

PRDM10-rearranged Soft Tissue Tumor:

A Clinicopathologic Study of Nine Cases

Florian Puls, MD, FRCPath, Nischalan Pillay, MB, ChB, FRCPath, PhD, †‡ Henrik Fagman, MD, PhD,* Anne Palin-Masreliez, BSc,* Fernanda Amary, MD, PhD, † Magnus Hansson MD, PhD,* Lars-Gunnar Kindblom, MD, PhD,* Tom McCulloch, MB, ChB, FRCP, FRCPath, § George Meligonis, MB, ChB, FRCPath, || Ronald Muc, MB, ChB, FRCPath, ¶ Pehr Rissler, MD, PhD, # Vaiyapuri P. Sumathi, FRCPath, ** Roberto Tirabosco, MD, † Jakob Hofvander, MSc, †† Linda Magnusson, MSc, †† Jenny Nilsson, MSc, †† Adrienne Flanagan MB, BCh, PhD, FRCPath, †‡ and Fredrik Mertens MD, PhD#††*

*Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital, Gothenburg, Sweden

†Department of Cellular and Molecular Pathology, Royal National Orthopaedic Hospital NHS Trust, Stanmore, UK

‡Research Department of Pathology, University College London Cancer Institute, London, UK

§Department of Histopathology, Nottingham City Hospital, Nottingham, UK

||Department of Histopathology, Addenbrooke's Hospital, Cambridge, UK

¶Department of Histopathology, Heartlands Hospital, Birmingham, UK

#Department of Clinical Genetics and Pathology, University and Regional Laboratories, Skåne University Hospital, Lund University, Lund, Sweden

**Department of Musculoskeletal Pathology, Royal Orthopaedic Hospital NHS Foundation Trust, Birmingham, UK

††Department of Clinical Genetics, Lund University, Lund, Sweden

Running title: *PRDM10*-rearranged soft tissue tumor

Word count: abstract: 245

main article: 2941

(plus references, tables and figure legends): 5087

Conflicts of interest and source of funding: The authors disclose that they have no relationship with, or financial interest in, any of the commercial companies pertaining to this article.

Correspondence: Florian Puls, MD, FRCPath, Department of Clinical Pathology and Genetics, Sahlgrenska University Hospital Gothenburg, Sweden (e-mail: florian.puls@vregion.se).

Abstract

Gene fusion transcripts containing *PRDM10* were recently identified in low grade undifferentiated pleomorphic sarcomas (UPS). Here, we describe the morphologic and clinical features of 9 such tumors from 5 men and 4 women (age 20-61 years). Three cases had previously been diagnosed as UPS, 3 as superficial CD34-positive fibroblastic tumor, 2 as pleomorphic liposarcoma, and 1 as pleomorphic hyalinising angiectatic tumor. The tumors were located in the superficial and deep soft tissues of the thigh/knee region (4 cases), shoulder (2 cases), foot, trunk and perineum (1 case each) ranging in size from 1-6 cm. All showed poorly defined cellular fascicles of pleomorphic cells within a fibrous stroma with frequent myxoid change and a prominent inflammatory infiltrate. All displayed highly pleomorphic nuclear features, but a low mitotic count. One of 9 tumors recurred locally, but none metastasized (follow up 12-231 months, median 54 months). Immunohistochemically, all were CD34-positive and showed nuclear positivity for PRDM10. PRDM10 immunoreactivity was therefore evaluated in 50 other soft tissue tumors that could mimic *PRDM10*-rearranged neoplasms, including 4 cases exhibiting histological features falling within the spectrum of superficial CD34-positive fibroblastic tumors. Except for 2/6 pleomorphic liposarcomas and 1/4 myxofibrosarcomas, other tumors did not show nuclear positivity but displayed weak to moderate cytoplasmic immunoreactivity. In conclusion, *PRDM10*-rearranged soft tissue tumors are characterized by pleomorphic morphology and a low mitotic count. Its morphological spectrum overlaps with superficial CD34-positive fibroblastic tumor. Clinical features of this small series suggest an indolent behavior justifying its distinction from UPS and other sarcomas.

Keywords: *PRDM10*, sarcoma, superficial CD34-positive fibroblastic tumor, translocation-associated sarcoma, immunohistochemistry.

Introduction

Complementing histological classification of tumors with genetic investigations is becoming increasingly important both for risk stratification, identification of targetable mutations predicting treatment response as well as confirmation of morphological diagnoses.¹ Subclassifications according to genetic alterations are formally integrated into diagnoses in an ever greater variety of lesions.^{2, 3} In soft tissue and bone sarcomas, several genetic subgroups with different prognoses and response rates to conventional treatment regimes have recently emerged within undifferentiated sarcomas with round cell morphology.^{4, 5}

Undifferentiated sarcoma with pleomorphic morphology (undifferentiated pleomorphic sarcoma, UPS), is genetically heterogeneous; most cases, however, display highly complex karyotypes with multiple copy number alterations, whereas driver mutations in the form of gene fusions and single nucleotide variants are less prominent.⁶⁻⁸

Recently, though, fusion genes involving the *PRDM10* gene as the 3'-partner were identified in 3 low grade UPS.⁹ The 5'-partner of *PRDM10* was either *MED12* or *CITED2*. None of these fusions had previously been identified in neoplasia and the information on the expression and function of *PRDM10* are limited. However, the structure of the predicted fusion proteins, with *PRDM10*-derived zinc-finger domains fused to transcriptional co-activators, suggests an important role in oncogenic transformation, a conclusion supported by the finding that these tumors had relatively simple karyotypes.⁹

The tumors with *PRDM10* fusions were characterized by their pleomorphic morphology with focal myxoid areas, a low mitotic count, and strong CD34 expression. None of the three *PRDM10*-rearranged tumors metastasized, suggesting that it is clinically important to distinguish them from conventional UPS and other morphologically similar lesions.⁹

Here, we studied an extended series of *PRDM10*-rearranged tumors to assess their morphological and immunohistochemical characteristics, as well as their clinical features. We hypothesized that these lesions may have previously been diagnosed as low-grade UPS or variants of low grade or borderline lesions, such as myxoinflammatory fibroblastic sarcoma (MIFS), pleomorphic hyalinizing angiectatic tumor (PHAT) or superficial CD34-positive fibroblastic tumor.¹⁰⁻¹⁴ Using fluorescence in situ hybridization (FISH), transcriptome sequencing (RNA-Seq), and/or whole genome sequencing (WGS) on archival material with similar morphology to the 3 previously described cases, we identified 6 additional tumors with *PRDM10* rearrangement. We also evaluated the diagnostic utility of *PRDM10* immunohistochemistry (IHC) for diagnosing *PRDM10*-rearranged tumors.

Materials and methods

Cases

Cases reported as low grade UPS, PHAT, MIFS, superficial CD34-positive fibroblastic tumor and low grade pleomorphic liposarcoma from the surgical pathology files of Sahlgrenska University Hospital Gothenburg, Sweden were retrieved, reviewed, and compared with previously published cases of *PRDM10*-rearranged tumors (cases 1-3).⁹ Prompted by the lipidization described in some superficial CD34-positive fibroblastic tumors, we also re-examined unusual pleomorphic liposarcomas with low mitotic counts.¹³ As morphological features mostly overlapped with superficial CD34-positive fibroblastic tumor, such cases were also retrieved from the Royal Orthopaedic Hospital, Birmingham, UK, the Nottingham City Hospital, Nottingham, UK and the Royal National Orthopaedic Hospital, London

Stanmore, UK. When possible, clinical information and follow-up were obtained. Sample collection and data analysis were conducted according to local ethical guidelines.

Histology and PRDM10 IHC

For IHC, 3 µm sections from formalin-fixed paraffin-embedded (FFPE) tumors were cut, dried and antigen-retrieved in epitope retrieval solution pH8 (RE7116, Novocastra, Newcastle, UK) at 68°C for 16 hours in a stirred water bath. For detection of PRDM10 the primary rabbit polyclonal PRDM10 antibody raised against the c-terminal portion of the protein (NBP1-81427 Novus Bio, Abingdon, UK) was used (1:100 dilution). Whole sections from normal duodenal/gastric wall and a tumor with known *PRDM10* rearrangement (case 1) were used for optimizing antibody dilution and antigen retrieval and served as positive control in each run.¹⁵ The DakoCytomation EnVision™ Detection System peroxidase/DAB visualization kit (K5007 Dako, Ely, UK) was used for visualization according to the manufacturer's instructions. For assessment of the diagnostic utility of PRDM10 IHC, whole sections of potential mimics of PRDM10-rearranged tumor were analyzed. These comprised 10 high-grade UPS, 5 myxofibrosarcomas, 6 pleomorphic liposarcomas, 2 dedifferentiated liposarcomas, 6 epithelioid sarcomas, 5 angiomatoid fibrous histiocytomas, 5 cases of dermatofibrosarcoma protuberans, 5 schwannomas, and 2 atypical fibrous histiocytomas. Intensity and extent of both nuclear and cytoplasmic PRDM10 immunoreactivity were graded semiquantitatively. The intensity of staining was graded as negative, weak, moderate or strong; the extent of immunoreactivity was graded to the percentage of immuno-positive tumor cell cytoplasm/nuclei (0, <5%; 1+, 5% to 25%; 2+, 26% to 50%; 3+, 51% to 75%; or 4+, >75%).

For detection of adipophilin, the primary mouse monoclonal adipophilin antibody, (clone AP125 OriGene, Herford, Germany) was used in 1:20 dilution and visualized using the Refine Detection kit on a Bond III platform (Leica Biosystems, Milton Keynes, UK).

Molecular genetic analyses

RNA-seq, RT-PCR, and FISH for *PRDM10* rearrangements were performed as described.⁹ RNA-seq was only attempted if RNA with a Dv200 value >30% was obtained, except for Case 6 (Dv200 23%). FISH results were considered positive if >15% of the nuclei showed split signals. The molecular results of cases 1-3 have been reported.⁹

Results

Clinicopathologic findings in tumors with *PRDM10* fusions

The cohort consisted of 9 patients, 5 men and 4 women, aged 20-61 years at diagnosis (mean = 42, median = 42). Three of the 9 cases have been published previously.⁹ Five tumors were located in the extremities (4 in the thigh or knee area and 1 in the foot), 2 in the shoulder, and 1 each on the trunk and in the groin/perineal area (Table 1). Tumor size ranged from 10-60 mm (mean = 36 mm). All tumors were at least partly subcutaneous, superficially at the dermal/subcutaneous junction or subcutis, 4 deeply, attached to or within the fascia, bulging equally into skeletal muscle and subcutis (Fig. 1).

Macroscopically, tumors were described as partly lobulated, yellowish to light grey with firm consistency (Fig. 1A). Focal myxoid areas were noted in some.

Histologically, all tumors were generally well circumscribed, often separated by artificial clefts from the surrounding tissue; however, focally ill-defined margins were noted in 4 cases (Table 2, Fig. 1C). The tumors typically had a dense collagenous matrix, sometimes with formation of pseudovascular clefts. Geographic myxoid areas were seen in 7 cases (Fig. 1D-

F). Apart from these areas, the tumors were moderately to highly cellular and composed of large spindled to polygonal cells arranged in long ill-defined fascicles. The tumor cells had eosinophilic, focally glassy and well-demarcated cytoplasm (Fig. 2A-E). The nuclei were hyperchromatic and pleomorphic with irregular outlines, coarse chromatin and distinct nucleoli (Fig. 2D). Bizarrely formed nuclei with inclusions with “virocyte”-like appearances were frequent (Fig. 2E). The mitotic count was very low (0-7 mitotic figures per 50 high power fields) and stood in contrast to the high cellularity and pleomorphism (Fig. 2 F). There was no necrosis. Invariably present was a scattered chronic inflammatory infiltrate of lymphocytes, plasma cells, and often also eosinophils (Fig. 2 B). Small lymphoid follicles were also frequently seen at the periphery. Areas with epithelioid tumor cell morphology were seen in some cases (Fig. 3D). Six tumors showed scattered cells with cytoplasmic vacuolization staining for adipophilin (Fig. 2F Fig. 4C). This was a prominent feature in 2 cases having led to an earlier diagnosis of pleomorphic liposarcoma (Fig. 3F). Three of 9 tumors contained tumor giant cells (Fig. 3F). There were a few muscularized and dilated vessels and focally areas with intracytoplasmic hemosiderin and hyalinized vessel walls resembling PHAT were noted, but these were not prominent features (Fig. 3E). One tumor contained metaplastic bone.

A basic immunohistochemical panel showed diffuse positivity for CD34 and weak positivity for EMA in all tumors tested. Five out of six tumors tested showed patchy positivity for cytokeratins (Table 3, Fig. 4 A, B). Expression of S100 protein, smooth muscle antigen or desmin was not seen. Ki-67 immunohistochemistry was available in 5 tumors showing immunoreactivity of less than 5% of cells (Fig. 4 D).

Clinically, one tumor (case 3), for which primary resection status was not available, recurred locally after 15 years. The patient is without disease after complete re-resection. All other

primary tumors were completely resected and none recurred (median follow up 54 months, 12-231 months).

PRDM10 IHC

On PRDM10 IHC, 7/9 with confirmed *PRDM10* rearrangement showed moderate to strong nuclear staining in at least 50% of tumor cell nuclei (4+ strong in 4 tumors, 3+ moderate in 2 tumors, 4+ moderate in 1 tumor, Table 3, Figs. 4 E-H, 5 A-B). Cytoplasmic immunoreactivity was weak in less than 25% of cells. The two negative cases were the only ones, on which immunohistochemistry was performed on archival, previously cut, unstained slides. After increasing antibody concentration from 1:100 to 1:25 similar immuno-positivity patterns to the other cases were observed (3+ moderate).

Immunohistochemistry patterns of histological mimics of *PRDM10*-rearranged soft tissue tumor are summarized in Table 4. In high grade UPS, weak to strong cytoplasmic immunoreactivity was noted, but no case showed nuclear positivity (Fig. 5 C-D). Two out of 6 pleomorphic liposarcomas and 1 of 5 myxofibrosarcomas showed moderate nuclear immunoreactivity and were considered positive (Fig. 5 F) however FISH with a break-apart probe for *PRDM10* failed in these three tumors and were morphologically different to the tumors with confirmed *PRDM10* rearrangement. All other entities tested showed highly variable cytoplasmic, but weak and patchy nuclear PRDM10 immunoreactivity (Table 4, Fig. 5 E).

FISH and RNA-Seq

In addition to the 3 index cases published previously, *PRDM10* fusions were identified by FISH and RNA-seq in 6 cases. Among the 9 tumors, 7 were positive for *PRDM10* fusions (4

MED12-PRDM12, 3 *CITED2-PRDM10*) at the transcript level using RNA-seq or RT-PCR, five of these fusions were verified also at the genomic level by whole genome sequencing (data not shown) and/or FISH (Table 3). Two tumors that were either negative or could not be analyzed by RNA-seq were scored as *PRDM10*-positive on FISH (Table 3).

Superficial CD34-positive fibroblastic tumors negative for *PRDM10* rearrangement

Two out of 4 additional tumors within the morphological spectrum of superficial CD34-positive fibroblastic tumor did not show evidence of *PRDM10* fusion by FISH and/or RNA seq (Table 5, Fig. 6). FISH of the two remaining cases failed and RNA-Seq was not performed due to poor RNA quality. All four tumors were negative on *PRDM10* IHC (Fig. 6 B, D, F). On retrospective comparison with *PRDM10*-rearranged tumors, none of the *PRDM10*-negative cases showed myxoid areas, they were less well circumscribed, and one tumor had a higher mitotic count (Table 5). However there was no single morphological feature allowing confident separation from tumors with confirmed *PRDM10* rearrangement.

Discussion

In this study we describe a series of mesenchymal tumors defined by *PRDM10* rearrangement. *PRDM10*-rearrangements were first described in low grade UPS.⁹ We therefore hypothesized that *PRDM10*-rearranged soft tissue tumors may have been previously classified as low grade malignant or borderline lesions, such as low grade UPS, superficial CD34-positive fibroblastic tumor, PHAT, MIFS, or unusual pleomorphic liposarcomas with low mitotic counts.⁹⁻¹³

When reviewed, this series of *PRDM10*-rearranged tumors displayed recurrent and distinctive morphological and clinical features. The majority of tumors occurred in middle aged adults (20-61 years, median 42 years). Tumors were typically located in the subcutis, although sometimes involving the underlying fascia or even pushing into the skeletal muscle. Histologically, the most salient feature was pronounced cellular pleomorphism of “MFH”-like type but with a very low level of mitotic activity, which, at first glance, appeared disproportionate given the striking pleomorphism. Other common features were a fascicular growth pattern, a fibrous matrix, myxoid changes and a chronic inflammatory infiltrate. The absence of metastases after a median of 54 months of follow-up adds further credence to the notion that these lesions have a low metastatic potential, possibly being benign.

More than half of the *PRDM10*-rearranged tumors in this series were initially classified as sarcomas. However, the combination of salient morphological characteristics outlined above, as well as circumscription, relatively small size and very low mitotic count, will be helpful in separating *PRDM10*-rearranged soft tissue tumor from its malignant mimics such as UPS, myxofibrosarcoma and pleomorphic liposarcoma.

UPS is typically a mitotically very active, high grade tumor, most often deep-seated.¹⁶ Myxofibrosarcoma, usually located in the deep subcutis with attachment to the fascia, is characterized by a diffuse and infiltrative growth, extensive myxoid matrix and a higher mitotic count than *PRDM10*-rearranged tumors.¹⁷ Pleomorphic liposarcoma is most often a deep-seated, high grade neoplasm with high mitotic count and frequent necrosis, although subcutaneous cases and lesions with very low mitotic count have been described.¹⁸⁻²⁰

Carter and colleagues were the first to draw attention to tumors within the morphological spectrum of *PRDM10*-rearranged soft tissue tumor by describing superficial CD34-positive

fibroblastic tumor, a borderline superficial lesion characterized by pronounced pleomorphism and low mitotic count.¹³ Tumors diagnosed as superficial CD34-positive fibroblastic tumors were therefore selected in search for *PRDM10*-rearranged tumors and 3 of 7 were found to harbor *PRDM10* rearrangements. Thus, it seems reasonable to assume that superficial CD34-positive fibroblastic tumor and *PRDM10*-rearranged tumor overlap. However, in contrast to the cases reported by Carter and colleagues, *PRMD10*-rearranged soft tissue tumors often show focal myxoid appearance and few mitoses. Also, some *PRDM10*-rearranged tumors appear to be located deeper than tumors reported in Carter's series. None of our cases metastasized, whereas Carter and colleagues report one metastasizing case out of 18.¹³ No evidence of *PRDM10* rearrangement or *PRDM10* nuclear staining was seen in 4 additional superficial CD34-positive fibroblastic tumors tested. A recently identified t(2;5)(q31;q31) translocation in one superficial CD34-positive fibroblastic tumor involves none of the *PRDM10*, *CITED2* or *MED12* loci.²¹ These findings indicate that superficial CD34-positive fibroblastic tumor could be genetically heterogeneous. Larger series need to be investigated in order to resolve if *PRDM10*-rearranged soft tissue tumor should be regarded as a subset of CD34-positive fibroblastic tumor or as a stand-alone entity.

Other borderline lesions that may mimic *PRDM10*-rearranged tumor are MIFS and PHAT.^{10-12,}
²² MIFS is usually a tumor of the distal extremities with relation to the tendons and aponeurosis and, in contrast to *PRDM10*-rearranged soft tissue tumor, displays extensive myxoid change and a highly infiltrative growth resulting in its characteristic propensity for local recurrence.²³

The shared features of *PRDM10*-rearranged tumor and PHAT are positivity for CD34, low mitotic rate and cellular pleomorphism.²² Lesional cells in PHAT are typically more spindled

and often the lesions are poorly circumscribed, with large gaping vessels being a prominent feature.¹² One of the tumors in this series focally displayed PHAT-like features and, as one case in Carter's series, had been originally considered to represent a variant of PHAT.¹³

PRDM10-rearranged tumor may also be confused with deep fibrous histiocytoma, which can show pleomorphism, immunoreactivity for CD34 and mitotic activity.²⁴ However, apart from small foci, the storiform architecture typical for fibrous histiocytoma was not present in the *PRDM10* rearranged tumors in this series.

In many gene fusion-associated neoplasms, immunohistochemical detection of components of the fusion protein has been shown to be diagnostically helpful.²⁵⁻²⁹ We therefore sought to test the diagnostic value of immunohistochemical detection of *PRDM10*, the common component in the known fusions. Little is known about the function and expression of *PRDM10*, but it appears to be widely expressed.³⁰ There is some variation among tissues with regard to intensity and localization of *PRDM10* expression, with the strongest nuclear expression being reported in the epithelial lining of the GI tract and gallbladder. In contrast, *PRDM10* is predominately present in the cytoplasm in hepatocytes, the exocrine pancreas, and renal proximal tubular epithelial cells.¹⁵ In healthy soft tissue components, immunostaining of *PRDM10* is comparatively weak.¹⁵

In all the *PRDM10*-rearranged soft tissue tumors, *PRDM10* immunohistochemistry showed distinct nuclear positivity, although archival slides (cut more than 3 years before immunohistochemical staining) required higher antibody concentrations than freshly cut sections, most likely due to decline of antigenicity in pre-cut sections of FFPE material.³¹⁻³³

Although weak nuclear and moderate cytoplasmic immunoreactivity was commonly seen in other mesenchymal neoplasms, moderate or strong nuclear positivity was only observed in

two pleomorphic liposarcomas and one myxofibrosarcoma. Clearly, larger studies are required to clarify the value of PRDM10 IHC as a diagnostic marker. Given the rarity of *PRDM10*-rearranged tumor, the positive predictive value of positive staining in soft tissue neoplasms is expected to be low. However, absence of PRDM10 staining in superficial CD34-positive fibroblastic tumors without evidence for *PRDM10* rearrangement suggests some value of PRDM10 IHC in the diagnosis of selected, morphologically ambiguous cases. Demonstration of *PRDM10* rearrangement may ultimately be required for definite diagnosis.

In other neoplastic tissues, strong nuclear immunostaining for PRDM10 is seen in a subset of ovarian, pancreatic, colonic and gastric carcinomas.^{15, 34} Further studies are required to address the mechanism of nuclear translocation of PRDM10 in *PRDM10*-rearranged tumor. One fusion partner, CITED2, shows nuclear localization, whereas MED12 can be found in the nucleus as well as in the cytoplasm.³⁵⁻³⁸ Both might therefore mediate nuclear translocation of the respective fusion protein.

In summary, the *PRDM10*-rearranged soft tissue tumors described here are relatively well circumscribed neoplasms arising in the deep subcutis of the extremities in young to middle-aged individuals. They are characterized by pleomorphic morphology, very low mitotic rate, and CD34 positivity and appear to overlap with superficial CD34-positive fibroblastic tumors. In view of their seemingly benign clinical course, they should be distinguished from high grade malignant mimickers, in particular UPS and pleomorphic liposarcoma. Immunohistochemical detection of nuclear PRDM10 and demonstration of *PRDM10* rearrangement are helpful in recognizing this rare soft tissue tumor.

Acknowledgments

The authors are indebted to Dr. C.D.M. Fletcher, Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA for providing material from cases 2 and 3. The authors acknowledge the RNOH Musculoskeletal Research Programme and Biobank, and the Wellcome Trust Sanger Institute - Cancer, Aging and Somatic Mutation Programme for sample preparation and whole genome sequencing. Dr. Pillay is funded through a Cancer Research UK Clinician Scientist fellowship. Dr. Fagman and Dr Puls are supported by grants from the Johan Jansson Foundation. Dr. Mertens is supported by grants from the Swedish Cancer Society (CAN2017/269) and Region Skåne. Dr Flanagan is a NIHR (UK) Senior Investigator.

References

1. Louis DN, Ohgaki H, O.D. W, et al. (Eds). *WHO classification of Tumours of the Central Nervous System*. Lyon: IARC Press 2016.
2. Louis DN, Perry A, Burger P, et al. International Society Of Neuropathology--Haarlem consensus guidelines for nervous system tumor classification and grading. *Brain Pathol*. 2014;24:429-435.
3. Lindeman NI, Cagle PT, Aisner DL, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med*. 2018;142:321-346.
4. Italiano A, Sung YS, Zhang L, et al. High prevalence of CIC fusion with double-homeobox (DUX4) transcription factors in EWSR1-negative undifferentiated small blue round cell sarcomas. *Genes Chromosomes Cancer*. 2012;51:207-218.
5. Pierron G, Tirorde F, Lucchesi C, et al. A new subtype of bone sarcoma defined by BCOR-CCNB3 gene fusion. *Nat Genet*. 2012;44:461-466.
6. Dei Tos AP. Classification of pleomorphic sarcomas: where are we now? *Histopathology*. 2006;48:51-62.
7. The Cancer Genome Atlas Research Network. Comprehensive and integrated genomic characterization of adult soft tissue sarcomas. *Cell*. 2017;171:950-965.
8. Mertens F, Fletcher CD, Dal Cin P, et al. Cytogenetic analysis of 46 pleomorphic soft tissue sarcomas and correlation with morphologic and clinical features: a report of the CHAMP study group. *Genes Chromosomes Cancer*. 1998;22:16-25.
9. Hofvander J, Tayebwa J, Nilsson J, et al. Recurrent PRDM10 gene fusions in undifferentiated pleomorphic sarcoma. *Clin Cancer Res*. 2015;21:864-869.

10. Meis-Kindblom JM, Kindblom LG. Acral myxoinflammatory fibroblastic sarcoma: a low-grade tumor of the hands and feet. *Am J Surg Pathol*. 1998;22:911-924.
11. Montgomery EA, Devaney KO, Giordano TJ, et al. Inflammatory myxohyaline tumor of distal extremities with virocyte or Reed-Sternberg-like cells: a distinctive lesion with features simulating inflammatory conditions, Hodgkin's disease, and various sarcomas. *Mod Pathol*. 1998;11:384-391.
12. Smith ME, Fisher C, Weiss SW. Pleomorphic hyalinizing angiectatic tumor of soft parts. A low-grade neoplasm resembling neurilemoma. *Am J Surg Pathol*. 1996;20:21-29.
13. Carter JM, Weiss SW, Linos K, et al. Superficial CD34-positive fibroblastic tumor: report of 18 cases of a distinctive low-grade mesenchymal neoplasm of intermediate (borderline) malignancy. *Mod Pathol*. 2014;27:294-302.
14. Jo VY, Hornick JL. Myxoinflammatory fibroblastic sarcoma. In: Hornick JL, (Ed). *Practical soft tissue pathology : A diagnostic approach, 2nd ed*. Philadelphia, PA: Elsevier 2019:p. 155-157.
15. The Human Protein Atlas. PRDM10. 2017. Available at: <https://www.proteinatlas.org/ENSG00000170325-PRDM10/tissue>. Accessed May 21, 2018.
16. Weiss SW, Enzinger FM. Malignant fibrous histiocytoma: an analysis of 200 cases. *Cancer*. 1978;41:2250-2266.
17. Weiss SW, Enzinger FM. Myxoid variant of malignant fibrous histiocytoma. *Cancer*. 1977;39:1672-1685.
18. Hornick JL, Bosenberg MW, Mentzel T, et al. Pleomorphic liposarcoma: clinicopathologic analysis of 57 cases. *Am J Surg Pathol*. 2004;28:1257-1267.
19. Downes KA, Goldblum JR, Montgomery EA, et al. Pleomorphic liposarcoma: a clinicopathologic analysis of 19 cases. *Mod Pathol*. 2001;14:179-184.
20. Gardner JM, Dandekar M, Thomas D, et al. Cutaneous and subcutaneous pleomorphic liposarcoma: a clinicopathologic study of 29 cases with evaluation of MDM2 gene amplification in 26. *Am J Surg Pathol*. 2012;36:1047-1051.
21. Yamaga K, Fujita A, Osaki M, et al. Detailed analysis of a superficial CD34-positive fibroblastic tumor: A case report and review of the literature. *Oncol Lett*. 2017;14:3395-3400.
22. Weiss SW, Dei Tos AP. Pleomorphic hyalinizing angiectatic tumour of soft parts. In: Fletcher CDM, Bridge JA, Hogendoorn PCW, et al., (Ed). *WHO Classification of Tumours of Soft Tissue and Bone*. Lyon: International Agency for Research on Cancer (IARC) 2013:200.
23. Laskin WB, Fetsch JF, Miettinen M. Myxoinflammatory fibroblastic sarcoma: a clinicopathologic analysis of 104 cases, with emphasis on predictors of outcome. *Am J Surg Pathol*. 2014;38:1-12.
24. Gleason BC, Fletcher CD. Deep "benign" fibrous histiocytoma: clinicopathologic analysis of 69 cases of a rare tumor indicating occasional metastatic potential. *Am J Surg Pathol*. 2008;32:354-362.
25. Argani P, Lal P, Hutchinson B, et al. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. *Am J Surg Pathol*. 2003;27:750-761.
26. Wang WL, Patel NR, Caragea M, et al. Expression of ERG, an Ets family transcription factor, identifies ERG-rearranged Ewing sarcoma. *Mod Pathol*. 2012;25:1378-1383.
27. Puls F, Niblett A, Marland G, et al. BCOR-CCNB3 (Ewing-like) sarcoma: a clinicopathologic analysis of 10 cases, in comparison with conventional Ewing sarcoma. *Am J Surg Pathol*. 2014;38:1307-1318.
28. Hung YP, Fletcher CD, Hornick JL. FOSB is a Useful Diagnostic Marker for Pseudomyogenic Hemangioendothelioma. *Am J Surg Pathol*. 2017;41:596-606.
29. Doyle LA, Fletcher CD, Hornick JL. Nuclear Expression of CAMTA1 Distinguishes Epithelioid Hemangioendothelioma From Histologic Mimics. *Am J Surg Pathol*. 2016;40:94-102.
30. Uhlen M, Fagerberg L, Hallstrom BM, et al. Proteomics. Tissue-based map of the human proteome. *Science*. 2015;347:1260419.

31. Wester K, Wahlund E, Sundstrom C, et al. Paraffin section storage and immunohistochemistry. Effects of time, temperature, fixation, and retrieval protocol with emphasis on p53 protein and MIB1 antigen. *Appl Immunohistochem Mol Morphol*. 2000;8:61-70.
32. Bertheau P, Cazals-Hatem D, Meignin V, et al. Variability of immunohistochemical reactivity on stored paraffin slides. *J Clin Pathol*. 1998;51:370-374.
33. Fergenbaum JH, Garcia-Closas M, Hewitt SM, et al. Loss of antigenicity in stored sections of breast cancer tissue microarrays. *Cancer Epidemiol Biomarkers Prev*. 2004;13:667-672.
34. Uhlen M, Zhang C, Lee S, et al. A pathology atlas of the human cancer transcriptome. *Science*. 2017;357.
35. Shioda T, Fenner MH, Isselbacher KJ. msg1, a novel melanocyte-specific gene, encodes a nuclear protein and is associated with pigmentation. *Proc Natl Acad Sci U S A*. 1996;93:12298-12303.
36. Huang S, Holzel M, Knijnenburg T, et al. MED12 controls the response to multiple cancer drugs through regulation of TGF-beta receptor signaling. *Cell*. 2012;151:937-950.
37. The Human Protein Atlas. CITED2. 2017. Available at: <https://www.proteinatlas.org/ENSG00000164442-CITED2/tissue>. Accessed May 21, 2018.
38. The Human Protein Atlas. MED12. 2017. Available at: <https://www.proteinatlas.org/ENSG00000184634-MED12/tissue>. Accessed May 21, 2018.
39. Örndal C, Rydholm A, Willen H, et al. Cytogenetic intratumor heterogeneity in soft tissue tumors. *Cancer Genet Cytogenet*. 1994;78:127-137.

FIGURE LEGENDS

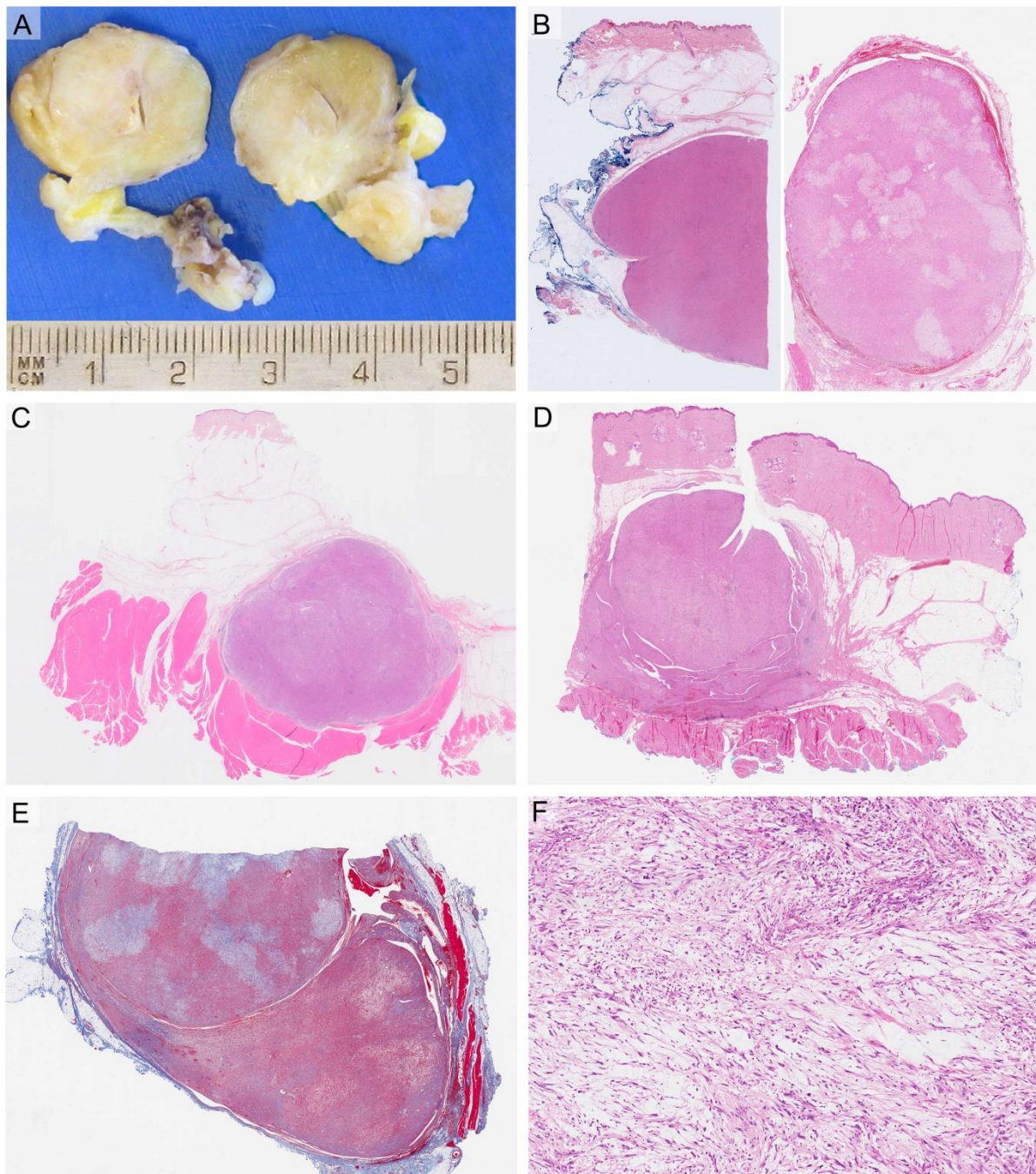


FIGURE 1. Macroscopic and low power appearances of *PRDM10*-rearranged tumor. A. A firm yellowish subcutaneous lesion (case 5). B. In general, tumors were well circumscribed and located in the subcutis (cases 7 and 6) C. Some tumors were attached to the fascia and showed extension into skeletal muscle (case 1). D. A recurrent lesion with more ill-defined borders (case 3). E. Myxoid areas were present in 7/9 tumors and were highlighted by Masson trichrome stain. Pseudovascular spaces were seen in 4 tumors (case 6). F. One case had a predominant myxoid appearance (case 9).

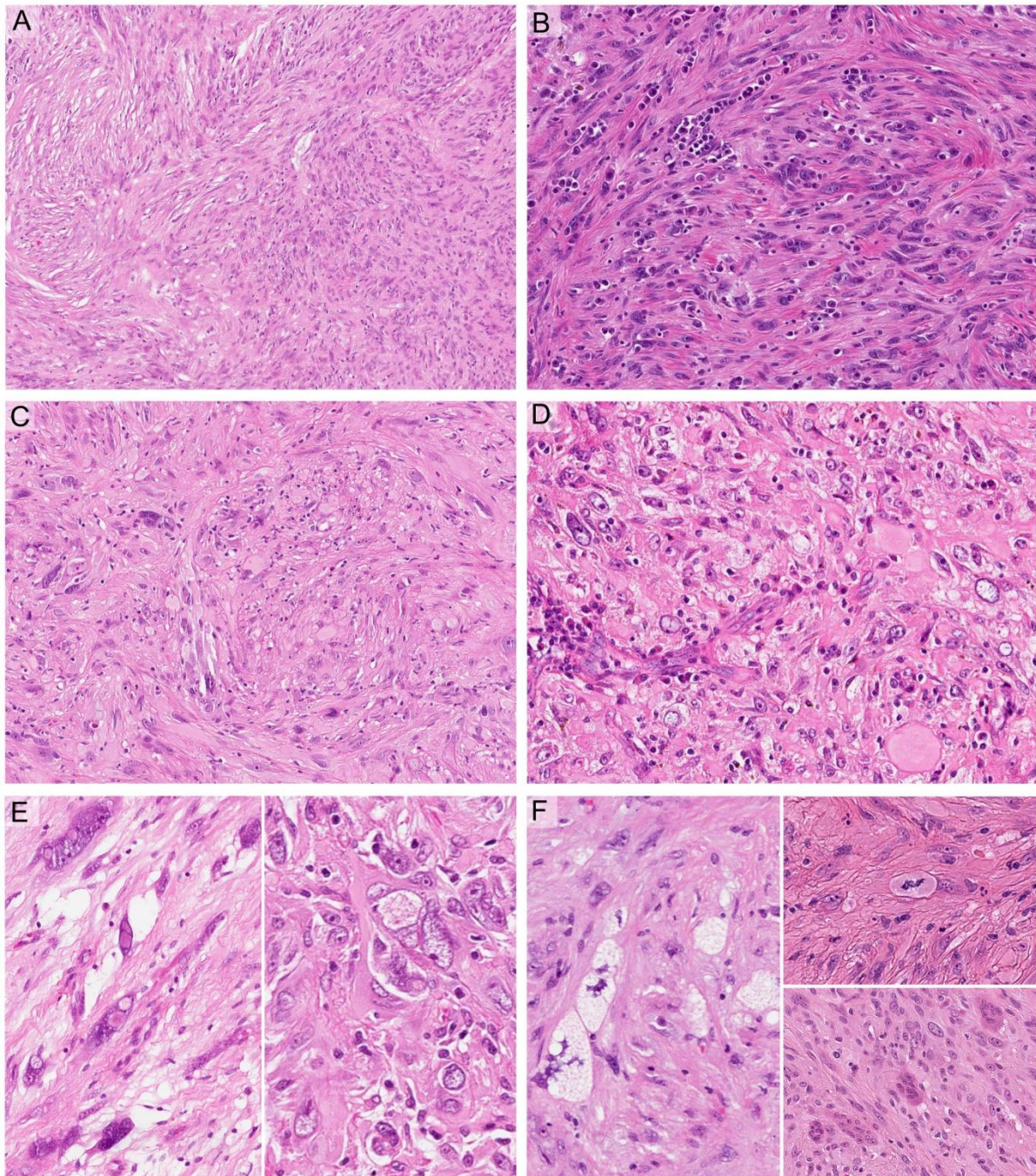


FIGURE 2. Medium and high power appearances of *PRDM10*-rearranged tumor. A. Generally, tumors were cellular, composed of spindle cells arranged in fascicles (case 8). B. An inflammatory infiltrate with plasma cells and lymphocytes was invariably present (case 4). C., D. The tumor cells showed marked pleomorphism; areas with glassy cytoplasm and well-defined cell borders were frequently seen (cases 7 and 2). E. “Virocyte”-like cells with intranuclear inclusions (cases 5 and 1). F. Focal cytoplasmic vacuolization was seen in 6 cases (case 8); all tumors showed a low mitotic count; multinucleated giant cells were seen in 3 cases (case 7).

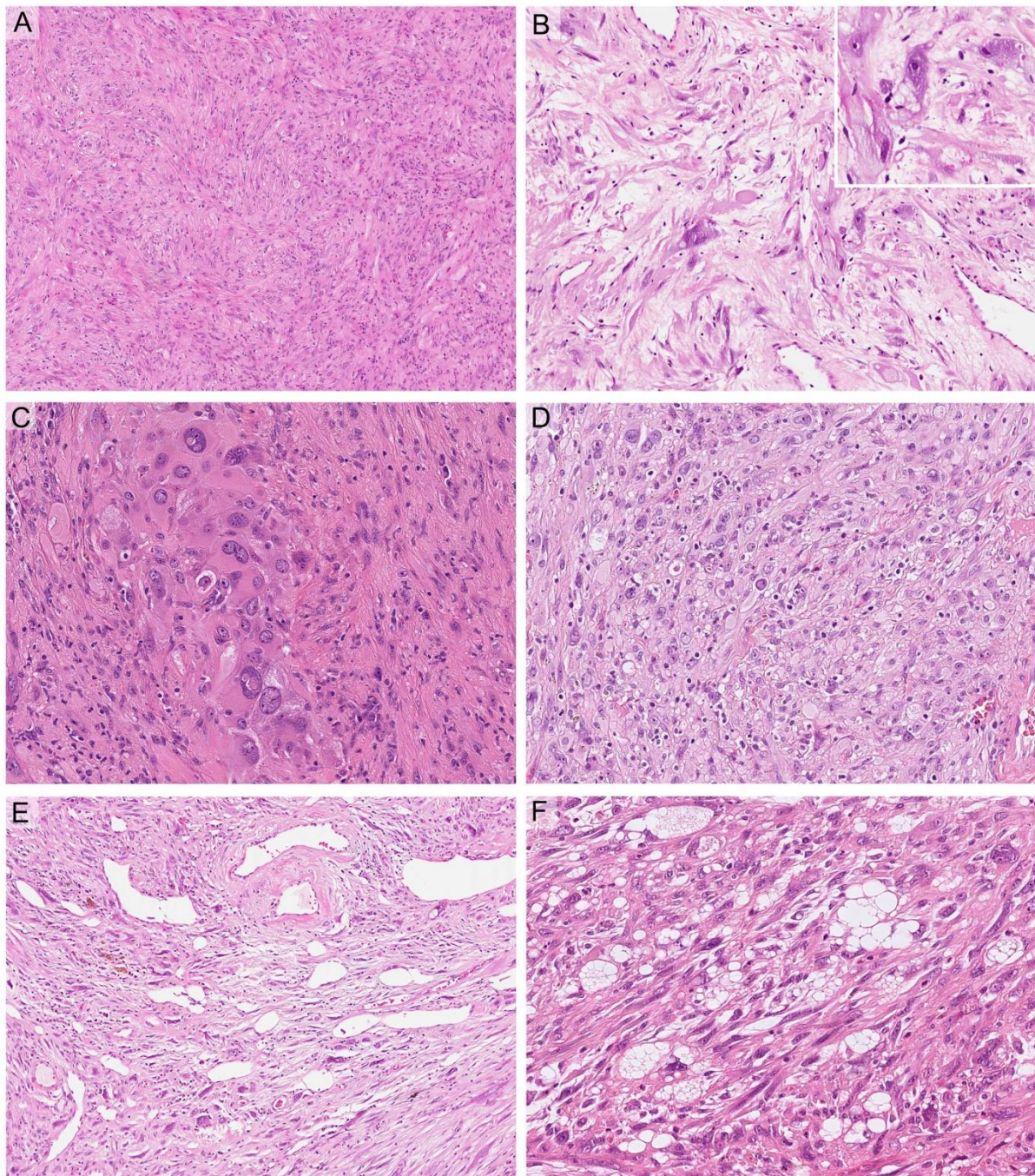


FIGURE 3. Morphological spectrum of *PRDM10*-rearranged tumor. A. Focal vaguely storiform architecture similar to deep fibrous histiocytoma with atypia (case 7). B Myxoid background with bizarre “virocytes” simulating myxoinflammatory fibroblastic sarcoma (case 8). C, D. Focal epithelioid morphology with glassy cytoplasm mimicking a carcinoma or epithelioid sarcoma (cases 7 and 1 respectively). E. Hyalinised gaping vessels and hemosiderin reminiscent of pleomorphic hyalinizing angiectatic tumor (case 5). F. Focally vacuolized cytoplasm suggestive of pleomorphic liposarcoma (case 4).

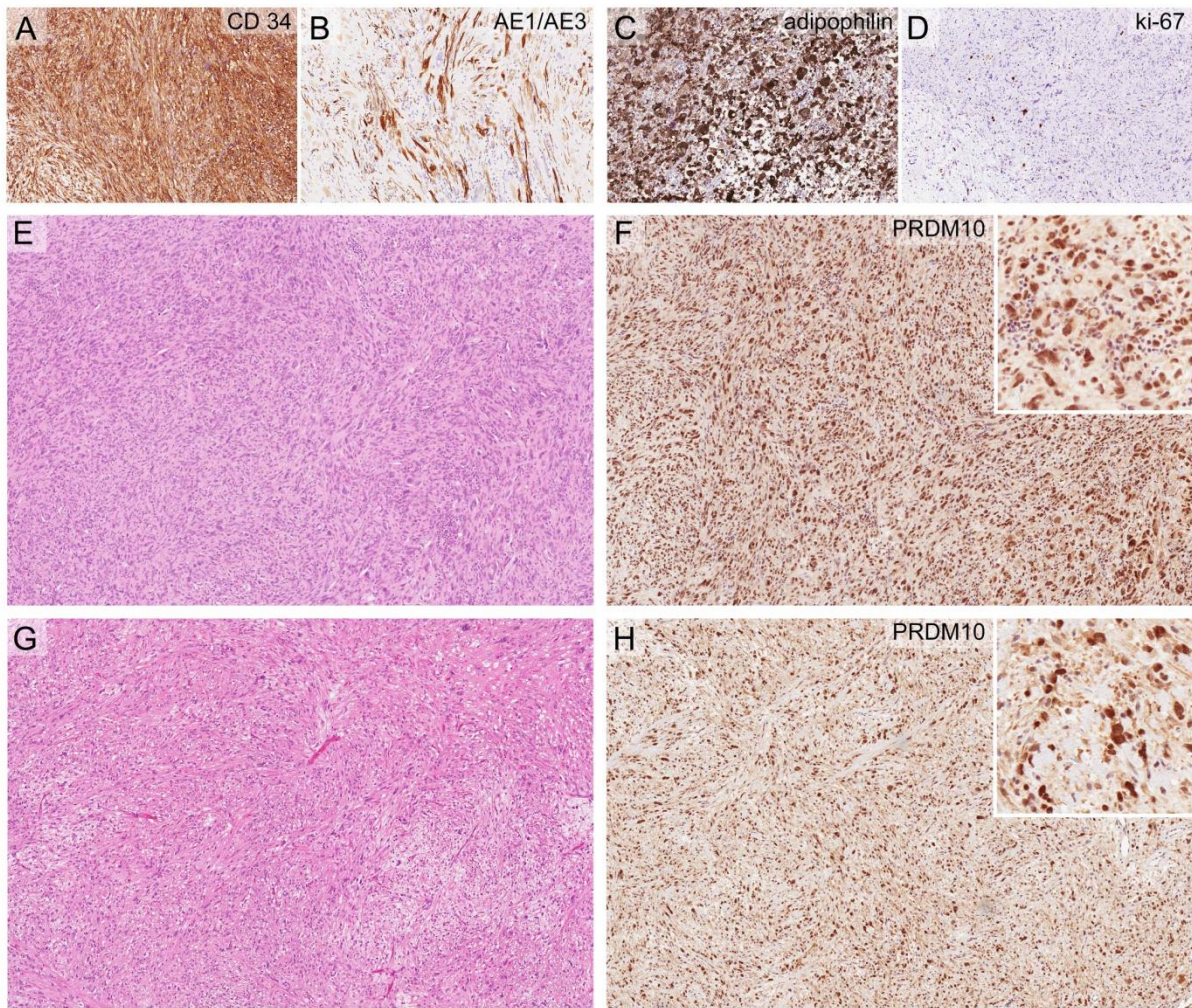


FIGURE 4. Immunohistochemical features of *PRDM10*-rearranged tumor. A., B. All tumors displayed uniform strong positivity for CD34 and focal positivity for cytokeratins was seen in 5/6 cases tested. C. 4 of 4 tumors showed scattered adipophilin-positive cells, focally confluent in 1 tumor. D. ki-67 labeling was low in all cases tested. E. to H. Diffuse strong nuclear positivity for PRDM10 (E., F. case 1; G., H. case 6).

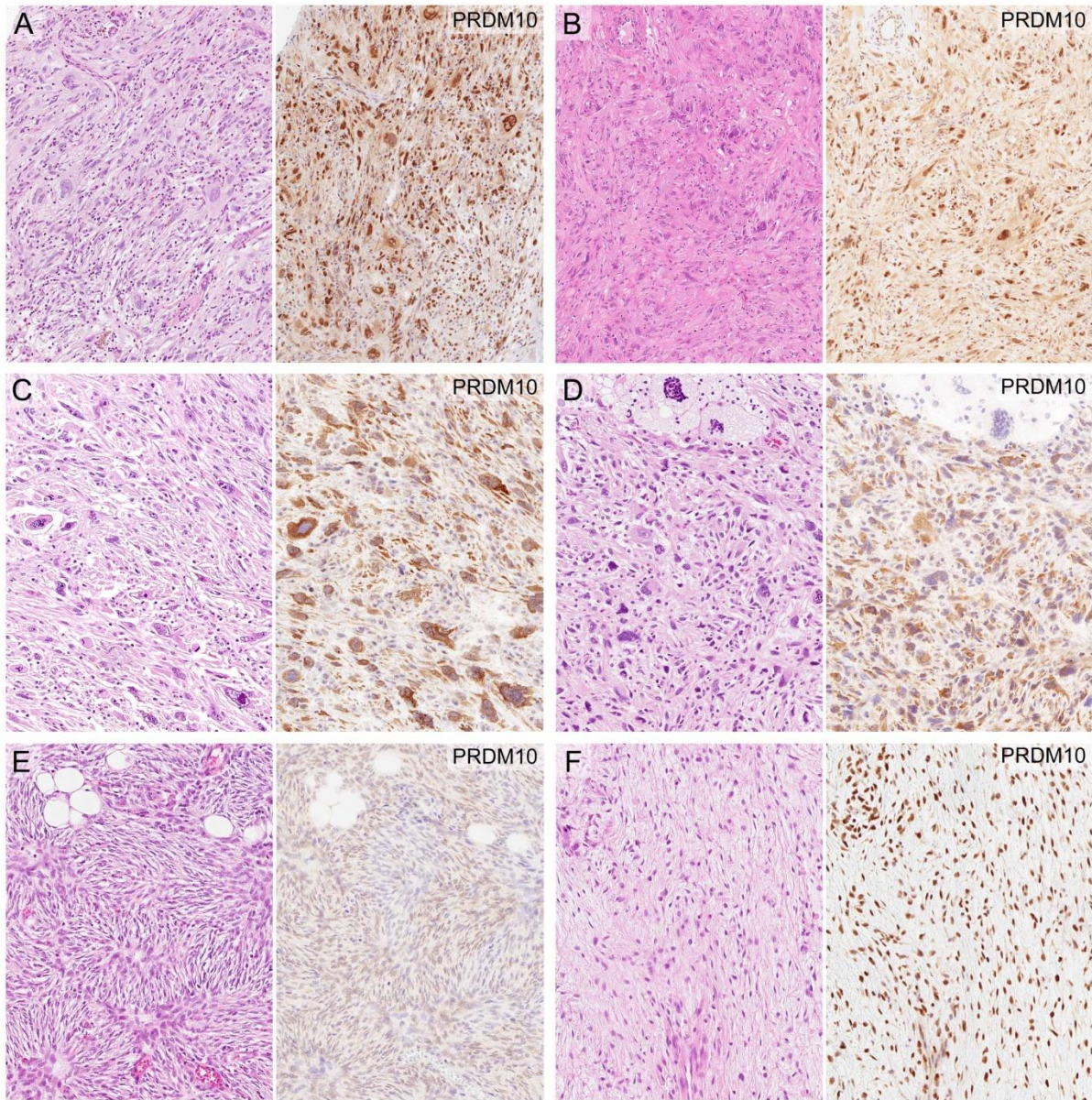


FIGURE 5. PRDM10 immunohistochemistry. A., B. Nuclear PRDM10 positivity in *PRDM10* rearranged tumor (A. case 4, B. case 7). C., D. Strong and moderate cytoplasmic PRDM10 positivity in UPS. The nuclei are negative. E. Weak nuclear positivity was noted in dermatofibrosarcoma protuberans. F. Low grade myxofibrosarcoma with nuclear PRDM10 positivity.

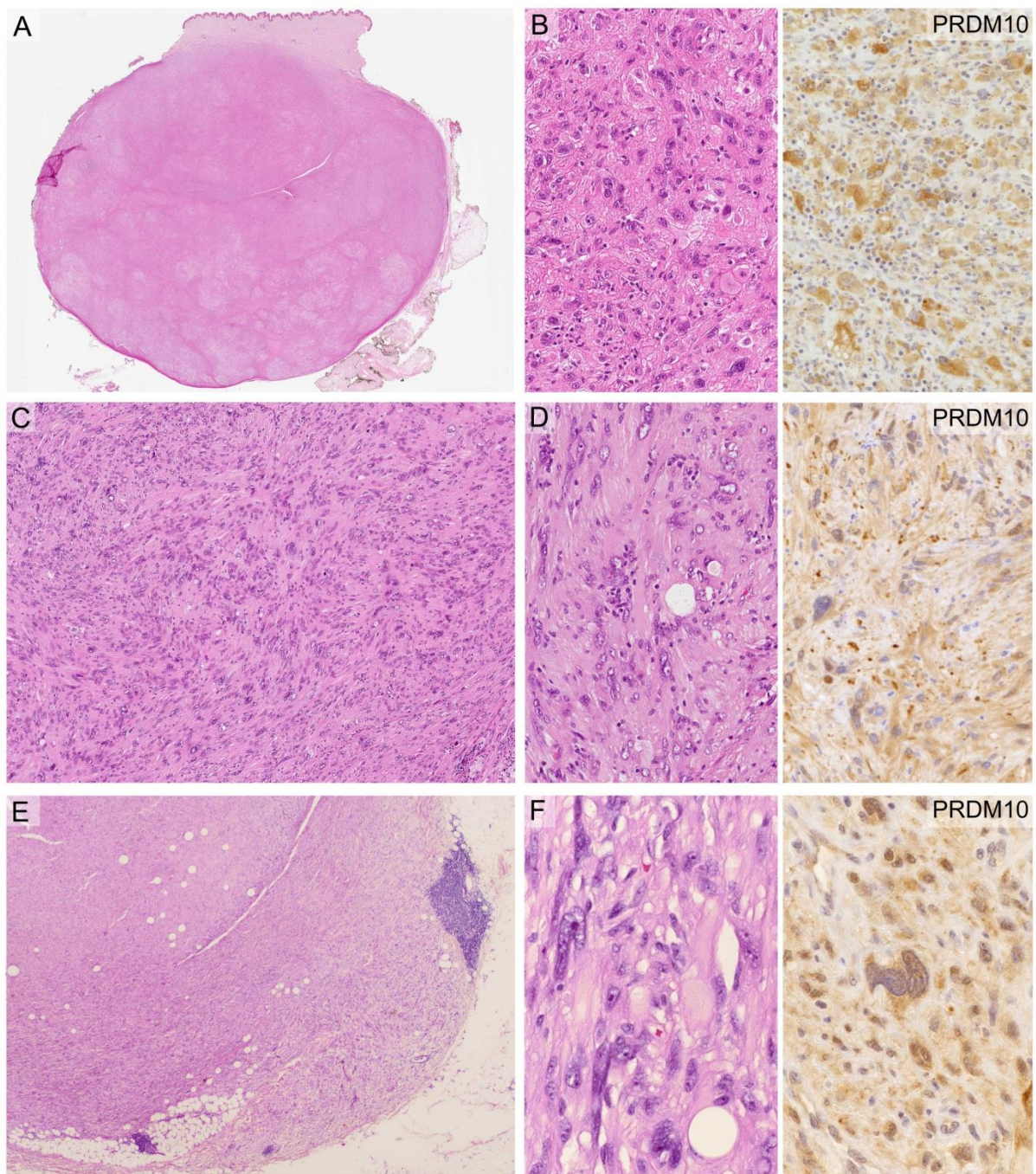


FIGURE 6. Three superficial CD34-positive fibroblastic tumors without evidence for *PRDM10* rearrangement. Although exhibiting the morphological spectrum of superficial CD34 fibroblastic tumor, nuclear PRDM10 immunoreactivity is not seen. A., B. case 1 in table 5. C., D. case 2 in table 5., E., F. case 3 in table 5.

Tables

TABLE 1: Clinical features of *PRDM10*-rearranged tumors

Case	Age* /sex	Site	Size, mm	Treatment	Follow up [months]
1**	41/F	Shoulder	30	CR	NED [61]
2**	58/F	Foot	30	CR	NED [41]
3**	30/M	Shoulder	10	CR of LR	NED [231], LR [180]
4***	61/M	Thigh	50	CR	NED [84]
5	42/F	Knee	30	CR	NED [18]
6	42/M	Trunk	20	CR	NED [204]
7	20/M	Thigh	35	CR	NED [12]
8	32/F	Thigh	45	CR	NED[48]
9	48/M	Perineum	60	CR	N/A

*age at diagnosis; M, male; F, female; CR, complete resection; LR, local recurrence; NED, alive; no evidence of recurrent disease/metastasis;

previously published⁹, *previously published as leiomyosarcoma, reclassified as pleomorphic liposarcoma^{8,39};

TABLE 2: Morphological features of *PRDM10*-rearranged tumors

Case	Demarcation	Myxoid areas	Multinucleated giant cells	Pseudovasc. spaces	Vacuolisation	Mitoses/50hpf*	Original diagnosis
1	Well demarcated	Present	Absent	Absent	Absent	7	UPS, low grade
2	Focal infiltrative	Absent	Present	Absent	Absent	7	UPS, low grade
3	Focal infiltrative	Present	Absent	Present	Single cells	1	UPS, low grade
4	Well demarcated	Present	Absent	Present	Prominent	0	PLS
5	Focal infiltrative	Present	Absent	Present	Single cells	3	PHAT
6	Well demarcated	Present	Absent	Present	Prominent	4	PLS
7	Well demarcated	Absent	Present	Absent	Single cells	7	SCD34FT
8	Focal infiltrative	Present	Present	Absent	Single cells	2	SCD34FT
9	Well demarcated	Entirely myxoid	Absent	Absent	Absent	3	SCD34FT

* mitoses were counted in 50 40x objective fields (field area of 0.238 mm²); PLS, pleomorphic liposarcoma; UPS, undifferentiated pleomorphic sarcoma;

PHAT, pleomorphic hyalinising angiectatic tumor; SCD34FT, superficial CD34-positive fibroblastic tumor

TABLE 3: Immunohistochemical and molecular features

Case*	CD34	Cytokeratins AE1/AE3 or MN116	S100 protein	Ki-67	PRDM10 cytoplasmic	PRDM10 nuclear	FISH†	Fusion transcript	Fusion junction‡
1	Pos	Pos (focal)	Neg	5%	1+ weak	4+ strong	Pos (68%)	<i>CITED2-PRDM10</i>	ex2-ex14
2	Pos	N/A	N/A	N/A	1+ weak	3+ moderate§	Pos (93%)	<i>MED12-PRDM10</i>	ex43-ex14
3	Pos	N/A	N/A	N/A	1+ weak	3+ moderate§	N/D	<i>MED12-PRDM10</i>	ex43-ex13
4	Pos	N/A	Neg	5%	1+ weak	4+ strong	N/D	<i>CITED2-PRDM10</i>	ex2-ex14
5	Pos	Pos (focal)	Neg	< 5%	1+ weak	3+ moderate	Pos (36%)	Neg	N/A
6	Pos	Pos (focal)	Neg	5%	1+ weak	4+ strong	Pos (17%)	N/D¶	N/A
7	Pos	Pos (focal)	Neg	5%	1+ weak	4+ moderate	Neg (8%)	<i>MED12-PRDM10</i>	ex42-ex14
8	Pos	Pos (focal)	Neg	N/A	1+ weak	3+ moderate	Pos (26%)	<i>MED12-PRDM10</i>	ex43-ex13
9	Pos	Neg	Neg	N/A	1+ weak	4+ strong	Neg (13%)	<i>CITED2-PRDM10</i>	ex2-ex14

*Cases 1, 2, and 3 correspond to cases 2, 27 and 1 in ref 9;

N/A, not available;

† Interphase FISH using a break-apart probe for *PRDM10*;

‡ Fusion junction based on RNA-seq and/or RT-PCR results;

§ *PRDM10* antibody dilution 1:25;

|| The karyotype supported the finding of a fusion between *CITED2* (maps to chromosome band 6q24) and *PRDM10* (11q24): 73-90,XXY,-Y,del(3)(p25)x2,del(6)(q23)x2,add(11)(q25)x2,add(20)(p13)x2,inc (Case 240 in ref 8);

¶ Extracted RNA was of too poor quality (Dv200 value 23%)

TABLE 4: Immunohistochemical staining patterns for PRDM10

Tumor type	n	PRDM10 cytoplasmic	PRDM10 nuclear
Undifferentiated pleomorphic sarcoma	10	3+ to 4+ variable, weak to strong	Negative
Pleomorphic liposarcoma	6	1+ to 2+ weak	2 cases 3+ to 4+ moderate to strong
Dedifferentiated liposarcoma	2	2+ weak	1+ negative to weak
Myxofibrosarcoma	5	3+ to 4+ weak to moderate	1 case 3+ moderate, all others 1+ negative or weak
Angiomatoid fibrous histiocyoma	5	3+ to 4+ weak to moderate	1+ negative to weak
Dermatofibrosarcoma protuberans	5	0 to 3+ negative to weak	1+ negative to weak
Atypical fibrous histiocyoma	2	3+ to 4+ weak to moderate	3+ negative to weak
Schwannoma	5	4+ moderate	Negative
Epithelioid sarcoma	6	3+ moderate	2+ to 3+ weak

TABLE 5: Features of superficial CD34-positive fibroblastic tumors without evidence of *PRDM10*-rearrangement

Case	Age*/sex	Location	Size, mm	CD34	Morphology	Mitoses/50hpf†	Cytoplasmic PRDM10 IHC	PRDM10 Nuclear	FISH	RNA-Seq	Follow up [months]
1	30/F	Shoulder	40	Positive	Circumscribed lesion with focally infiltrative margins, pleomorphic, glassy cytoplasm, virocytes, distinct cell borders	11	3+ moderate	Negative	Failed	Negative	NED [48]
2	42/F	Thigh	30	Positive	Circumscribed lesion, pleomorphic, glassy cytoplasm, virocytes, distinct cell borders nuclear vacuolisation	4	3+ weak	Negative	Failed	N/D¶	NED [48]
3	40/F	Chest wall	30	Positive	Focally infiltrative, moderate pleomorphism, glassy cytoplasm	2	3+ moderate	Negative	Negative	Negative	N/A
4	25/M	Lateral foot	35	Positive	Infiltrative lesion, fascicular, glassy cytoplasm, non-myxoid, inflammatory infiltrate not prominent.	1	3+ strong	2+ weak	Failed	N/D¶	N/A

*age at diagnosis; † mitoses were counted in 50 40x objective fields (field area of 0.238 mm²); M, male; F, female; NED, no evidence of disease; N/A, not available; N/D, not done.