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Novel *SRF-ICA1L* Fusions in Cellular Myoid Neoplasms with Potential for Malignant Behavior

Albert J. Suurmeijer[1], **Brendan C. Dickson**[2], **David Swanson**[2], **Yun-Shao Sung**[3], **Lei Zhang**[3], **Cristina R. Antonescu**[3]

[1]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands [2]Department of Pathology & Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada [3]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Abstract

Pericytic tumors comprise a histologic continuum of neoplasms with perivascular myoid differentiation, which includes glomus tumors, myopericytoma, myofibroma and angioleiomyoma. Despite their morphologic overlap, recent data suggest a dichotomy in their genetic signatures, including recurrent *NOTCH* gene fusions in glomus tumors and *PDGFRB* mutations in myofibromas and myopericytomas. Moreover, *SRF-RELA* fusions have been described in a subset of cellular variants of myofibroma and myopericytoma showing myogenic differentiation. Triggered by an index case of an unclassified cellular myoid tumor showing a a novel *SRF-ICA1L* fusion we have investigated our files for cases showing similar histology and screened them using a combined approach of targeted RNA sequencing and FISH. An identical fusion between *SRF* exon 4 and *ICA1L* exon 11 was identified in a total of 4 spindle cell tumors with similar clinicopathological features. Clinically, the tumors were deep seated and originated in the trunk or proximal lower extremity of adult patients (age range 23–55 years). Histologically, the tumors were composed of cellular fascicles of monomorphic eosinophilic spindle cells showing increased mitotic activity, harboring densely hyalinized stroma, often with focal areas of necrosis. All 4 tumors had similar immunoprofiles with positivity for smooth muscle actin, calponin, and smooth muscle myosin heavy chain. Tumors were negative for desmin and caldesmon, markers often seen in *SRF-RELA* positive tumors with similar morphology. Follow-up information was available in 3 patients. Two patients had no evidence of disease, 2 and 5 years after surgical resection. One patient, a 35-year-old male patient with a 19 cm deep-seated tumor with brisk mitotic activity (>20 mitoses in 10 HPF), developed lung metastases 7 years after initial diagnosis. In summary, we report a series of 4 cellular myoid tumors with novel S*RF-ICA1L* gene fusions, characterized by bland spindle cell fascicular growth, expression of specific smooth muscle markers, elevated mitotic activity, marked stromal hyalinization, focal coagulative necrosis, and potential for malignant behavior. Given the morphologic overlap with related cellular myopericytic tumors with *SRF-RELA* fusions, it is likely that *SRF-ICA1L* fusions define a similar subset of neoplasms composed of immature smooth muscle cells.

Correspondence: Cristina R. Antonescu, MD, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10021, antonesc@mskcc.org.

Keywords

SRF; ICA1L; RELA; fusion; myoid; pericytic; spindle cell sarcoma

INTRODUCTION

Historically, four pathologic entities: glomus tumor, myofibroma, myopericytoma, and angioleiomyoma, have been grouped together under one family of perivascular myoid tumors, based on overlapping or hybrid histologic features[1]. Their genetic abnormalities have been only recently elucidated[2] showing a dichotomy in two molecular subsets, with *PDGFRB* mutations defining myofibroma and myopericytomas[3,4], while *miR143-NOTCH* fusions occurring in most glomus tumors[5]. Aside from these 4 major categories, a small subset of cellular perivascular myoid neoplasms has been difficult to classify due to their worrisome histologic features and variable myogenic differentiation. *SRF–RELA* fusions were recently described in a group of cellular spindle cell neoplasms with histologic features reminiscent of cellular myofibroma or cellular myopericytoma[6]. These tumors had a wide age range and presented in children and adults at various anatomic locations (extremities, trunk, head and neck and intra-abdominal). Despite the dense cellularity and variable mitotic activity, none of the lesions displayed nuclear pleomorphism or necrosis. Immunohistochemically, these tumors had a smooth muscle-like phenotype with abundant expression of smooth muscle actin, desmin, and caldesmon. As a result these lesions may be misdiagnosed as sarcomas with myogenic differentiation, including leiomyosarcomas. However, the few cases with available follow-up did not indicate an aggressive or metastatic behavior. In this study, we describe the clinicopathologic and molecular features of four myoid spindle cell tumors with novel *SRF-ICA1L* fusions. These tumors showed a partial or incomplete smooth muscle immunophenotype and their morphology overlapped with the group of cellular myopericytic tumors harboring *SRF-RELA* fusions.

MATERIAL AND METHODS

Based on an index case, the senior authors files were searched among a group of unclassified smooth muscle/myopericytic neoplasms characterized by a cellular, often fascicular growth, lacking nuclear pleomorphism and a variable smooth muscle immunophenotype. The cohort encompassed four myoid spindle cell tumors showing recurrent *SRF-ICA1L* fusions, which were identified by targeted RNA sequencing. Clinical data, including age, gender, anatomic site, and gross features of tumors were retrieved from pathology reports. All tumors had been surgically removed and hematoxylin and eosin-stained slides from resection specimens were reviewed by two of us (AS, CRA). In all four cases immunohistochemical (IHC) stains for smooth muscle actin (SMA), calponin, smooth muscle myosin heavy chain I (SM-MHC, SMMS-1), desmin, caldesmon, S100, SOX10, CD34, EMA, and cytokeratins were available. IHC staining was performed on a Leica-Bond-3 (Leica, Buffalo Grove, IL) or a Ventana Benchmark (Ventana Medical Systems, Tucson, AZ) automated immunostaining platform using a heat-based antigen retrieval method and high pH buffer.

Targeted RNA Sequencing.

All four cases were analyzed by targeted RNA sequencing. RNA was extracted from FFPE tissue using Amsbio's ExpressArt FFPE Clear RNA Ready kit (Amsbio LLC, Cambridge, MA). RNA-seq libraries were prepared using 20 to 100 ng total RNA with the TruSight RNA Fusion Panel (Illumina, San Diego, CA)[7]. Targeted RNA sequencing was performed on an Illumina MiSeq platform. Reads were independently aligned with STAR (version 2.3) against the human reference genome (hg19) and analyzed by STAR-Fusion.

Fluorescence In Situ Hybridization.

All cases were tested by FISH for *SRF* and *ICA1L* gene abnormalities. Custom probes made by bacterial artificial chromosomes (BAC) clones flanking the genes of interest according to UCSC genome browser (http://genome.ucsc.edu) and obtained from BACPAC sources of Children's Hospital of Oakland Research Institute (Oakland, CA; http://bacpac.chori.org) (Supplem Table 1). DNA from each BAC was isolated according to the manufacturer's instructions. The BAC clones were labeled with fluorochromes (fluorescent-labeled dUTPs, Enzo Life Sciences, New York, NY) by nick translation and validated on normal metaphase chromosomes. The 4 μm-thick FFPE slides were deparaffinized, pretreated, and hybridized with denatured probes. After overnight incubation, the slides were washed, stained with 4', 6-diamidino-2-phenylindole, mounted with an antifade solution, and then examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) controlled by Isis 5 software (Metasystems).

RESULTS

Clinicopathological features of myoid tumors with SRF-ICA1L gene fusions

The cohort was composed of three males and one female patient, with an age range at diagnosis of 23–55 years (mean 42; median 40.5 years)(Table 1). All four tumors arose within the deep soft tissue of the proximal lower extremity and trunk (buttock, hip and thoracic wall). In three cases, there was a long clinical duration, with a tumor mass being present for many years (range 4–7 years). The tumors were large; tumor size varied from 8.5–19 cm (mean 12 cm). Grossly, the tumors had a uniform gross appearance, being tan-white and well demarcated, but non-encapsulated on cut surface (Figure 1).

Histologically, all 4 tumors showed similar morphology, being composed of monomorphic spindle cells arranged in streaming fascicles or cords containing a variable amount of hyalinized collagenous stroma (Figure 2). In one case, a hemangiopericytoma-like vascular network was observed. Concentric perivascular arrangements, typical of myopericytoma, was absent. Tumor cells had a moderate amount of well-demarcated eosinophilic cytoplasm and ovoid to fusiform uniform nuclei, with fine or vesicular chromatin and only minimal cytologic atypia. No hyperchromasia or marked nuclear pleomorphism was noted. However, in 3 cases mitotic activity was higher than 10 MF/10 HPFs (Figure 2), whereas one case had a mitotic count of up to 6 MF/10 HPFs. Focal areas of coagulative tumor necrosis were noted in 3 cases, 2 being associated with dystrophic calcification (Figure 2). Clinical follow-up was available in 3 patients, with a range of 2–7 years. One patient (case 4) developed lung metastases, being alive with disease 7 years since diagnosis.

SRF-ICA1L positive tumors showed a partial smooth muscle immunoprofile

By IHC, the 4 tumors showed diffuse expression of smooth muscle actin, calponin, and smooth muscle myosin heavy chain (SMM-HC), as well as variable focal to multifocal expression of cytokeratins and S100 protein (Table 2, Figure 2). However, markers of differentiated smooth muscle cell lineage (desmin and caldesmon) and myoepithelial markers (EMA and SOX10 in 4/4 cases, and p63 and basal type cytokeratins in 2/2 cases) were negative. In addition, CD34 staining was negative.

SRF-ICA1L fusion results in transcriptional upregulation of *SRF*

By targeted RNA sequencing, all 4 tumors had an identical fusion transcript, in which *SRF* exon 4 was fused to either exon 10 (3 cases) or exon 11 (1 case) of *ICA1L* gene (Figure 3). By RNA sequencing, *SRF* mRNA expression was upregulated, at levels even higher than observed in cellular myopericytic tumors with *SRF-RELA* fusion. By hierarchical clustering analysis, all 4 tumors formed a tight genomic group compared to other soft tissue tumors available on the similar platform (Figure 3). FISH confirmed that all 4 tumors had *SRF* and *ICA1L* gene rearrangements (Supplem Fig. 1).

DISCUSSION

We identified 4 unusual cellular myoid neoplasms sharing an identical *SRF-ICA1L* fusion. Prior to RNA sequencing profiling, these tumors remained unclassified, showing in-between features of myopericytic and smooth muscle neoplasms. Their common morphologic denominator included a cellular fascicular growth of bland ovoid myoid spindle cells often with pronounced stromal hyalinization. The tumor cells lacked nuclear pleomorphism, but frequently showed a brisk mitotic activity and focal necrosis. Immunohistochemically, the *SRF-ICA1L* positive tumors displayed an incomplete smooth muscle cell differentiation, with consistent and diffuse expression of alpha-smooth muscle actin (ASMA), calponin, and smooth muscle heavy myosin isoform (SM-MHC). *SRF-ICA1L* fused tumors occurred in adults, presenting as large tumors in deep soft tissue locations, including trunk and extremities. None occurred in the pediatric age group or in visceral location.

Our group has recently defined a somewhat related molecular group of cellular myofibroma/myopericytoma characterized by *SRF-RELA* fusions[6]. The histologic appearance of *SRF-ICA1L* fusion positive tumors showed significant overlap with tumors with *SRF-RELA* fusions. In contrast, the latter was characterized by co-expression for SMA and desmin, often with a strong and diffuse pattern of staining, mimicking sarcomas with myogenic differentiation. Although the follow-up in that study was limited, none of the patients developed distant metastasis.

The ubiquitously expressed SRF (serum response factor) belongs to the MADS box family of transcription factors, which can bind to promotor regions (serum response elements) of many different target genes, including muscle-specific genes[8]. At the confluence of complex signaling pathways, SRF is considered to be a cell phenotypic modulator, involved, amongst other, in transformation of fibroblasts into SMA-positive myofibroblasts[9,10] and embryonic development of smooth muscle[8]. At the molecular level, it has been shown that *SRF*

interaction with myocardin-related transcriptional coactivators is critical during embryonic development of smooth muscle[8,11]. Of note, both *SRF-RELA* and *SRF-ICA1L* fusion positive tumors have similar *SRF* breakpoints, resulting in *SRF* mRNA upregulation at the transcriptional level. Based on the common *SRF* breakpoint in exon 4, the projected fusion protein retains the MADS box domain and DNA binding potential of wild-type SRF. *SRF* gene fusions (with *NCOA2* gene partner) have been also reported in rare examples of congenital/infantile spindle cell rhabdomyosarcoma, often associated with a favorable outcome[12,13].

The *ICA1L* (*islet cell antigen-like 1*) gene encodes a member of the interacting BAR domain protein family. ICA1L and its binding partner, PICK1, play a key role in cellular functions that require dynamic remodeling of the actin cytoskeleton and membranes, such as organelle trafficking[14]. In testis, ICA1L and PICK1 are localized in spermatids, being implicated in vesicle trafficking from the Golgi region and formation of acrosomes of mature spermatozoa[15]. However, in the *SRF-ICA1L* fusion gene, the *ICA1L* breakpoint in exon 10 or 11 predicts that the exons encoding the BAR domain protein are lost, whereas the ICA1L activating domains are retained.

As shown in mouse embryogenesis and induced pluripotent mesenchymal stem cells, SM-MHC is a robust and specific marker of smooth muscle differentiation[16,17] and expression of SM-MHC is not compatible with myofibroblastic differentiation[18]. Within the spectrum of perivascular myoid tumors, smooth muscle differentiation as defined by SMM-HC expression is typically found in myopericytoma and angioleiomyoma[19–21]. The latter also being positive for desmin. However, classic examples of myopericytoma and angioleiomyoma present as small tumors, usually less than 2 cm in diameter, within the dermis and subcutis. None of our study group cases showed histologic features of classic myopericytoma or angioleiomyoma. Moreover, both sporadic and familial cases of myopericytoma and myofibroma are characterized by recurrent activating *PDGFRB* mutations rather than *SRF* related fusions[3,6].

Based on the coexpression of myoid markers and variable expression of cytokeratins and S100 protein, the differential diagnosis of *SRF-ICA1L* positive tumors also included myoepithelial tumors. However, the histologic appearance of myoepithelial tumors includes alternating areas of spindle cells and/or epithelioid cells embedded in a variable amount of fibrous, myxoid or myxohyaline stroma. Moreover, by IHC, other myoepithelial markers (EMA, SOX10, basal-type cytokeratins and P63) were consistently negative in our study group. One may argue whether *SRF-ICA1L* fused tumors represent low-grade leiomyosarcomas. However, in soft tissue locations, the nuclei of low-grade leiomyosarcomas are usually more elongated, hyperchromatic and pleomorphic and often show immunoreactivity for desmin and caldesmon. The latter smooth muscle markers were completely negative in our cohort. *SRF-ICA1L* tumors lacked nuclear pleomorphism or hyperchromasia, but their high mitotic activity was a worrisome feature. One patient, who had a 7-year clinical history of a tumor mass and a mitotic rate exceeding 20 MF/10 HPFs developed lung metastases, which indicates that these cellular myoid neoplasms have the potential for malignant behavior. Clearly, a larger tumor series is necessary for more adequate prediction of clinical outcome.

In summary, we report a series of 4 myoid tumors with novel S*RF-ICA1L* gene fusions, characterized by bland spindle cell fascicular growth, expression of specific smooth muscle markers, moderate to high mitotic activity, marked stromal hyalinization, focal necrosis, and potential for malignant behavior. Given their morphologic overlap with similar tumors with *SRF-RELA* fusions that also express desmin and caldesmon, we postulate that *SRF-ICA1L* tumors are neoplasms showing incomplete smooth muscle differentiation, being composed of immature, nonterminally differentiated smooth muscle cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Disclosures:

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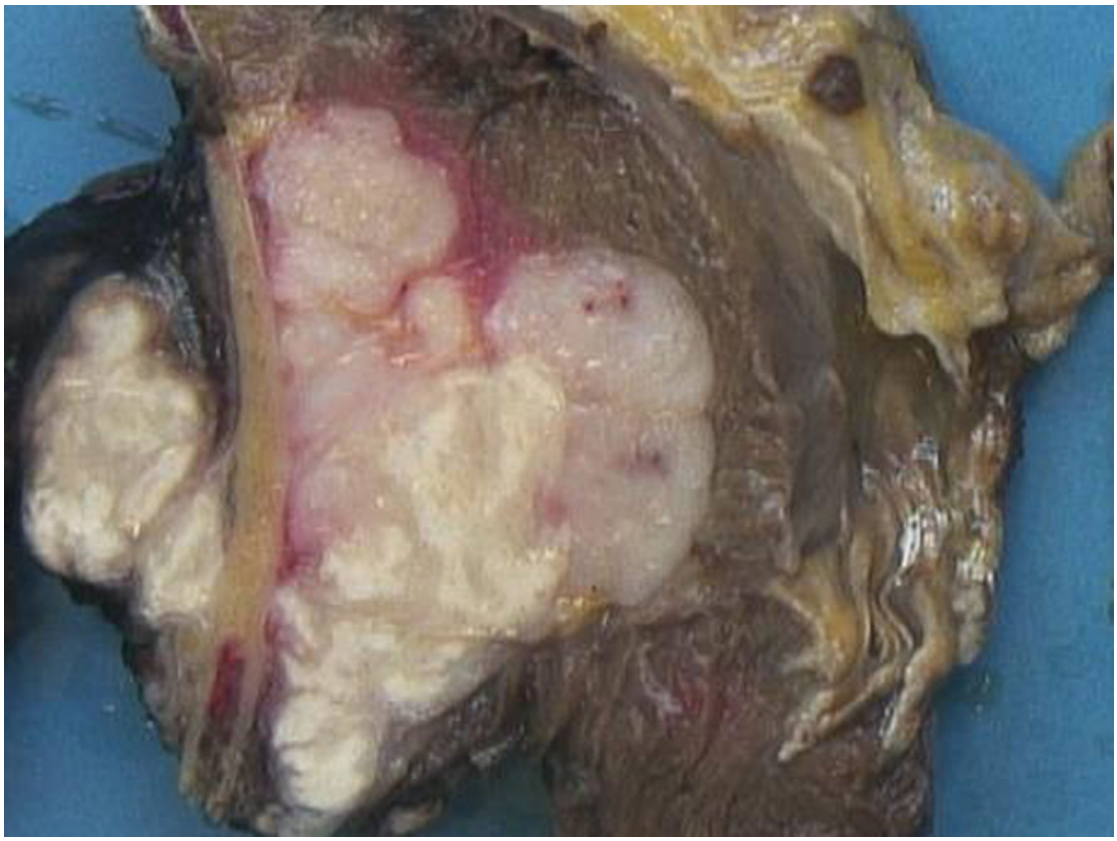

Figure 1. Gross appearance of *SRF-ICA1L* positive tumor

arising in the thoracic wall of a 49-year-old female (case 2) showing a white-grey lobulated cut surface, involving skeletal muscle on both sides of the rib.

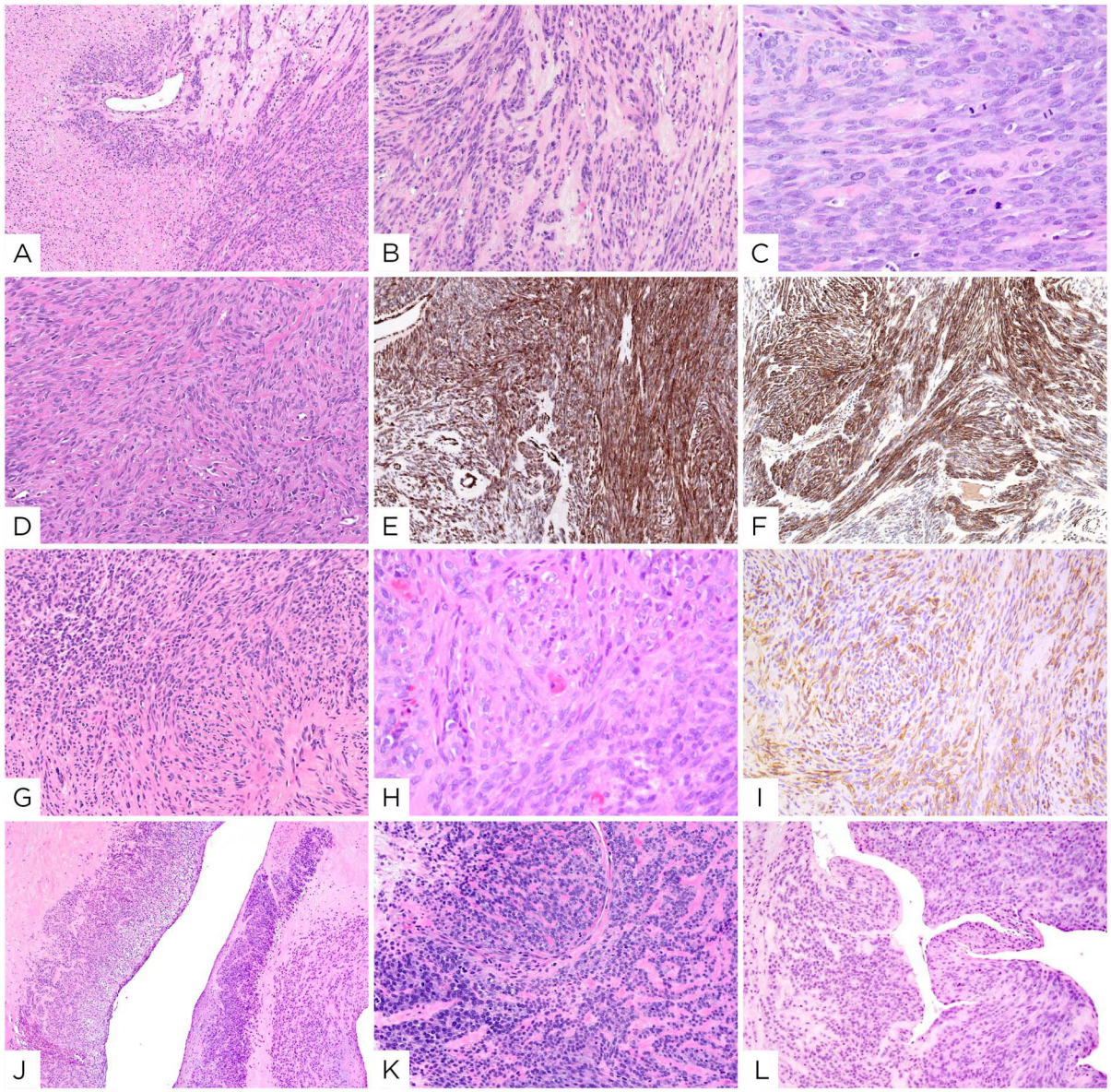

Figure 2. Histologic features of SRF-ICA1L fusion positive tumors.

A-C (case 1). Low power showing areas of variable cellularity and pathcy necrosis (lower left corner)(A); medium power showing uniform, ovoid to spindle cells arranged in files separated by hyalinized stromal component (B); at high power showing a monomorphic proliferation of ovoid cells with fine chromain and lack of nuclear pleomorphism, but displaying a brisk mitotic acitvity (C). **D-F (case 2)**. Uniformly cellular spindle cell neoplasm associated with coarse collagen bands; tumor lacked necrosis (D); IHC diffuse staining for smooth muscle actin (E) and for smooth muscle myosin heavy chain (F). **G-I (Case 3)** Tumor is associated with variable areas of cellularity and hyalinization, entrapping cells in cords (G,H); and diffuse reactivity for smooth muscle actin. **J-L (case 4)** Low power showing hypercellular components alternating with acellular hyalinized stroma. The increased cellularity often showed a perivascular distribution (J); at high power magnification revealed monomorphic ovoid cells separated by wispy collagen; cells had

open chromatin, with inconspicuous nucleoli and a high mitotic activity (K); focal areas also showed a prominent hemangiopericytic vascular pattern (L).

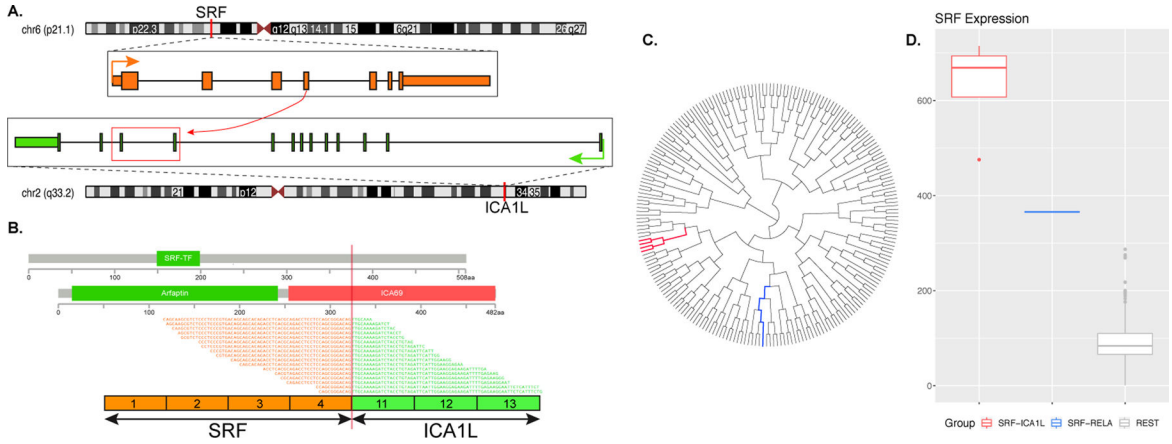

Figure 3. Diagrammatic representation of the *SRF-ICA1L* fusion based on targeted RNA sequencing results.

A. Chromosomal location of *SRF* gene locus on 6q21.1 and *ICA1L* on 2q33.2; red vertical lines depict the genomic breakpoint locus. Arrows show the direction of transcription of each gene. Red curved arrow and red box shows the exonic locations of the break in *SRF* and *ICA1L* genes, respectively. **B**. *SRF-ICA1L* transcript is composed of the first 4 exons fused to the last 3 exons of *ICA1L* (exons 11–13). The protein domains of each of the genes involved is also schematically depicted. **C**. Unsupervised hierarchical clustering shows that the 4 *SRF-ICA1L* fusion positive cases group together in a tight cluster (red), away from all the other types of sarcomas (gray), as well as from the single case with *SRF-RELA* fusion (blue). **D**. *SRF* mRNA expression is upregulated in the 4 cases investigated on the targeted RNA sequencing (red), even at higher levels than one case wtih *SRF-RELA*-positive cellular myopericytoma (green), compared to low levels of other sarcoma types available on the same platform.

Table 1.

Clinicopathological features of *SRF-ICA1L* fusion positive tumors

#	Case number	Age/Sex	Location	Size	MC	Necrosis	Clinical history	Clinical follow-up
1	ME365	23/M	buttock	11 cm	12	+	several months	NED 5 years
2	ME652	49/F	thoracic	8,5 cm	6	−	4 years	NED 2 years
3	ME700	55/M	buttock	10 cm	13	+	6 years	NA
4	ME701	35/M	hip	19 cm	>20	+	7 years	AWD 7 years

MC, mitotic count; M, male; F, female; NED, no evidence of disease NA, not available; AWD, alive with disease.

Table 2.

Immunohistochemistry of *SRF-ICA1L* fusion positive tumors

#	SMA	calponin	SMMS-1	desmin	caldesmon	CKs	EMA	S100	SOX10
1	pos	pos	pos	neg	neg	foe	neg	pos	neg
2	pos	pos	mfoc	neg	neg	mfoc	neg	mfoc	neg
3	mfoc	pos	pos	neg	neg	mfoc	neg	neg	neg
4	mfoc	pos	mfoc	neg	neg	foe	neg	foe	neg

SMA, smooth muscle actin; SMMS-1, smooth muscle myosin heavy chain I; CK, cytokeratin; EMA, epithelial membrane antigen; pos, positive; neg, negative; focal, focal; mfoc, multifocal.