 2–5 of sufficient quality and quantity extracted from FFPE samples was analyzed by array CGH as described in Supporting Information.

3 | RESULTS

3.1 | Clinical findings

Relevant clinical data are summarized in Table 1. Case 1 was in an adult (50 years old), and cases 2–5 were in children ranging from 1 to 2.5 years old. All patients presented with a mass lesion in the cerebral hemisphere; cases 1, 4, and 5 were located in the superficial portion, and the others were in the deep portion involving the lateral ventricle. Tumors demonstrated iso- to high intensity on T2-weighted images (Figure 1A,D), iso-intensity on T1-weighted images (Figure 1B,E), and were heterogeneously enhanced after gadolinium injection (Figure 1C,F). Cases 1–3, and 5 possessed cystic components, and cases 1 and 4 with available computed tomography images had calcification. Patients in cases 1, and 3–5 underwent primary gross-total resection, whereas that in case 2 underwent two-staged resection over 3 months. Of four patients with a follow-up period longer than 2 years, those in cases 2 and 3 died of the disease (3.5 and 2.2 years, respectively), and those in cases 4 and 5 were alive without evidence of disease at 4.5 and 3.5 years after initial surgery, respectively.

**Figure 1** Radiological images of representative cases; (A–C) case 3, (D–F): case 5. Both tumors consist of a cystic component and solid component. The solid components exhibit iso- to high intensity in the cerebral cortex on T2-weighted images (A and D) and iso-intensity on T1-weighted images (B and E), and were heterogeneously enhanced after gadolinium injection (C and F). Meningeal enhancement surrounding the surface of the brain and spinal cord is observed, suggesting meningeal dissemination (C).
3.2 | Histopathological findings

All five cases demonstrated a mixed histology; the major components observed in all cases were embryonal-appearing components and spindle-cell mesenchymal components (Figure 2A). The embryonal-appearing components were characterized by variably sized and shaped tumor cell nests separated mostly by the mesenchymal components (Figure 2B). Thin cord-like structures and minute small clusters were also observed (Figure 2C). The components exhibited a highly cellular, poorly differentiated, hyperchromatic, and mitotically active histological appearance composed of small tumor cells with scant cytoplasm (Figure 2D). Small to large tubular structures were found in limited parts of the components, with some containing eosinophilic amorphous material (Figure 2E). The mesenchymal components were composed of relatively monotonous spindle cells in a fascicular or diffuse pattern, ranging from low- to high-grade sarcoma-like histologies (Figure 2F); the former exhibited mitotically indolent tumor cells with low cellularity in a collagenous, edematous, or myxoid background (Figure 2G), whereas the latter demonstrated a dense proliferation of spindle cells with larger nuclei and higher mitotic activity (Figure 2H). PAM staining revealed abundant pericellular reticulin in the mesenchymal components (Figure 2I). The third element was glioneuronal components, observed in cases 2, 3, and 5, consisting of astrocyte-like tumor cells with oval nuclei and eosinophilic cytoplasm with processes and neurocyte-like tumor cells with round nuclei and clear cytoplasm (Figure 2J). A small number of ganglioid tumor cells with relatively large nuclei with prominent nucleoli were also observed (Figure 2K). The components occasionally assumed acinus-like structures with the mesenchymal component trapping the glioneuronal tumor cells (Figure 2K). Mitoses were rare in the glioneuronal components. A small area with lipomatous metaplasia was found only in case 4 (Figure 2L).

On immunohistochemistry, tumor cells of the embryonal-appearing components were diffusely positive for CAM5.2 (Figure 3A), GFAP and Olig2 immunoreactivity in the components was focally identified only in cases 4 and 3, respectively (Figure 3B,C). EMA staining exhibited a dot-like pattern of cytoplasmic positivity and linear positivity along the apical surface of some of the tubular structures in the embryonal-appearing components (Figure 3D). The mesenchymal components were positive for vimentin in all five cases (Figure 3E) and αSMA was negative in the two cases tested (cases 3 and 4). A limited number of spindle tumor cells was positive for GFAP and podoplanin in all cases. In the glioneuronal components, the astrocyte-like tumor cells were positive for GFAP and the neurocyte-like tumor cells were positive for synaptophysin (Figure 3F,G). The neurocyte-like tumor cells were weakly positive for NeuN (Figure H). Reactivity for Olig2 was observed in the astrocyte-like tumor cells to varying degrees. CD99 was negative in all cases. LICAM expression was almost exclusively found in the embryonal-appearing components in all cases (Figure 3I). Nuclear accumulation of p65/RelA was detected in cases 1 and 4, but not in cases 2, 3, or 5 (Figure 3J–L). Nuclear expression of INI1 and BRG1 was retained throughout the tumor tissue in all cases. MIB-1 labeling indices were high in the embryonal-appearing components and high-grade mesenchymal components, with the highest ranging from 30% to 57%.

Most of the specimen from the second operation in case 2, besides the components described above, displayed an ependymoma-like histology, that is, the proliferation of tumor cells with round to ovoid nuclei and eosinophilic cytoplasmic processes, exhibiting perivascular pseudorosettes with anuclear zones (Figure 4A–C). One mitosis was detected in 10 high-power fields in this element. The ependymoma-like tumor cells were immuno reactive for GFAP, with perivascular cytoplasmic processes having particularly strong staining (Figure 4D). Dot-like and ring-like patterns of cytoplasmic EMA positivity were observed in this component (Figure 4E). The components were negative for CAM5.2 staining. LICAM expression was limited in the embryonal-appearing components (Figure 4F). Nuclear accumulation of p65/RelA was not detected. MIB-1 labeling index was 3% in the ependymoma-like components.

3.3 | Genetic analysis

RNA sequencing identified in-frame fusions of C11orf95 (exon 5) and NCOA1 (exon 15), C11orf95 (exon 5) and NCOA2 (exon 14), and C11orf95 (exon 5) and NCOA1 (exon 14) in cases 2, 3, and 5, respectively (Figure 5A, Table 1, and Figure S1). The breakpoints in C11orf95 for these fusions were within exon 5. The fusion in case 5 was confirmed by RT-PCR and Sanger sequencing (Figure 5A).

FISH analysis using break-apart C11orf95 probes revealed positive signals of C11orf95 rearrangement in all five cases (Figure 5B–D). In cases 1 and 4, break-apart signals of RELA and fusion signals of C11orf95-RELA were observed (Figure 5E,H, Table 1). In the remaining cases, break-apart signals of NCOA1 (cases 2 and 5) or NCOA2 (case 3) and fusion signals of C11orf95-NCOA1 (cases 2 and 5) or C11orf95-NCOA2 (case 2) were observed (Figure 5F,G,I,J and Table 1).

Based on analysis of cases 2-5 by WES, variants shared by more than two cases are listed in Table S2. No variants, including COSMIC database-registered variants, were assigned as pathogenic in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and we did not observe any obvious oncogenic variants. C11orf95-NCOA1/2 detected by RNA sequencing in cases 2, 3, and 5 were also identified by WES (Figure S2), whereas C11orf95-RELA
Figure 2  Microscopic appearance of the ependymoma-like tumors with mesenchymal differentiation (ELTMDs). (A) The tumor is mainly composed of embryonal-appearing components and spindle-cell mesenchymal components (case 1). (B–E) Microscopic appearance of the embryonal-appearing components. Variably sized and shaped tumor cell nests separated by the mesenchymal components (B, case 5). Tumor cell nests (right), thin cord-like structures, and minute small clusters (left) (C, case 1). The components exhibit a highly cellular, poorly differentiated, hyperchromatic, and mitotically active histological appearance composed of small tumor cells with scant cytoplasm (D; left, case 2; right, case 5). Small to large tubular structures, with some containing cosinophilic amorphous material (E; top left, case 5; bottom left, case 1; right, case 4). (F–H) Microscopic appearance of the mesenchymal components. A transition between low- (top right) to high-grade sarcoma-like (bottom left) histologies (F, case 4). The low-grade area includes mitotically indolent spindle tumor cells with low cellularity (G, case 3). The high-grade sarcoma-like area exhibits the dense proliferation of spindle cells with larger nuclei and high mitotic activity (H, case 1). (I) Periodic acid–methenamine silver staining exhibits abundant pericellular reticulin in the mesenchymal components. The arrows indicate the embryonal-appearing components (case 5). (J and K) Microscopic appearance of the glioneuronal components. Astrocyte-like tumor cells in top right and neurocyte-like tumor cells in bottom left (J, same area as Figure 3F, G, case 5). Acinus-like structures with the mesenchymal component trapping the glioneuronal tumor cells (K, left, case 2). The circle indicates a ganglioid tumor cell (K, right, case 2). Lipomatous metaplasia found is limited (L, case 4). Original magnification: A x40; B, E right, F x100; C, E bottom left, I, J, K left, L x200; D, G, H, K right x400; E top left x600.
detected by FISH analysis in case 4 was not identified, possibly because neither of the breakpoints in *C11orf95* or *RELA* is within or near an exon.

By methylation analysis using the DKFZ methylation classifier, case 3 was classified as no matching methylation classes with a confidence threshold of the calibrated score.
≥0.9, and as methylation class ependymoma, RELA fusion with a low calibrated score (0.65) (Table 1). Case 5 was classified as no matching methylation classes with a calibrated score ≥0.3 (Table 1). t-distributed stochastic neighbor embedding analysis of DNA methylation data from cases 3 and 5 and a reference set of 380 CNS tumors demonstrated that cases 3 and 5 were clustered together and distinct from all subgroups of ependymomas (Figure 5K).

By array CGH, no apparent copy number changes other than small deletions and gains in regions of known benign copy number variants (polymorphisms) reported in the Database of Genomic Variants (DGV) (http://dgv.tcag.ca/dgv/app/home) were found in cases 2-5 (Figure S3A). Copy number analysis using the DKFZ methylation classifier also demonstrated stable chromosomal status with no apparent copy number changes in cases 3 and 5 (Figure S3B).

4 | DISCUSSION

In this report, we described five high-grade CNS tumors exhibiting distinct histopathological and molecular features, analyzed them as a group of tumors under the tentative label of ELTMD, and demonstrated that fusion genes involving C11orf95 are not restricted to histologically defined ependymomas.

The tumors collected for this study displayed a mixed histology, and one of the major components demonstrated embryonal-appearing histology (Figure 2B–D), being the most similar to anaplastic ependymoma considering the minimal ependymal differentiation observed in the components and the detected fusion genes, C11orf95-NCOA1/2 or -RELA. Histopathological features of ependymal differentiation in the embryonal-appearing components of ELTMDs include a dot-like pattern of cytoplasmic EMA positivity and small to large tubular structures with linear EMA positivity along the apical surface resembling ependymal rosettes and tubules, which are histological characteristics of ependymoma (Figures 2E and 3D). Meanwhile, ependymal differentiation is not restricted to ependymoma, but is generally accepted in several other primary CNS tumors, including angiocentric glioma, astroblastoma, chordoid glioma, and papillary tumor of the pineal region [reviewed in reference (20)], and the former 3 are known to be associated with specific genetic alterations: MYB-QKI fusion, MNI fusions, and PRKCA D463H mutation, respectively (21–25).

Despite the above-mentioned ependymal differentiation and genetic associations, we think that the embryonal-appearing components histologically did not fit with anaplastic ependymoma because of the following points. (1) Perivascular pseudorosettes with perivascular cytoplasmic processes exhibiting particularly strong GFAP staining can be found, almost by definition, in practically all (anaplastic) ependymomas.
(1,26,27); however, these formations were not present in the embryonal-appearing components throughout the tumor tissues in all cases. (2) In (anaplastic) ependymoma, staining for CAM5.2 is focal at best, and diffuse and strong staining for CAM5.2, which was observed in the embryonal-appearing components of all ELTMDs in the current study (Figure 4A), is not consistent with a diagnosis of (anaplastic) ependymoma (26,28). In addition, the embryonal-appearing components lacked clear cell morphology with branching vessels, histological features often observed in ST ependymomas with C11orf95-RELA (8). Microvascular proliferation or palisading
necrosis, findings indicative of malignancy in ependymoma (27), were not noted. From a genetic standpoint, ST ependymomas with C11orf95-RELA were reported to typically have abundant copy number changes; frequent changes were focal losses and gains on chromosome 1q (including chromothripsis), losses involving chromosomes 3, 9 (often resulting in homozygous deletion of CDKN2A), 10, and 22, and gain of chromosome 1q (3,29). However, although only one case of ELTMD with C11orf95 has not been examined in primary ependymosarcomas; however, C11orf95-RELA was detected both in primary anaplastic ependymoma and recurrent sarcoma in a patient; the latter developed after chemotherapy and radiation (32).

In this study, we identified C11orf95-NCOA1/2 in three ELTMDs. Fusion genes involving NCOA1/2 have been recurrently identified in several types of soft tissue tumors and acute leukemia (33,34). In the CNS, the case of ST anaplastic ependymoma presenting clear cell morphology with C11orf95-NCOA1 was reported (5). Quite recently, Keenan et al reported three cases of “infratentorial” ependymomas with C11orf95-NCOA2, -MAML2, or -RELA showing histological features closely resembling ST ependymomas with C11orf95-RELA (35). DNA methylation analysis demonstrated that all these infratentorial ependymomas clustered together with ST ependymomas with C11orf95-RELA (35). On the contrary, our study revealed that two ELTMDs with C11orf95-NCOA1/2 were epigenetically clearly distinct from ST ependymomas with C11orf95-RELA (Figure 5K). Taken together, histologically defined ependymomas with C11orf95 fusion including C11orf95-NCOA2 may be epigenetically different from ELTMDs with C11orf95-NCOA1/2. Further DNA methylation analysis of ELTMDs, especially those with C11orf95-RELA, is necessary to clarify the epigenetic relationships between ELTMDs with C11orf95-RELA and ependymomas with C11orf95-RELA, and between ELTMDs with C11orf95-RELA and ELTMDs with C11orf95-NCOA1/2.

By immunohistochemistry, although LICAM was reported to be typically expressed in a diffuse and strong manner in ST ependymomas with C11orf95-RELA (10,11,36–38), LICAM expression was almost exclusively found in the embryonal-appearing components in all cases of primary ELTMD and the recurrent tumor in case 2 regardless of the fusion partners of C11orf95 (Figure 3I). The function of LICAM may be required in the most proliferative components with ambiguous differentiation in ELTMDs, and its expression may be lost along with mesenchymal, glioneuronal, and ependymal (in the recurrent tumor of case 2) differentiation with lower proliferative activity. LICAM expression, though in few cases, was also

FIGURE 5 (A) C11orf95-NCOA1 fusion in case 5 was identified by target RNA sequencing, and confirmed by RT-PCR and Sanger sequencing. Reference sequence: C11orf95, NM_0011444936; NCOA1, NM_003743. (B–J) Representative fluorescence in situ hybridization results. (B–G) Rearrangement with fused (normal) and break-apart signals in each case. (B–D) 5’C11orf95, orange signal; C11orf95-3’, green signal. In case 1, one fused signal and one isolated orange signal are observed, and isolated green signals are lost (B). (E) 5’C11orf95, orange signal; RELA-3’, green signal. (F) 5’NCOA2, orange signal; NCOA2-3’, green signal. (G) 5’NCOA1, orange signal; NCOA1-3’, green signal. (H–J) Fusion signals in each case: 5’C11orf95-RELA-3’ (H), 5’C11orf95-NCOA2-3’ (I), and 5’C11orf95-NCOA1-3’ (J). (K) t-distributed stochastic neighbor embedding analysis of DNA methylation data from cases 3 and 5 and a reference set of 380 CNS tumors. Reference methylation classes: EPN_PFA, ependymoma, posterior fossa group A; EPN_PFB, ependymoma, posterior fossa group B; EPN_RELA, ependymoma, RELA fusion; EPN_SUBEPN_PF, subependymoma, posterior fossa; EPN_SUBEPN_ST, subependymoma, supratentorial; EPN_YAP, ependymoma, YAP fusion; BCOR, high-grade neuroepithelial tumor (HGNET) with BCOR alteration; EFT-CIC, Ewing sarcoma family tumor with CIC alteration; FOXR2, neuroblastoma with FOXR2 activation; MBL, midline pilocytic astrocytoma; RELA, (high-grade) astrocytoma, IDH-mutation; ANA_P, anaplastic pilocytic astrocytoma; K27M, diffuse midline glioma H3 K27M-mutant; G34R, glioblastoma, H3.3 G34-mutant; GBM_MES, glioblastoma, IDH-wildtype, subclass mesenchymal; GBM_MID, glioblastoma, IDH-wildtype, subclass midline; GB_MYC, glioblastoma, IDH-wildtype, subclass MYC; GB_MRTK1, glioblastoma, IDH-wildtype, subclass RTK-IIII; O_IDH, oligodendroglioma, IDH-mutant and 1p/19q-codeleted; LGG_DIG, desmoplastic infantile ganglioglioma; DLGNT, diffuse leptomeningeval glioneuronal tumor; LGG_DNT, dysembryoplastic neuroepithelial tumor; LGG_HGNET, infantile hemispheric glioma LGG_GG, ganglioglioma; LGG_MG, low-grade glioma with MYB/MYBL1 rearrangement; LGG_MID, midline pilocytic astrocytoma; LGG_PF, permanent fossa pilocytic astrocytoma; LGG_PAGGST, supratentorial/hemispheric pilocytic astrocytoma/ganglioglioma; LGG_RGNT, rosette-forming glioneuronal tumor; LGG_SEGA, subependymal giant cell astrocytoma; LGG_PX, pleomorphic xanthoastrocytoma.
reported in ST ependymomas with C11orf95-YAP1 and C11orf95-MAML2 (4,12). In ST ependymomas with YAP1-MAML2, another molecular subgroup of ST ependymoma, no positivity for LICAM was observed in any of the 11 cases tested (39). Together with our results, LICAM expression may be more related to C11orf95 than to RELA in ST ependymomas and ELTMDs with fusion genes involving C11orf95. On the contrary, nuclear accumulation of p65/RelA was detected only in cases with C11orf95-RELA (cases 1 and 4), but not in cases with C11orf95-NCOA1/2 (cases 2, 3, or 5) in this study (Figure 3J–L), consistent with the fusion protein C11orf95-RELA leading to NF-κB pathway activation (4).

Although primary ELTMDs cannot be regarded as anaplastic ependymoma or ependymosarcoma, the recurrent tumor in case 2 predominantly displayed a classic low-grade ependymoma histology, including perivascular pseudorosettes with an accentuated perivascular staining pattern of GFAP, and dot-like and ring-like patterns of cytoplasmic EMA positivity (Figure 4). Chemotherapy performed after the first surgery may be responsible for the morphological and phenotypical changes; however, this phenomenon may reflect the intrinsic ependymal nature of ELTMD.

In conclusion, although ELTMDs demonstrated minimal ependymal differentiation and genetic association with ST ependymoma with C11orf95-RELA, they cannot be regarded as (anaplastic) ependymoma or ependymosarcoma by the current WHO classification. Given the small number of cases examined in the current study, further clinicopathological and genetic analyses of more cases are needed to clarify their differences and similarities, and the possibility of them being included in the spectrum of ependymoma by the more molecularly oriented definition of ependymoma in the future cannot be excluded.

ACKNOWLEDGMENTS

We thank Ms. Machiko Yokota and Mr. Tatsuya Yamazaki (Gunma University) for their excellent technical assistance. We thank the Laboratory for Analytical Instruments, Education and Research Support Center, Gunma University Graduate School of Medicine. This study was supported by the Fostering Health Professionals for Changing Needs of Cancer by MEXT of Japan and Gunma University Initiative for Advanced Research (GIAR).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Sumihito Nobusawa designed the study; Ran Tomomasa, Takanori Hirose, Atsushi Sasaki, Junko Hirato, and Sumihito Nobusawa performed the pathological analysis; Ran Tomomasa, Yasuhiro Arai, Reika Kawabata-Iwakawa, Kohei Fukuoka, Yoshihiko Nakano, Natsuko Hama, Masahiko Nishiyama, Koichi Ichimura, Tatsuhiro Shibata, and Sumihito Nobusawa performed the laboratory research; cases and clinical data were provided by Nozomi Suzuki, Yukitomo Ishi, Shinya Tanaka, Jun A. Takahashi, Yoshiaki Yuba, Mitsutaka Shiota, Atsushi Natsume, Michihiro Kurimoto, Yoshihi Shiba, Mikiko Aoki, Kazuki Nabeshima, Toshiyuki Enotomo, Tooru Inoue, Junya Fujimura, Akiiide Kondo, and Takashi Yao; Ran Tomomasa, Satoshi Nakata, Naoki Okura, and Sumihito Nobusawa analyzed and interpreted the data; Ran Tomomasa, Satoshi Nakata, and Sumihito Nobusawa wrote the manuscript; Junko Hirato and Hideaki Yokoo participated in construction of the manuscript and revised it critically; and all authors accepted the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Derived data supporting the findings of this study are available from the corresponding author on request.

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REFERENCES

1. Ellison DW, McLendon R, Wiestler OD, Kros JM, Korshunov A, Ng HK, et al. Ependymoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. World Health Organization classification of tumours of the central nervous system. Revised, 4th ed. Lyon: IARC Press; 2016:106–11.
2. Mack SC, Witt H, Piro RM, Gu L, Zuyderduyn S, Stütz AM, et al. Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. Nature. 2014;506:445–50.
3. Pajtler KW, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, et al. Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. Cancer Cell. 2015;27:278–83.
4. Parker M, Mohankumar KM, Punchihewa C, Weinrich L, Dalton JD, Li Y, et al. C11orf95-RELA fusions drive oncogenic NF-κB signalling in ependymoma. Nature. 2014;506:451–5.
5. Pietsch T, Wohlers I, Kaszczik T, Dreschmann V, Denkhaus D, Dörner E, et al. Supratentorial ependymomas of childhood carry C11orf95-RELA fusions leading to pathological activation of the NF-κB signaling pathway. Acta Neuropathol. 2014;127:609–11.
6. Wani K, Armstrong TS, Vera-Bolanos E, Raghunathan A, Ellison D, Gilbertson R, et al. A prognostic gene expression signature in infratentorial ependymoma. Acta Neuropathol. 2012;123:727–38.
7. Witt H, Mack SC, Ryzhova M, Bender S, Sill M, Isserlin R, et al. Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. Cancer Cell. 2011;20:143–57.
8. Ellison DW, Korshunov A, Witt H. Ependymoma, RELA fusion-positive. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. World Health Organization classification of tumours of
the central nervous system. Revisited, 4th ed. Lyon: IARC Press; 2016:112.

9. Lindner J, Rathjen FG, Schachner M. L1 mono- and polyclonal antibodies modify cell migration in early postnatal mouse cerebellum. Nature. 1983;305:427–30.

10. Gessi M, Giagnacovo M, Modena P, Elefante G, Gianno F, Buttarelli FR, et al. Role of immunohistochemistry in the identification of supratentorial C11ORF95-RELA fused ependymoma in routine neuropathology. Am J Surg Pathol. 2019;43:56–63.

11. Wang L, Liu L, Li H, Wang P, Hu Z, Wei Y, et al. RELA fusion in supratentorial extraventricular ependymomas: a morphologic, immunohistochemical, and molecular study of 43 cases. Am J Surg Pathol. 2019;43:1674–81.

12. Tamai S, Nakano Y, Kinoshita M, Sabit H, Nobusawa S, Arai Y, et al. Ependymoma with C11orf95-MAML2 fusion: presenting with granular cell and ganglion cell features. Brain Tumor Pathol. 2021;38:64–70.

13. Flucke U, Töps BB, de Saint Aubain Somerhausen N, Bras J, Creyten DH, Küsters B, et al. Presence of C11orf95-MLK2 fusion is a consistent finding in chordoid lipomas: a study of eight cases. Histopathology. 2013;62:925–30.

14. Huang D, Sumegi J, Dal Cin P, Reith JD, Yasuda T, Nelson M, et al. C11orf95-MK2L2 is the resulting fusion oncogene of t(11;16) (q13;p13) in chordoid lipoma. Genes Chromosom Cancer. 2010;49:810–8.

15. Nakazato Y, Ishizeki J, Takahashi K, Yamaguchi H, Kamei T, Mori T. Localization of S-100 protein and glial fibrillary acidic protein-related antigen in pleomorphic adenoma of the salivary glands. Lab Invest. 1982;46:621–6.

16. Yokoo H, Nobusawa S, Takebayashi H, Ikenaka K, Isoda K, Kamiya M, et al. Anti-human Olig2 antibody as a useful immunohistochemical marker of normal oligodendrocytes and glia-mas. Am J Pathol. 2004;164:1717–25.

17. Nobusawa S, Yokoo H, Hirato J, Kakita A, Takahashi H, Sugino K, et al. Analysis of chromosome 19q13.42 amplification in embryonal brain tumors with ependymoblastic multilayered rosettes. Brain Pathol. 2012;22:689–97.

18. Nobusawa S, Hirato J, Sugai T, Okura N, Yamazaki T, Yamada S, et al. Atypical teratoid/rhabdoid tumor (AT/RT) arising from ependymoma: a type of AT/RT secondarily developing from other primary central nervous system tumors. J Neuropathol Exp Neurol. 2016;75:167–74.

19. Sumegi J, Streblow R, Frayer RW, Dal Cin P, Rosenberg A, Meloni-Ehrig A, et al. Recurrent t(2;2) and t(2;8) translocations in chordoid glioma of the third ventricle. Nat Commun. 2018;9:810.

20. Lehman NL. Central nervous system tumors with ependymal features: a broadened spectrum of primarily ependymal differentiation? J Neuropathol Exp Neurol. 2008;67:177–88.

21. Bandopadhayay P, Ramkissoon LA, Jain P, Bergthold G, Wala J, Zied R, et al. MYB-QKI rearrangements in angiocentric glioma target RNA sequencing in cases 2 and 3. Sequence reads spanning the breakpoints are illustrated. The breakpoint.

22. Burger PC, Scheithauer BW. Ependymoma. In: Silverberg SG, Sobin LH, eds. AFIP atlas of tumor pathology fourth series fascicle 7, tumors of the central nervous system. Washington, DC: Armed Forces Institute of Pathology; 2007:145–65.

23. Ellisson DW, McLendon R, Wiestler OD, Kros JM, Korshunov A, Ng HK, et al. Anaplastic ependymoma. In: Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, eds. World Health Organization classification of tumours of the central nervous system. Revised, 4th ed. Lyon: IARC Press; 2016:113–4.

24. Vege KD, Giannini C, Scheithauer BW. The immunophenotype of ependymomas. Appl Immunohistochem Mol Morphol. 2000;8:25–31.

25. Bemore M, Andreoulo F, Varlet P, Tauriozzi-Espariat A, Jünger S, Dörrer E, Dreschmann V, et al. Childhood supratentorial C11orf95-fused gliomas share histologic, immunohistopathogenic, and molecular characteristics of supratentorial RELA fused ependymoma. Acta Neuropathol. 2020;140:963–5.

26. Figarella-Branger D, Lechapt-Zalcman E, Tabouret E, Jünger S, de Paula AM, Bouvier C, et al. Supratentorial clear cell ependymomas with branching capillaries demonstrate characteristic clinicopathological features and pathological activation of nuclear factor-kappaB signaling. Neuro Oncol. 2016;18:919–27.

27. Maligulwbar PB, Nambirajan A, Pathak P, Faruq M, Rajeshwari M, Singh M, et al. C11orf95-RELA fusions and upregulated NF-KB signalling characterise a subset of aggressive supratentorial ependymomas that express LiCAM and nestin. J Neurooncol. 2018;138:29–39.

28. Suzuki A, Hirato J, Hirose T, Fukuoka K, Kanemura Y, Hashimoto N, et al. Review of ependymomas: assessment of consensus in pathological diagnosis and correlations with genetic profiles and outcome. Brain Tumor Pathol. 2019;36:92–101.

29. Ahmad S, Whisenhunt H, Leach J, Jünger S, Dörrer E, Dreschmann V, et al. Childhood supratentorial C11orf95-fused gliomas with YAP1-MAMLD1 fusion: an entity with characteristic clinical, radiological, cytogenetic and histopathological features. Brain Tumor Pathol. 2019;29:205–16.

SUPPORTING INFORMATION
Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 C11orf95-NCOA1/2 OR-RELA
junctons contain 2- and 11-bp insertions, respectively. Reference sequence: C11orf95, NM_001144936; NCOAI, NM_003743; NCOA2, NM_006540

**FIGURE S2** Identification of C11orf95-NCOAI2 fusion events by whole exome sequencing. Fusions between exon 5 of C11orf95 and introns 14 and 13 of NCOAI (cases 2 and 5, respectively), and intron 13 of NCOA2 (case 3) are observed. Reads are sorted and colored based on the location of their mate reads: orange (cases 2 and 5) and purple (case 3), mate reads in chromosome 11 (C11orf95); brown, mate reads in chromosome 2 (NCOAI, cases 2 and 5) and in chromosome 8 (NCOA2, case 3)

**FIGURE S3** (A) In case 4 with C11orf95-RELA, array comparative genomic hybridization shows no apparent copy number changes in chromosomes 1, 3, 9, 10, 11, or 22, where supratentorial ependymomas with C11orf95-RELA were reported to have abundant copy number changes. (B) Copy number analysis using the DKFZ methylation classifier demonstrated stable chromosomal status with no apparent copy number changes in cases 3 and 5

**Supplementary Material**

**TABLE S1** Fluorescence in situ hybridization probes

**TABLE S2** Variants observed in more than two cases by whole exome sequencing

**How to cite this article:** Tomomasa R, Arai Y, Kawabata-Iwakawa R, et al. Ependymoma-like tumor with mesenchymal differentiation harboring C11orf95-NCOAI2 or -RELA fusion: A hitherto unclassified tumor related to ependymoma. *Brain Pathology*. 2021;31:e12943. [https://doi.org/10.1111/bpa.12943](https://doi.org/10.1111/bpa.12943)