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Recurrent *SRF-RELA* Fusions Define a Novel Subset of Cellular Variants in the Myofibroma/Myopericytoma Spectrum: A Potential Diagnostic Pitfall with Sarcomas with Myogenic Differentiation

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Abstract

Cellular myofibroblastic tumors other than desmoid-type fibromatosis are often diagnostically challenging due to their relative rarity, lack of known genetic abnormalities, and expression of muscle markers which may be confused with sarcomas with myogenic differentiation. In this study we investigate the molecular alterations of a group of cellular myofibroblastic lesions showing morphologic overlap with the myofibroma and myopericytoma spectrum for better sub-classification. Two index cases were studied by paired-end RNA sequencing for potential fusion gene discovery. One chest wall soft tissue tumor in a 3 month-old girl case showed a *SRF-C3orf62* fusion, while the other, a forearm lesion in an 8 year-old female, showed a *SRF-RELA* fusion. Further screening of 42 cellular examples of myofibroma/myopericytoma by FISH identified additional 8 cases with recurrent *SRF* gene rearrangements, 6 of them showing identical *SRF-RELA* fusions. The cohort was composed of 7 females and 3 males, with a wide age range of 3 months–63 years (mean 17). All tumors showed a densely packed growth of oval to spindle cells with fibrillary eosinophilic cytoplasm, arranged either in intersecting fascicles or with a distinct nested pattern around a rich vascular network. Despite the dense cellularity and variable mitotic activity none of the lesions displayed nuclear pleomorphism or necrosis. All tumors showed co-expression for SMA and desmin, in most cases with a strong and diffuse pattern of staining, while myogenin was consistently negative. No distant metastases were seen in the few cases with follow-up information. A control group of 34 well-characterized myofibroblastic tumors, including 10 typical myofibromas and 3 myopericytomas, were also investigated for *SRF* gene abnormalities by FISH and were negative. In summary, we report a subset of cellular variants of myofibroma and myopericytoma showing a smooth muscle-like immunophenotype and harboring recurrent *SRF-RELA* gene fusions, which mimic sarcomas with myogenic differentiation.

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Keywords

SRF; RELA; fusion; myofibroma; myofibroblastic; sarcoma

INTRODUCTION

Mesenchymal tumors of myofibroblastic lineage represent a morphologically diverse group of sometimes locally aggressive soft tissue tumors that encompass a wide spectrum of pathologic entities. Classification of these tumors can be controversial, due to their relatively rare incidence, morphologic features and clinical presentation overlapping with true sarcomatous processes. Especially challenging are lesions with increased cellularity, which based on their immunoexpression of SMA and/or desmin may be misdiagnosed as either leiomyosarcoma or rhabdomyosarcoma. Particularly difficult are cellular myofibroblastic proliferations in children, i.e. the cellular myofibroma spectrum, which closely resemble pediatric malignancies, such as infantile fibrosarcoma or spindle cell rhabdomyosarcoma. Additionally, most of these myofibroblastic lesions lack well-defined genetic abnormalities, hampering their objective classification.

In the present WHO classification, myofibroma(tosis) belongs to the family of pericytic/perivascular tumors, which also includes myopericytoma, glomus tumor, and angioleiomyoma¹. However, only glomus tumors have been reported to harbor recurring gene fusions involving members of the *NOTCH* family, while myofibromas and myopericytomas did not, suggesting different pathogenesis². Additionally a small subset (15%) of myopericytomas, mainly located in the skin and thyroid, harbored *BRAF* mutations, including one case with multifocal/infiltrative clinical presentation³, while familial examples of infantile myofibromatosis were reported to have *PDGFRB* gain-of-function mutations^{4–6}.

In this study we investigate a group of cellular myofibroblastic tumors, in the spectrum of cellular myofibroma and myopericytoma, in an attempt to further our understanding of their genetic alterations using various methodologies, including whole transcriptome sequencing and fluorescence in situ hybridization (FISH).

MATERIAL AND METHODS

Case selection

Based on the 2 index cases subjected to RNA sequencing and found to have recurrent *SRF* gene rearrangements, cases with similar morphology were searched from the personal consultation files or the in-house material of the 2 senior authors (CRA, CDF), previously diagnosed as cellular myofibroma, cellular myopericytoma, or cellular myofibroblastic neoplasms, NOS. Together with the two index cases a total of 44 lesions were selected after re-review of H&E and immunohistochemical slides. The study group displayed increased cellularity, with variable mitotic activity, but lacked overt features of malignancy, such as hyperchromasia, nuclear pleomorphism and necrosis. The expression of one or more muscle markers by immunohistochemistry (SMA, desmin, caldesmon) was required for diagnosis as

indication of myofibroblastic lineage, but no myogenin staining was present in any of the cases selected. A control group of 34 various myofibroblastic lesions, typically included in the differential diagnosis, were also investigated by FISH for *SRF* gene abnormalities, such as: 10 typical myofibromas (5 adult, 5 infantile variants), 3 myopericytomas, 6 malignant myopericytomas, 5 low grade myofibroblastic sarcomas, 10 myofibroblastomas, including 7 mammary-type. The study was approved by the Institutional Review Board.

RNA sequencing

Total RNA was extracted from frozen tissue from 2 index cases using RNeasy Plus Mini (Qiagen), followed by mRNA isolation with oligo(dT) magnetic beads and fragmentation by incubation at 94°C in fragmentation buffer (Illumina) for 2.5 minutes. After gel size-selection (350–400bp) and adapter ligation, the library was enriched by PCR for 15 cycles and purified. Paired-end RNA sequencing at read lengths of 50 or 51 bp was performed with the HiSeq 2000 (Illumina). All reads were independently aligned with STAR (ver 2.3)⁷ against the human reference genome (hg19). The reads were then analyzed by FusionSeq for potential gene fusions.⁸

Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR for confirming the fusion candidates was performed to validate the fusion transcript found by RNA sequencing. An aliquot of the RNA material extracted above for RNA sequencing was reverse transcribed by SuperScript III First-Strand Synthesis System (Invitrogen) into complementary DNA. PCR was performed by Advantage 2 PCR kit (Clontech, Mountain View, CA) using *SRF* exon 3 forward 5'-GCCAATGGGACTGTGCTGAAGAG -3' and *RELA* exon 11 reverse 5'-CCTCTGACAGCGTTCCTTCCC -3' primers, designed according to the RNA sequencing of the candidate fusion transcript.

Fluorescence in situ hybridization (FISH)

Formalin-fixed paraffin-embedded tissues were available in each case for FISH analysis. FISH for *SRF* and *RELA* was performed on all cases. FISH was performed on 4 µm-thick formalin-fixed paraffin-embedded (FFPE) tissue sections. Custom probes were made by bacterial artificial chromosomes (BAC) clones (Supplementary Table 2) flanking the target genes, 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>). DNA from each BACs was isolated according to the manufacturer's instructions. The BAC clones were labeled with fluorochromes by nick translation and validated on normal metaphase chromosomes. The slides were deparaffinized, pretreated, and hybridized with denatured probes. After overnight incubation, the slides were washed, stained with DAPI, mounted with an antifade solution, and then examined on a Zeiss fluorescence microscope (Zeiss Axioplan, Oberkochen, Germany) controlled by Isis 5 software (Metasystems).

RESULTS

Pathologic Findings of Cellular Myofibroma/Myopericytoma with Recurrent *SRF* Gene Fusions

The cohort was composed of 7 females and 3 males, with a wide age range at diagnosis, of 3 months – 63 years (mean 17; median 14), with most aged 18 or under. The anatomic distribution was quite broad, including 3 in the trunk (chest wall, abdominal wall, inguinal area), 2 in the upper and 2 in the lower extremities, 2 in the head and neck and one intra-abdominal, involving the small bowel wall. The tumors size varied from 1.9–7 cm (mean of 4 cm) and occurred preferentially within deep soft tissues (skeletal muscle). None of the tumors were encapsulated, 4 were well-circumscribed, while the remaining had a multinodular or focally infiltrative growth within skeletal muscle or adipose tissue (Fig. 1). Histologically, 2 cytomorphic and growth patterns were discerned: one being composed of monomorphic spindle cell arranged in intersecting fascicles (Fig. 1–2), while the other showing rounded to ovoid cells arranged in either a syncytial growth or a nested architecture around a rich capillary network (Fig. 2). Three cases showed a distinctive biphasic appearance (cases# 1, 5, 9), reminiscent of myofibroma, with a more primitive component of round to ovoid cells, in sharp demarcation from a differentiated myoid component of spindle cells with fibrillary eosinophilic cytoplasm (Fig 1). Three cases (cases #3, 6, 8) were composed predominantly of round to ovoid cells arranged in a distinctive nested arrangement, more reminiscent of a cellular myopericytoma, although no whorling pattern was discerned. The remaining cases had mostly a spindle cell appearance arranged in long fascicles. The tumor cells showed ovoid to fusiform nuclei with smooth nuclear contour, fine or vesicular chromatin, and minimal cytologic atypia. There was no hyperchromasia or marked nuclear pleomorphism noted. The mitotic activity was typically low (range 1–3 MF/10HPFs) seen in 7 cases, one case had an intermediate mitotic count of up to 8 MF/10HPFs and there were 2 outlier cases showing 10 MF/10 HPFs (Table 1). None of the cases showed tumor necrosis, but areas of infarction were noted in 4 cases, 3 being associated with dystrophic calcification (Fig. 2). Follow-up was available in 5 patients, range 24–44 months (mean 36 months). Only one patient developed two local recurrences, and he is now NED, after 44 months since original diagnosis.

There were no significant differences clinically or histologically between the *SRF*-fusion positive cellular myofibroblastic tumors and the ones that lacked *SRF* gene abnormalities.

SRF-Rearranged Cellular Myofibroblastic Tumors Display a Smooth Muscle-like Immunophenotype

All tumors showed reactivity for both SMA and desmin (Table 1). SMA expression was strong and diffuse in all cases tested (Fig. 1), while desmin was diffuse in 4 cases (Fig. 1), multifocal in 5 cases, and focally positive in one case. Caldesmon performed in 3 cases, showed diffuse positivity in one and multifocal reactivity in the other two. CD34 showed focal positivity in 3 cases. Myogenin was negative in all cases. Due to their increased cellularity and diffuse expression of muscle markers, 3 cases were initially misinterpreted as sarcomas with myogenic differentiation, including congenital spindle cell rhabdomyosarcoma, low grade leiomyosarcoma, and low grade myofibroblastic sarcoma.

RNA Sequencing Discovery of Novel and Recurrent *SRF-RELA* Gene Fusions

RNA sequencing was performed in case #1 and 2 with available frozen tissue. Case 1 showed the presence of a *SRF-C3orf62* fusion, which resulted from a t(3;6), while case 2 showed an *SRF-RELA* fusion, resulting from a t(6;11)(p21.1;q13.1) translocation (Fig 3A). The latter fusion candidate was detected by FusionSeq algorithm and the chimeric reads were confirmed by RT-PCR, showing a fusion between *SRF* exon 4 and *RELA* exon 11 (Fig 3B). Further FISH analysis confirmed both *SRF* and *RELA* break-apart signals (Fig 3 C). The fusion resulted in a significant downregulation of *SRF* mRNA distal to the break-point and an upregulation of the *RELA* last exon (exon 11) retained in the fusion (Fig 3 D).

FISH studies confirmed *SRF* gene rearrangements in 9 of 42 (21%) cases, while *RELA* gene break-part was identified in 7 of the 9 cases with *SRF* gene rearrangements (Table 1). No *SRF* gene abnormalities were identified in any of the myofibroblastic tumors included in the control group, suggesting a different molecular pathogenesis.

DISCUSSION

We report herein a group of cellular myofibroblastic tumors with morphologic features in keeping with cellular variants of myofibroma and myopericytoma harboring recurrent gene rearrangements in the *SRF* (*serum response factor*) gene, which in the majority of these cases resulted in *SRF-RELA* gene fusions. The clinical presentation included a wide age-range at diagnosis, but occurred mostly in children and young adults within the deep-seated soft tissues and skeletal muscle at various anatomic sites. The tumors displayed some worrisome histologic features, such as increased cellularity, solid or focally infiltrative growth, and in 2 cases had an increased mitotic activity of >10 MF/10HPFs and areas of infarction-type necrosis. By immunohistochemistry tumors consistently showed diffuse reactivity for SMA and in most cases for desmin as well, suggestive of a myoid/smooth muscle immunophenotype. Although there were no notable clinicopathologic differences between the *SRF*-positive and *SRF*-negative groups of cellular myofibroblastic lesions tested, the tumors with *SRF* fusions displayed a more consistent smooth muscle-like immunophenotype, with often strong and diffuse co-expression of SMA and desmin, raising the possibility of a sarcoma with myogenic differentiation. Indeed, in 3 of the *SRF*-fusion positive tumors, alternative diagnoses of low grade leiomyosarcoma, spindle cell rhabdomyosarcoma or low grade myofibroblastic sarcoma were suggested on the initial biopsies. The presence of *SRF* gene abnormalities identified only in a subset (22%) of the cellular myofibroblastic tumors tested further reinforces the genetic heterogeneity within the family of pericytic/perivascular tumors, which encompasses not only myofibroma and myopericytoma, but also glomus tumors and angioleiomyoma. Myofibroma and myopericytoma exist on a morphologic continuum, and any separation is to some extent artificial, most cases being defined based on the classic ends of the spectrum. All lesions investigated here were more cellular than 'classic' examples of myofibroma or myopericytoma, having more prominent thin-walled vessels than usual myofibroma, hence exaggerating the overlap with myopericytoma. The distinct smooth muscle-like phenotype seen in the present subset of cellular myofibroma/myopericytoma harboring *SRF* gene fusions suggests a genetically distinct group of tumors associated with a more differentiated

immunophenotype toward myoid lineage. Furthermore none of these *SRF* genetic abnormalities were noted in the control group including classic examples of both myofibroma and myopericytoma. Additionally a recent study has demonstrated that in fact >70% of both classic adult and infantile myofibromas are associated with distinct genetic abnormalities, i.e. *PDGFRB* mutations, different from our cohort of cases⁹. Moreover, our previous studies have shown that recurrent *NOTCH* gene rearrangements were only identified in glomus tumors, while the other pericytic/ perivascular tumors do not.²

The *serum response factor (SRF)* gene on 6p21.1 encodes a ubiquitous nuclear protein, which is a member of MADS (MCM1, Agamous, Deficiens and SRF) box superfamily of transcription factors, which binds to the serum response element (SRE) in the promoter region of target genes. This conserved transcription factor during evolution is at the confluence of multiple signaling pathways controlling the transcription of immediate-early response genes and muscle-specific genes, including the homeostasis of cardiac, smooth and skeletal muscle tissues. SRF is required for normal cardiac development and maturation; mouse models of tamoxifen inducible cardiac specific *SRF* knockout led to heart failure with dilated cardiomyopathy, while constitutive overexpression of *SRF* gene leads to concentric hypertrophic cardiomyopathy.¹⁰ Furthermore, inactivation of SRF in smooth muscle cells during embryonic development leads to a decrease in the number of perivascular progenitor cells as well as defects in the cyto-architecture, suggesting a crucial role for SRF in vascular smooth muscle cell differentiation.¹¹ Several lines of evidence suggest a role for SRF in modulating muscle plasticity; including *SRF*-mutant mice developing growth retardation and a major decrease in muscle mass, the latter resulting from downregulation of skeletal actin and other contractile genes. *SRF*-related gene fusions have been previously reported in one case of congenital/spindle cell rhabdomyosarcoma, showing an *SRF-NCOA2* fusion.¹²

RELA proto-oncogene, a.k.a. *nuclear factor-kappa-B (NF-kB) subunit*, encodes a ubiquitous transcription factor involved in several biological processes. Upon activation through inhibitor degradation, RELA translocates from the cytoplasm to the nucleus and activates transcription of specific genes. Similar to *SRF*, *RELA* has also been implicated in oncogenic transformation through recurrent chromosomal translocations, with two-thirds of supratentorial ependymomas containing oncogenic fusions between *RELA* and an uncharacterized gene, *C11orf95*. Similar to *RELA* fusion-positive ependymomas, our index case (Case 2) with *SRF-RELA* fusion also showed up-regulation of the exon 11 *RELA* mRNA distal to the breakpoint, containing the transactivation domain (TAD) (Fig 3). In ependymomas, the RELA fusion protein accumulates preferentially in the nucleus of ependymoma cells compared to the wild-type RELA protein, which is sequestered in the cytoplasm of unstimulated controls¹³, indicating pathologic activation of the NF-kB pathway.¹⁴

With the advent of next-generation sequencing, an increasing number of novel gene fusions have emerged recently in the spectrum of atypical/malignant myofibroblastic tumors. In one such study, Haller et al¹⁵ described 4 patients (2 children and 2 adults) with soft tissue tumors harboring *NTRK1*-related fusions, displaying a variety of morphologies and immunophenotype, including infantile HPC, myofibroblastic sarcoma and myopericytoma-

like. Their cases showed consistently high mitotic activity, ranging from 12–17 MF/ 10 HPFs. It remains unclear if these *NTRK1*-rearranged atypical/malignant myofibroblastic tumors have any relationship to our cohort of cellular myofibroblastic lesions, since all 20 cases of myofibromas/myopericytomas, spanning classic, cellular and malignant examples, from our files lacked *NTRK1* genetic abnormalities¹⁶. We believe that the cases described recently by Linos and colleagues¹⁷, as ‘myofibroma with atypical features’, based on increased cellularity, infiltrative borders and increased mitotic activity (but not increased pleomorphism), resemble closely our cohort and may represent *SRF*-positive cellular variants of myofibroma or myopericytoma as described here.

The differential diagnoses of cellular myofibroblastic tumors typically encompass low grade sarcomas with variable myogenic differentiation by immunohistochemistry. Low grade leiomyosarcoma might be difficult to distinguish from our cohort due to the smooth muscle-like immunophenotype exhibited, however, none of the *SRF*-positive cellular myofibroblastic tumors showed the typical elongated cigar-shaped nuclei with nuclear hyperchromasia and overt pleomorphism. Although some of the *SRF*-positive tumors had a distinct fascicular growth pattern and diffuse desmin reactivity reminiscent of spindle cell rhabdomyosarcoma, none of the cases showed myogenin positivity expected with a skeletal muscle lineage. Another lesion often considered in the differential diagnosis is a low grade myofibroblastic sarcoma, which may show significant overlap in both cytomorphology and immunoprofile, but which displays cytologic atypia at least focally, which was completely lacking in our study group.

In conclusion, we describe recurrent *SRF* gene abnormalities, resulting in novel *SRF-RELA* gene fusions in a subset of cellular myofibroblastic tumors in the spectrum of cellular myofibroma/myopericytoma. In fact none of the *SRF*-positive lesions fit the criteria for typical myofibroma or myopericytoma, all displaying atypical and worrisome features, such as: diffuse hypercellularity, increased mitotic figures and in some cases a distinct immunoprofile, with often diffuse and strong co-expression of desmin and SMA, reminiscent of a smooth muscle phenotype. This constellation of findings may represent a potential diagnostic pitfall in children and young adults, causing possible confusion with sarcomas with myogenic differentiation. The follow-up available to date in these patients suggests a benign clinical course, with rare local recurrences if incompletely excised and lack of metastatic potential. The RNA sequencing from one of the index cases indicated that the *SRF-RELA* fusion triggers opposite effects on the fusion gene partners, with downregulation of *SRF* mRNA distal to the fusion, while the *RELA* last exon retained in the fusion showed marked overexpression. Thus further functional studies are required to determine if the pathogenesis of these cellular myofibroblastic tumors is induced by the truncated expression of the muscle specific SRF transcription factor, or driven through *RELA* upregulation and subsequent NF-κB activation, similar to ependymomas, or through a combination of both processes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Biphase morphologic appearance in cellular myofibroblastic tumors with *SRF* gene rearrangements

(A) Solid and multinodular growth infiltrating into skeletal muscle and protruding into dilated blood vessels (case 5, 100×); (B,C) Tumor showed a biphasic phenotype, with predominant areas of round-ovoid cells with high N/C ratio and vague nesting (B) and a focal myoid component with cells showing more abundant eosinophilic cytoplasm (C); (case 5, 200×); (D–I) A second tumor with biphasic growth (case 1) showing an abrupt transition (D) between a more primitive cellular component (E) and cells with fibrillary cytoplasm,

resembling smooth muscle (F). Both components displayed a fascicular growth and were diffusely positive for both SMA (G) and desmin (H), and focally positive for CD34 (I).

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Figure 2. Morphologic features of cellular myofibroblastic tumors with recurrent *SRF-RELA* fusions

(A) Bland round to ovoid cells arranged in a distinctive nested architecture separated by a rich vascular component with a sinusoidal-like pattern (case 6, 200×); (B) Ovoid cells with ill-defined cell borders, fibrillary eosinophilic cytoplasm and uniform vesicular nuclei, arranged in a solid growth pattern (case 6, 200×); (C) Bland spindle cells arranged in intersecting fascicles (case 4, 200×) (D) Ovoid to fusiform cells with vesicular nuclei and small nucleoli, arranged in a syncytial growth (case 2, 200×); (E) Monomorphic round to

ovoid cells arranged in a loose myxoid stroma (case 8, 200×); (F) area of infarction associated with dystrophic calcification (case 6, 100×).

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Figure 3. RNA sequencing discovery of *SRF-RELA* novel gene fusion and experimental validation

(A,B) Transcriptome sequencing detected a fusion between SRF on 6p21.1 and RELA on 11q13.1. The domains retained in the projected fusion protein includes the MADS domain of SRF and the TAD domain of RELA (B, upper panel). RT-PCR confirming *SRF* exon 4 being fused to *RELA* exon 11 (B, lower panel). (C) FISH showing break-apart signals (arrows) for both SRF (left panel) and RELA (right panel) (red, centromeric, green, telomeric). (D) The exon expression showed a significant decrease in the SRF mRNA level distal to the breakpoint, while no mRNA changes are noted in RELA exons, compared to other myofibroma cases lacking this fusion.

Table 1

Clinicopathologic features of cellular variants of myofibroma/myofibroblastoma spectrum with *SRF*-related fusions

#	Age Sex	Location	Histologic Features/ MF/10HPFs	IHC-positivity	Gene abnormality	FU
1 [*]	0mo /F	Chest wall (deep, 1.9 cm)	Spindle cells, intersecting fascicles; 3/10 HPFs	SMA D, desmin D, CD34 Fp	<i>SRF-C3orf62</i>	NED, 24 mo
2 [*]	8/F	Forearm (deep, 2.8 cm)	Spindle cells, intersecting fascicles; 2/10 HPFs	SMA D, caldesmon D, desmin MF	<i>SRF-RELA</i>	NED, 28 mo
3	3/F	Abdominal wall (deep, 3 cm)	Ovoid cells, nested 1/10 HPFs	SMA D, desmin MF, caldesmon MF	<i>SRF-RELA</i>	NED, 42 mo
4 [#]	6/M	Knee	Spindle cells, intersecting fascicles; 1/10 HPFs	SMA D, desmin Fp, CD34 Fp	<i>SRF-RELA</i>	NED, 44 mo
5	18/ M	Upper arm (deep, 4.5 cm)	Ovoid-spindle, nested to short fascicles; 10/10HPFs	SMA D, desmin D	<i>SRF</i>	NED, 42 mo
6	28/ M	Inguinal area (s- c, 7 cm)	Ovoid cells, nested, infarction; 1/10 HPFs	SMA D, desmin MF, Caldesmon Fp	<i>SRF-RELA</i>	NA
7	40/F	Neck (deep)	Spindle cells, intersecting fascicles, infarction; 14/10 HPFs	SMA D, desmin MF	<i>SRF-RELA</i>	NA
8	63/F	Small bowel wall (4.5 cm)	Round-ovoid, discohesive; 1/10 HPFs	desmin D, CD34 MF	<i>SRF-RELA</i>	NA
9	13/F	Chin (3.5 cm, deep, abuts bone)	Ovoid-spindle, infarction, calcification; 8/10 HPFs	SMA D, desmin MF	<i>SRF-RELA</i>	NA
10	0/F	Foot (5.0 cm)	Spindle cells, intersecting fascicles, central infarction, calcification; 2/10 HPFs	SMA D, desmin D	<i>SRF</i>	NA

^{*} tested by RNA seq;

[#] history of whole body irradiation for AML at age 1, developed 2 local recurrences s/p incomplete excision;

mo, months; F, female; M, male; MF/10HPFs, mitotic figures/10 high power fields; Fp, focal; MF, multifocal; D, diffuse; FU, follow-up; mo, months; NED, no evidence of disease, NA, not available.