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CASE REPORT

C11orf95–RELA fusion present in a primary supratentorial ependymoma and recurrent sarcoma

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Abstract Ependymomas are rare glial tumors of the central nervous system that arise from the cells lining the ventricles and central canal within the spinal cord. The distribution of these tumors along the neuroaxis varies by age, most commonly involving the spinal cord in adults and the posterior fossa in children. It is becoming evident that ependymomas of infratentorial, supratentorial, and spinal cord location are genetically distinct which may explain the differences in clinical outcomes. A novel oncogenic fusion involving the *C11orf95* and *RELA* genes was recently described in supratentorial ependymomas that results in constitutive aberrant activation of the nuclear factor-kB signaling pathway. Ependymosarcomas are rare neoplasms in which a malignant mesenchymal component arises within an ependymoma. We here describe a case of a sarcoma developing in a patient previously treated with chemotherapy and radiation whose original ependymoma and recurrent sarcoma were both shown to carry the type 1 *C11orf95–RELA* fusion transcript indicating a monoclonal origin for both tumors.

Keywords Ependymoma · *C11orf95* · *RELA* · Fusion transcript · L1CAM

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Introduction

Ependymomas are glial tumors that show morphologic features resembling those of ependymal cells lining the ventricles of the central nervous system. They may develop supra- and infratentorially, as well as in the spinal cord [1]. It is now evident that ependymomas of infratentorial, supratentorial, and spinal cord location are genetically distinct and may in fact represent distinct tumor entities [2, 3]. Recently, a novel oncogenic fusion involving the *C11orf95* and *RELA* genes was described in supratentorial ependymomas that results in constitutive aberrant activation of the nuclear factor-kB (NF-κB) signaling pathway [4]. Several different *C11orf95–RELA* fusion transcripts have been identified in supratentorial ependymomas, with the type 1 fusion involving *C11orf95* exon 2 and exon 2 of *RELA,* and the type 2 fusion with *C11orf95* exon 3 and *RELA* exon 2 being the most commonly identified aberrant transcripts.

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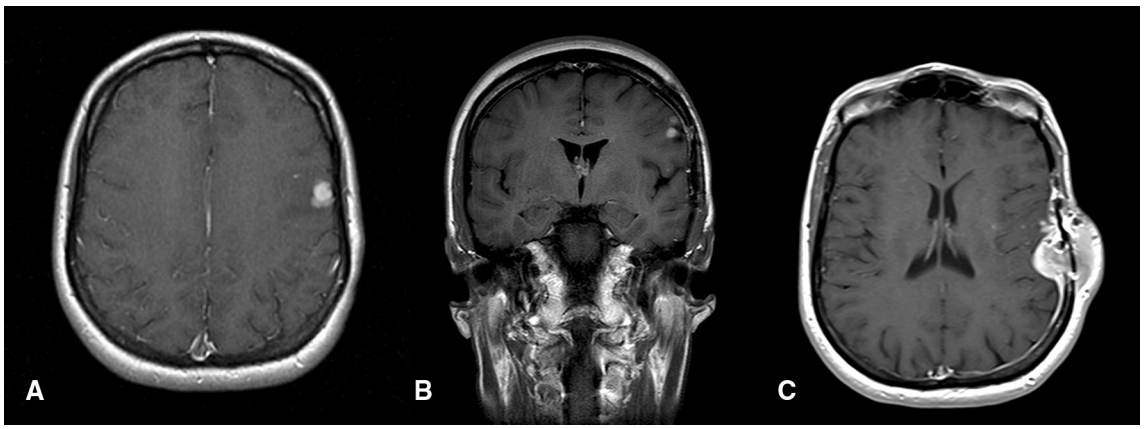

Fig. 1 MRI T1-weighted post-contrast sequences show a gadolinium-enhancing left frontal lobe mass at the time of presentation (**a** axial section, **b** coronal section) with pathology consistent with an anaplastic ependymoma. At the time of first recurrence, two new enhancing lesions (**b**) in the left frontal lobe within the surgical bed were seen. Imaging findings at the time of sarcomatous transformation show recurrence of an enhancing mass extending through the craniotomy site into the surrounding soft tissues (**c**)

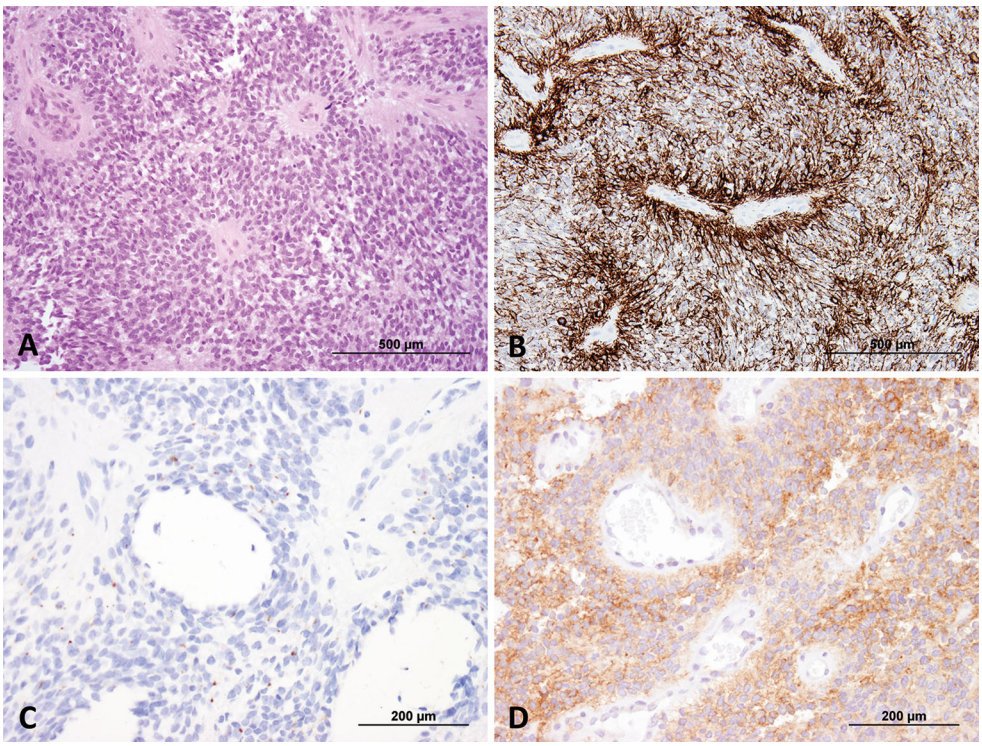

Fig. 2 Primary anaplastic ependymoma with hypercellularity, perivascular pseudorosettes, microvascular proliferation, and mitotic activity (**a** hematoxylin and eosin, ×100). The tumor cells diffusely immunoexpressed GFAP in an accentuated perivascular staining pattern (**b** GFAP, ×100), EMA in a "dot-like" perinuclear pattern (**c** EMA, ×200), and L1CAM in a regionally diffuse cytoplasmic and focal membranous pattern (**d** L1CAM, ×200)

We present a case of sarcoma developing after radiation in a patient with a prior diagnosis of supratentorial ependymoma. Both the initial supratentorial ependymoma and subsequent sarcoma had the type 1 *C11orf95–RELA* fusion. This unique finding strongly suggests that the sarcoma likely developed from the malignant transformation of the primary ependymoma.

Clinical summary

A 35-year-old woman presented with recurrent episodes of right jaw tingling and dysarthria. MRI of the brain revealed a left frontal enhancing mass (Fig. 1a). Gross total resection revealed a WHO grade III anaplastic ependymoma with characteristic hypercellularity, perivascular pseudorosettes,

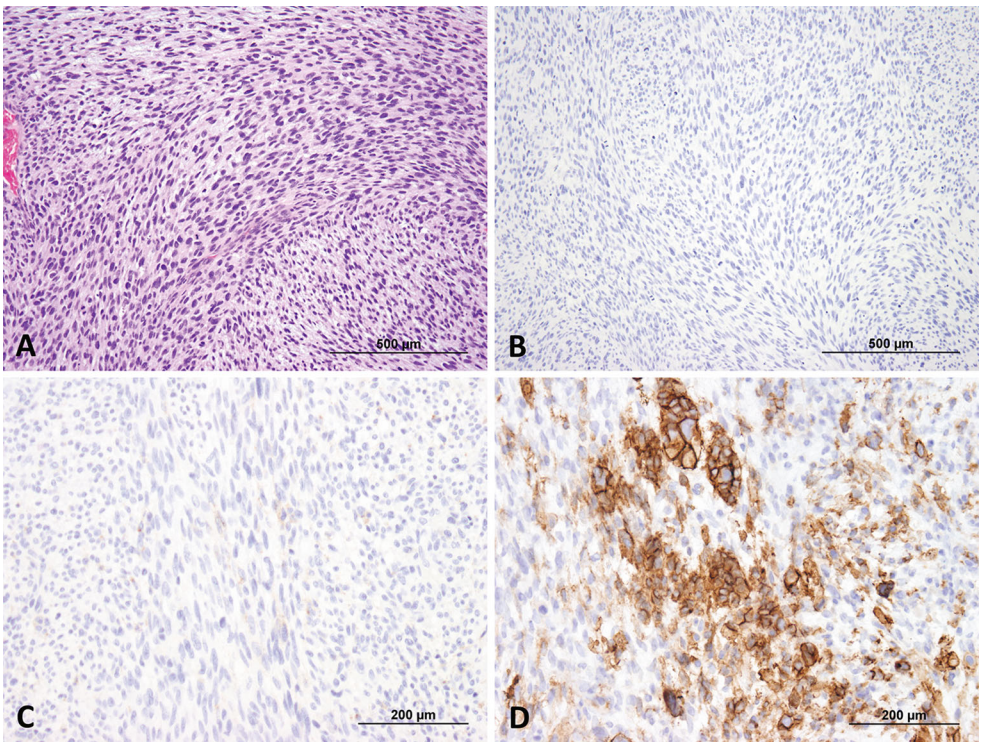

Fig. 3 Sarcoma recurrence with interlacing hypercellular fascicles with brisk mitotic activity (**a** hematoxylin and eosin, ×100). The tumor cells lost GFAP (**b** GFAP, ×100) and EMA (**c** EMA, ×200) immunoexpression but kept strong regional cytoplasmic and membranous L1CAM immunoreactivity (**d**– L1CAM, ×200) suggestive of the *C11orf95–RELA* fusion transcript

microvascular proliferation, mitotic activity, diffuse positive immunohistochemical staining for glial fibrillary acidic protein (GFAP), focal S-100 immunoreactivity, and regional "dot-like" perinuclear epithelial membrane antigen (EMA) immunoreactivity (Fig. 2). Subsequent staging of the tumor did not show evidence of tumor spread along the spinal axis. The patient received 54 Gy in 30 fractions of involved field intensity-modulated radiation therapy (IMRT) postoperatively. Three years after diagnosis, MRI demonstrated two new enhancing lesions adjacent to the original tumor location (Fig. 1b). The two tumors were resected demonstrating recurrent anaplastic ependymoma, similar in appearance to the original tumor but with a higher proliferative index (MIB-1 of 27 % compared with 18.4 % originally) and greater degree of tumor infiltration into the surrounding brain. Hypofractionated stereotactic radiosurgery to the tumor bed (25 Gy in 5 fractions) was then undertaken.

One year later, imaging revealed a new area of nodular enhancement. The patient was treated with a combination of lapatinib and dose-dense temozolomide, remaining clinically and radiographically stable on this combination, completing 12 cycles. Two years after completing treatment, a new enhancing mass was found near the original tumor but extending through a skull defect into the temporalis muscle (Fig. 1c). The mass was resected, with pathology showing a highly mitotic, densely cellular spindle cell neoplasm negative for GFAP, S-100 protein, EMA, and pancytokeratin on immunohistochemistry, with a MIB-1 proliferative index of 72 %, consistent with sarcoma (Fig. 3). The patient was treated with high-dose methotrexate followed by liposomal doxorubicin, with recurrence requiring surgical resection after 7 months with pathology once again confirming the tumor to be sarcomatous in nature. The patient passed away after developing a large cerebral hemorrhage 9 months following the sarcomatous transformation.

Pathological findings

Methods

C11orf95–RELA fusion transcript detection

RNA was extracted from representative areas of formalin-fixed paraffin-embedded tissue obtained from the anaplastic ependymoma at the time of initial resection and the sarcoma tissue at second recurrence. This was done using the Master Pure Complete DNA and RNA Purification Kit (Illumina Inc., San Diego, CA, USA). The RNA quality

Table 1 RT-PCR primers used for the detection of the *C11orf95–RELA* fusion

Primer	Sequence 5′ → 3′
TYPE 1 forward	GGGGGCTGAGGAGGAGGAG
TYPE 1 reverse	TGTGGAGATCATTGAGCAGC
TYPE 2 forward	CCTGCACCTGGACGACAT

was evaluated using a real-time PCR assay for *RPS27*, a housekeeping control gene on an Applied Biosystems 7500 Real-Time PCR (Life Technologies Inc., Grand Island, NY, USA). cDNA was synthesized using the High-capacity cDNA synthesis kit (Life Technologies Inc., Grand Island, NY, USA). All procedures were done according to the manufacturers' recommendations.

RT-PCR was used to investigate the presence of the *C11orf95–RELA* type 1 and type 2 fusion transcripts. The primers used are described in Table 1. The PCR cycles were as follows: initial denaturation at 95 °C for 5 min followed by 40 cycles of 40 s at 95 °C and 40 s at 60 °C, 1 min at 72 °C and a final extension was performed for 7 min at 72 °C. The PCR mix contained 4 µl of cDNA, 0.2 µM forward primer, 0.2 µM reverse primer and 12.5 µl Amplitaq gold PCR master mix (Life Technologies Inc., Grand Island, NY, USA). The PCR product was purified using the Qiagen MinElute PCR purification Kit (Qiagen, CA, USA). Direct sequencing of the purified PCR product was performed using an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). Obtained sequence data were analyzed using BLASTN software (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Immunohistochemistry

Immunohistochemical staining for L1CAM, a downstream marker for the *C11orf95–RELA* fusion [4], was carried out using a mouse monoclonal antibody (clone UJ127.11; Sigma Aldrich, St. Louis, MO, USA; 1:1500). Antigen retrieval was done using heat-induced antigen retrieval in 10 mM sodium citrate buffer, pH 6.0. Staining was detected using the Envision kit (Dako, CA, USA). Normal kidney tissue was used as a positive control.

Results

Immunohistochemical staining for L1CAM was strongly positive in a regional cytoplasmic and focal membranous pattern in both the supratentorial ependymoma and the sarcoma recurrence, indicating the presence of the *C11orf95–RELA* fusion (Figs. 2d, 3d). We then performed RT-PCR for the *C11orf95–RELA* type 1 and 2 fusion transcripts followed by Sanger sequencing. Both the supratentorial ependymoma and the subsequent sarcoma contained the type 1 fusion transcript indicative of a common genetic origin for both tumors (Fig. 4a–b).

Discussion

Classified as glial tumors, ependymomas make up 3.3 % of malignant primary brain and CNS tumors [5]. Their incidence is higher in children and young adults, in whom it is the second most common malignant brain tumor. Due to their glial nature, ependymomas usually display GFAP immunoreactivity in pseudorosette patterns. Other immunostains that are characteristically positive include S-100 protein, vimentin and EMA. Recent molecular analyses strongly suggest that although ependymomas from the supratentorial compartment, infratentorial region, and spinal cord have similar histologic features, biologically they are distinct, possibly explaining the regional differences in prognosis [6]. Molecular and genetic data demonstrate distinct expression profiles and DNA copy number alterations. [7–9] Importantly, Parker et al. recently described a novel oncogenic fusion on chromosome 11 involving a previously uncharacterized gene, *C11orf95* and RELA, a transcriptional factor in the NF-kB pathway. This fusion transcript was estimated to occur in two-thirds of supratentorial ependymomas, but is absent in posterior fossa tumors [4, 10].

The term ependymosarcoma was first coined by Rodriguez et al. [11] who described 11 cases of ependymal tumors with sarcomatous features. To date, 19 cases of ependymal tumors classified as WHO Grade II or III with sarcomatous changes have been published in the literature, with 10 of these cases occurring at recurrence as in our case [12]. The histogenesis of ependymosarcomas, especially the sarcomatous component, is controversial. This controversy extends also to gliosarcomas where initially the mesenchymal component was thought to have arisen from malignant transformation of the proliferative vasculature of astrocytomas [13]. However, genetic and mutational analyses have shown that identical genetic mutations occur in both the glial and sarcomatous tissues. This indicates that, though histologically distinct, the two components have a common genetic origin, favoring a monoclonal origin for both components. [14–17] Chromosomal imbalances in gliosarcomas include gains on chromosome X, 7, 9q, 12q and 20q and losses on chromosomes 9p, 10, 13q and 17. These chromosomal abnormalities are seen both in the glial as well as in the sarcomatous components, which are also genetically identical in terms of *TP53* mutations, *PTEN* mutations, *p16INK4a* deletion, *CDK4* amplification and

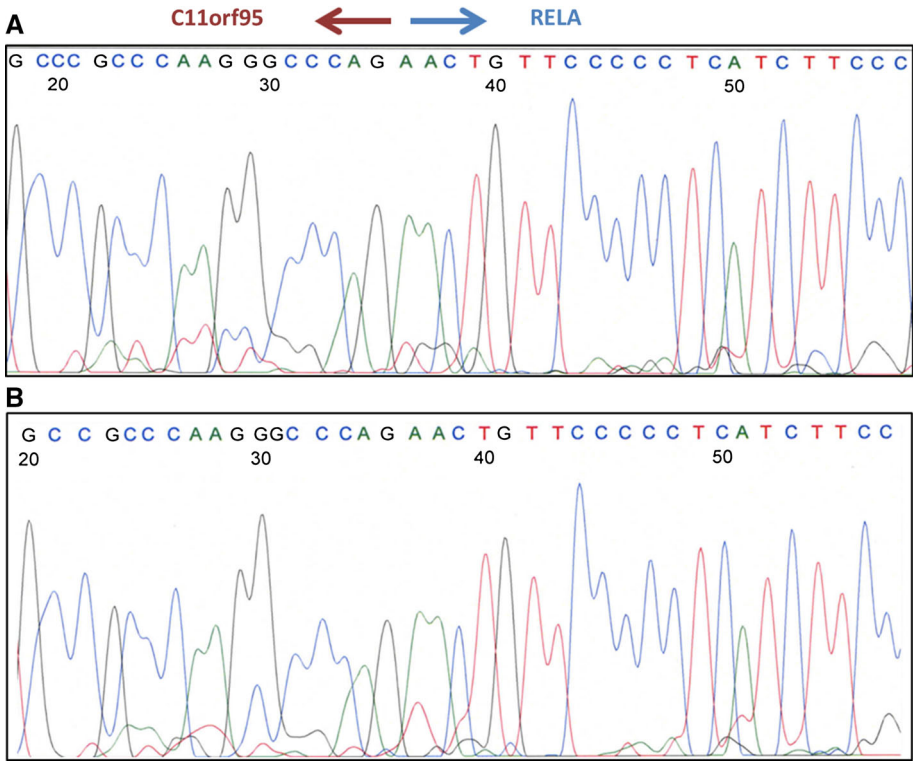

Fig. 4 Sanger sequencing of the supratentorial ependymoma (**a**) and the subsequent sarcoma (**b**) confirms the presence of the *C11orf95–RELA* fusion transcript

MDM2 amplification [14–16, 18, 19]. Similarly, in the reported ependymosarcomas, chromosome imbalances, such as gains on chromosome 1q, deletions of 22q and 6p, monosomy 18 and polysomies/polyploidy, were found in both the mesenchymal and glial tissues [11]. Extensive genomic instability of glial tumors and mechanisms involved in epithelial to mesenchymal transition as seen in epithelial neoplasms have been proposed as possible mechanisms [20–22]. These processes might also occur in ependymosarcomas, though an as yet unidentified driver mutation might be responsible for this histological transformation.

Since our patient received both IMRT and stereotactic radiosurgery to the same area where the recurrent sarcomatous transformation occurred, the sarcoma was initially thought to represent a post-radiation sarcoma. Though one cannot definitely rule out the role of radiation in the sarcomatous transformation, the presence of the *C11orf95–RELA* fusion in the sarcoma clearly indicates that it arose from the previous ependymoma rather than the surrounding normal tissue as is the case with most post-radiation sarcomas.

This case demonstrates an important phenotype–genotype dissociation. While by morphology and immunohistochemistry the recurrent tumor was clearly a sarcoma, the presence of the characteristic fusion in both the primary ependymoma and subsequent sarcoma indicates a common origin for both tumors, supporting a monoclonal theory for ependymosarcomas. As discussed above, the *C11orf95–RELA* fusion transcript is thought to be the driver genetic alteration leading to activation of the NF-kB pathway in supratentorial ependymomas, with evidence that the activation of the NF-kB pathway is also often activated in sarcomas. [23, 24] Sarcomatous change in ependymoma is rare, but this finding may provide additional opportunities to explore the molecular mechanisms underlying the malignant transformation. In light of the increasing recognition of the link of the NF-kB pathway to the pathogenesis in many cancers, this finding may lead to greater understanding of the underlying molecular mechanisms and a potential therapeutic target [25, 26].

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Conflict of interest D Cachia, K Wani, M Penas Prado, A Olar, IE McCutcheon, RS Benjamin, KD Aldape report no disclosures. T.S. Armstrong serves as consultant for Immunocellular therapeutics; is on the advisory board for Roche; receives research support from Merck to Genentech. M.R. Gilbert reports research support from Genentech, Merck, Glaxo Smith Kline; receives honoraria from Merck, Genentech, AbbVie; and serves on the advisory board for Genetech, AbbVie, Heron Therapeutics.

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