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Identification of a novel translocation producing an in-frame fusion of *TAF15* and *ETV4* in a neonatal form of extrasosseous Ewing sarcoma

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Abstract

Ewing sarcoma (ES) is a highly malignant round cell sarcoma, characterized by gene fusion involving FET (*FUS*, *EWSR1*, *TAF15*) and ETS family genes, respectively. The involvement of the *EWSR1* gene has been reported in approximately 90% of cases of ES, with the *EWSR1::FLI1* fusion being the most frequent. We report the case of a newborn with a localized soft tissue paravertebral neoplasm discovered during the prenatal period. Histopathology and immunophenotype were consistent with a CD99+ undifferentiated round cell sarcoma (URSC), whole-exome RNA-sequencing demonstrated an undescribed in-frame *TAF15::ETV4* fusion transcript, while consensus clustering analysis showed high transcriptomic proximity to the ES group. Given clinical context, high tumor chemosensitivity to ES conventional drugs, morphological characteristics, nature of the fusion partners involved and high transcriptomic proximity to *bona fide* ESs, we believe that this case represents a new genetic variant of ES.

Introduction

Ewing sarcoma (ES) is the second most common bone tumor in childhood and adolescence and can also occur in soft tissue with a wide anatomical distribution. ES is an aggressive tumor with a survival rate approaching 80% for localized standard-risk patients, and only 30% for metastatic cases[1, 2]. This sarcoma is characterized by a driver chromosomal translocation that fuses the amino-terminal domain of a member of the FET family protein (encoded by *FUS*, *EWSR1*, or *TAF15*) with the carboxy-terminal domain of a member of the ETS (E26 Transformation-Specific) transcription factor family, with the *EWSR1::FLI1* fusion oncoprotein being the first described and the most common fusion (85% of cases). Alternative fusions were described afterwards, involving *EWSR1* with *ERG* (10% of cases), *FEV*, *ETV1*, and *ETV4* (<1%) or *FUS* with *ERG*, *FEV* and *ETV4* (<1%)[3–7].

Given their similar structure to the *EWSR1::FLI1* fusion protein, each of these chimeric proteins is likely to act as aberrant transcription factors with similar biological functions. The *TAF15::ETS* gene fusion in ES have not been described to date.

In this report, we present the case of a newborn presenting with a localized paraspinal soft tissue tumor discovered on prenatal ultrasound, with the morphological and

immunohistochemical characteristics of an undifferentiated round cell sarcoma (URCS) and diffuse expression of CD99. Whole-exome RNA-sequencing demonstrated an in-frame *TAF15::ETV4* fusion transcript, which had not yet been reported. As the clinical response to conventional chemotherapy, morphology, phenotype, nature of the fusion partners, and transcriptomic profile are highly suggestive of ES, this novel fusion transcript expands the molecular alterations known to be associated with ES.

Materials and methods

Immunohistochemistry

Immunohistochemistry was performed on 4 µm sections cut from the paraffin block using an autostainer (Benchmark ULTRA; Ventana Medical System Inc., Tucson, AZ) using the following antibodies: CD99 (clone 12E7, prediluted, Dako), epithelial membrane antigen (EMA) (clone E29, 1:50; Dako), Ki67 (clone MIB-1, 1: 100; Dako), desmin (clone DE-R-11, prediluted; Roche), myogenin (clone F5D, 1: 2; Dako), smooth muscle actin alpha (clone 1A4, prediluted; Microm), PS100 (polyclonal, 1: 400; DBS), PHOX2B (clone, NC, 1:200, Finetest), BCOR (clone C-10, 1:50, Cliniscience), BRG1 (clone EPNCIR111A, 1:100, Abcam), ETV4 (clone 16, 1:25, Santa-Cruz), NUT (clone C52B1, 1:100, Ozyme), ERG (clone EPR3864, prediluted, Roche).

Whole-exome RNA sequencing

Whole-exome RNA- sequencing (WERS), expression profiles analyses including several clustering methods, detection of fusion genes, and small nucleotide variations has been performed at the Centre Leon Bérard (CLB) in Lyon using exome-based RNA capture sequencing on the archival material, as previously described[8].

Case report

A newborn was referred shortly after birth for a right intramuscular paravertebral dorsal mass discovered on prenatal ultrasound at 28 weeks of gestation. She was the second child of unrelated parents of Tunisian origin, without a significant familial history of cancer. Ultrasound and magnetic resonance imaging (MRI) performed after birth showed an

extraskelatal heterogeneous compartmentalized 37 mm-large lesion with liquid levels and microcalcifications, consistent with a vascular malformative process (**Fig. 1A**).

At one-month of life, a percutaneous intralesional sclerosis was performed. As there was no functional consequence and that the mass size was slowly decreasing at this time, the girl was seen at the age of 6 months and a follow-up consultation was then scheduled at the age of 12 months. She was referred at the age of 9 months for a worrying clinical evolution. The intramuscular mass was indeed 70 mm high (vs 37 mm) and firm, with a hypoechogenic tissue aspect in MRI (**Fig. 1B/1C**).

Histopathological analysis of the biopsy showed a high-grade URCS, with high cellularity and necrosis (**Fig. 2A/2B**). The immunohistochemical phenotype revealed a strong and diffuse membranous expression of CD99, suggestive of ES (**Fig. 2C**), while CD45, Desmin, Myogenin, keratins, PHOX2B, ERG, BCOR, NUT, and ETV4 were negative, with physiological nuclear expression of INI1 and BRG1. The rearrangement of the *EWSR1* locus was lacking by FISH analysis, while WERS demonstrated an in-frame *TAF15::ETV4* fusion transcript, with expression profile aggregating with Ewing sarcomas (**Fig. 3A/3B**).

Given the clinical setting and the fusion partners involved, the diagnosis of ES was favored. The tumor was localized and she received neoadjuvant chemotherapy according to the European strategy for the treatment of ES that included vincristine, doxorubicin, cyclophosphamide, alternating with ifosfamide, etoposide. There was an excellent radiological response to chemotherapy with a 80% reduction of tumor volume (**Fig 1D**).

A marginal oncologic surgical resection was then decided that found a regressive lesion with only few residual tumor cells (**Fig 2D**). Radiotherapy was avoided because of the patient's age and the excellent response to neoadjuvant chemotherapy. She is currently receiving adjuvant chemotherapy with good tolerance and no signs of tumor recurrence.

Discussion

We report a novel in-frame fusion involving *TAF15* and *ETV4* in an URCS for which clustering analysis was strongly consistent with Ewing sarcoma (ES). This case extends the known *FET/ETS* fusions involved in ES.

ES is an aggressive sarcoma that affects bone and soft tissue in children and young adults, with a peak incidence at the age of 15 years[1, 2]. It rarely occurs during the neonatal period. The Ewing family tumors were first described after the description of a pathognomonic translocation t(11;22)(q12;q24) resulting in the fusion of *EWSR1*, member of the *FET* family gene, and *FLI1*, present in approximately 85% cases of ES. In the remaining 15-20% of cases, variant fusions involve other members of the ETS transcription factor family, mostly *ERG*, but also *ETV1*, *ETV4* or *FEV*[3–7]. Similarly, rare fusions were described that involve *EWSR1* paralogues (mainly *FUS*) and members of the ETS family genes. ES was finally defined as a round cell sarcoma with CD99 expression and a driver fusion involving members of the FET and ETS family. Although molecular studies have focused mainly on *EWSR1::FLI1*, the oncogenic function of alternative FET/ETS fusions involved in ES likely has comparable biological properties, as demonstrated for the recently described *FUS::ETV4* fusion[9].

TAF15 belongs to the FET family of genes that also comprise *EWSR1* and *FUS*. These genes are mostly known to be components of fusion oncogenes after rearrangements with various transcription factors, predominantly in mesenchymal neoplasms, but also in rare hematopoietic and epithelial cancers[10]. FET genes encode highly conserved RNA-binding proteins, whose functional roles in transcription and splicing are partially known. In oncogenic aberrations of the FET genes, the RNA-binding domain is generally replaced by the DNA-binding domain of transcription factors such as members of the ETS family (ie. *FLI1*, *ERG*, *ETV1*, *ETV4*, *FEV*), the latter determining the tumor phenotype[11].

These fusion oncoproteins are believed to act as an aberrant transcription factor that deregulate hundreds of genes involved, for examples, in cell cycle regulation or cell proliferation. While *EWSR1* and *FUS* have been found to be involved in various tumors, including Ewing and various mesenchymal tumors including sarcomas, *TAF15* has only been reported fused with *NR4A3* in extraskeletal myxoid chondrosarcoma and in different forms of acute leukemia to date [12, 13]. *TAF15::ETS* gene fusion in ES has not been described so far, but its high structural similarity to other FET/ETS fusions makes its occurrence likely possible [2].

The pathological characteristics and immunohistochemistry in conventional ES lacks specificity, belonging to the spectrum of URCS but with intense membranous expression of CD99 [14]. The *TAF15::ETV4* fusion sarcoma described here was consistent with this pattern,

composed of round cells with monotonous nuclei and scant eosinophilic cytoplasm, separated by fibrous septa. Furthermore, high-grade features were present such as high mitotic activity, apoptosis, and areas of confluent necrosis. CD99 was strongly positive in a membranous pattern which suggests ES, albeit not entirely specific.

Although *ETV4* was involved in the fusion, *ETV4* immunohistochemistry was negative, suggesting an absence of upregulation in contrast to *CIC*-fused sarcoma[15]. In cases of URCS, the pathological work-up should include molecular analysis to confirm the diagnosis, with primarily FISH-based detection of *EWSR1* (and *FUS*) rearrangements, RT-PCR or targeted sequencing methods. When this first step cannot confirm the diagnosis of ES and when the morphological presumption is strong, the use of an untargeted sequencing method is essential to detect rare gene fusions.

This report helps to decipher and expand the ES spectrum with a novel FET/ETS gene fusion. Given the classical histomorphology of the tumor, as well as the nature of the partners involved in this fusion, it is likely that *TAF15::ETV4* translocation is a very rare combination that binds and regulates target genes critical for ES oncogenesis. This case highlights the importance of WERS (including expression profiles analyses) for theranostic purposes in URCS with neonatal presentation.

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Figure legends

Figure 1. Radiological presentation of the case. Postnatal sagittal MRI (**1A**) showing a compartmentalized and heterogeneous intramuscular tumefaction located in the right dorsal paraspinous area. A macrocystic lymphatic malformation was initially suggested. Transversal and coronal MRI (**1B & 1C**) performed given a sudden evolution of the mass at the age of 9-month-old, showed a lesion on T2 hypersignal with a strong restricted diffusion that had doubled in size. Transversal MRI after neoadjuvant chemotherapy showed an excellent response on the tumor volume, with a 22 mm-large residual lesion displaying a T2 central hypersignal (**1D**).

Figure 2. Histomorphology of the tumor. Hematoxylin & eosin staining of biopsies showed a densely cellular proliferation alternating with area of necrosis (*) and broad fibrous septa (**2A** x200). The proliferation was composed of sheets of closely packed and uniform cells with small and round nuclei (**2B**, H&E, x400), that displayed a strong and diffuse membranous expression of CD99 (**2C**, x400). Histological analysis of the specimen after chemotherapy by ES conventional drugs showed a regressive lesion with sheet of macrophages, hemosiderin and very few residual tumor cells (**2D**, x100).

Figure 3. A. Schematic representation of the fusion gene and protein. Protein domains and the sequence at the fusion point are indicated. **B.** Unsupervised consensus hierarchical clustering of various FET-fused tumor types. Position of our TAF15::ETV4 is indicated by the arrow. AFH: Angiomatoid Fibrous Histiocytoma, CCS: Clear Cell Sarcoma, NFATc2: EWSR1/FUS::NFATC2 Sarcoma, EMCS: Extraskelatal Myxoid Chondrosarcoma, PATZ1: EWSR1::PATZ1 Spindle Cells Fibrous Sarcoma, MLPS: Myxoid Liposarcoma, MET: Myoepithelial Tumor, LGFMS: Low Grade Fibromyxoid Sarcoma, DSRCT: Desmoplastic Small Round Cell Tumor, TFPC2: FET::TFPC2 Epithelioid Rhabdomyosarcoma, EwS: Ewing Sarcoma.