



# Histiocyte-rich rhabdomyoblastic tumor: rhabdomyosarcoma, rhabdomyoma, or rhabdomyoblastic tumor of uncertain malignant potential? A histologically distinctive rhabdomyoblastic tumor in search of a place in the classification of skeletal muscle neoplasms

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## Abstract

Skeletal muscle tumors are traditionally classified as rhabdomyoma or rhabdomyosarcoma. We have identified an unusual adult rhabdomyoblastic tumor not clearly corresponding to a previously described variant of rhabdomyoma or rhabdomyosarcoma, characterized by a very striking proliferation of non-neoplastic histiocytes, obscuring the underlying tumor. Ten cases were identified in nine males and one female with a median age of 43 years (range 23–69 years). Tumors involved the deep soft tissues of the trunk ( $N = 4$ ), lower limbs ( $N = 4$ ), and neck ( $N = 2$ ). Tumors were well-circumscribed, nodular masses, frequently surrounded by a fibrous capsule containing lymphoid aggregates and sometimes calcifications. Numerous foamy macrophages, multinucleated Touton-type giant cells, and sheets/fascicles of smaller, often spindled macrophages largely obscured the underlying desmin, MyoD1, and myogenin-positive rhabdomyoblastic tumor. Cases were wild type for *MYOD1* and no other mutations or rearrangements characteristic of a known subtype of rhabdomyoma or rhabdomyosarcoma were identified. Two of four cases successfully analyzed using a next-generation sequencing panel of 170 common cancer-related genes harbored inactivating *NFI* mutations. Next-generation sequencing showed no gene fusions. Clinical follow (nine patients; median 9 months; mean 23 months; range 3–124 months) showed all patients received wide excision; four patients also received adjuvant radiotherapy and none received chemotherapy. At the time of last follow-up, all patients were alive and without disease; no local recurrences or distant metastases occurred. We hypothesize that these unusual tumors represent rhabdomyoblastic tumors of uncertain malignant potential. Possibly over time they should be relegated to a new category of skeletal muscle tumors of intermediate (borderline) malignancy.

## Introduction

It is now well-recognized that many soft tissue tumors cannot be neatly categorized as simply “benign” or “malignant”. Thus, the current WHO Classification of Tumors of Soft Tissue and Bone recognizes an “intermediate” (borderline) category for several types of soft tissue tumors (e.g., atypical lipomatous tumor in adipocytic tumors and hemangioendothelioma in endothelial tumors) [1]. Some soft tissue tumors are by definition considered to be of intermediate malignancy, either because of their capacity for locally aggressive growth (e.g., desmoid-type fibromatosis and hemosiderotic fibrolipomatous tumor) or because of their low but unpredictable risk of distant metastasis (e.g., angiomatoid fibrous histiocytoma and ossifying fibromyxoid tumor) [1]. In other soft tissue

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neoplasms, such as glomus tumors [2] and perivascular epithelioid cell tumors [3], classification systems have been developed that not only predict clinically benign and clinically malignant tumors with reasonable sensitivity and specificity, but also classify subsets of such tumors as having “uncertain malignant potential,” usually reflecting limited clinical data. Finally, selected soft tissue tumors, such as gastrointestinal stromal tumors [4] and solitary fibrous tumors [5], are best viewed from a “risk assessment” perspective rather than as benign or malignant.

Tumors showing skeletal muscle differentiation, however, continue to be dichotomized as either benign rhabdomyomas or fully malignant rhabdomyosarcomas (RMSs). Rhabdomyomas are rare tumors typically involving the head/neck [6, 7] or genital region [8], consisting of non-infiltrative proliferations of well-differentiated skeletal muscle with epithelioid (adult rhabdomyoma) or spindled (fetal and genital rhabdomyoma) morphology. RMSs are more common, occurring most often in children, and can be classified as embryonal, alveolar, pleomorphic, and spindle cell/sclerosing subtypes [1, 9]. Skeletal muscle tumors of intermediate malignancy or of uncertain malignant potential are not currently recognized.

Over the past several years, we have seen in consultation a small number of very unusual tumors of skeletal muscle origin in adults, which do not clearly correspond to a previously described variant of rhabdomyoma or RMS, showing distinctive morphological features including a striking histiocytic infiltrate. Although we originally regarded these tumors as variants of RMS, we have come to appreciate that these tumors lack the typical features of RMS, such as diffuse infiltration or mitotic activity, and instead show morphological features suggestive of a slowly growing, indolent tumor. We therefore undertook this study, in order to more fully elucidate the clinicopathological features of these rare tumors, herein provisionally termed “histiocyte-rich rhabdomyoblastic tumors”, and to define their proper place in the classification of skeletal muscle neoplasms.

## Materials and methods

### Case selection

Approval for the study was granted by the Institutional Review Boards of Mayo Clinic, Emory University, St. Jude Children’s Research Hospital, Erlangen University and Chang Gung Hospital. The initial group of seven cases were identified through search of the Mayo Clinic institutional and consultation archives (January 1990–November 2017) for adult cases previously classified as “rhabdomyosarcoma” and noted to contain large numbers of histiocytes.

Previously treated tumors were excluded. All available slides and blocks were retrieved and re-reviewed. Following poster presentation of these seven cases at the 2018 United States and Canadian Academy of Pathology annual meeting (Vancouver, BC, Canada), three similar cases were identified from the consultation archives of Drs Weiss, Agaimy, and Huang.

### Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections from each case using antibody-specific epitope retrieval techniques with Dako Envision (Dako, Carpinteria, CA, USA) automated system for detection of the following primary antigens: Desmin (DE-R-11, 1:100, Leica, Newcastle Upon Tyne, UK), MyoD1 (EP212, 1:25–1:100, Cell Marque, Rocklin, CA, USA), Myogenin (F5D, 1:25–1:50, Dako), and CD163 (10D6, 1:200, Leica, Newcastle Upon Tyne, UK).

### MyoD1 mutational analysis

Mutational analysis targeting the spindle cell/sclerosing RMS-associated *MyoD1* mutations was performed at St. Jude Children’s Research Hospital for cases 1–5. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue sections using the Maxwell 16 formalin-fixed, paraffin-embedded Tissue LEV DNA Purification Kit (Promega, Madison, WI), according to the manufacturer’s protocol. A recurrently mutated site in *MYOD1* was amplified with GoTaq Long PCR Master Mix (Promega) with primers forward 5′-CAAGCGCAAGACCACCAAC-3′ and reverse 5′-GGTTTGGATTGCTCGACGTG-3′ for 45 cycles at 95 °C for 15 s, at 62 °C for 20 s, and at 68 °C for 30 s. PCR reaction products were purified with ExoSAP-IT (Thermo Fisher Scientific, Waltham, MA) and directly sequenced by Sanger sequencing. Wild-type or mutant sequences were visualized at the location of p.L122 with CLC Main Workbench sequence analysis software version 6.0.2 (CLC bio, Cambridge, MA).

### Archer sarcoma FusionPlex next-generation sequencing

Case 8 was tested at Emory University with the Archer Sarcoma FusionPlex next-generation sequencing assay for rearrangements involving the *ALK*, *CAMTA*, *CCNB*, *CIC*, *EPC*, *EWSR1*, *FOXO1A*, *FUS GLI*, *HMGA*, *JAZF*, *MEAF6*, *MKL2*, *NCOA2*, *NTRK2*, *PDGFB*, *PLAG1*, *ROS1*, *SS18*, *STAT6*, *TAF15*, *TCF12*, *TFE3*, *TFG*, *USP6*, and *YWHAE* genes using standard laboratory methods.

## NGS analysis

Initially, molecular analysis (aiming to identify gene fusions) was performed on Cases 8–10 using the TruSight RNA Fusion panel (Illumina, Inc., San Diego, CA, USA). RNA was isolated from formalin-fixed, paraffin-embedded tissue sections using the RNeasy FFPE Kit (Qiagen, Hilden, Germany) and quantified spectrophotometrically using NanoDrop-1000 (Thermo Fisher Scientific, Wilmington, USA). A total of 500 ng RNA was used as a starting material. Specifically, cDNA was first synthesized followed by adaptor ligation, amplification of the total cDNA, hybrid capture of 507 fusion-associated genes, and sequencing on the MiSeq instrument using MiSeq Reagent Kit v3 (150 cycles) according to the protocol of Illumina (San Diego, USA). The resulting raw data (fastq files) were then processed by the Illumina's BaseSpace RNA-Seq Alignment workflow with STAR aligner. No fusion event was identified in four cases. Therefore, further molecular testing was initiated on tumor DNA extracted from formalin-fixed, paraffin-embedded tissue using the TST170 cancer panel (Illumina). Briefly, DNA was extracted from formalin-fixed, paraffin-embedded tissue sections with the QIAamp DNA FFPE Tissue Kit (Qiagen) and quantified with NanoDrop-1000 (Thermo Fisher Scientific). Approximately 40 ng DNA was used for library preparation using the TruSight Tumor 170 gene panel (Illumina). Libraries were sequenced on a NextSeq 550 machine (Illumina), and alignment and variant calling was done using the TST170 BaseSpace app (Illumina). Variants were manually filtered against population databases and non-synonymous variants were visually controlled using the integrative genome viewer.

## Results

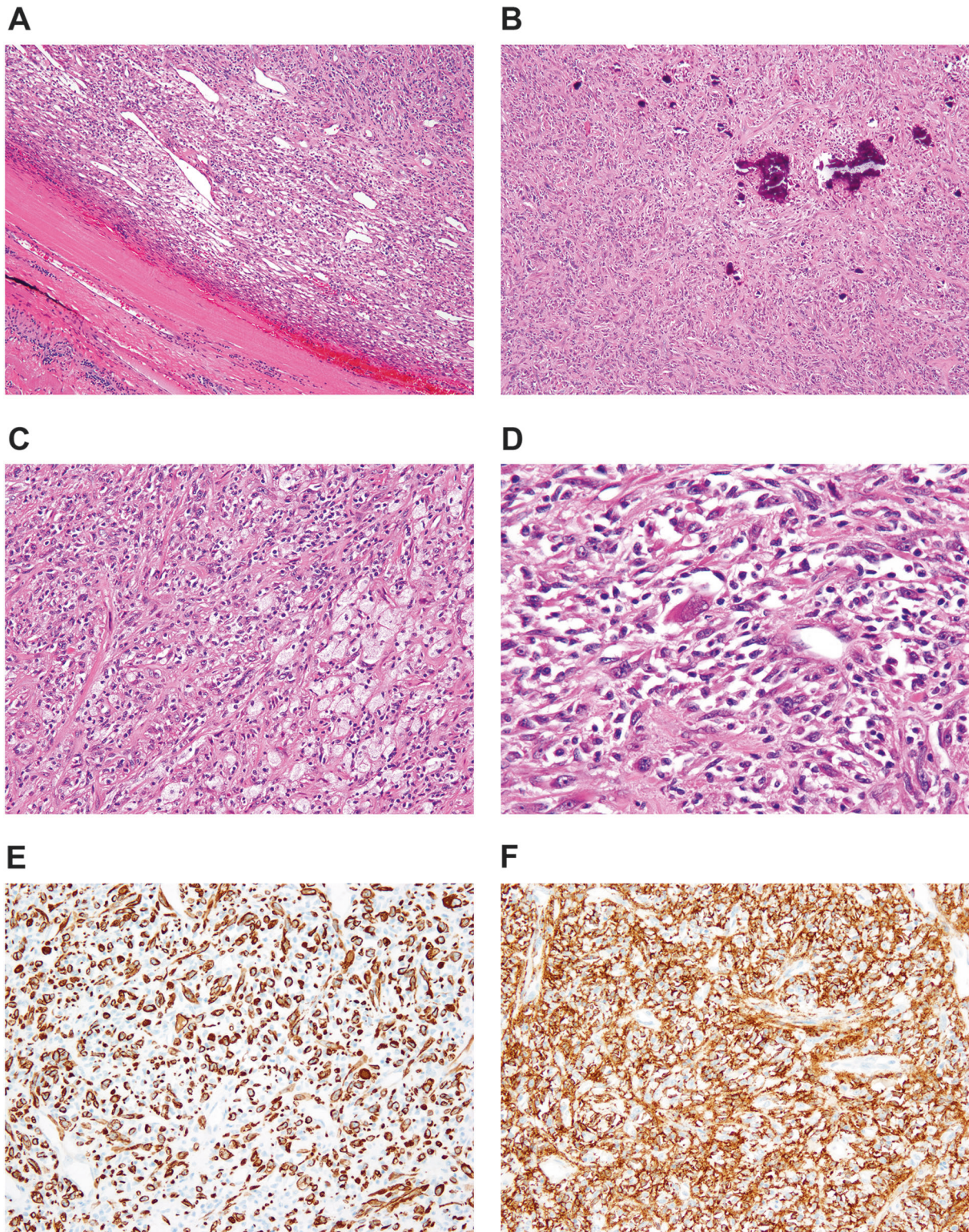
Table 1 summarizes the clinicopathologic features of these ten cases. The tumors occurred in adults ranging from 23 to 69 years of age (median age 43 years), showed a striking male predilection (9M:1F), and involved the deep soft tissues of the trunk ( $N=4$ ), lower limbs ( $N=4$ ), and neck ( $N=2$ ). Patients presented with nonspecific soft tissue masses, slowly growing over a period of 1–2 years in three cases. The tumors ranged from 2.9 to 8.0 cm (median 3.5 cm). The cases were referred in consultation with a range of differential diagnoses, which included RMS in only a minority of cases.

The morphologic and immunohistochemical features of four histiocyte-rich rhabdomyoblastic tumors are illustrated in Fig. 1 (Case 7), Fig. 2 (Case 4), Fig. 3 (Case 1), and Fig. 4 (Case 5). Microscopically, the tumors grew as well-circumscribed, nodular masses, frequently surrounded by a hyalinized fibrous capsule containing lymphoid aggregates

**Table 1** Clinicopathological, immunohistochemical, and molecular genetic findings

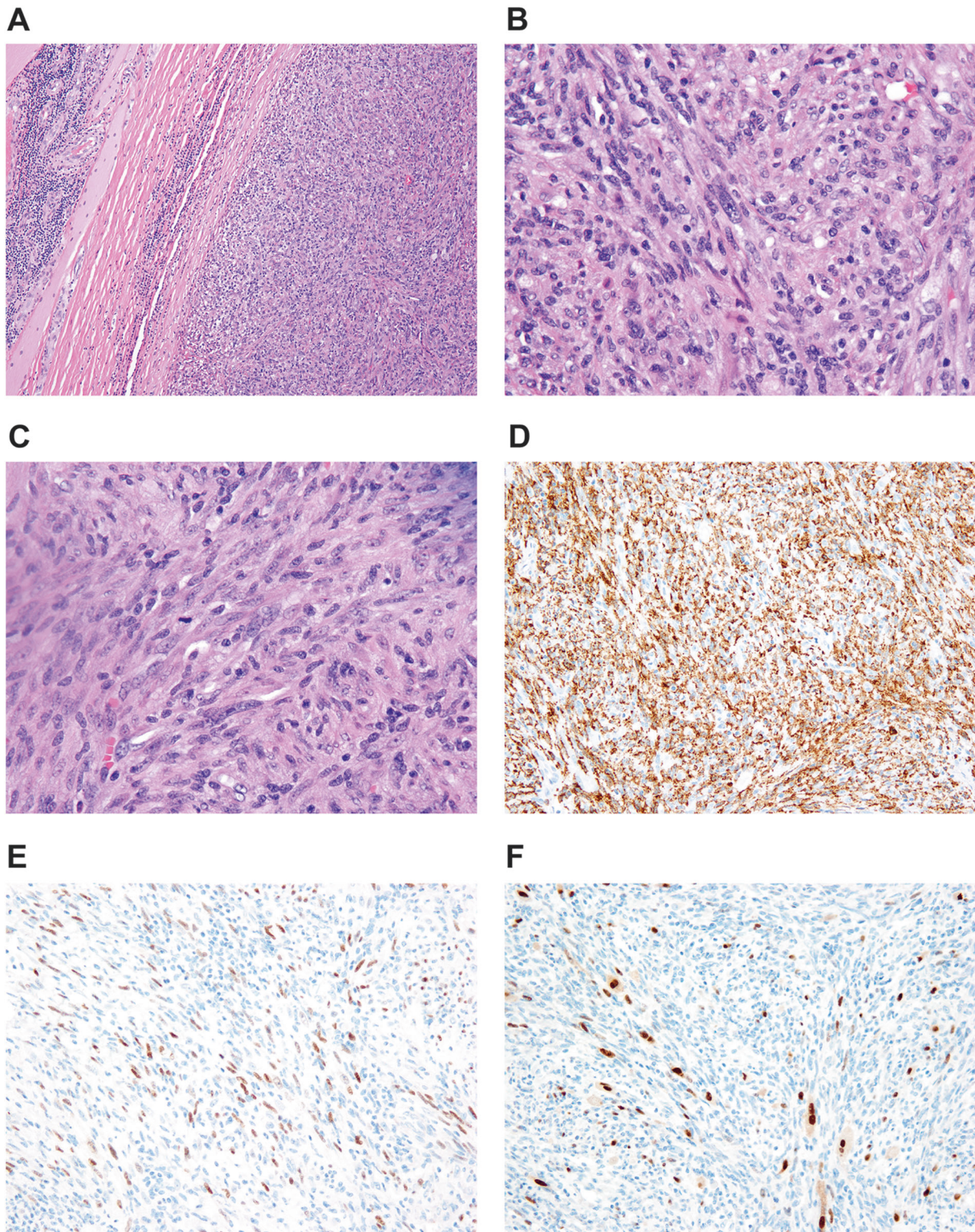
| Case | Age/ sex | Site/size (cm)         | Desmin | MyoD1 | Myogenin | S100/<br>SOX10 | F/U (months) | LR | Metis | Outcome | Other findings   |
|------|----------|------------------------|--------|-------|----------|----------------|--------------|----|-------|---------|--|
| 1    | 69/M     | Buttock/NA             | 3+     | 2+    | Positive | ND             | 13           | No | No    | ANED    | Negative for <i>MYOD1</i> mutations by Sanger sequencing   |
| 2    | 64/F     | Abdominal wall/<br>8.0 | 3+     | 2+    | Rare     | 0              | 14           | No | No    | ANED    | Negative for <i>MYOD1</i> mutations by Sanger sequencing   |
| 3    | 26/M     | Thoracic wall/NA       | 3+     | 2+    | Rare     | ND             | 124          | No | No    | ANED    | Negative for <i>MYOD1</i> mutations by Sanger sequencing; no genetic alterations identified by NGS |
| 4    | 44/M     | Left knee/3.3          | 3+     | 2+    | 1+       | 0              | 24           | No | No    | ANED    | <i>NF1</i> mutation identified by NGS  |
| 5    | 41/M     | Left thigh/2.9         | 3+     | 2+    | 1+       | 0              | 5            | No | No    | ANED    | Negative for <i>MYOD1</i> mutations by Sanger sequencing; <i>NF1</i> mutation identified by NGS    |
| 6    | 53/M     | Back/3.5               | 3+     | 2+    | 1+       | 0              | 8            | No | No    | ANED    | Negative for <i>MYOD1</i> mutations by Sanger sequencing   |
| 7    | 45/M     | Left neck/NA           | 3+     | 2+    | 1+       | ND             | 9            | No | No    | ANED    | ND   |
| 8    | 33/M     | Thigh/7.0              | 3+     | 0     | 1+       | ND             | NA           | NA | NA    | NA      | No genetic alterations identified by ARCHER  |
| 9    | 23/M     | Thigh/7.2              | 3+     | 1+    | 1+       | 0              | 4            | No | No    | ANED    | No genetic alterations identified with TST170 panel  |
| 10   | 26/M     | Left neck/3            | 3+     | 1+    | 1+       | 0              | 3            | No | No    | ANED    | Concurrent schwannoma of the thigh; no clinical features of NF1                                    |

ANED alive, no evidence of disease. F/U follow-up. LR local recurrence, Metis distant metastasis, NA not available, ND not done, *NF1* neurofibromatosis type-1, NGS next-generation sequencing, WT wild type, 0 negative, 1+ positive in 5–10% of cells, 2+ positive in 11–50% of cells, 3+ positive in >51% of cells



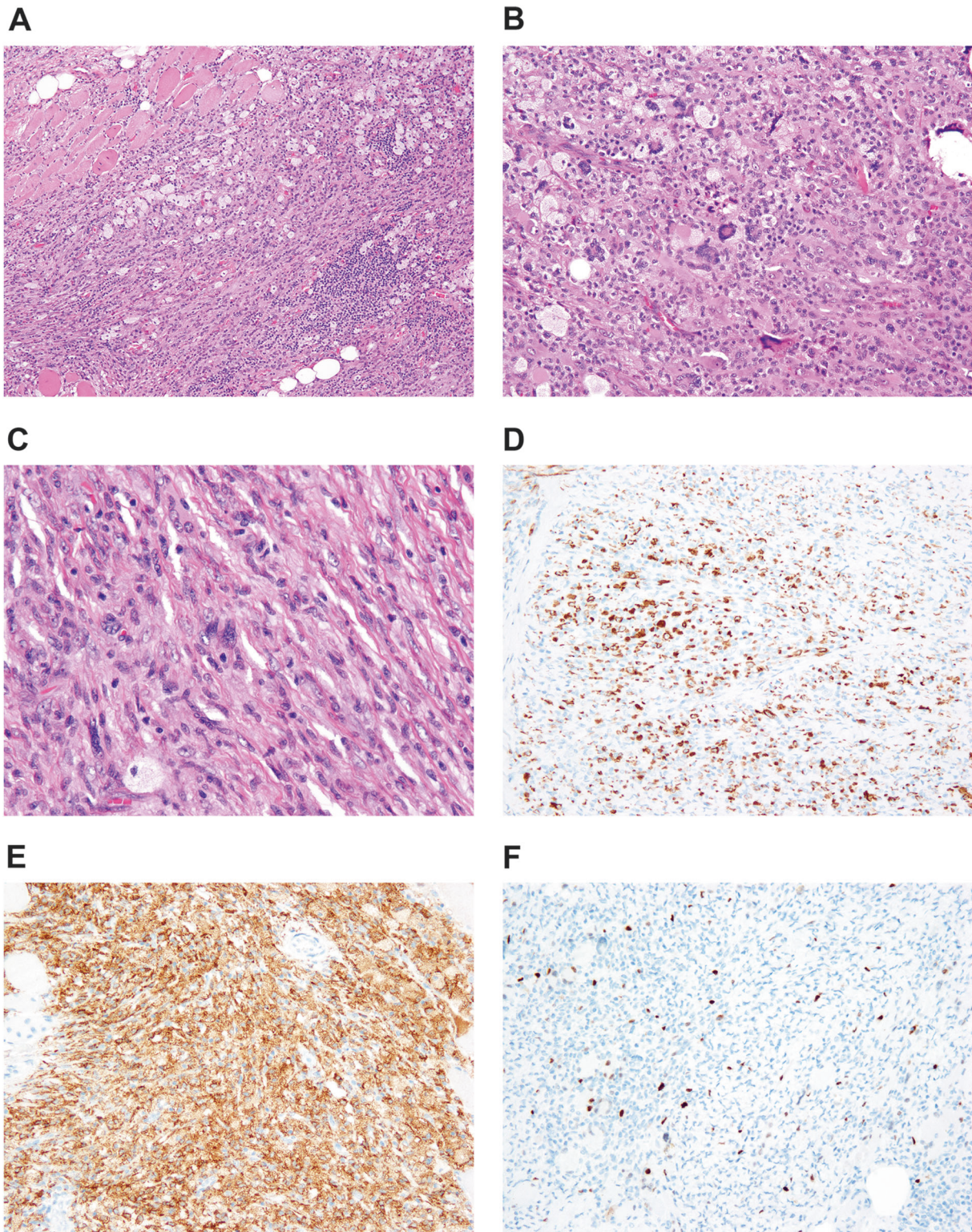
**Fig. 1** The tumors typically grew in a circumscribed manner, often with a well-formed, hyalinized fibrous capsule (**a**). Other features suggesting a slowly growing tumor, such as calcifications, were often present (**b**). Large numbers of foamy macrophages partially obscured

the tumor cells (**c**). Close examination, however, showed atypical rhabdomyoblastic cells with enlarged, hyperchromatic nuclei (**d**). Desmin (**e**) and CD163 (**f**) immunostains highlight the neoplastic cells and macrophages



**Fig. 2** A well-circumscribed, encapsulated mass with peripheral lymphoid aggregates is seen (**a**). The tumor consisted of a fascicular proliferation of spindled cells, with occasional cells having nuclear enlargement and hyperchromatism (**b**). Mitotic activity was extremely

low, however (**c**). Immunohistochemistry showed the overwhelming majority of these spindled cells to represent CD163-positive macrophages (**d**), with only a very small minority of cells positive for desmin (not shown), MyoD1 (**e**), and myogenin (**f**)

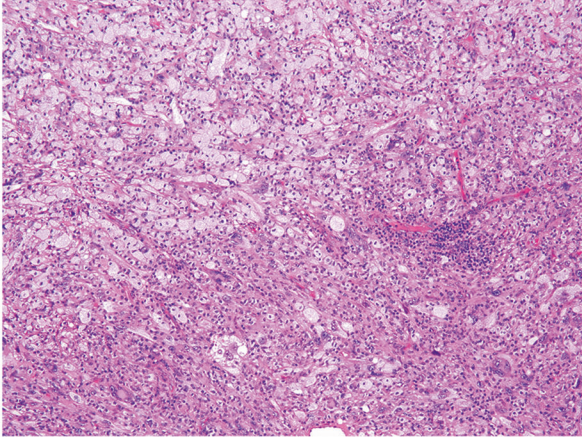
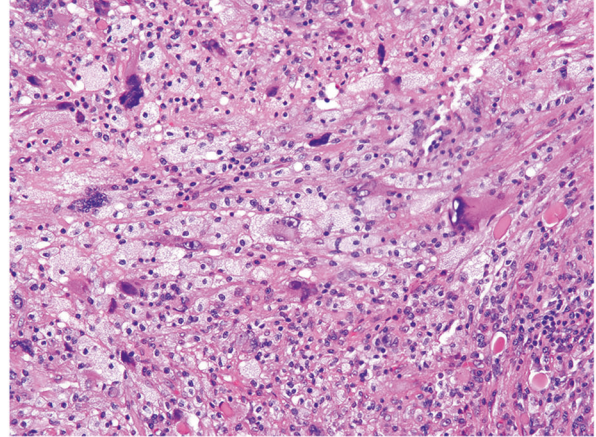
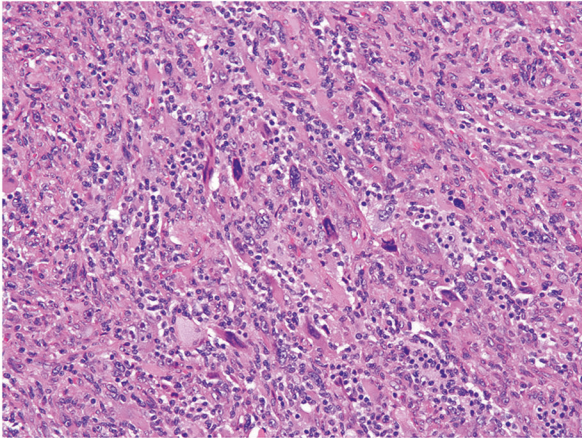
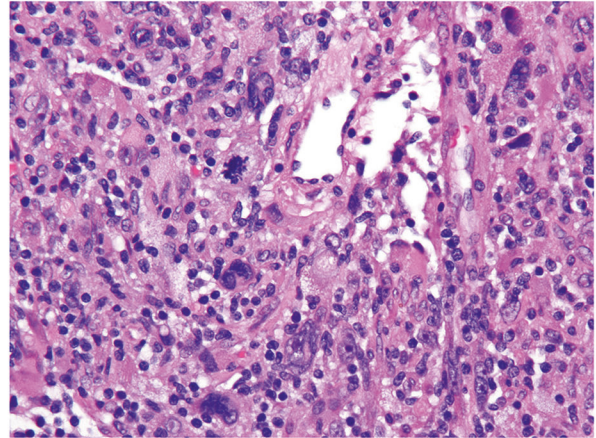
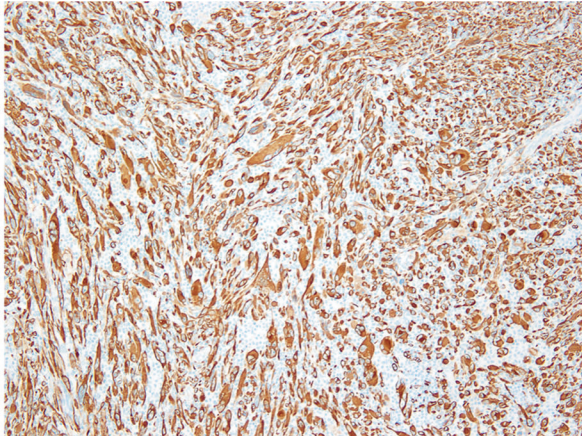
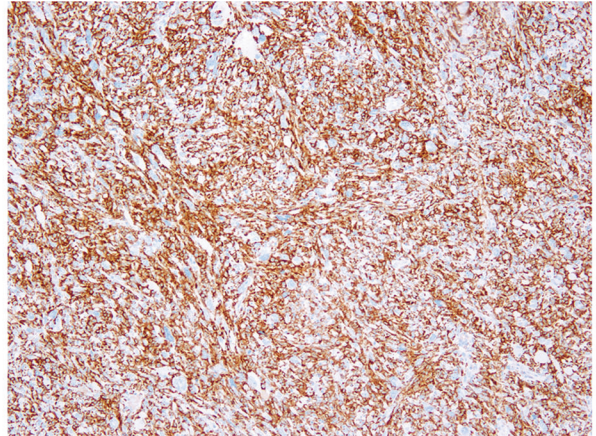


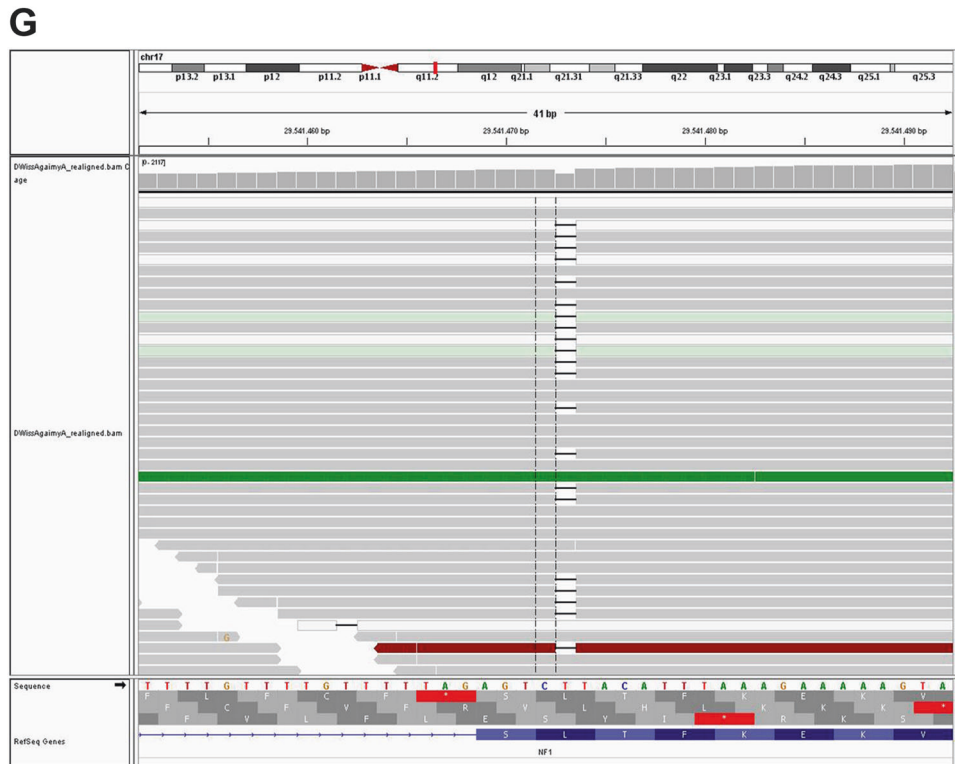
**Fig. 3** Although the tumors tended to grow in a circumscribed manner, as illustrated in Figs. 1 and 2, infiltrative growth into muscle and fat was also present (a). The neoplastic cells were frequently obscured by foamy macrophages and Touton-type giant cells (b). At higher magnification, spindled cells with enlarged, hyperchromatic nuclei were visible. Mitotic activity was extremely low, less than 1/50 HPF in all

tumors (c). These spindled cells were highlighted on desmin immunostains (d). The overwhelming majority of the mass represent CD163-positive histiocytes (e). Myogenin (shown) and MyoD1 expression, although present in all cases, was typically very limited in extent (f)

and sometimes calcifications. Closer examination of all cases, however, showed at least small areas with infiltrative growth into the surrounding skeletal muscle and fat. Perhaps the most striking feature of these tumors was the presence of numerous large foamy macrophages,

multinucleated Touton-type giant cells, and sheets/fascicles of smaller, often spindled macrophages. In several cases, this histiocytic infiltrate largely obscured the underlying neoplastic cell population. However, careful inspection of all cases disclosed distinctly eosinophilic tumor cells, either

**A****B****C****D****E****F**



**Fig. 4** A diffuse proliferation of foamy histiocytes almost entirely obscures the underlying rhabdomyoblastic tumor (**a, b**). Most lesions tended to demonstrate a low power histiocytic nature (**a**) with admixed foamy macrophages (**b**). The tumor itself was composed of variably pleomorphic spindled cells with abundant, often “glassy”-appearing, eosinophilic cytoplasm. Numerous small histiocytes and other chronic

inflammatory cells were also typically present (**c**). Mitotic activity, although present, was extremely low (< 1/50 HPF (**d**). Desmin (**e**) and CD163 (**f**) immunostains highlighted the rhabdomyoblastic cells and histiocytes, respectively. Next-generation sequencing showed mutation of the *NF1* gene (**g**)

with epithelioid or spindled morphology, and often having unusual “glassy”-appearing cytoplasm. A variable degree of nuclear pleomorphism was present and cells with large, irregular, hyperchromatic nuclei and prominent nucleoli could be identified in all cases. Cross-striations were not seen in any tumor. Mitotic activity was extremely low, with typically < 1 mitotic figure/50 high-powered fields. Most cases, in fact, contained no more than a single mitotic figure in an entire tissue section. Other microscopic features suggestive of a slowly growing tumor, such as stromal calcification, hyalinized blood vessels, and hemosiderin deposition were commonly present.

By immunohistochemistry, the overwhelming majority of the cells comprising the masses were strongly positive for CD163, confirming histiocytic lineage. The large, eosinophilic tumor cells were diffusely positive for desmin and showed much more limited expression of MyoD1 and myogenin. Myogenin expression, in particular, was typically confined to a very small number of tumor cells. Immunohistochemistry for other markers, including keratins and S100 protein/SOX10, had been performed in an ad hoc manner by the submitting pathologists; all were negative in the lesional cells.

Sanger sequencing was negative for the known hotspot *MYOD1* mutations in RMS in the five cases for which material was available for analysis. Five cases tested for gene fusions (1 case tested with the Archer FusionPlex next-generation sequencing and 4 cases using a 170 and/or 517 gene fusion next-generation sequencing assay) were negative for gene rearrangements, including those involving *EWSR1*, *FUS*, and *FOXO1A*. Molecular genetic testing performed at Erlangen University showed two of four analyzed cases to harbor oncogenic pathogenic inactivating mutations in the *NF1* gene. One sample (case 5) harbored a single base pair deletion with a mutated allele frequency of 17% that resulted in a truncating frameshift alteration (c.1398delT; p.Thr467HisfsTer6) (Fig. 4g). Another sample (case 4) had a single base pair missense mutation (c.4600 C > T; p.Arg1534Ter) that resulted in a premature stop codon, which was detected at an allele frequency of 24%. The second case also displayed a likely benign *PTCH1* polymorphism (rs115556836; c.2183 C > T; p.Thr728Met) at an allele frequency of 64%. The 3rd and 4th samples (cases 3 and 9) analyzed with the 170 gene panel did not show any pathogenic alterations.

Clinical follow-up was available for 9 patients (median 9 months, mean 23 months, range 3–124 months). Follow-



up of >24 months was available for two patients. All patients were known to have received wide excision showing negative margins. Four patients received adjuvant radiotherapy; none was known to have received chemotherapy. At the time of last follow-up, all patients were alive and without disease; no local recurrences or distant metastases were known to have occurred. No patient was known to have a history of an inherited genetic disorder, in particular neurofibromatosis type-1.

## Discussion

We herein have presented the clinicopathological and molecular genetic features of an unusual, hitherto unreported skeletal muscle neoplasm, characterized by circumscribed growth, a diffuse histiocytic infiltrate that largely obscures the underlying tumor cells, extremely low mitotic activity, and microscopic features suggestive of a slowly growing neoplasm, such as encapsulation, calcification, fibrosis, and hemosiderin deposition. As will be discussed below, the morphologic and genetic features of these lesions do not seem to us to correspond to any of the described variants of rhabdomyoma or, more importantly, RMS.

The generally circumscribed growth pattern and the extremely low mitotic activity that characterize histiocyte-rich rhabdomyoblastic tumor raise the possibility that these tumors may be related to rhabdomyoma. However, the clinicopathological features of these lesions are clearly different from those of adult rhabdomyoma, a tumor that usually occurs in the head and neck of older males (median 60 years of age), and is composed of a lobulated proliferation of large polygonal or round cells with abundant eosinophilic cytoplasm, a large nucleus, and a prominent nucleolus, as well as so-called “spider cells” with predominantly clear cytoplasm and thin strands of eosinophilic cytoplasm extending from the nucleus to the cytoplasmic membrane [7, 10–15]. Fetal rhabdomyomas, which typically occur as a solitary mass in the subcutaneous tissue of the head and neck region of young boys (<5 years of age) are also morphologically quite different from the tumors that comprise the present series, consisting in the “myxoid type” of a variably myxoid growth of bland spindled cells, immature skeletal muscle fibers, and peripherally located mature skeletal muscle, or in the “intermediate type” of differentiated skeletal muscle fibers with little myxoid matrix, ganglion-like rhabdomyoblasts, mature-appearing skeletal muscle cells with easily identified cross-striations, and vacuolated cells [6, 7, 11, 15–19]. Genital rhabdomyomas resemble “intermediate” fetal rhabdomyomas but involve the cervix or vagina in middle-aged women, or rarely the paratesticular region or epididymis of men [20–22]. Fetal rhabdomyomas are characterized genetically

by *PTCH1* mutations [23], events we did not identify in any studied tumor of our present series, although one case contained a likely benign *PTCH1* polymorphism.

Histiocyte-rich rhabdomyoblastic tumor also does not seem to correspond to a previously described variant of RMS. Embryonal RMS most often involves the head/neck of young children, and is characterized by primitive mesenchymal cells showing varying degrees of rhabdomyoblastic differentiation, with primitive “small round blue” cells, undifferentiated spindled cells, ganglion-like rhabdomyoblasts, and strap cells with brightly eosinophilic cytoplasm and cross-striations [24–27]. Mitotic activity is invariably present and necrosis is frequently identified. At the cytogenetic level, embryonal RMS are characterized by complex structural and numerical abnormalities, including trisomies of chromosomes 2, 8, and 13 [28–31]. Molecular analyses commonly show allelic loss at chromosome 11p15, a site containing a number of tumor suppressor genes, including *IGF2*, *H19*, and *CDKN1C*. *RAS* pathway alterations, including *NFI* mutations, are seen in subsets of embryonal RMS, including highly differentiated ones resembling intermediate forms of fetal rhabdomyoma [32, 33].

The morphological features of histiocyte-rich rhabdomyoblastic tumor are obviously also quite different from those of alveolar RMS, a tumor that usually arises in older children and young adults, and is composed of a solid to pseudoalveolar proliferation of highly malignant-appearing round cells, with scattered rhabdomyoblasts and multinucleated neoplastic giant cells [24, 25, 34]. Genetically, most ARMS are characterized by *PAX3/PAX7-FOXO1A* fusions, genetic events not present in any of our studied cases.

Although the older patient age, extremity location, and variably pleomorphic eosinophilic tumor cells of histiocyte-rich rhabdomyoblastic tumor might suggest pleomorphic RMS, there are a number of important differences. Pleomorphic RMS typically present as large, destructive, partially necrotic masses, and consist microscopically of a fascicular to sheet-like proliferation of highly malignant-appearing, large, eosinophilic cells with frequent mitotic figures, including atypical forms [35–40]. Encapsulation, diffuse histiocytic infiltration, and features of chronicity such as fibrosis and calcification would not be expected in pleomorphic RMS.

Finally, although histiocyte-rich rhabdomyoblastic tumors occur in similar locations and patient ages as spindle cell/sclerosing RMS, their morphological features are obviously quite different. Spindle cell RMS shows a spectrum of appearances, with some well-differentiated tumors consisting of relatively bland-appearing spindled cells arranged in a fascicular or storiform pattern, and other tumors having a much higher grade, fibrosarcoma-like

appearance [41, 42]. Rhabdomyoblasts are typically few in number. Sclerosing RMS is characterized by the presence of a strikingly sclerotic, osteochondroid-like stroma, micro-alveolar and pseudovascular growth patterns, primitive round cells with high nuclear grade, and occasionally small clusters of differentiating rhabdomyoblasts [43–46]. Spindle cell/sclerosing RMS (in particular tumors showing sclerosing morphology) are known to harbor the *MYOD1* L122R mutation [47–50], or less commonly *PIKC3A* mutations [47, 48]. Mutations of these genes were not identified in any studied case of histiocyte-rich rhabdomyoblastic tumor.

In addition to rhabdomyoma and RMS, we also considered whether histiocyte-rich rhabdomyoblastic tumor might be related to other desmin-positive mesenchymal tumors, in particular angiomatoid fibrous histiocytoma and diffuse-type tenosynovial giant cell tumor. Angiomatoid fibrous histiocytoma shares some morphologic features with the tumors we have presented, including a fibrous capsule with lymphoid aggregates and intralesional histiocytes [51]. Angiomatoid fibrous histiocytomas may occasionally contain pleomorphic cells, similar to those seen in histiocyte-rich rhabdomyoblastic tumor, although these are essentially always seen alongside more typical areas, composed of bland, histiocytoid cells often arranged in distinctive whorls [52]. Although roughly 50% of angiomatoid fibrous histiocytomas are desmin-positive, they are negative for myogenin and myoD1, distinguishing them from RMSs and the present tumors [51]. Furthermore, angiomatoid fibrous histiocytomas show rearrangements involving the *EWSR1* and *FUS* loci, most often with *CREB1* or *ATF1* [53], which histiocyte-rich rhabdomyoblastic tumors do not. Although diffuse-type tenosynovial giant cell tumors may contain large numbers of desmin-positive cells and foamy histiocytes, they would not be expected to be encapsulated, contain pleomorphic tumor cells, or express myogenin/MyoD1 [54–56]. In addition, the desmin-positive cells present in our tumors lacked dendritic morphology, as is typically seen in the desmin-positive cells of tenosynovial giant cell tumors, and no tumor analyzed with next-generation sequencing techniques showed evidence of *CSF1* rearrangements, the molecular hallmark of tenosynovial giant cell tumors. Finally, although *NF1* mutations were identified in two cases, the morphological and immunohistochemical features of histiocyte-rich rhabdomyoblastic tumors are quite clearly different from malignant peripheral nerve sheath tumors with rhabdomyoblastic differentiation (malignant Triton tumor).

Our study strongly suggests that histiocyte-rich rhabdomyoblastic tumor is clinically and morphologically distinct from other tumors of skeletal muscle, but it is not clear where in the nosological classification of skeletal muscle tumors they fall. Are they benign, malignant, or possibly rhabdomyoblastic tumors of uncertain malignant potential

or even intermediate malignancy? Whereas the long pre-clinical duration, circumscription, partial encapsulation, exceedingly low mitotic activity, and indolent clinical behavior to date suggest they are benign (and thus best classified as “rhabdomyoma”), we are concerned about the cytologic atypia that characterizes these lesions and their lack of well-differentiated skeletal muscle cells (a common feature in rhabdomyomas). In fact, some of us initially thought these were variants of pleomorphic or spindle cell RMS based on these features. We are also uncomfortable labeling these lesions as definitely benign in the absence of larger case numbers and longer clinical follow-up. It is also, however, very difficult to unequivocally label these lesions “rhabdomyosarcoma,” as they lack the morphologic and genetic features of any described RMS subtype, and show instead features suggesting slow growth, including partial encapsulation, extremely low mitotic activity, hyaline fibrosis, and calcifications. Furthermore, none of these tumors have to date presented with metastatic disease, or subsequently resulted in local recurrence or distant metastasis, despite arguably sub-optimal therapy for RMS, with only four patients receiving adjuvant radiotherapy and none chemotherapy.

The molecular pathogenesis of the tumors we are reporting remains to be further characterized. Detection of likely somatic pathogenic mutations in the *NF1* gene resulting in protein truncation indicates a likely role for this genetic defect in their pathogenesis, although the absence of clinical stigmata or family history of NF1 in any of these patients suggests that these mutations were likely somatic, given the low allelic frequencies and absence of NF1 phenotype in the patients. The presence of inactivating *NF1* mutations in some of our cases might suggest a possible link to embryonal RMS, although the morphology of these tumors would seem to argue strongly against this hypothesis.

In summary, we have reported the clinicopathologic and molecular genetic features of a previously undescribed, distinctive rhabdomyoblastic tumor, characterized by diffuse histiocytic infiltration, pleomorphic, eosinophilic tumor cells lacking cross-striations, morphologic features suggestive of chronicity, and to date favorable outcome. The diffuse histiocytic infiltration and peripheral lymphoid aggregates that characterize these tumors are interesting and unexplained, and it is tempting to speculate that their presence in some way reflects immune modulation of the tumor, as has been described in other mesenchymal tumors. We propose the term “histiocyte-rich rhabdomyoblastic tumor of uncertain malignant potential” for this unusual neoplasm, reflective of its uncertain place in the current nosology of skeletal muscle neoplasms. Study of additional cases with longer follow-up will be necessary to determine whether this tumor is best considered “rhabdomyoma”,

“rhabdomyosarcoma”, or perhaps instead a skeletal muscle tumor of intermediate (borderline) malignancy.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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