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Cutaneous Syncytial Myoepithelioma is Characterized by Recurrent *EWSR1-PBX3* Fusions

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Abstract

Cutaneous syncytial myoepithelioma (CSM) is a rare but distinctive benign variant in the family of myoepithelial neoplasms of skin and soft tissue. CSM has unique morphologic and immunohistochemical features, characterized by intradermal syncytial growth of spindled, ovoid, and histiocytoid cells and consistent staining for S-100 protein and EMA, and differs from other myoepithelial tumors by showing only infrequent keratin staining. Rearrangement of the *EWSR1* gene is now known to occur in up to half of all skin and soft tissue myoepithelial tumors, with a wide family of documented fusion partners. In 2013, we reported frequent (80%) *EWSR1* rearrangements in CSM, but were unable to identify the fusion partner using available studies at that time. After recent identification of an index case of CSM harboring an *EWSR1-PBX3* fusion, we used a combination of targeted RNA sequencing and fluorescence in situ hybridization (FISH) studies to investigate the genetic features of a cohort of CSM. An *EWSR1-PBX3* fusion was identified in all 13 cases successfully tested. RNA sequencing was successful in 8/13 cases, all of which were found to have identical breakpoints fusing exon 8 of *EWSR1* to exon 5 of *PBX3*. FISH confirmed both *EWSR1* and *PBX3* rearrangements in 9/9 cases tested, which included 4 confirmed to have *EWSR1-PBX3* fusion by RNA-Seq, 3 cases that failed RNA-Seq, and 2 cases examined by FISH alone. 2 cases failed RNA sequencing but had no additional tissue remaining for FISH studies. Our findings demonstrate that *EWSR1-PBX3* fusions occur in most (and possibly all) cases of CSM.

Keywords

myoepithelioma; syncytial; skin; soft tissue; *EWSR1*; *PBX3*; RNA-Seq

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INTRODUCTION

Cutaneous syncytial myoepithelioma (CSM) is a rare but distinctive variant in the family of myoepithelial neoplasms, having unique morphologic and immunohistochemical features. CSM most commonly arises in the extremities of adult patients (although a wide age range is observed) and occurs more frequently in men than women(1). In contrast to the characteristic morphologically heterogeneous appearance of myoepithelial neoplasms of skin and soft tissue(2–10), CSM is characterized by syncytial intradermal growth of spindled, ovoid, and histiocytoid cells(1, 8, 11). Tumors are benign and show no metastatic risk; local recurrence occurs only rarely, usually after incomplete excision. CSM shows consistent positivity for S-100 protein and EMA. However, unlike most myoepithelial neoplasms, keratin staining is infrequent, being at most focal or multifocal in only up to 10% of cases(1). Notably, CSM is distinct from cutaneous mixed tumor (i.e. chondroid syringoma), which shows ductal differentiation and frequent *PLAG1* gene rearrangement (similarly to their salivary counterparts)(12, 13).

The genetic features of myoepithelial neoplasms of skin and soft tissue have increasingly been characterized over the past decade. Rearrangements of the *EWSR1* gene are now known to occur in up to half of all benign and malignant myoepithelial tumors of skin, soft tissue, and bone(14, 15). To date, fusion partners have been identified in about 20% of cases, and the varied group of documented partners include *POU5F1*, *PBX1*, *PBX3, ZNF444, ATF1,* and *KLF17*(14, 16–20), as well as fusion genes with alternate *FUS* rearrangement(19, 21). In 2013, in a series of 38 CSM, we reported *EWSR1* rearrangements in approximately 80% (14/17) of cases tested (1), and subsequent case reports of CSM have also confirmed the presence of *EWSR1* rearrangement(11, 22). However, the fusion partner has remained unknown, and in our prior series of CSM, testing was negative for all known fusion partners at that time(1).

Recently, we identified an index case of CSM harboring an *EWSR1-PBX3* fusion. Our aim in this current study was to evaluate a cohort of CSM to investigate the frequency of *EWSR1-PBX3* fusions and to detect the presence of any alternative fusion genes.

MATERIALS AND METHODS

Index Case and Selection of CSM Cohort

The index case (case 1) was a 59-year-old woman who underwent biopsy of a skin lesion on the left shin. Immunohistochemical workup showed EMA positivity and focal weak S-100 staining (Table 1). Targeted RNA sequencing was performed on formalin-fixed paraffin embedded (FFPE) tissue sections, as part of the diagnostic work-up. Detection of *EWSR1-PBX3* fusion prompted a retrospective search for a cohort of CSM. Fourteen additional cases of CSM diagnosed between 2013–2018 were selected from the consultation archives of one author (C.D.M.F.; Brigham and Women’s Hospital, Boston, MA), for which FFPE sections were available for RNA sequencing (n=12) and/or fluorescence in situ hybridization (FISH) (n=9). Immunohistochemical studies performed during diagnostic workup were reviewed. None of these cases were included in the prior study (1).

RNA Sequencing

For case 1, RNA was extracted from 4 10 μm FFPE tissue scrolls. For the remaining 12 cases, 4 μm FFPE sections were cut onto positively charged glass slides and 3–5 slides scraped to provide tissue for RNA extraction. RNA was prepared using the ExpressArt FFPE Clear RNA Ready kit (Amsbio, Cambridge, MA), and total RNA quantified using the Qubit RNA HS Assay kit (Thermofisher Scientific, Mississauga, ON). RNA-Seq libraries were prepared with the TruSight RNA Fusion Panel (Illumina, San Diego, CA), and an input of 20–100 ng RNA. Each sample was sequenced with 76 base-pair paired-end reads on an Illumina MiSeq at 8 samples per flow cell (~3 million reads per sample). Results were analyzed using two pipelines; STAR aligner with Manta fusion caller, and BOWTIE2 alignment with the JAFFA fusion caller(23, 24).

Fluorescence in Situ Hybridization—FISH was performed on 9 cases from FFPE 4-μm sections by applying custom probes using bacterial artificial chromosomes (BAC), covering and flanking *EWSR1* and *PBX3*, using previously detailed methods(18). Briefly, BAC clones were chosen according to the USCS genome browser (http://genome.uscs.edu) and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (CHORI) (Oakland, CA) (http://bacpac.chori.org). DNA from individual BACs was isolated according to the manufacturer's instructions, and labeled with different fluorochromes in a nick translation reaction, denatured, and hybridized to pretreated slides. The slides were then incubated, washed, and mounted with DAPI in an antifade solution. The genomic location of each BAC set was verified by hybridizing them to normal metaphase chromosomes. 200 successive tumor cell nuclei were examined using a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany), controlled by Isis 5 software (Metasystems). A positive score was interpreted when at least 20% of interphase nuclei showed the break-apart signal. Nuclei with incomplete signal sets were not scored.

RESULTS

Clinicopathologic Features

The clinicopathologic features of the cohort are summarized in Table 1. The cohort consisted of 7 men and 8 women, with an age range of 17 to 70 years (median, 36 years). Tumors arose on the lower extremity (n=7, including 1 on toe), arm (n=4), and trunk (n=4). Lesions ranged from 0.3 cm to 0.9 cm (median, 0.5 cm). Tumors were well-circumscribed, but unencapsulated, dermal lesions comprised of solid and sheet-like syncytial growth of ovoid-to-spindled and histiocytoid cells with palely eosinophilic cytoplasm (Fig 1A). Tumor cells had uniform appearing ovoid or round nuclei with fine chromatin, smooth nuclear membranes, and small or inconspicuous nucleoli (Fig 1B–C). No significant atypia was seen, and necrosis was absent. Mitotic activity was generally low (range, 0–2 per 10 high-power fields (median count, 0). Five cases showed adipocytic metaplasia (Fig 1D). Entrapment of adnexal structures was common, as were lymphocytic infiltrates within and around the tumor, often surrounding vessels.

All cases of CSM showed expression of EMA and S-100. EMA staining was diffuse in all cases (15/15), while S-100 protein was strong in most cases but with some cases showing

focal (n=4) or multifocal (n=2) staining. Keratin staining was focal in 2/11 cases tested, all others being negative. GFAP expression was present in 1/8 cases examined. SOX10 was negative in two cases tested (0/2).

RNA Sequencing

13 cases of CSM underwent RNA sequencing (including the index case). *EWSR1-PBX3* fusions were identified in 7 additional cases (cases 4, 8, 9, 10, 11, 12 and 13), for a total of 8/13 cases confirmed by RNA-Seq. Five cases failed due to low sequencing quality metrics; of these, 3 cases had material available for FISH (see below) and 2 had insufficient material for further molecular testing. All 8 cases with *EWSR1-PBX3* fusions had identical breakpoints involving exon 8 of *EWSR1* fused to exon 5 of *PBX3* (Fig 2).

EWSR1 and *PBX3* FISH Findings

FISH testing for *EWSR1* and *PBX3* were performed to independently verify the presence of the fusion gene in 9 cases, which included 3 cases that failed RNA-Seq (cases 2, 3, 7), 4 confirmed to have *EWSR1-PBX3* fusion (cases 4, 8, 10, 12), and 2 cases examined by FISH alone (cases 14, 15). All 9 cases showed rearrangement of both *EWSR1* and *PBX3* (Fig 3).

DISCUSSION

Herein we report that *EWSR1-PBX3* fusions characterize the vast majority of CSMs. In 2013, we first reported the presence of *EWSR1* rearrangement in 82% (14/17) cases of CSM(1). We had excluded all known fusion partners in myoepithelial neoplasms at the time, including *PBX1*, *ZNF444, POU5F1*, *DUX4, ATF1*, *CREB1*, *NR4A3, DDIT3,* and *NFATc2*, and concluded that CSMs were likely characterized by a novel fusion gene. Subsequently in 2015, *EWSR1-PBX3* fusions were identified in 3 myoepithelial tumors (2 in bone and 1 in soft tissue)(18), however, no *PBX3* rearrangements were detected in the 3 CSM cases with *EWSR1*-rearrangements included in that study(18). While it is possible that these cases may harbor separate, unidentified novel fusions, it is also likely that tissue degradation of older archival material was a factor.

Myoepithelial neoplasms of skin and soft tissue are characterized by histologic heterogeneity, both intratumorally, and between different tumors, with patterns including spindled, ovoid, epithelioid, and clear cell morphology and a combination of reticular, trabecular, and nested growth, as well as variable expression of keratin, EMA, S-100 protein, and GFAP (2, 3, 5, 6, 8, 9, 15). A variably chondromyxoid or hyalinized stroma is common and often a useful diagnostic feature. Heterologous differentiation occurs in up to 15% of myoepithelial neoplasms and is most commonly chondro-osseous(2, 6, 9). In contrast, CSM show a distinctive appearance that is relatively constant between tumors. While showing variably spindled, ovoid, and histiocytoid morphology, CSM tumor cells overall appear uniform with palely eosinophilic cytoplasm and consistent syncytial growth. CSM lacks ductal differentiation, a feature of cutaneous mixed tumors. Moreover, unlike other myoepithelial tumors, adipocytic metaplasia is the most commonly observed type of heterologous differentiation (1, 8, 11), and the immunophenotype is reliably positive for EMA and S-100 protein, and usually negative for keratin.

Given the consistent morphology and immunophenotype of CSM, their genetic uniformity, with nearly all cases showing *EWSR1-PBX3* gene fusions, is not entirely surprising. Studies have suggested that certain fusion genes are associated with specific morphologic features, such as tumors with *EWSR1-POU5F1* showing epithelioid cells with clear cytoplasm in a solid and nested growth pattern surrounded by thin fibrous septa(14). Among myoepithelial neoplasms with *EWSR1-PBX1* fusions, a subset have either a predominant appearance (or foci) of a deceptively bland spindle cell proliferation(14), as was also seen in 2008 in the first ever reported myoepithelial tumor of soft tissue with a fusion gene (*EWSR1-PBX1*) (16). To date, *EWSR1-PBX3* fusions are rare in neoplasia, having been reported in 3 intraosseous myoepitheliomas, arising in the tibia, fibula(18), and fourth metatarsal(25), and one soft tissue myoepithelial tumor of the finger(18), as well as a single case of retroperitoneal leiomyoma(26), thus any typical morphologic features characterizing these tumors requires a larger sample size to ascertain with any certainty.

The PBX transcription factors (PBX1, PBX2, PBX2, PBX4) are members of the TALE (three amino acid loop extension) homeobox gene family and are involved in regulating gene expression during development through their ability to form DNA-binding heterodimers that interact with a wide range of transcription factors, including the HOX family(27). The *PBX* genes show a high degree of homology(28) and are involved in the differentiation of urogenital system and steroidogenesis in the adrenal cortex(27). *PBX1*, the most extensively characterized PBX transcription factor, was first discovered as part of the *E2A-PBX1* fusion characteristic of a subset of pre-B-cell acute lymphoblastic leukemia(29). The oncogenic role of *PBX3* is currently less well understood, but this gene has been shown to be overexpressed in cell lines of colorectal cancer(30), prostate cancer(31), and cervical cancer(32), and upregulated in gastric cancer resections(33). The *EWSR1-PBX3* gene fusion in CSM is predicted to result in a chimeric protein containing the transactivation domain of *EWSR1* fused to the DNA binding homeodomain of *PBX3* (Figure 2). This is presumed to lead to dysregulation of PBX3 target genes.

Despite the distinctive morphologic and immunohistochemical features of CSM, the diagnosis may be challenging due to the rarity of these tumors, and, on rare occasion, detection of *EWSR1-PBX3* fusion may potentially be diagnostically helpful, particularly in distinguishing CSM from more clinically significant lesions. The differential diagnosis includes epithelioid benign fibrous histiocytoma, juvenile xanthogranuloma, epithelioid sarcoma, and melanocytic lesions. Immunohistochemistry is useful in most cases in resolving the differential diagnosis. Epithelioid benign fibrous histiocytoma shows frequent EMA positivity, but is negative for S-100 and harbors *ALK* rearrangements that can be identified by immunohistochemistry, FISH, and/or RNA-Seq(34, 35). Juvenile xanthogranuloma (especially “early stage” lesions) are positive for histiocytic markers and do not express conventional myoepithelial markers. Epithelioid sarcoma is positive for EMA, but typically shows more keratin expression as well as CD34 positivity (in 50% of cases) and S-100-negativitiy. Most cases of epithelioid sarcoma show loss of INI1/SMARCB1 expression(36), frequently correlating with homozygous deletions of *SMARCB1*(37). Among melanocytic tumors, Spitz nevus and spitzoid melanoma may arise in the differential; both express S-100 and melanocytic differentiation can be confirmed by HMB-45 and Mart1.

In summary, we identified recurrent *EWSR1-PBX3* fusions in CSM. In contrast to the broad spectrum of myoepithelial neoplasms, CSM shows more uniform immunophenotypic and genetic features. Whether other fusion variants exist in CSM remains to be determined, and the molecular spectrum, if any, may be expanded in the future.

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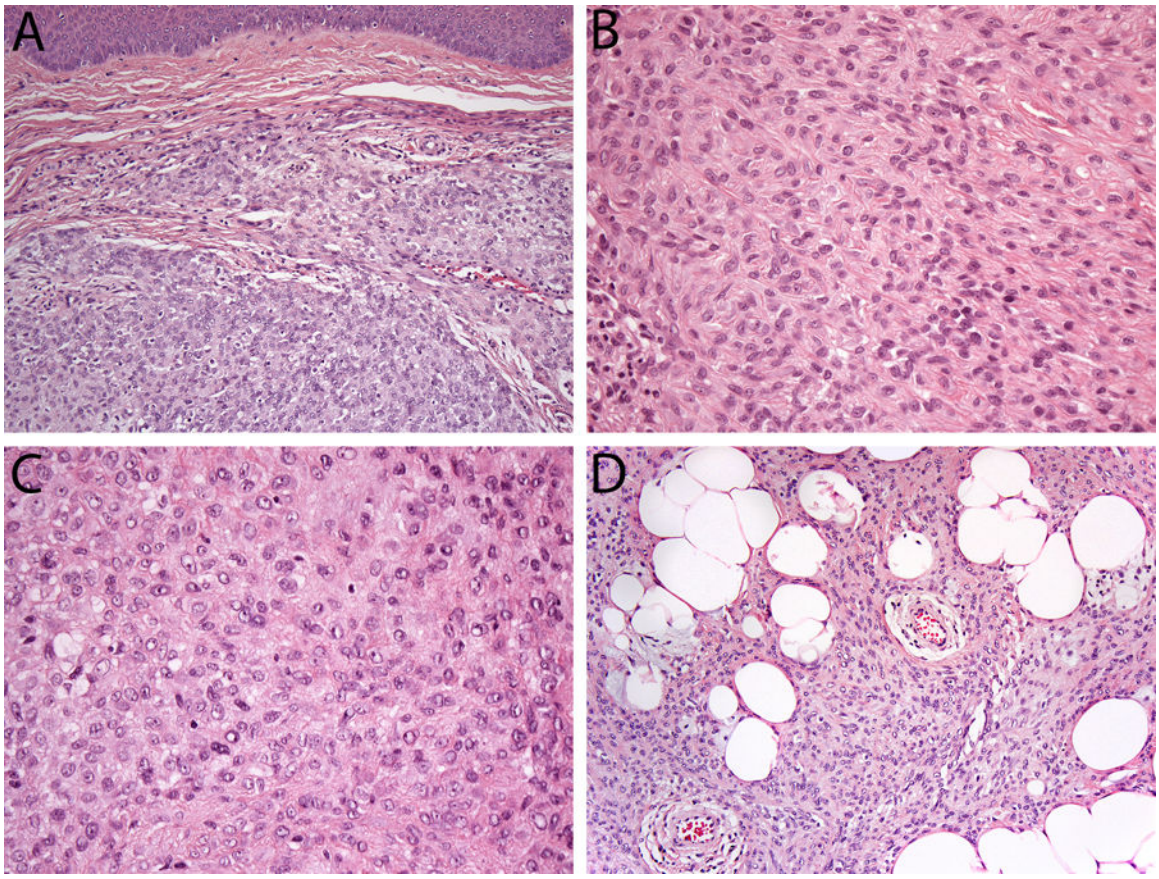

Figure 1.

Features of cutaneous syncytial myoepithelioma. (A) Tumors are well-circumscribed and show syncytial growth of uniform ovoid, spindled, and histiocytoid cells (Case 8). Tumors cells have palely eosinophilic cytoplasm, and uniform nuclei that are ovoid (B, case 14) or round (C, case 7), with small or inconspicuous nuclei. Heterologous adipocytic metaplasia was present in a subset of cases (D, Case 11).

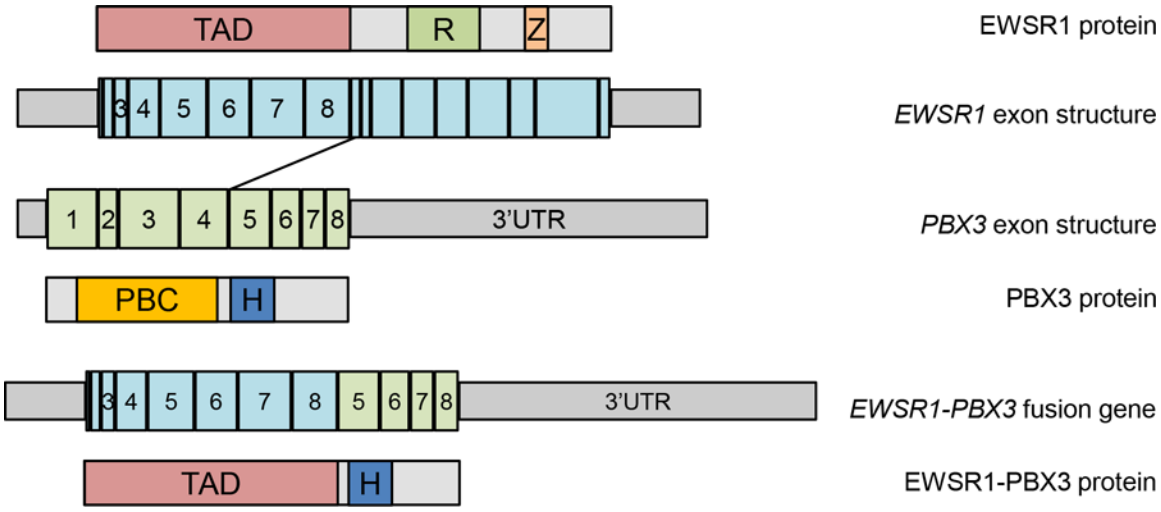

Figure 2.

Schematic illustrating exon and protein structure of *EWSR1* (top), *PBX3* (middle), and the *EWSR1-PBX3* fusion product found in cutaneous syncytial myoepithelioma (bottom). Grey boxes in the exon structure indicate 5' and 3' untranslated regions (UTRs). TAD – Transactivation domain, R – RRM RNA recognition motif, Z- Zinc Finger, PBC – PBC domain, H- Homeodomain DNA binding domain. The *EWSR1-PBX3* gene fusion between *EWSR1* exon 8 and *PBX3* exon 5 results in a chimeric protein containing the transactivation domain of *EWSR1* fused to the DNA binding homeodomain of PBX3.

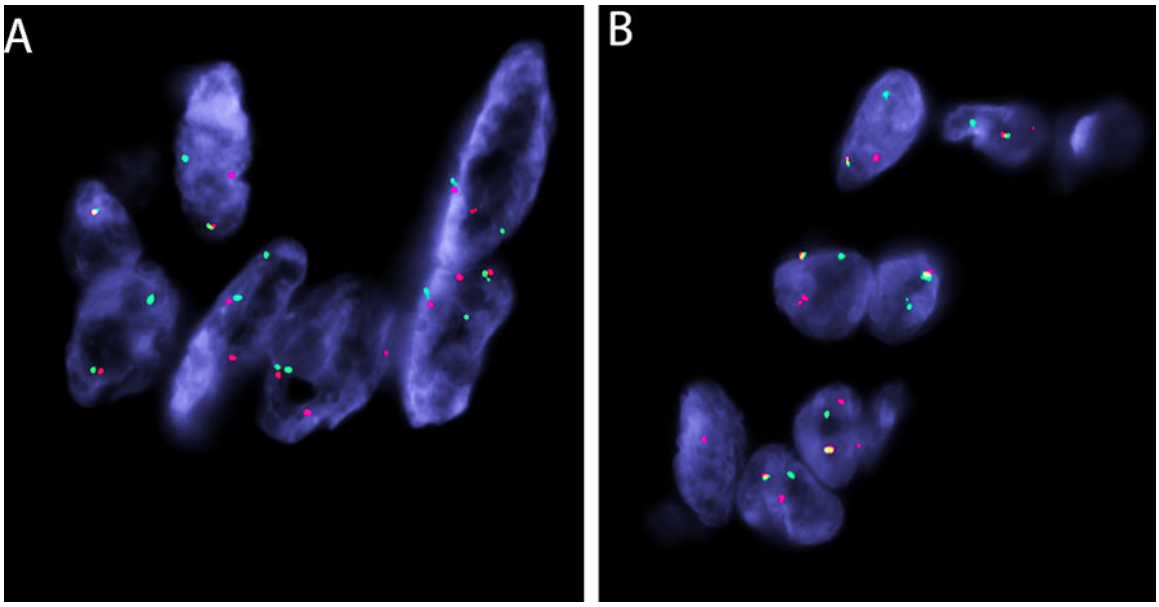

Figure 3.
FISH break-apart assays demonstrating split signals for *EWSR1* gene (A) and *PBX3* (B) genes (B) (red, centromeric; green telomeric, for both genes).

Table 1:

Clinicopathologic Features of this Cutaneous Syncytial Myoepithelioma Cohort

Case	Clinicopathologic Features				Immunohistochemistry			Molecular Results	
	Sex	Age (yrs)	Site	Tumor size (cm)	EMA	S-100	Keratin	RNA-Seq	FISH
1	F	59	Leg	0.7	Pos	Focal	Neg	*EWSR1-PBX3*	-
2	F	19	Back	0.6	Pos	Pos	Neg	Failed	EWSR1, PBX3
3	M	47	Buttock	0.6	Pos	Pos	Focal	Failed	EWSR1, PBX3
4	F	30	Thigh	0.4	Pos	Pos	-	*EWSR1-PBX3*	EWSR1, PBX3
5	M	17	Arm	0.4	Pos	Pos	Neg	Failed *	-
6	F	38	Thigh	0.3	Pos	Pos	Neg	Failed *	-
7	M	70	Shoulder	0.4	Pos	Pos	Neg	Failed	EWSR1, PBX3
8	M	36	Back	0.5	Pos	Pos	Neg	*EWSR1-PBX3*	EWSR1, PBX3
9	M	56	Thigh	0.5	Pos	Focal	Neg	*EWSR1-PBX3*	-
10	F	47	Back	0.3	Pos	Pos	Focal	*EWSR1-PBX3*	EWSR1, PBX3
11	M	30	Thigh	0.9	Pos	Focal	-	*EWSR1-PBX3*	-
12	F	35	Arm	0.5	Pos	Focal	Neg	*EWSR1-PBX3*	EWSR1, PBX3
13	F	46	Toe	0.3	Pos	MF	Neg	*EWSR1-PBX3*	-
14	M	22	Arm	0.5	Pos	Pos	-	-	EWSR1, PBX3
15	F	24	Thigh	0.6	Pos	MF	-	-	EWSR1, PBX3

F, female; M, male; Pos, positive; Neg, negative; MF, multifocal.

* Insufficient material left for FISH analysis