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CLINICAL STUDY

Specificity and sensitivity of differentiation antigens in superficial soft tissue tumors: comparison of SMA, calponin, H-caldesmon, C-Kit, PLAP and HPL

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Abstract: We examined the expression pattern of smooth muscle actin (SMA), h-caldesmon (HCD), calponin (CALP), placental alkaline phosphatase (PLAP) and human placental lactogen (HPL) in benign and malignant spindle cell superficial soft tissue tumors in order to determine the role of these markers in differential diagnosis. Archival tissue from 38 patients with superficial smooth muscle cell and so-called fibrohistiocytic tumors (8 benign fibrous histiocytomas (BFHs), 6 dermatofibrosarcoma protuberans (DFPT), 9 malignant fibrous histiocytomas (MFHs), 9 leiomyomas (LMs) and 6 leiomyosarcomas (LMSs)) were immunostained with antibodies against SMA, HCD, CALP, PLAP and HPL. smooth muscle cell (SMC) tumors showed significantly high immunopositivity for HCD than that of so-called fibrohistiocytic tumors (p is less than or equal to 0.05) but 1/3 of DFPT and MFH cases and half of BFH cases also showed HCD immunopositivity; thus, this difference is debatable and not highly discriminative as expected. All tumor groups showed 100% immunopositivity for CALP. SMC tumors displayed significantly stronger and more widespread immunostaining pattern for PLAP than so-called fibrohistiocytic tumors ($p<0.05$). Superficial soft tissue tumors did not express c-kit. In conclusion, HCD and PLAP can be used as ancillary immunomarkers in differential diagnosis of SMC tumors *(Tab. 2, Fig. 7, Ref. 37)*. Full Text in free PDF *www.bmj.sk.*
Key words: Immunohistochemistry, h-caldesmon, placental alkaline phosphatase, soft tissue tumors.

Identifying benign and malignant spindle cell superficial soft tissue tumors is often challenging. Differential diagnosis of neoplasms of smooth muscle cell (SMC) origin and fibroblastic/myofibroblastic and so-called fibrohistiocytic tumors may be extremely difficult. Leiomyomas (LMs) and leiomyosarcomas (LMSs) are common and distinctive tumors showing SMC differentiation. Recent immunohistochemical studies have shown that some other soft tissue tumors, such as dermatofibroma, dermatofibrosarcoma protuberans (DFPT), malignant fibrous histiocytoma (MFH) (Undifferentiated pleomorphic sarcoma) (1) also show features of and have overlap with neoplasms of SMCs; these researchers detected smooth muscle actin (SMA) positive cells within these tumors (2–9). These immunoreactive cells are thought to be myofibroblasts but the origin of the myofibroblasts has not been elucidated (10). Although SMA is one of the most specific markers of SMC, myofibroblasts also express SMA (11–13).

H-caldesmon and calponin are cytoskeleton-associated proteins and both are expressed in smooth muscle and myoepithelial cells, similar to actins. Caldesmon is a Ca^{2+}, calmodulin-binding and actin-binding protein involved in the regulation of smooth muscle contraction. Its high molecular weight isoform, HCD, is thought to be specific to smooth muscle and myoepithelial cells (14). CALP is a calmodulin, F-actin, and tropomyosin-binding protein and is considered important in the regulation of smooth muscle contraction (14).

C-kit is a tyrosin kinase growth factor receptor expressed in diverse cell types, including Cajal cells of the gastrointestinal tract, mast cells and subsets of hematopoietic stem cells. Although it has been reported that c-kit staining was limited in soft tissue sarcomas (15), in some recent reports it has been stated that sarcomas showed c-kit immunopositivity (16).

Placental alkaline phosphatase (PLAP) is a 70-kDa alkaline phosphatase that is normally expressed in syncytiotrophoblasts and primordial germ cells (17). PLAP immunopositivity has been reported in SMC tumors recently (18).

Human placental lactogen (HPL), a member of the GH/prolactin gene family, is thought to alter maternal metabolism such that the pool of nutrients available for the fetus is increased (19).

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To the best of our knowledge there is not any previous report studying HPL expression in soft tissue tumors.

In this study in addition to SMA; we evaluated HCD, CALP, c-kit, PLAP and HPL as immunohistochemical markers for differential diagnosis of superficial soft tissue spindle cell neoplasms, and their distribution in LM, LMS, MFH, benign fibrous histiocytomas (BFH) and DFPT cases.

Materials and methods

Tissue Collection

Eight BFH, 6 DFPT, 9 MFH, 9 LM and 6 LMS cases were examined immunohistochemically. All cases were fixed in 10% formaline and embedded in paraffin wax. Sections 3–4 μm thick were cut and stained with hematoxylin and eosin.

Immunohistochemistry

Immunohistochemical studies using the streptavidin-biotin-peroxidase complex method were performed on the paraffin-embedded sections (20). The primary antibodies are: caldesmon (Clone TD107, Novo Castra, Newcastle, UK, monoclonal, dilution 1:50), calponin (Clone CALP, Novo Castra, Newcastle, UK, monoclonal, dilution 1:30), c-Kit receptor (Clone T595, Novo Castra, Newcastle, UK, monoclonal, 1:40), Placental lactogen (Novo Castra, Newcastle, UK, polyclonal, dilution 1:200), Placental Alkaline Phosphatase (Clone 8A9, Novo Castra, Newcastle, UK, monoclonal, dilution 1:20), Alpha Smooth Muscle Actin (Clone ásm-1, Novo Castra, Newcastle, UK, monoclonal, dilution 1:50).

Following fixation with 10 % formaldehyde, the samples were embedded in paraffin and 5 μm-thick sections were obtained from the selected paraffin blocks. The sections were deparaffinized in xylene, rehydrated through graded alcohols. Rehydration was followed by incubation with 2 % hydrogen peroxide and methanol for 5 min to prevent intrinsic peroxidase activity. After the samples were washed 3 times using PBS (phosphate-buffered saline, pH: 7.4), they were warmed in a microwave oven in 0.1 nM sodium citrate for 10 min and the antigen retrieval procedure was completed. Following the incubation and blocking of the non-immune serum at room temperature for 20 min (Novocastra, Novostatin Universal detection Kit, Newcastle, UK), the sections were incubated with the specific primary antibodies against SMA, HCD, CALP, PLAP and HPL for 60 minutes and for c-kit overnight at 4 °C. The sections washed in PBS were incubated with the biotin-conjugated secondary antibody for 30 min and then kept at room temperature with streptavidin horse- raddish peroxidase macromolecular complex (Novocastra, Novostatin Universal detection Kit, Newcastle, UK) for 30 min. AEC substrate system (3 Amino-9-Ethylcarbazole) solution (Novocastra, Novostatin Universal detection Kit, Newcastle, UK) was then used for color development in the tissues and Mayer's hematoxylin was used as contra-stain.

In each case the entire tumor area was evaluated at x400 magnification. We evaluated the immunoreactivities of HCD, CALP, and SMA in the cytoplasm of the tumor cells. For PLAP membranous, for HPL and for c- kit, plasma membranous and/ /or cytoplasmic staining was considered positive. Appropriate positive controls were performed at the same time, while tissue sections in which no primary antibody was applied were used as negative controls. Mast cells and Cajal cells in appendix and a GIST case were used as positive controls for c-kit.

The distribution of positive cells was graded as follows: 1) 0–25 % of stained; 2) 26–75 % of stained; and 3) more than 75 % of stained. The staining intensity was scored as follows: 1) absent or weak; 2) moderate; and 3) strong (clearly identified by x40 magnification). The sum of both parameters gave the immunohistochemical score. Category I corresponds to a score of 2, category II to a score of 3 or 4, and category III to a score of 5 or 6. Category I tumors were considered as immunonegative, whereas category II and III tumors were considered as immunopositive (21).

Statistical analysis

Data analysis was performed using SPSS Version 8.0 (Chicago, IL) statistical package. Frequency data were evaluated by the Kruskal-Wallis-nonparametric test. A p value = 0.05 was considered to be statistically significant.

Results

The immunohistochemical results are summarized in Tables 1 and 2.

All tumor groups displayed SMA immunostaining to some extent. BFHs showed less immunopositivity for SMA than the other groups in a statistically significant manner (p is less than or equal to 0.05). Besides SMC tumors, which showed 100 % positivity, DFPTs (Fig. 1) and MFHs also displayed SMA positivity in very high proportions (100 % and 78 % respectively).

So-called fibrohistiocytic tumors showed less immunopositivity for HCD than that of SMC tumors, and the difference was statistically significant (p is less than or equal to 0.05) although 2 of 6 DFPT and 3 of 9 MFH, and 4 of 8 BFH (Fig. 2) cases also showed HCD immunopositivity. In LMs, the intensity of HCD immunopositivity was strong and the distribution was diffuse (Fig. 3) whereas in LMSs these were moderate and less widespread, respectively. However, although immunopositivity decreased, the immunostaining score did not differ significantly ($p<1$).

All tumor groups showed 100 % immunopositivity for CALP (Figs 4 and 5) and 100 % immunonegativity for C-kit (Fig. 6).

SMC tumors displayed significantly stronger and more widespread immunostaining pattern for PLAP than so-called fibrohistiocytic tumors ($p<0.05$). Only 2 of 8 BFH cases showed PLAP positivity and none of DFPT and MFH cases contained PLAP positive tumor cells. Two of LMS cases were immunopositive for PLAP whereas 8 of 9 LM cases were positive (Fig. 7). LMs differed significantly from LMSs in relation to PLAP expression ($p <0.05$).

All tumor groups displayed HPL immunostaining to some extent and the evaluation of HPL expression among the differ-

Tab. 1. Expression of SMA, CALP, HCD, C-KIT, PLAP and HPL.

		SMA			CALP			HCD			C-KIT			PLAP			HPL		
		I	D	T	I	D	TS	I	D	T	I	D	T	I	D	T	I	D	T
BFH	1	3	3	6	2	2	4	2	2	4	1	1	2	1	1	2	2	2	4
	2	1	1	2	2	3	5	2	3	5	1	1	2	1	1	2	1	1	2
	3	1	1	2	2	2	4	2	2	4	1	1	2	1	1	2	2	2	4
	4	1	1	2	3	3	6	1	1	2	1	1	2	1	1	2	2	2	4
	5	1	1	2	2	3	5	1	1	2	1	1	2	2	2	4	2	3	5
	6	3	2	5	3	3	6	1	1	2	1	1	2	2	3	5	2	3	5
	7	1	1	2	3	3	6	1	1	2	1	1	2	1	1	2	1	1	2
	8	3	2	5	3	3	6	2	2	4	1	1	2	1	1	2	1	1	2
DFPT	9	3	2	5	3	3	6	1	1	2	1	1	2	1	1	2	2	3	5
	10	3	2	5	3	2	5	1	1	2	1	1	2	1	1	2	2	3	5
	11	3	3	6	3	2	5	2	2	4	1	1	2	1	1	2	2	2	4
	12	2	2	4	3	3	6	1	1	2	1	1	2	1	1	2	2	2	4
	13	2	2	4	2	2	4	1	1	2	1	1	2	1	1	2	1	1	2
	14	2	2	4	3	3	6	2	2	4	1	1	2	1	1	2	1	1	2
MFH	15	1	1	2	2	2	4	1	1	2	1	1	2	1	1	2	2	2	4
	16	2	2	4	2	2	4	1	1	2	1	1	2	1	1	2	2	2	4
	17	1	1	2	2	2	4	1	1	2	1	1	2	1	1	2	3	3	6
	18	2	2	4	2	2	4	1	1	2	1	1	2	1	1	2	2	2	4
	19	2	2	4	2	2	4	1	1	2	1	1	2	1	1	2	2	2	4
	20	3	3	6	3	2	5	1	1	2	1	1	2	1	1	2	3	3	6
	21	2	2	4	3	3	6	2	2	4	1	1	2	1	1	2	3	3	6
	22	3	3	6	3	3	6	2	2	4	1	1	2	1	1	2	2	2	4
	23	2	2	4	3	3	6	2	2	4	1	1	2	1	1	2	2	2	4
LM	24	3	3	6	3	3	6	3	3	6	1	1	2	1	1	2	2	2	4
	25	3	3	6	3	3	6	3	2	5	1	1	2	2	2	4	3	3	6
	26	3	3	6	3	3	6	3	3	6	1	1	2	3	3	6	2	3	5
	27	2	2	4	3	3	6	3	3	6	1	1	2	3	3	6	3	3	6
	28	3	3	6	3	3	6	3	3	6	1	1	2	3	3	6	3	3	6
	29	3	3	6	3	3	6	3	3	6	1	1	2	2	3	5	2	2	4
	30	2	3	5	2	3	5	3	3	6	1	1	2	2	2	4	2	3	5
	31	3	3	6	3	3	6	3	3	6	1	1	2	3	2	5	2	3	5
	32	3	3	6	3	3	6	3	3	6	1	1	2	3	3	6	3	3	6
LMS	33	2	2	4	3	3	6	2	2	4	1	1	2	1	1	2	2	2	4
	34	2	2	4	3	2	5	2	2	4	1	1	2	1	1	2	1	1	2
	35	2	2	4	3	3	6	2	2	4	1	1	2	1	1	2	3	3	6
	36	2	2	4	3	3	6	2	2	4	1	1	2	2	2	4	1	1	2
	37	3	3	6	3	3	6	2	2	4	1	1	2	3	2	5	2	3	5
	38	3	3	6	3	3	6	2	2	4	1	1	2	1	1	2	2	3	5

I – intensity; D – distribution; T – total score.

ent tumor groups did not reveal any statistically significant result ($p<1$).

Discussion

Diagnosis of the spindle cell soft tissue tumors of the skin often necessitates immunohistochemistry. In recent WHO classification of tumors, some entities are defined more clearly (1, 22). MFH was accepted to be a diagnosis of exclusion and it was stated that these lesions appear to have nothing to do with true histiocytes, therefore they have been given a new term of undifferentiated pleomorphic sarcoma (UPS) (23). Thus, immunohistochemistry became even more important in differential diagnosis.

SMA was thought to be a specific marker of SMC and widely used as definitive criteria for pathologic diagnosis of SMC tumors but recent evidence has shown that this practice would be problematic (4, 6–8, 24, 25). Although SMA is an important part of the basic panel, it is often required to be complemented by new markers, because myofibroblasts can be a part of the tumor. Several reports have shown SMA immunopositivity in non-SMC tumors (5, 9, 26–31). It has been stated that myofibroblastic differentiation occurred in 10–20 % of all dermatofibromas, affecting <25 % of cells (3). Furthermore it has been shown that more

Tab. 2. Summary of immunohistochemical profiles.

	SMA (%)	CALP	HCD	c-kit	PLAP	HPL
BFH	3/8 (37,5)	8/8 (100)	4/8 (50)	0/8 (0)	2/8 (25)	5/8 (62,5)
DFPT	6/6 (100)	6/6 (100)	2/6 (33)	0/6 (0)	0/6 (0)	4/6 (67)
MFH	7/9 (78)	9/9 (100)	3/9 (33)	0/9 (0)	0/9 (0)	9/9 (100)
LM	9/9 (100)	9/9 (100)	9/9 (100)	0/9 (0)	8/9 (89)	9/9 (100)
LMS	6/6 (100)	6/6 (100)	6/6 (100)	0/6 (0)	2/6 (33)	4/6 (67)

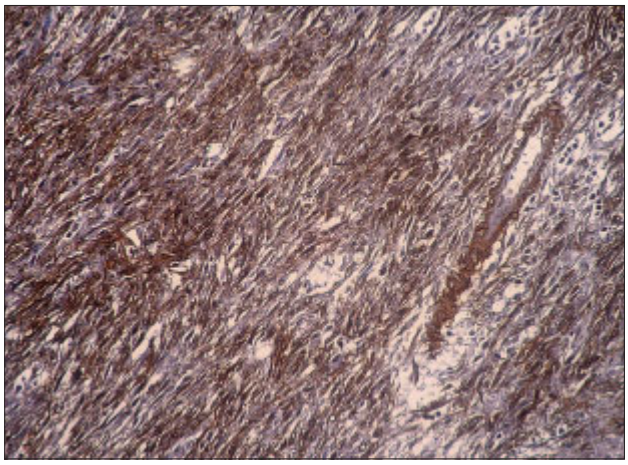

Fig. 1. SMA immunopositivity in DFPT (x200, SMA).

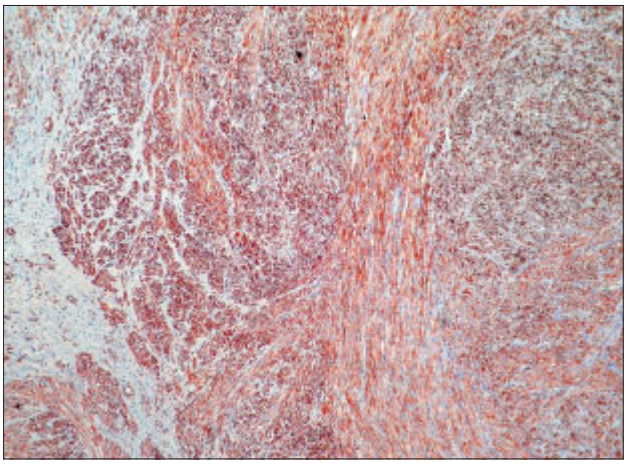

Fig. 3. HCD immunopositivity in LM (x100, HCD).

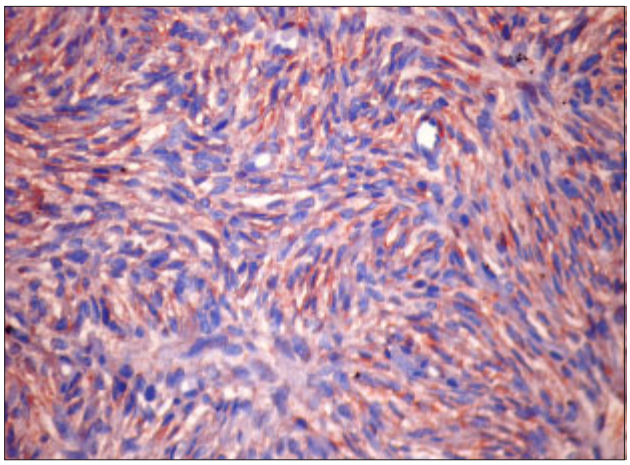

Fig. 2. HCD immunopositivity in BFH (x200, HCD).

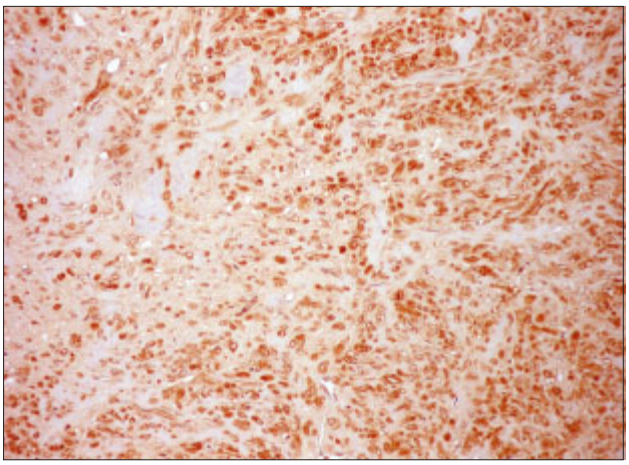

Fig. 4. CALP immunopositivity in MFH (x200, CALP).

prominent to extensive (>90 %) content may occur in 2 and 0.2 % respectively (4). There are reports describing DFPT cases showing SMA immunopositive myoid areas which are thought to be hyperplasia of myofibroblasts (6, 7, 24). Montgomery and Fisher detected myofibroblastic feature in 7 of 35 MFH cases (8). They stated that there were no light microscopic differences between pleomorphic sarcomas with myofibroblastic differentiation and those without (8). In our series, we could not detect a difference in SMA staining pattern between SMC tumors and DFPTs-MFHs. Only BFHs significantly differed from the other tumor groups, however 3 of 8 BFH cases were also positive, all of them showing strong intensity. This result further supported the statement that SMA was a sensitive but not a specific immunomarker for SMC tumors.

It has been reported that myofibroblastic proliferations had not been stained with HCD (13). Watanabe et al. stated that HCD was specifically expressed in SMC and myoepithelial cells but not in myofibroblasts (32). They suggested that HCD could be used as a specific marker of SMCs and tumors originating in SMC (32). Further studies supported this opinion (9, 26, 28).

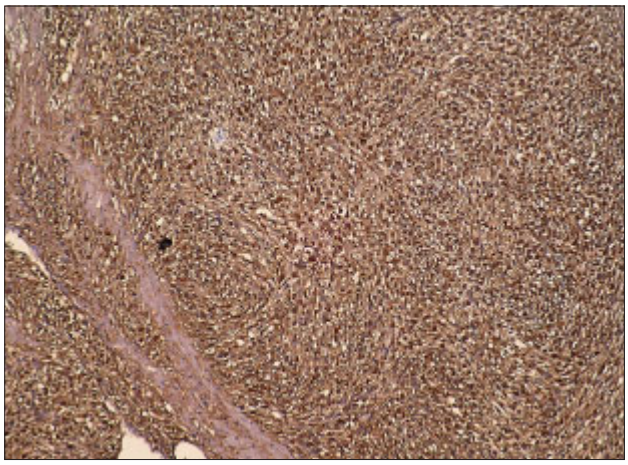

Fig. 5. CALP immunopositivity in LMS (x100, CALP).

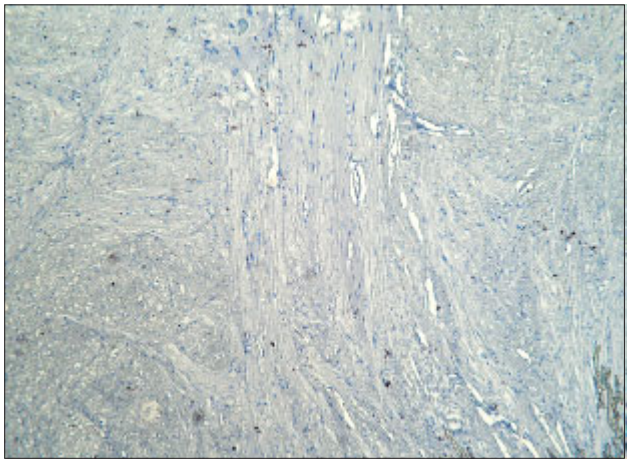

Fig. 6. C-Kit immunonegativity in LM (x100, c-kit).

However some researchers found that HCD expression was in a specific but variable fashion although they could not detect HCD immunopositivity in MFH cases (27, 33). They suggested that frequency of expression of HCD depends on the location of the LMSs. Retroperitoneal tumors showed the highest frequency (27, 33). Oda et al. further stated that the expression of HCD in ordinary LMS was significantly higher than those in pleomorphic areas of pleomorphic LMS cases (33). Miettinen et al found high frequency of HCD expression in MFHs besides CALP immunopositivity (34). They proposed that the coexpression of these markers suggested traits of smooth muscle differentiation in some MFHs (34). We have found HCD positivity in all our LM and LMS cases and this staining pattern differed significantly from that of so-called fibrohistiocytic tumors. However because 1/3 of DFPT and MFH cases and half of BFH cases also showed HCD immunopositivity, this difference is debatable and not highly discriminative as expected.

Calponin is an actin-binding protein which was used as a marker for myoepithelial and smooth muscle cells (34). Calponin was consistently expressed in smooth muscle tumors (32, 34). However, some researchers have reported CALP immunopositivity in so-called fibrohistiocytic tumors in high ratios. Miettinen et al. found immunopositivity for CALP in 80 % of MFHs (34). Fanburg-Smith and Miettinen reported immunopositivity for CALP in 85 % of angiomatoid MFHs (31). Sakamoto et al. found that 3 of 10 atypical fibroxanthoma and 11 of 17 BFH cases were positive for CALP (28). However, in some other reports, MFH cases expressed CALP in very low ratios or did not expressed at all (27, 30, 33). CALP was also expressed in myofibroblastic lesions. It has been stated that lesions with myofibroblastic differentiation, such as nodular fasciitis, aggressive fibromatosis and myofibroblastic sarcoma, were positive for calponin and negative for HCD (13). Therefore, for tumors with myofibroblastic differentiation the use of both antibodies has been proposed (13, 34). We have detected 100 % CALP immunopositivity in benign and malignant so-called fibrohistiocytic and SMC tumors of the skin including BFH, DFPT, MFH, LM and LMS cases. These findings may be explained by myofibroblastic differentiation in so-called fibrohistiocytic tumors. In conclusion we think that CALP is not a specific marker for SMC and should not be used alone but instead with other immunomarkers such as HCD, as a supplementary marker.

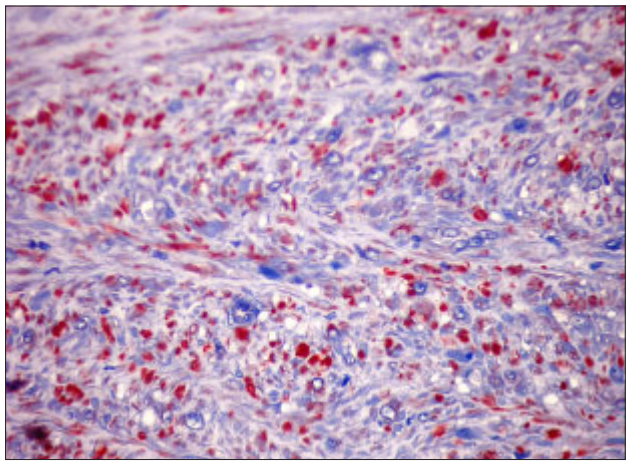

Fig. 7. PLAP immunopositivity in LM (x200, PLAP).

C-kit is reported to be rarely positive in soft tissue tumors (34). In some recent reports it has been stated that c-kit expression in soft tissue sarcomas was very limited (15, 35). Sato et al reported that weak staining was observed in 3 of 11 LMSs and in 1 of 97 MFHs (35). Hornick and Fletcher could not detect c-kit immunopositivity in none of their LMS and DFPT cases (15). In an immunohistochemical study analyzed by a tissue microarray format, all LM, LMS, DFPT and MFH cases were c-kit negative also (36). However, Barisella et al reported c-kit positivity in 3 of their 7 MFH cases (16) and Horie et al reported a cutaneous LMS with overexpression of c-kit (37). We could not detect c--kit immunopositivity in any of our tumors. In conclusion, we think that in spindle cell soft tissue tumors of the skin, c-kit immunopositivity is not a prominent feature.

It has been reported that LMs expressed PLAP strongly, and approximately 50% of LMSs showed PLAP immunostaining (18). Goldsmith et al. suggested that PLAP could be useful as a

myoid marker (18). Almost all of our leiomyoma cases expressed PLAP, however in our LMS cases we have observed moderate PLAP immunopositivity in 2 of 6 tumors. Among non-muscle tumors only 2 BFH cases showed slight PLAP positivity. SMC tumors displayed significantly stronger and more widespread immunostaining pattern for PLAP than so-called fibrohistiocytic tumors and immunostaining pattern of PLAP in LMs also differed significantly from that of LMSs supporting the statement that PLAP could be an ancillary immunomarker in differential diagnosis of smooth muscle tumors especially of LMs.

All tumor groups expressed HPL in a variable fashion; therefore we could not detect any significant difference between SMC and so-called fibrohistiocytic tumors.

The major conclusions of this study are as follows: 1) HCD is a sensitive immunomarker for SMC tumors but it is not highly discriminative in differential diagnosis between SMC tumors and so-called fibrohistiocytic tumors. 2) CALP is not a specific immunomarker for SMC tumors. 3) C-kit is immunonegative in spindle cell superficial soft tissue tumors. 4) PLAP can be an ancillary immunomarker in differential diagnosis of smooth muscle cell tumors especially of LMs.

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