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CD99-positive undifferentiated round cell sarcoma diagnosed on fine needle aspiration cytology, later found to harbour a *CIC-DUX4* translocation: a recently described entity

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Introduction

Poorly differentiated, small round cell sarcomas often represent a serious differential diagnostic problem. CD99 expression was initially considered to be specific for Ewing sarcoma (ES); however, it may also be seen in many histologically similar neoplasms. The differential diagnosis of these tumours includes mesenchymal chondrosarcoma, poorly differentiated synovial sarcoma and lymphoblastic lymphoma. However, rarely, CD99 expression may appear in other neoplasms of a similar morphology such as the blastemal component of Wilms' tumour, small cell osteosarcoma, rhabdomyosarcoma, small cell carcinomas, etc., further expanding the list of differentials.[1] Here we describe a case of CD99-positive, *EWSR1*-translocation negative undifferentiated small round cell sarcoma with plasmacytoid morphology, which was initially diagnosed on fine needle aspiration (FNA) cytology and subsequently found on molecular analysis of a resection specimen to harbour the *CIC-DUX4* translocation.

Correct recognition of these entities is facilitated by cytogenetic or molecular genetic studies. Some of the above neoplasms are characterized by specific cytogenetic abnormalities; primary examples are synovial sarcoma characterized by *SYT*-translocations and ES harbouring *EWSR1*-translocations in the majority of cases.[1] Recently, a *BCOR–CCNB3* gene fusion described in undifferentiated sarcoma of the bone has been added to the list of such cytogenetic abnormalities.[2]

A proportion of small blue round cell tumours (SBRCT) lack a characteristic phenotype and genotype, and cannot be diagnosed with confidence on morphological grounds alone and are often labelled as undifferentiated sarcoma. It is likely that several of these will be reclassified based on genetic changes associated in recently described cases. Next to the TET/ETS fusions cases of 'Ewing-like sarcomas' have been identified with fusions between EWSR1 and other non TET-proteins such as NFATc2, ZSG, POU5F1 and others.[3,4] Moreover cases have been identified without similarities to EWSR1-based fusions. Such an example is a recently described recurrent translocation t(4;19)(q35;q13) in a proportion of undifferentiated sarcomas.[5–10] The translocation results in the fusion of the *CIC* gene to the *C*-terminal part of the *DUX4* gene located at 19q13 or 4q35 regions, respectively.[10]

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Case history

A 62-year-old woman detected a small nodule in her right inguinal region in 2010, which showed rapid progression within 3 months. FNA was performed, followed by the resection of a 110 × 85 × 80-mm necrotic tumour. No association with the peripheral nerves was seen. No lymph node or distant metastases were detected. The patient received radiotherapy and chemotherapy. Another resection was performed 4 months after the first because of local recurrence. Significant progression was seen in 3 months with the appearance of multiple pulmonary metastases and an additional mass in the left thigh. Radiother-

apy was administered resulting in partial regression of both lesions. The patient succumbed to the disease 2 months later; an autopsy was not performed.

Materials and methods

Using the FNA sample, direct smears were prepared for haematoxylin and eosin (H&E) and Giemsa staining. The syringe was rinsed with 4% buffered formaldehyde, and a cell block was prepared for immunophenotyping and fluorescence *in situ* hybridization (FISH). The resection specimens were fixed in 4% buffered formaldehyde, tissue samples were embedded in paraffin and 4-μm thick sections were cut. Immunohistochemistry was performed with a large panel of antibodies (Table 1). FISH analysis was performed using commercially available probes for *EWSR1*-translocation and *FUS* (Vysis-Abbott), and for the detection of the CIC/DUX4 translocation probes published by Italiano *et al.*[10]

Table 1. Antibodies used for immunohistochemistry

Antibody	Manufacturer	Dilution	Result
Bcl2	DAKO	1 : 100	Positive
CD99	TS	1 : 100	Positive
Vimentin	DAKO	1 : 1000	Positive
Synaptophysin	TS	1 : 200	Negative/Positive*
AE1/AE3	DAKO	1 : 300	Negative
BerEP4	DAKO	1 : 100	Negative
CD10	LN	1 : 50	Negative
CD138	DAKO	1 : 50	Negative
CD30	TS	1 : 50	Negative
CD34	DAKO	1 : 100	Negative
CD38	LN	1 : 150	Negative
CD56	LN	1 : 50	Negative
CD79a	DAKO	1 : 75	Negative
Desmin	DAKO	1 : 100	Negative
EMA	DAKO	1 : 2400	Negative
HMB45	DAKO	1 : 250	Negative
Inhibinα	DAKO	1 : 20	Negative
Kp1 (CD68)	DAKO	1 : 1000	Negative
LCA (CD45)	DAKO	1 : 400	Negative
Myf4	DAKO	1 : 10	Negative
S100	DAKO	1 : 3000	Negative
SMA	DAKO	1 : 200	Negative

LC, Leica Novocastra; TS, Thermo Scientific
For BerEP4 and CD38, Tris/EDTA buffer, pH6 was used for antigen retrieval, in all other cases, Tris/EDTA buffer, pH 9.0 was used for 20 min. The reactions were visualized using the Envysion System (DAKO), in case of Myf4, the Envysion FLEX visualization system (DAKO) was used.
*Synaptophysin was positive in the second biopsy.

Results

The smears revealed a tumour composed of round cells forming few, loose cell clusters surrounding capillaries. The tumour cells had round, moderately pleomorphic nuclei and scant, bluish, vacuolated cytoplasm (Figure 1). Immunohistochemical staining performed on cell block sections demonstrated cytokeratin and desmin negativity, vimentin and strong, membranous CD99 positivity (Figure 1). FISH did not show *EWSR1*-translocation. The cytological diagnosis was of an undifferentiated small round cell sarcoma, type uncertain.

Histological investigation of the first resection specimen showed that the tumour consisted of small cells showing a solid growth pattern, demarcated by fibrous septae. The cell nuclei were eccentric, often harboured enlarged nucleoli and appeared plasmacytoid in most of the areas without PAS positivity (Figure 2). The mitotic activity was high (47 per 10 high-power fields). The cells showed diffuse vimentin, Bcl2 and CD99 positivity. Additional FISH studies were performed and showed neither *SYT*- nor

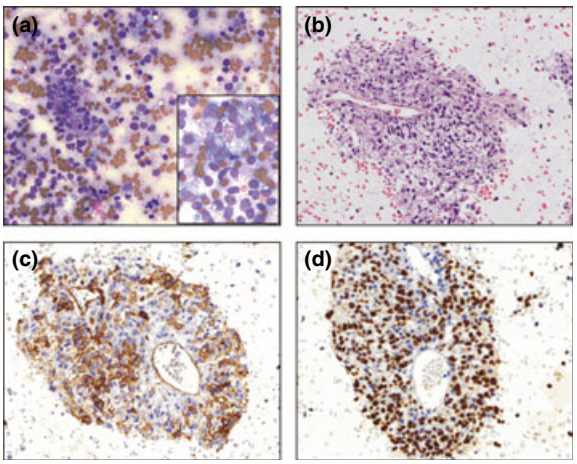

Figure 1. Fine needle aspiration cytology. (a) Among small tumour cells with scant cytoplasm, scattered plasmacytoid cells are visible with irregular nuclei and cytoplasmic vacuolation (Giemsa ×200, inset ×400). (b) The cell blocks showed tumour cells arranged around blood vessels. Many cells with plasmacytoid morphology are seen (haematoxylin and eosin ×200). (c) A proportion of cells show membranous CD99 positivity. (d) The majority of cells are labelled with Mib1 (c and d: immunohistochemistry ×200).

FUS-translocations characteristic of synovial sarcoma and *EWSR1*-translocation negative ES, respectively.

Histology of the second resection specimen showed a microscopic morphology very similar to what was previously seen; however, a minority of the cells showed synaptophysin positivity. Also, several larger cells with atypical nuclei appeared, possibly indicating chemotherapy-related changes. Based on the CD99 expression, *EWSR1*-translocation negativity and the ambiguous differentiation of the sample, additional FISH was performed and detected a *CIC-DUX4* translocation (Figure 2).

Discussion

Soft tissue sarcomas account for approximately 20% of paediatric and 1% of adult malignancies.[5] In spite of significant advancement in classification of these tumours, approximately 5% of sarcomas are unclassifiable, diagnosed by exclusion as undifferentiated soft tissue sarcomas. Understanding the genetic background of these tumours may help us to understand sarcomagenesis, as these tumours may correspond to the malignant transformation of uncommitted mesenchymal progenitors or stem cells.

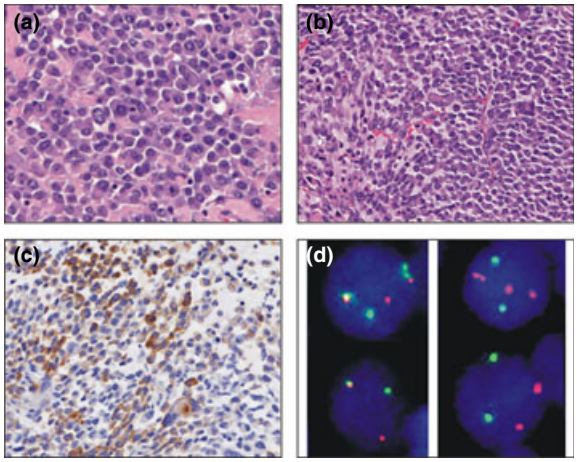

Figure 2. Histology of the resected tumour. (a) The cells have a plasmacytoid appearance, forming sheaths (haematoxylin and eosin ×400). (b) Large, multinucleated cells can be found (H&E ×200). (c) A few tumour cells demonstrate synaptophysin positivity (immunohistochemistry ×200). (d) Fluorescence *in situ* hybridization using probes specific for *CIC* (green) and *DUX4* at 4q35 (red) (left panel) showed fusion signals indicating *CIC-DUX4* fusion by t(4;19) translocation (630×), while using *CIC* (green) and *DUX4* at 10q26 (red) (right panel) showed the lack of fusion signals excluding the possibility of t(10;19) translocation.

Table 2. Important genetic aberrations in undifferentiated sarcomas[1]

Neoplasm	Genetic abnormality	Chromosomal aberration	Fusion gene
Ewing family tumours	EWSR1 fusion to ETS gene family	t(11;22)(q24;q12) t(21;22)(q22;q21) t(7;22)(q22,q24)	EWSR1-FLI1 EWSR1-ERG EWSR1-ETV1
	FUS fusion to ETS gene family	t(16;21)(p11;q22) t(2;16)(q35;q11)	FUS-ERG FUS-FEV
Ewing-like sarcoma	BCOR-CCNB3 fusion	inv(X)(p11.4p11.2)	BCOR-CNNB3
	EWSR1-NFATc2 translocation	Complex rearrangement leading to amplification of the translocated segment with ring chromosome formation	EWSR1-NFATc2
Undifferentiated round cell sarcoma	CIC-DUX4 fusion	t(4;19)(q35;q13) t(10;19)(q26;q13)	CIC-DUX4
Desmoplastic small round cell tumour	EWSR1-WT1 translocation	t(11;22)(p13;q12)	EWSR1-WT1
Mesenchymal chondrosarcoma	HEY1-NCOA2 fusion	inv(8)(q13q21)	HEY1-NCOA2
Alveolar rhabdomyosarcoma	FOXOA1 fusion	t(2;13)(q35;q14) t(1;13)(p36;q14)	PAX3-FOXOA1 PAX7-FOXOA1
Extraskeletal myxoid chondrosarcoma	NR4A3 fusion to TET gene family	t(9;22)(q22;q12) t(9;17)(q22;q11)	EWSR1-NR4A3 TAF2N-NR4A3
Synovial sarcoma	SYT fusion	t(X;18)(p11;q11)	SYT-SSX1 or SYT-SSX2

Recently, a novel translocation involving *CIC* at 19q13 and *DUX4* at 4q35 was reported in undifferentiated sarcomas that may represent a variant of Ewing sarcoma, or a similar entity.[5–10] The *CIC* gene is a member of the HMG-box superfamily of transcription factors, its expression is important for the development of immature granule cells of the nervous system and has been shown to play a role in the pathogenesis of medulloblastoma.[10] The *DUX4* gene belongs to the Dux family of double homeobox-genes. Its physiological function is poorly understood and its expression is normally suppressed in differentiated cells.[10] DUX4 may be involved in the pathogenesis of fascioscapulohumeral muscular dystrophy, as the disease is associated with an inherited shortening of repetitive elements of *D4Z4*. The *CIC-DUX4* fusion harbours the majority of the *CIC* gene, and does not contain the homeodomains of *DUX4*. There is evidence that the presence of the fusion transcript results in upregulation of some downstream transcription factors of the Ets (E-26) family similar to what may be seen in Ewing sarcoma.[9]

Here we have reported a case of undifferentiated sarcoma with vimentin, strong membranous CD99 and focal synaptophysin positivity. The cytological and histological appearance did not allow unambiguous classification. The strong CD99 expression raised the possibility of ES; however, the characteristic *EWSR1* and the rare *FUS*-translocations were absent. Further FISH studies revealed *CIC-DUX4* translocation which indicated a recently described entity.[5]

Twenty-one cases of undifferentiated soft tissue sarcomas with a *CIC-DUX4* fusion have been published so far. The majority showed small round cell morphology with a solid, sheath-like growth pattern and demonstrated only weak-to-moderate CD99 expression. Most of the reported cases were not classifiable based on the morphology and immunophenotype.[5] Synaptophysin expression was not reported, and only one case featured focal plasmacytoid differentiation.[10] The present case expands the phenotypic spectrum associated with a *CIC-DUX4* fusion further than previously reported.

Cytogenetic and molecular genetic investigations are invaluable ancillary studies when diagnosing poorly differentiated SBRCT[3]. The tests may be performed on cytological samples, as well. Besides providing an aid for correct diagnosis, establishing the genetic background of a neoplasm may reveal potential therapeutic targets. The results may influence patient management, as SBRCTs are treated with loco-regional therapy (radiation and resection) while chemotherapy is administered for adult Ewing sarcoma and rhabdomyosarcoma. As molecularly targeted treatment modalities are becoming reality, a better understanding of the cytogenetic background of soft tissue tumours is essential. Thus, when an undifferentiated neoplasm is found, it is imperative to first rule out carcinoma, lymphoma and melanoma, taking the age of the patient in to consideration while setting up the differential. When the diagnosis is undifferentiated round cell sarcoma, especially with CD99 positivity, molecular analysis of an EWSR1, FUS, SYT, FOXOA1, BCOR-CCNB3 and CIC-DUX4 rearrangement should be performed.

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