Original paper

Immunohistochemical positive regulatory domain member 10 expression in soft tissue sarcomas

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Positive regulatory domain member (PRDM) proteins play a critical role in the transmission of signals that control cell proliferation and differentiation, and neoplastic transformation. Positive regulatory domain member 10 (tristanin) is a poorly studied member of PRDM protein family. Gene fusion transcripts containing PRDM10 were recently identified in low-grade undifferentiated pleomorphic sarcomas (UPS), and associated with pleomorphic morphology and low mitotic index. The aim of this study was to investigate the immunohistochemical staining of PRDM10 in a larger sample of soft tissue sarcomas. Therefore, the study included 118 soft tissue sarcomas from different classes, and PRDM10 antibody was applied to all of them.

Immuno-histochemically, staining was observed in 22 (19%) cases, while 96 (81%) showed no staining. When PRDM10 expression was compared with clinico-pathological features, there was a statistically significant correlation between PRDM10 expression and myxoid changes, multi-nucleated giant cells, and surgical margin ($p = 0.017$, $p = 0.034$, $p = 0.032$, respectively). No statistically significant association was found between PRDM10 expression and other parameters.

Based on the obtained data, it can be said that PRDM10-positive-stained tumors (tumors with PDRM10 expression) are mostly myxoid, containing multi-nucleated giant cells, and can be removed with well-circumscribed margins.

Key words: undifferentiated pleomorphic sarcoma, PRDM10, tristanin, soft tissue.

Introduction

Soft tissue can be defined as non-epithelial extra-skeletal tissues of the body, excluding the reticulo-endothelial system, glia, and supporting tissues of various parenchymal organs [1]. Soft tissue tumors represent a highly heterogeneous group of rare malignancies, classified according to the mature tissue they resemble, with an overall incidence of about 60/1,000,000 per year [1, 2]. These tumors occur in the extremities in 75% of cases, in the abdomen in 30% of cases, and in the trunk, head and neck region in 15% of cases [1–3].

Soft tissue sarcomas tend to behave aggressively and metastasize in the majority of cases. Tumor size, location, depth, and histologic type are prognostic factors for metastatic risk and overall survival. With few exceptions, histological typing does not provide sufficient information to predict the clinical course of the disease. Therefore, grading systems based on histologic parameters have been developed to provide a more accurate estimate of the degree of tumor malignancy [3, 4].

In its' fifth guideline published in 2020, World Health Organization (WHO) classified soft tissue sarcomas into 11 groups according to their histological

differentiation. In general, the WHO classification divides each group into four categories based on their biological behavior: benign, intermediate (locally aggressive), intermediate (rarely metastatic), and malignant. The new classification includes more detailed cytogenetic and molecular data consistent with the rapidly growing knowledge in tumor genetics [5].

Although immuno-histochemistry (IHC) was previously used mainly to determine lineage of differentiation, it is now evolving into newer markers that directly or indirectly detect tumor-specific genetic abnormalities to identify specific molecular alterations with new molecular genetic discoveries [6, 7]. The spectrum of diagnostic immunohistochemical markers for proteins formed by repetitive molecular genetic aberrations in soft tissue tumors is expanding. Moreover, immuno-histochemistry can also guide targeted therapy for these molecular alterations because it is cheap and easy to administer. In selected cases, IHC can replace molecular diagnostic confirmation of specific genetic events. As molecular advances continue, so will immunohistochemical and related studies [7].

Positive regulatory domain member (PRDM) is a family of proteins characterized by the presence of a PR domain and a variable number of zinc finger repeats. It takes its' name from two founding members: positive regulatory domain I-binding factor 1 [(PRD1-BF1)/PRDM1]; is defined by the presence of zinc finger protein 1 [(RIZ1)/PRDM2], which interacts with retinoblastoma, and by the presence of a PR domain, which is 20–30% identical at the amino acid level to SET domain found in many histone lysine methyltransferases [8]. The PRDM protein family has a unique structure with an N-terminal PR domain that has potential methyltransferase activity and zinc finger proteins at the C-terminal end, which mediate protein-protein, protein-RNA, or protein-DNA interactions [9]. Currently, 16 family members have been identified in mice and 17 in humans [8, 10].

The PRDM10 protein is a poorly studied member of this family that has been shown to control corneal endothelial cell differentiation and proliferation, and contains the PR domain (PRDI-BF1-RIZ1 homology domain) shared by many histone lysine methyltransferases [11, 12].

Analysis of soft tissue sarcomas using RNA sequencing and other methods suggests that PRDM10 has a gene fusion with MED12 or CITED2, and that these re-arrangements are specific to a sub-set of low-grade UPS [13].

PRDM10 protein plays a role in the development, progression, and drug resistance of many malignancies, such as hepato-cellular, prostate, and naso-pharyngeal, gastric, and rectal carcinomas [14–19]. In addition, PRDM10-re-arranged soft tissue tumors are characterized by pleomorphism and a low mitotic count [20]. Positive regulatory domain member 10 protein may influence apoptosis by stimulating BCL2 gene expression at the transcriptional level. The expression of Bcl-2 and PRDM10 correlates in cancers that over-express PRDM10. It has been argued that the upregulation of PRDM10 in cancers over-expressing PRDM10 may be a potential mechanism for tumorigenesis, and may play an oncogenic role [2].

An attempt was made to evaluate the diagnostic utility of immunohistochemical expression of PRDM10 without molecular studies. In the current study, 118 soft tissue sarcomas were examined to detect tumors with immunohistochemical expression of PRDM10, and to evaluate the morphologic and clinical features and prognosis. A specific tumor group was not selected, and all cases (n = 118) diagnosed with soft tissue sarcoma between 2013 and 2020 were included in the study.

Material and methods

All cases (n = 118) diagnosed with soft tissue sarcoma between 2013 and 2020 at Mersin University Faculty of Medicine, Department of Medical Pathology, were included in the study. Hematoxylin-eosin-stained slides of formalin-fixed, paraffin-embedded blocks of the cases were retrieved from the archives, re-evaluated by two investigators, and the most suitable blocks for the immunohistochemical method, which had sufficient tumor tissue, good fixation, and little or no necrosis, were selected for investigation. Using IHC, we studied PRDM10 in all cases. Patients clinico-pathological data, including sex, age, location, tumor size, and diagnosis, were obtained from the hospital operating system and pathology reports. This study was approved by Clinical Research Ethics Committee of the Mersin University Faculty of Medicine by decision number of 2019/ 333 on 07/08/2019.

Immunohistochemical staining

For the application of PRDM10 immunohistochemical antibody, 2.5 – 3 μm thick sections of paraffin-embedded blocks fixed in formalin were collected on positively charged slides, and then de-paraffinized with xylene after one hour in an oven at 68°C. A primary rabbit polyclonal PRDM10 antibody (NBP1-81427 Novus Bio, Abingdon, UK) to the C-terminal portion of protein and chromogen UltraWiev DAB were used to detect PRDM10. The antibody was treated at a dilution of 1/125 for 2 hours in a Ventana BenchMark Ultra automated staining instrument (Ventana Medical Systems; Roche, USA). Duodenum and testicular tubules were selected as positive controls to optimize antibody dilution and antigen uptake.

Immunohistochemical evaluation

Two pathologists assessed the stained slides under Olympus BX53 double-head light microscope. Immunohistochemical staining was evaluated by the percentage and intensity of positive cells staining [21]. The intensity and extent of both nuclear and cytoplasmic PRDM10 immunoreactivity were graded semi-quantitatively:

- according to cytoplasmic/nuclear staining percentage of positive tumor cells immuno-histochemically:
  – staining in 1–5% of tumor cells – 1+,
  – staining in 6–50% of tumor cells – 2+,
  – staining in > 50% of tumor cells – 3+;
- according to staining intensity:
  – negative,
  – weak,
  – moderate,
  – strong;
- for statistical analysis, the values obtained by multiplying staining percentage in the tumor by staining intensity (0, 1, 2, 3, 4, 6, 9) were grouped and categorized:
  – category 1 – 0, 1, 2,
  – category 2 – 3, 4,
  – category 3 – 6, 9.

Statistical analysis

SPSS-16 package program was used for statistical analysis of the data. Clinico-pathological parameters and staining results of PRDM10 antibody were analyzed using χ^2 test. Results with χ^2 significance values at $p < 0.05$ and $p \geq 0.05$ were considered statistically significant and not statistically significant, respectively.

Results

Of the 118 patients diagnosed with soft tissue sarcoma in the study, 63 (53.4%) were males and 55 (49.6%) were females. The youngest patient was 3 years old, and the oldest was 96 years old. The mean age was 50.6 years, and the median age was calculated as 52 years. Among the 118 cases, 18 (15.2%) were diagnosed as undifferentiated pleomorphic sarcoma, 14 (11.8%) as leiomyosarcoma, 11 (9.3%) as synovial sarcoma, 11 (9.3%) as Ewing/PNET sarcoma, 7 (5.9%) as myxoid liposarcoma, 7 (5.9%) as dedifferentiated liposarcoma, 6 (5.1%) as epithelioid sarcoma, 6 (5.1%) as angiosarcoma, 6 (5.1%) as malignant peripheral nerve sheath tumors, 5 (4.2%) as rhabdomyosarcoma, 4 (3.4%) as low-grade fibro-myxoid sarcoma, 4 (3.4%) gastrointestinal stromal tumors, 2 (1.7%) low-grade myxofibrosarcoma, 2 (1.7%) as high-grade myxofibrosarcoma, 2 (1.7%) as atypical lipomatous tumor/well-differentiated liposarcoma, 2 (1.7%) as extra-skeletal myxoid chondrosarcoma, 2 (1.7%) as extra-skeletal osteosarcoma, 2 (1.7%) as unclassified malignant mesenchymal tumors, 1 (0.8%) as angiomatoid fibrous histiocytoma, 1 (0.8%) as low-grade malignant myxoid mesenchymal tumor, 1 (0.8%) as malignant glomus tumor, 1 (0.8%) as fibrosarcoma that developed from dermato-fibrosarcoma protuberans, 1 (0.8%) as pleomorphic liposarcoma, 1 (0.8%) as desmoplastic small-cell round tumor, and 1 (0.8%) as malignant rhabdoid tumor.

While the tumor persisted at surgical margin in 68 (57.7%) cases, no persistence of the tumor at surgical margin was observed in 47 cases (39.8%), and surgical margin could not be reached in three cases (2.5%).

Myxoid changes were seen in 37 (31.4%) cases included in the study, and no myxoid changes were found in 81 (68.6%) cases. Multi-nucleated tumor giant cells were observed in 43 (36.4%) cases, while they were not observed in 75 (63.6%) cases. In addition, while there were vacuolar changes in 18 (15.3%) of soft tissue tumors, this change was not detected in 100 (84.7%) of them. Clinico-pathological features of the cases included in the present study are summarized in Table 1.

Considering the ratio of cells stained with PRDM10 to all tumor cells, 22 tumors (18.6%) had positive results, with staining detected in more than 50% of cells (3+ PRDM10-stained) in ten cases, 6–50% of cells (2+ PRDM10-stained) in six cases, and less than 5% of cells (1+ PRDM10-stained) in six cases; staining was negative in 96 cases. Eleven of the 22 positive cases had weak staining, four had moderate staining, and seven had strong staining.

While 9 of the 22 positive-stained sarcoma cases were diagnosed as undifferentiated pleomorphic sarcoma, 2 cases contained myxoid areas, and 1 case showed myogenic differentiation. Of the remaining 13 positive-stained tumor cases, 2 were diagnosed as myxoid liposarcoma, 2 as dedifferentiated liposarcoma, 1 as monophasic fibrous synovial sarcoma, 1 as extra-skeletal myxoid chondrosarcoma, 1 as embryonal type rhabdomyosarcoma, 1 as angiosarcoma, 1 as epithelioid malignant peripheral nerve sheath tumor, 1 as epithelioid sarcoma, 1 as leiomyosarcoma, 1 as low-grade fibro-myxoid sarcoma, and 1 as high-grade myxofibrosarcoma. Distribution of immunohistochemical PRDM10-positive-stained cases by diagnosis is shown in Figure 1. A comparison of the percentage and intensity of PRDM10 immunohistochemical staining according to tumor type is presented in Table 2.

Of the 22 soft tissue sarcomas that were PRDM10-positive-stained, half were females and half were males. The mean age at diagnosis was 58.5 years. No significant difference was found between cases with positive and negative PRDM10 expression in relation to gender ($p = 0.381$).

The location could not be determined as one of the positive cases came with a consultation from an external center. Fifteen of them were located in the

Table 1. Clinico-pathological features of cases included in the present study

Age (years)	
Youngest age	3.0
Oldest age	96.0
Mean age	50.6
Median age	52.0
Location, *n* **(%)**	
Extremity location	68 (57.6)
Intra-abdominal location	16 (13.5)
Other areas	34 (28.8)
Largest diameter of the tumor, *n* **(%)**	
≤ 5 cm	29 (24.6)
> 5 cm to ≤ 10 cm	37 (31.4)
> 10 cm to ≤ 15 cm	26 (22.0)
> 15 cm	19 (16.1)
Pleomorphism, *n* **(%)**	
Present	46 (39.0)
Not present	72 (61.0)
Number of mitoses (in 10 high-magnification fields), *n* **(%)**	
0–9 pieces	46 (39.0)
10–19 pieces	27 (22.9)
≥ 20 pieces	45 (38.1)
Necrosis, *n* **(%)**	
Present	75 (63.6)
Not present	43 (36.4)
Myxoid change, *n* **(%)**	
Present	37 (31.4)
Not present	81 (68.6)
Surgical margin, *n* **(%)**	
Tumor present	68 (57.7)
Tumor not present	47 (39.8)
Multi-nuclear giant cells, *n* **(%)**	
Present	43 (36.4)
Absent	75 (63.6)
Nuclear vacuolization, *n* **(%)**	
Present	18 (15.3)
Absent	100 (84.7)

extremities, four in the abdomen, and two in other regions. There was no significant difference between tumors with positive and negative PRDM10 expression in terms of location ($p = 0.489$).

The size of two of the positive cases could not be determined; one was 5 cm or smaller, eight were larger than 5 cm, smaller than 10 cm, seven were larger than 10 cm, smaller than 15 cm, and four were larger than 15 cm. There was no significant difference between tumor diameter and PRDM10 expression ($p = 0.065$).

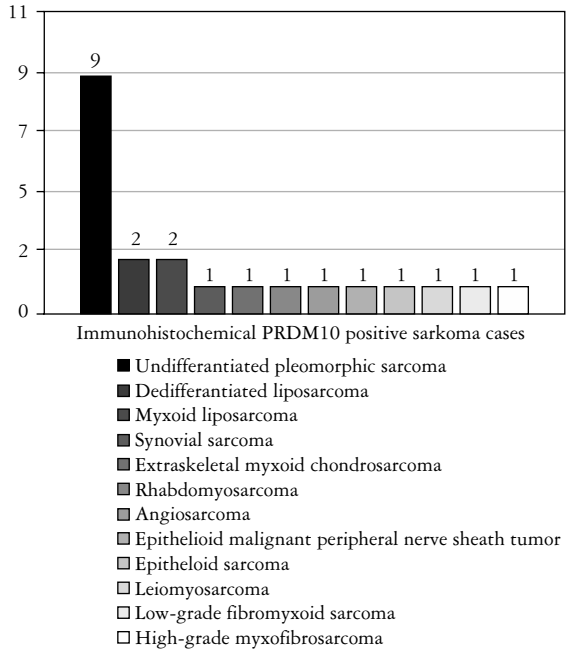

Fig. 1. Distribution of immunohistochemical positive regulatory domain member 10-positive-stained cases by diagnosis
PRDM – positive regulatory domain member

While no pleomorphism was observed in seven of the cases with PRDM10 staining, pleomorphism was observed in fifteen cases. There was no significant difference in pleomorphism between PRDM10 expression of positive and negative tumors ($p = 0.186$).

While the percentage of necrosis and PRDM10 staining in the tumor was only marginally significant ($p = 0.050$), a statistically significant difference was found in terms of staining score ($p = 0.024$).

Thirteen of the 22 soft tissue sarcomas that were PRDM10-positive-stained had myxoid changes, and nine did not have. When PRDM10 expression was compared with clinico-pathological features, a statistically significant correlation was found between PRDM10 expression and myxoid changes' staining percentage, staining intensity, and staining score, which was obtained by multiplying staining intensity by staining percentage ($p = 0.018$, $p = 0.017$, $p = 0.017$, respectively). It was determined that PRDM10 expression was higher in tumors with myxoid changes (Fig. 2).

Multi-nucleated tumor giant cells were observed in 12 of these cases, and not in 10. A significant correlation was found between multi-nucleated tumor giant cells and staining intensity and staining percentage ($p = 0.034$). Tumors with multi-nucleated tumor giant cells showed more PRDM10 expressions (Fig. 3). While the surgical margins were intact in 14 cases, the tumor persisted in the surgical margins of 8 cases. While there was no significant difference

Table 2. Comparison of percentage and intensity of positive regulatory domain member 10 immunohistochemical staining according to tumor type

Number of cases	Tumor type	Staining intensity	Staining percentage		
			One positive (+)	Two positive (++)	Three positive (+++)
11,598-18	UPS, containing myxoid areas	Moderate			X
698-17	UPS, containing myxoid areas	Strong	X		
12,225-19	UPS, showing myogenic differentiation	Strong			X
10,934-15	UPS	Weak		X	
669-18	UPS	Weak			X
967-18	UPS	Weak	X		
6,705-20	UPS	Weak		X	
11,903-19	UPS	Weak	X		
211-20	UPS	Strong			X
15,860-19	Dedifferentiated liposarcoma	Weak	X		
8,010-19	Dedifferentiated liposarcoma	Strong			X
585-14	Myxoid liposarcoma	Weak	X		
5,825-18	Myxoid liposarcoma	Moderate		X	
7,703-20	Monophasic synovial sarcoma	Moderate			X
13,654-17	Extra-skeletal myxoid chondrosarcoma	Weak			X
4,266-20	Rhabdomyosarcoma	Strong		X	
15,125-19	Angiosarcoma	Weak		X	
1,045-19	Epithelioid malignant peripheral nerve sheath tumor	Moderate			X
7,286-16	Epithelioid sarcoma	Weak	X		
2,384-20	Leiomyosarcoma	Strong			X
16,509-19	Low-grade fibro-myxoid sarcoma	Weak	X		
1,579-20	High-grade myxofibrosarcoma (malignant fibrous histiocytoma, myxoid type)	Strong			X

UPS – undifferentiated pleomorphic sarcomas

in the percentage of PRDM10 staining ($p = 0.087$), a significant difference was found in terms of staining intensity ($p = 0.032$).

A statistically significant difference was observed, when surgical margin and staining intensity were compared in tumors with PRDM10 expression ($p = 0.032$). PRDM10 expression was lower in cases with positive surgical margins. Additionally, there was no statistically significant association between other parameters and PRDM10 expression (mitosis: $p = 0.575$; nuclear vacuolization: $p = 0.590$).

When comparing prognosis, staining percentage, and staining intensity in tumors with PRDM10 expression, a significant statistical difference was observed ($p = 0.040$, $p = 0.035$). Tumors expressing PRDM10 were found to be associated with longer survival. This implies that PRDM10 can be used as a marker for predicting patient's prognosis. Clinicopathological features of the cases with immunohistochemical staining of PRDM10 (PRDM10-positive-stained tumors) are shown in Table 3.

Discussion

The spectrum of diagnostic immunohistochemical markers for protein markers of recurrent molecular genetic abnormalities in soft tissue tumors is expanding, and currently provides essential diagnostic and prognostic information. Immunohistochemical markers specifically designed for molecular markers can guide targeted therapy as they are cheap and easy to use. In some conditions, they can also replace molecular diagnostic confirmation of specific genetic alterations [7].

Positive regulatory domain member 10 has been shown to be associated with many epithelial tumors. Zhang *et al*. found that PRDM10 was over-expressed in hepato-cellular carcinomas, and therefore may play a critical role in tumor formation and progression [14].

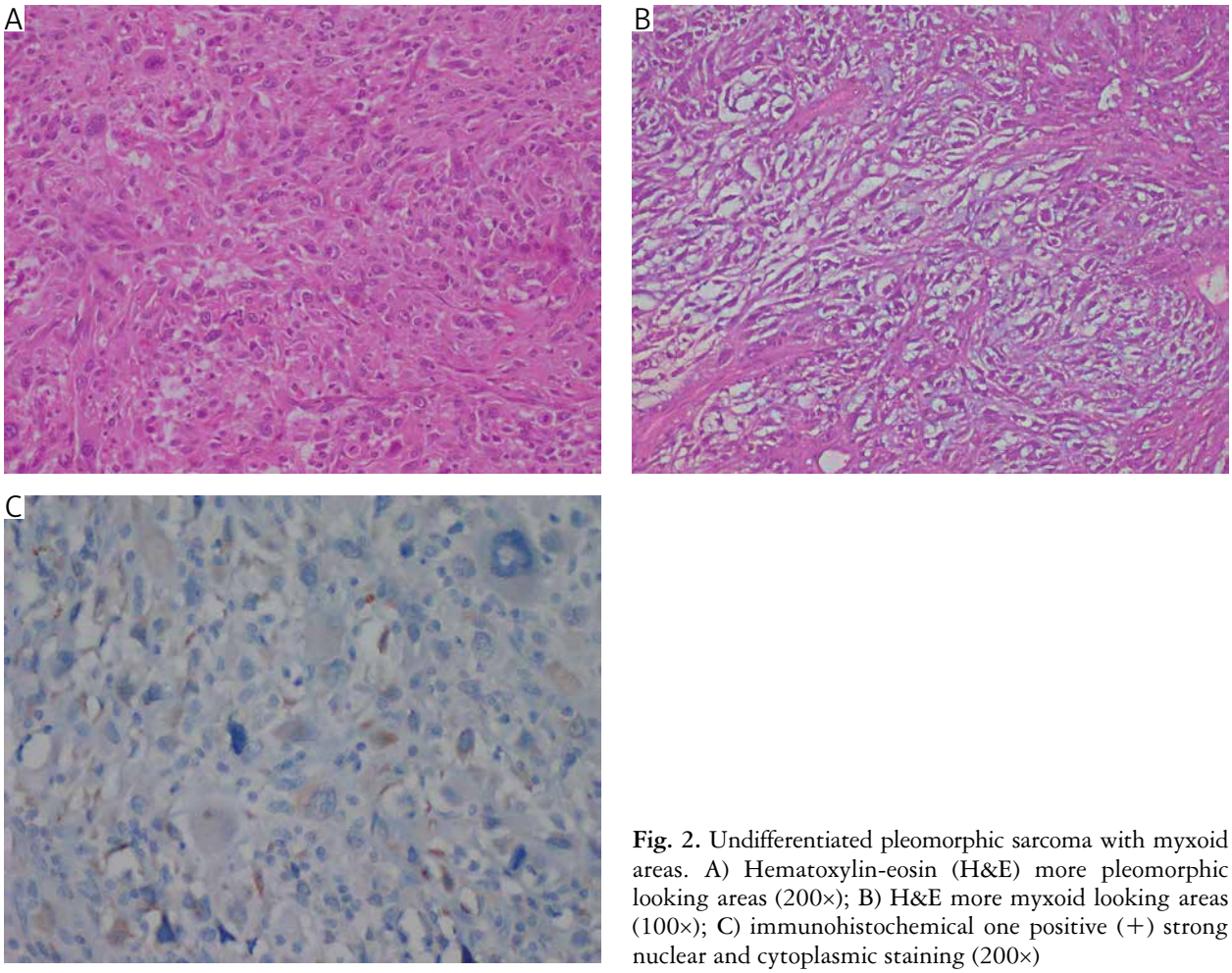

Fig. 2. Undifferentiated pleomorphic sarcoma with myxoid areas. A) Hematoxylin-eosin (H&E) more pleomorphic looking areas (200×); B) H&E more myxoid looking areas (100×); C) immunohistochemical one positive (+) strong nuclear and cytoplasmic staining (200×)

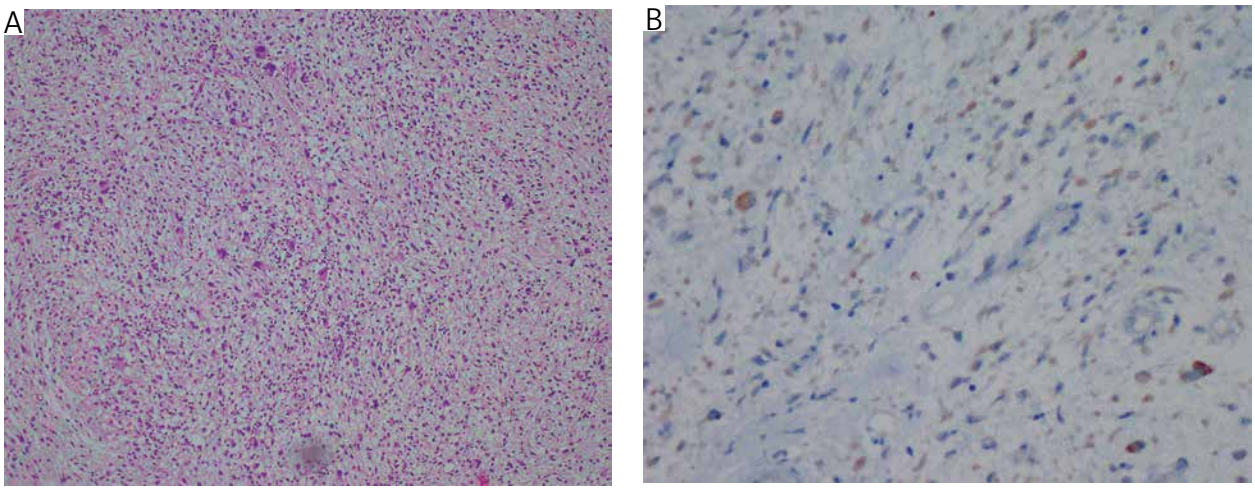

Fig. 3. The appearance of undifferentiated pleomorphic sarcoma with giant cells on hematoxylin-eosin-stained sections (H&E, 200×) (A), and immunohistochemical three positive (+++) cytoplasmic and nuclear staining (400×) (B)

Lou *et al*. reported that breast cancer cells have up-regulated PRDM10 expression. This suggests that PRDM10 plays a role in breast cancer cell proliferation and invasion. Moreover, these authors considered that its' association with drug resistance should be investigated [15]. Mansouri *et al*. stated that PRDM10 is a common biomarker in the development of gastric cancer and chronic gastritis, and can be used for drug targets [16]. Rostami-Nejad *et al*. found that PRDM10 plays a role in the transition from grade 2 to grade 3 in rectal cancers [17]. Wu *et al*. demonstrated that PRDM10 functions as an oncogene in

Table 3. Clinico-pathological features of cases with immunohistochemical staining of positive regulatory domain member 10 (tumors with positive regulatory domain member 10 expression)

Gender	
Female	11
Male	11
	$p = 0.381$
Age (years)	
Mean age	58.5
Location	
Extremity	15
Intra-abdominal	4
Other areas	2
	$p = 0.866$
Largest diameter of the tumor	
≤ 5 cm	1
> 5 cm to ≤ 10 cm	8
> 10 cm to ≤ 15 cm	7
> 15 cm	4
	$p = 0.230$
Pleomorphism	
Present	15
Not present	7
	$p = 0.544$
Number of mitoses (in 10 high-magnification fields)	
0–9 pieces	10
10–19 pieces	5
≥ 20 pieces	7
	$p = 0.408$
Necrosis	
Present	13
Not present	9
	$p = 0.050$
Myxoid change	
Present	13
Not present	9
	$p = 0.018$
Surgical margin	
Tumor present	8
Tumor not present	14
	$p = 0.087$
Multi-nuclear giant cells	
Present	12
Absent	10
	$p = 0.030$
Nuclear vacuolization	
Present	5
Absent	17
	$p = 0.590$

molecular mechanisms of prostate cancer initiation and development, and shows nuclear staining by IHC [18]. Bein *et al*. revealed that PRDM10 plays a role in the development and progression of lung cancer [22]. Azodi *et al*. found that PRDM10 might play an important role in the development and progression of nasopharyngeal carcinoma, and argued that new treatment approaches could be achieved by targeting this protein [19]. Ye *et al*. indicated that PRDM10 expression is higher in squamous cell carcinomas of the esophagus than in normal tissues, and that high PRDM10 expression is associated with higher survival. They also claimed that PRDM10 could be used as a prognostic parameter in squamous cell carcinomas of the esophagus [23]. In addition, using the Cancer Genome Atlas project, Sorrentino *et al*. detected PRDM10 expression in carcinomas of the ovary, prostate, lung, kidney, colon, and breast [24].

As mentioned earlier, PRDM10 has been studied in a variety of epithelial tumors and lesions in the literature. However, studies in soft tissue sarcomas are limited. Gene fusion transcripts containing PRDM10 have recently been identified in low-grade UPS [20, 23]. Undifferentiated pleomorphic sarcomas are soft tissue sarcomas that do not have a clear differentiation lineage, and can be detected by current diagnostic technological methods. It has a patternless appearance, and often contains bizarre multinucleated tumor giant cells. It is common in adults, and occurs most frequently in the lower extremities. Typically, the local recurrence rate ranges 19–31% and the metastasis rate is 31–35% [25].

In 2015, Hofvander *et al*. first found that two UPS had novel gene fusions, including PRDM10, MED12, and CITED2, which were not previously counted as neoplasia-related gene fusions [13]. The authors then examined 82 other soft tissue sarcomas, and detected MED12/PRDM10 fusion in one additional case. They concluded that since these three gene fusions had not been detected in any other neoplasm, they may be specific to a small sub-set of UPS. They noted that all three cases were morphologically and immunophenotypically characteristics of UPS, which had no specific lineage of differentiation, consisted morphologically of bizarre, irregular, spindle-shaped, oval, or multi-nucleated cells with eosinophilic cytoplasm and vesicular nuclei, and contained multifocally scattered lymphocytes in a collagenous stroma; interestingly, they had less than one mitosis and no necrosis in ten high-magnification fields. The authors detected a focal myxoid matrix in one case, prominent pseudo-vascular clefts in one case, and a large number of multi-nucleated giant cells in one case, with CD34 staining detected in all three tumors [13].

In 2018, Puls *et al*. investigated PRDM10 immunoreactivity in a series of 50 cases. They detected PRDM10 expression by IHC in 9 of this series, and

PRDM10 fusion by molecular methods in 7 cases. Three immunohistochemically positive cases were diagnosed as UPS, 3 cases as superficial CD34-positive fibroblastic tumor, 2 cases as pleomorphic liposarcoma, 1 case as pleomorphic hyalinized angioectatic tumor [20]. Three cases of UPS, which were immunohistochemically positive in this series, are the cases published by Hofvander *et al*. [13]. In the present study, nuclear and cytoplasmic immunohistochemical staining was considered positive. Similar to a study by Hofvander *et al*. [13], the tumors typically had dense collagenous stroma, myxoid areas, marked cellularity, irregular hyperchromatic nuclei, distinct nucleoli and pleomorphic nuclei; sometimes with pseudo-vascular cleft formation and diffuse chronic inflammatory infiltrates consisting of lymphocytes, plasma cells, and often eosinophils, showing strong CD34 positivity. Our study examined 118 soft tissue sarcomas, and 18 were diagnosed with UPS. Staining with the immunohistochemical antibody PRDM10 was performed in 22 of these tumors. Nine of these 22 cases were diagnosed with UPS. In all 9 cases, oval or multi-nucleated tumor cells with bizarre vesicular nuclei were observed, similar to the study by Hofvander *et al*. [13] and Puls *et al*.; scattered lymphocytes were seen in the stroma in all cases. Similar to the literature, pleomorphism was noted in 15 of the 22 cases with PRDM10 immunoreactivity, and multi-nucleated tumor giant cells were observed in 12 cases, with a statistically significant association ($p = 0.034$). Tumors with multi-nucleated giant cells show more PRDM10 expression.

In the study by Hofvander *et al*. [13], myxoid changes were detected in one out of three cases. Puls *et al*. [20] observed geographic myxoid areas in 7 out of 9 cases. In our study, myxoid changes were observed in 13 out of 22 cases, which was statistically significant ($p = 0.017$). It can be said that myxoid changes are associated with lower-grade tumors.

Hofvander *et al*. [13] also found in their study that less than one mitosis and no necrosis occurred in ten high magnification fields. In the study by Puls *et al*. [20], the number of mitoses was very low (0–7/50 high-magnification fields), and no necrosis was observed. However, in contrast to the literature, no mitosis was observed in only 3 of our cases, 1–7 mitoses in 7 cases, 11–16 mitoses in 7 cases, and > 20 mitoses in the remaining 5 cases, with no statistically significant difference noted. Again, necrosis was present in the majority of cases, although to a lesser extent. This could be due to the co-existence of low- and high-grade areas in the tumor.

In the study by Puls *et al*. [20], PRDM10 showed marked nuclear positivity immunohistochemically in all tumors with PRDM10 re-arrangement. Although weak nuclear and moderate cytoplasmic immunoreactivity is common in other mesenchymal neoplasms, moderate or strong nuclear positivity was observed only in two pleomorphic liposarcomas and one myxofibrosarcoma. All other entities tested showed highly variable cytoplasmic but weak and irregular nuclear PRDM10 immunoreactivity [20]. This is the first study to investigate PRDM10 protein in soft tissue sarcomas immunohistochemically. In our study, immunohistochemical positivity was detected in 1 monophasic fibrous-type synovial sarcoma, 1 extraskeletal myxoid chondrosarcoma, 1 rhabdomyosarcoma, 1 angiosarcoma, 1 epithelioid malignant peripheral nerve sheath tumor, 2 dedifferentiated liposarcomas, 2 myxoid liposarcomas, 1 epithelioid sarcoma, 1 low-grade fibro-myxoid sarcoma, 1 leiomyosarcoma, and 1 high-grade myxofibrosarcoma. Some of these tumors are actually high-grade tumors, contrary to the literature. In our immunohistochemical study, we also failed to detect staining in most of the PRDM10-applied soft tissue neoplasms. Therefore, we considered both cytoplasmic and nuclear staining positive in our evaluation. The inconsistency between our study and the literature may be due to the fact that the immunohistochemical evaluation method used in our study (nuclear and cytoplasmic staining) was different from the methods used in the studies in the literature (strong nuclear staining).

Puls *et al*. reported that of 9 tumors expressing PRDM10, 5 were located on the extremities, 2 on the shoulder, 1 on the trunk, and 1 on the perineum; tumor size ranged 1–6 cm, all tumors were generally well-circumscribed, and only four cases had focal marginal deterioration [20]. In our study, 15 of the cases were located in the extremities, 4 in the intra-abdominal region, and 2 in the gluteal region, which is in agreement with this study, and the tumor was mostly observed on the extremities. Also in our study, the tumor was completely removed in 14 of the cases, and the tumor did not persist at surgical margin, which was statistically significant ($p = 0.032$). However, the tumor size in our study ranged 6–26 cm.

In the literature, tumors with PRDM10 re-arrangement were found to have a lower metastatic tendency, and were associated with a favorable outcome. However, it has been emphasized that there is no characteristic morphologic feature that distinguishes them from other UPS [13, 20]. In a study by Carter *et al*., a superficial CD34-positive fibroblastic tumor characterized by marked pleomorphism and a low mitotic count was described [26]. In the study by Hofvander *et al*., a substantial overlap between superficial CD34-positive fibroblastic tumors and tumors re-arranged by PRDM10 was reported [13]. In another study by Foot *et al*., both CD34-positive fibroblastic tumors and tumors re-arranged by PRDM10 were described as pleomorphic tumors with low mitotic index, staining with CD34, and of-

ten with lipidized cells and chronic infiltrate. It has also been said that tumors showing re-arrangement with PRDM10 differ from superficial CD34-positive fibroblastic tumors in that they contain myxoid alterations and show PRDM10/MED12 or PRDM10/CITED2 gene fusion [27].

Comparison between morphologic features of the cases and cytogenetic data in UPS indicates that PRDM10 fusions are rare. Therefore, the incidence of tumors with a PRDM10 fusion is estimated to be approximately 5% of all UPS [13]. Undifferentiated pleomorphic sarcomas are a heterogeneous group, and their genetic features have been poorly studied. Although cases showing PRDM10 re-arrangement represent only a small proportion of all UPS, it may be important to know this from a clinical and survival point of view. Therefore, UPS indeed requires aggressive treatment, with a metastasis rate of 30%. From the literature, tumors that have a PRDM10 re-arrangement appear to have a lower propensity to metastasize. Hence, detection of tumors with PRDM10 may be important to avoid aggressive treatment. However, evaluation should be done in a much larger case series to determine the PRDM10 expression ratio among all UPS, and whether it makes a difference in treatment.

In the literature, studies of tumors with PRDM10 re-arrangement in soft tissue tumors have been started in recent years. There are very few studies in the literature on this topic; moreover, the number of PRDM10-re-arranged tumor studies, in which IHC has been evaluated, is limited. To the best of our knowledge, the present research is the second study in the literature to investigate PRDM10 protein in soft tissue tumors immunohistochemically, and it is the largest study performed with 118 soft tissue sarcomas. Consistent with the literature, our study showed that myxoid changes can be observed in tumors expressing PRDM10, and that these tumors may contain multi-nucleated giant cells and have cleaner surgical margins. However, some of our results, such as mitotic activity, were not consistent with the literature. Therefore, we thought that this could also be due to technical reasons.

When PRDM10 expression was compared with clinico-pathological features, a statistically significant correlation was found between PRDM10 expression and the staining percentage, staining intensity, and staining score of myxoid changes. It was found that PRDM10 expression was higher in tumors with myxoid changes. A significant correlation was observed between multi-nucleated tumor giant cells and staining intensity and percentage. Tumors with multi-nucleated tumor giant cells showed more PRDM10 expression.

A statistically significant difference existed when surgical margin and staining intensity were compared in tumors with PRDM10 expression. PRDM10 expression is lower in cases with positive surgical margins. No statistically significant association was found between PRDM10 expression and gender, location, pleomorphism, size, mitosis, nuclear vacuolization, and hemorrhage.

Nine (50%) of 18 UPS cases and some high-grade sarcomas showed immunohistochemical staining with PRDM10. According to data in the literature, tumors with PRDM10 re-arrangement are expected to occur in about 5% of UPS cases, have an indolent course, and be tumors with low mitotic activity. The discrepancy between our results and the literature could be due to technical reasons.

The fact that the immunohistochemical evaluation method used in our study (cytoplasmic and nuclear staining) differs from the methods used in literature studies (strong nuclear staining) may have resulted in different values obtained in our data. Subjectivity can be a major confounding factor in such studies, as there is no clearly accepted cut-off point for assessing expression. Developing a consensus for scoring cases could help produce more reliable results. However, studies with larger samples are needed, in which immunohistochemical and molecular evaluations are performed in soft tissue tumors.

Conclusions

There are few studies in the literature that evaluate PRDM10 protein in soft tissue sarcomas by IHC. The present study has the largest sample size in soft tissue compared with studies in the literature, and is the second study to perform immunohistochemical evaluation.

It is clear that studies with larger cohorts are needed to clarify the immunohistochemical value of PRDM10 as a diagnostic marker that may be effective in determining prognosis and regulating treatment of soft tissue sarcomas, especially in UPS. Previous studies and our study suggested that the specificity of molecular methods for PRDM10 is higher than that of immunohistochemical methods, and that IHC should be studied in correlation with molecular methods.

The authors declare no conflict of interests.

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