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CD a potential adjunct in rnoma from morphologic m

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Summary Although the morphologic diagnosis of hibernoma is usually str
nomas have atypical morphologic features, mimicking atypical lipom
ated liposarcomas (ALT/WDLs). In addition, the multivacuolated br
for lipoblasts by pathologists, especially those without significant
we continue to receive in consultation cases of hibernoma s
hybridization testing to *exclude ALT/WDL*. Testing hiberno
adds cost and delays the final diagnosis. Recently, we ha
CALLA), a zinc-dependent metalloproteinase involve
mones, in brown fat cells, and wished to explore th
pensive ancillary test in the differential diagnosis
tissue sections from well-characterized cases
WDLs (n = 17), pleomorphic liposarcom
(n = 5) were immunostained for CD10,
ratory protocols. CD10 expression was
The hibernomas occurred in 28 men
the extremities (n = 25), pelvis
region (n = 6), back (n = 2
multivacuolated brown fat
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contrast, CD10 expre
was absent in lipo
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☆ Disclosures: None.

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morphologic features favor hibernoma. CD10 expression in adipocytes, however, should be rigorously distinguished from fibroblastic stromal cell CD10 expression, a nonspecific finding.

1. Introduction

Hibernomas, benign lipomatous tumors with brown fat differentiation, typically present as subcutaneous masses in middle aged patients, in the trunk, extremities, and chest. Originally felt to be non-neoplastic and termed *pseudolipomas* by Merkel in 1906, hibernomas are now known to be clonal neoplasms, most often showing alterations of the 11q13 locus, with codeletions of *MEN1* and *AIP* [1–5]. Morphologically, hibernomas are highly vascular and contain multivacuolated brown fat cells showing a variable degree of cytoplasmic eosinophilia and a centrally placed normochromatic nucleus, juxtaposed to an inconstant percentage of univacuolated white fat. Some hibernomas consist largely of brown fat, while others are composed predominantly of univacuolated adipocytes, with only scattered brown fat cells. While most hibernomas are easily recognized, some show atypical features including irregular fibrous septa, myxoid change, a modestly increased number of stromal cells and *lipoblast-like cells*, mimicking atypical lipomatous tumors/well-differentiated liposarcomas (ALTs/WDLs) [6]. Furthermore, pathologists continue to confuse multivacuolated brown fat cells with lipoblasts, raising concern for liposarcoma. These morphologic pitfalls often lead to unnecessary testing for ALT/WDL-associated molecular genetic events, such as *MDM2* amplification, increased cost, and delaying diagnosis.

Over the past several years, we have noted brown fat cells to be commonly positive with antibodies to CD10. CD10 antibodies recognize neprilysin (also known as CALLA), a widely distributed zinc-dependent metalloproteinase involved in the inactivation of various peptide hormones. Prompted by these observations, we explored the utility of CD10 immunohistochemistry in the differential diagnosis of hibernoma.

Table 1 Immunohistochemical results.

Tumor Type	CD10-positive adipocytes/ lipoblasts (%)	CD10-positive stromal cells (%)
Brown fat (n = 21)	21 (100)	21 (100)
Hibernoma	48 (100)	13 (28)
Lipoma (n = 5)	0 (0)	0 (0)
Fat necrosis (n = 5)	0 (0)	1 [20]
Atypical lipomatous tumor/ well-differentiation liposarcoma (n = 17)	3 [18]	10 (59)
Pleomorphic liposarcoma (n = 6)	3 (50)	6 (100)

2. Material and methods

2.1. Case selection

This study was performed with approval of the Institutional Review Board at the Mayo Clinic. Archived histological slides from biopsied or surgically resected hibernomas and control cases were retrieved from our institutional and consultation archives. Control cases included ALTs/WDLs, pleomorphic liposarcomas (all containing numerous, multivacuolated lipoblasts) (PLPSs), lipomas, non-neoplastic white fat with fat necrosis and multivacuolated histiocytes (mimicking brown fat), and non-neoplastic brown fat. All tested ALTs/WDLs were known to be *MDM2* amplified (17/17), whereas tested cases of hibernoma (0/11) and fat necrosis (0/1) were MDM2 negative.

2.2. Immunohistochemistry

Automated immunohistochemical analysis was performed on formalin-fixed paraffin-embedded tissue sections (4 μm in thickness) using a commercially available monoclonal mouse anti-CD10 antibody (Commercial source: Leica [Novocastra], 56C6 clone, Catalog Number: NCL-CD10-270) with dilution of 1:200 (diluted with DAKO Background Reducing Diluent). The platform used to stain the selected tissue sections was the Ventana Benchmark XT (32 min. CC1 @95C, followed by primary antibody for 16 min @37C, Optiview DAB detection with AMP, Hematoxylin II 8 min, Bluing Reagent 4 min) with tonsillar tissue used as a control tissue. CD10 expression was evaluated in both white fat and brown fat components, as well as the stromal cells. The presence of any positive cells was scored as *positive*.

3. Results

In total, we evaluated 102 lipomatous specimens including hibernomas (n = 48), ALTs/WDLs (n = 17), PLPSs (n = 6), non-neoplastic brown fat (n = 21), lipomas (n = 5), and fat necrosis (n = 5). The hibernomas occurred in 28 men and 20 women ranging from 11 to 76 years of age (median 44 years) and involved the extremities (n = 25), pelvis (n = 7), head and neck region (n = 6),

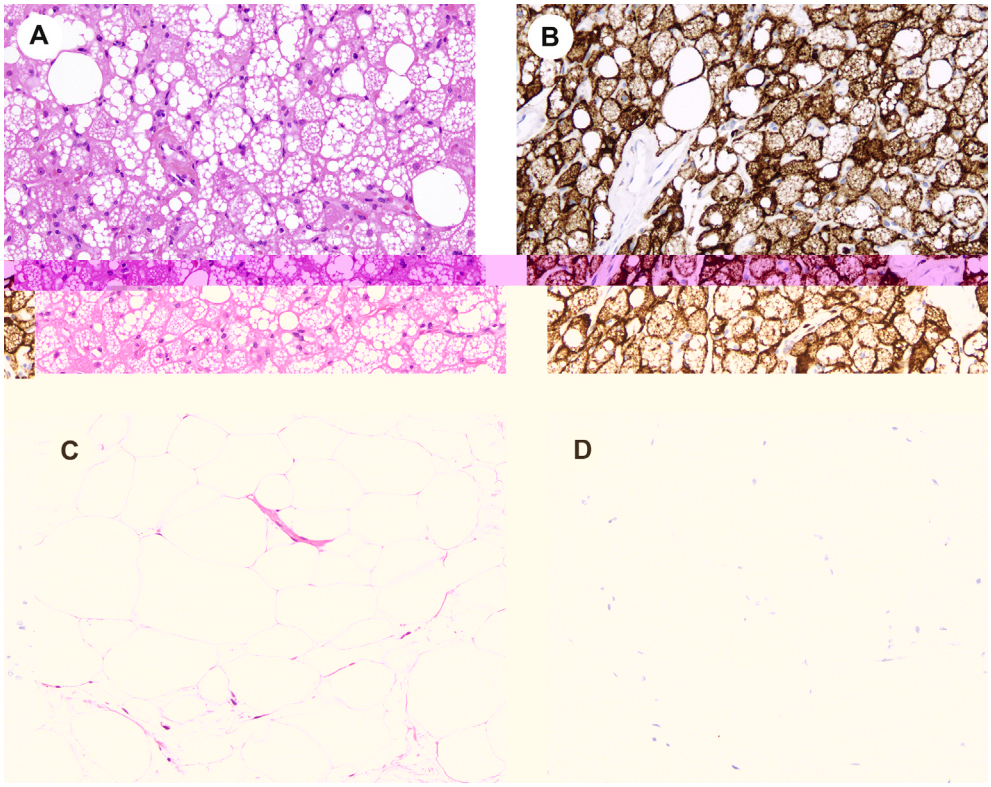

Fig. 1 Non-neoplastic brown fat from the axillary region of an infant (A), showing diffuse expression of CD10 (B). In contrast, white fat (here from a lipoma) (C) was consistently CD10-negative (D).

retroperitoneum (n = 5), abdomen (n = 2), back (n = 2), and chest (n = 1).

Table 1 summarizes the immunohistochemical results. Robust cytoplasmic expression of CD10 expression was present in all examples of non-neoplastic brown fat (21/21, 95%) (Fig. 1A and B) but was absent in white fat from lipomas (0/5, Fig. 1C and D). Similarly, diffuse, strong CD10 expression was present in the multivacuolated brown fat cells in all hibernomas (48/48, 100%) (Fig. 2A–F). The univacuolated adipose tissue present in these tumors was also commonly CD10 positive (36/48, 76%), whereas the fibroblastic stromal cells of these lesions showed only infrequent CD10 expression (13/48, 28%). In contrast, adipocyte CD10 expression was present in only 3 of 17 ALTs/WDLs (18%) (Fig. 3A–D), one of which showed a focus of definite brown fat differentiation (Fig. 4A–D). This case was known to be *MDM2* amplified. The other positive ALT/WDL did not show morphologic features suggestive of brown fat differentiation. CD10 expression was, however, more widespread in the enlarged, hyperchromatic, nonlipogenic fibroblastic stromal cells located within the fibrous septa of ALT/WDL (10/17, 59%). Interestingly, CD10 expression in the pleomorphic lipoblasts of PLPSs was *all or nothing*, with 3 cases demonstrating uniform, intense CD10 expression in both lipoblasts and stromal cells and 3 cases showing expression only in stromal cells (Fig. 5A–D). CD10 expression was seen only rarely in the stromal cells of fat necrosis and was absent in adipocytes and *pseudolipoblastic* or foamy macrophages (adipocytes: 0/5, 0%; stromal cells: 1/5, 20%) (Fig. 6A and B).

4. Discussion

There are three types of adipocytes found in the body: brown, white, and beige. Brown fat, which primarily has a thermogenic function and is predominantly present in the axillary and subpleural regions in newborns, develops embryonically from cells expressing myogenic nuclear regulatory genes such as *MYOD1* and *MYF5*, thought to represent skeletal muscle precursors [7]. Brown fat cells are rich in mitochondria and express high levels of UCP1, the enzyme critical for the uncoupling of oxidative respiration and the generation of heat [8]. Beige adipocytes are also involved in the regulation of body temperature but develop after birth either de novo or by white-to-brown adipocyte transdifferentiation (also known as *browning* of white adipose tissue) [8–10]. The morphologic features of brown and beige fat are identical, and these tissues cannot be distinguished by microscopic evaluation alone. The function of white adipose tissue is chiefly the storage of excess chemical energy as triacylglycerol (fat), although

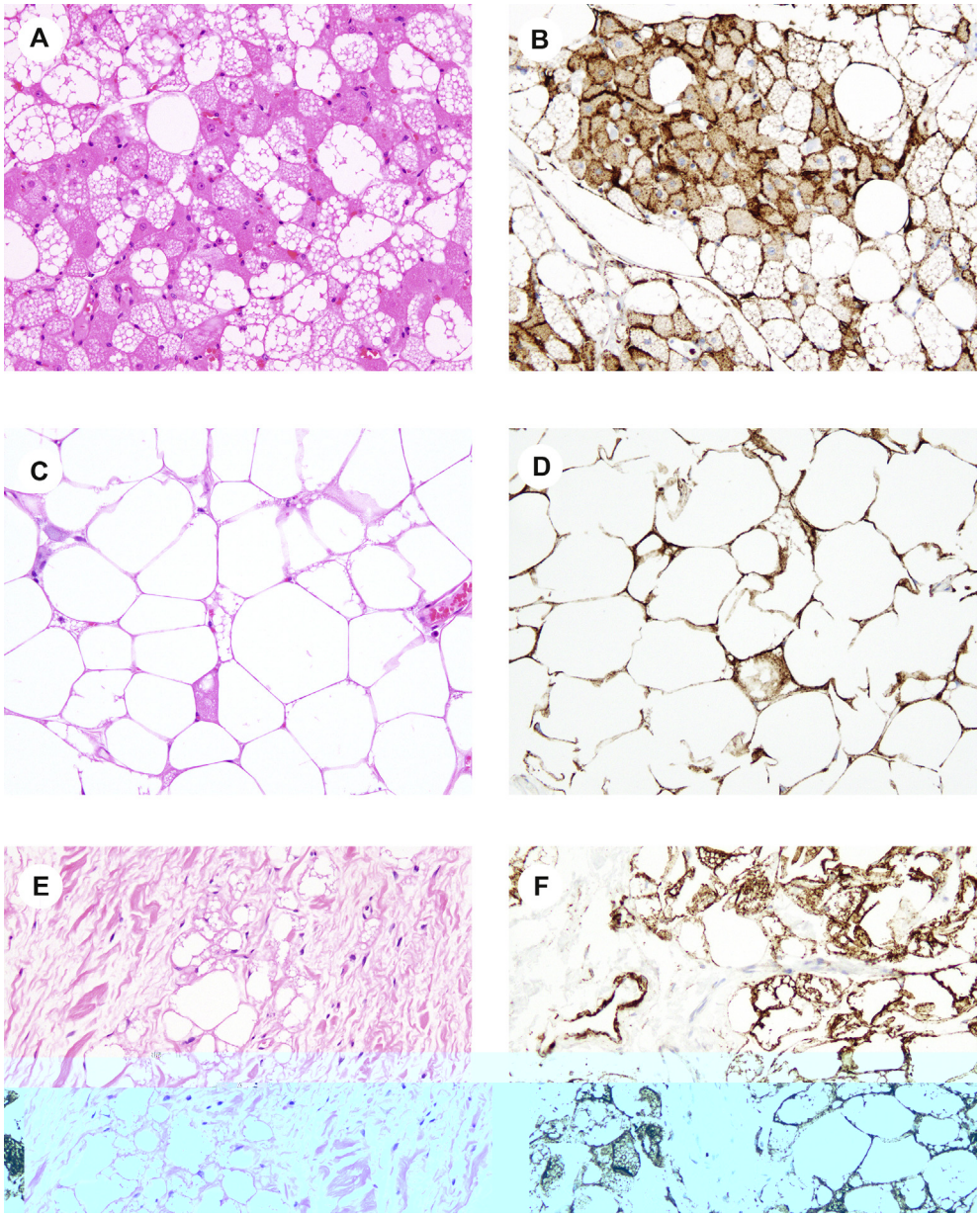

Fig. 2 Conventional hibernoma, consisting chiefly of multivacuolated brown fat but also containing scattered univacuolated cells (A). Both multivacuolated and univacuolated cells were strongly CD10 positive (B). *Lipoma-like* hibernomas contain only scattered multivacuolated cells and are most often referred in consultation to exclude atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDL) (C). As in conventional hibernoma, diffuse CD10 expression characterizes the *lipoma-like* variant as well (D). *Atypical* hibernomas demonstrate greater collagen deposition and an increased number of stromal cells, mimicking ALT/WDL (E). In such cases, demonstration of diffuse CD10 expression may be reassuring (F). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

it also provides mechanical cushioning in areas such as the plantar foot.

Hibernomas are benign adipocytic neoplasms that show brown fat differentiation. At the genetic level, hibernomas usually harbor 11q13 alterations with codeletion of the *MEN1* and *AIP* genes [5]. With the widespread use of FDG-PET for the detection of metastatic disease, these benign lipomatous tumors appear, in our experience, to be more often coming to the attention of surgical pathologists, as they are intensely FDG avid, mimicking malignancy [11–13]. Thus, it is important for general surgical pathologists to be aware of the morphologic features of hibernoma and their potential to mimic other adipocytic tumors.

Far and away the largest study to date of hibernomas is that of Furlong et al. [14], who studied 170 such cases,

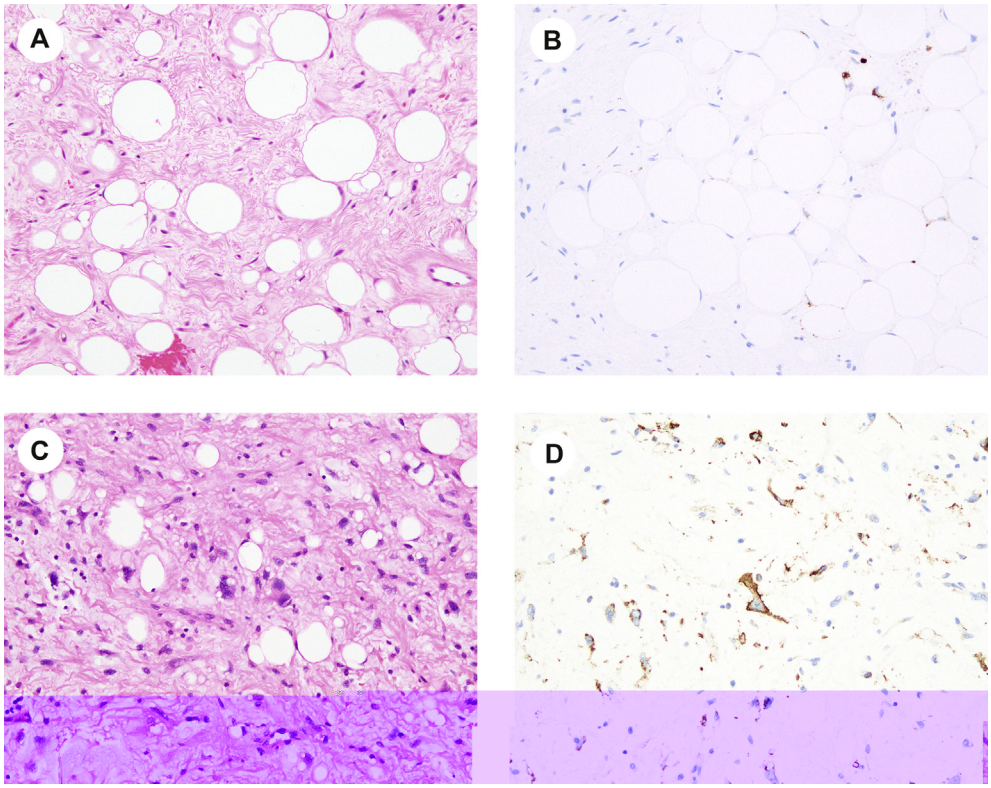

Fig. 3 ALT/WDL (A), negative for CD10 (B). In contrast to hibernomas, ALT/WDL was much less frequently CD10 positive. This example of ALT/WDL contains an increased number of hyperchromatic, nonlipogenic, fibroblastic stromal cells (C), which demonstrated strong CD10 expression (D). CD10 expression in stromal cells should be carefully distinguished from CD10 expression in adipocytes. ALT/WDL, atypical lipomatous tumor/well-differentiated liposarcoma.

classifying hibernomas as *typical*, *myxoid*, *spindle cell*, and *lipoma-like* based on their relative percentage of brown and white fat, myxoid matrix, and spindled stromal cells. Interestingly, 23% of hibernomas in this series were submitted in consultation out of concern for the possibility of an atypical lipomatous tumor (well-differentiated liposarcoma), in particular *lipoma-like* tumors where isolated multivacuolated brown fat cells mimicked lipoblasts, and extensively myxoid lesions, often confused with myxoid liposarcoma and myxoid well-differentiated liposarcoma. Appropriately, Furlong et al [14] emphasized the importance of careful morphologic observation in the distinction of hibernoma from malignant mimics, noting that the "nucleus of the hibernoma cell may be indented but is usually central or slightly eccentric and often has a prominent nucleolus, features distinguishing it from a lipoblast. Furthermore, it lacks the hyperchromasia and scalloping of a lipoblast." [14]. A more recent series of "hibernomas mimicking atypical lipomatous tumor", reported by Al Hmada et al [6], further stressed the potential for hibernomas to display potential worrisome features, including lipoblast-like cells and an increased number of modestly atypical stromal cells, resembling those seen in true atypical lipomatous tumors. In additional to careful morphologic study, Al Hmada et al. [6] emphasized the potentially useful role for MDM2 and CDK4 immunohistochemistry in this differential diagnosis, noting that all studied hibernomas were negative for these markers.

Neprilysin (CD10), a zinc metalloproteinase involved in the inactivation of various peptide hormones, is found in greatest concentration in the kidneys but is expressed in essentially every organ [15–17]. This enzyme performs critical roles in a wide variety of organs, including inactivation of enkephalins in the brain, clearance of cardiac bradykinin, inactivation of tachykinin in the airways, and metabolism of various neurotransmitters in the presynaptic axonal terminals [18]. Neprilysin is known to be expressed by white adipocytes, where it is involved in control of the regulation of local levels of angiotensin-II; the role of neprilysin in brown fat does not appear to have been studied to date [19]. In the arena of surgical pathology, a very large number of immunohistochemical studies have shown CD10 expression to be quite widespread across various human tissues and tumor types to the extent that the utility of CD10 as a diagnostic reagent has been questioned by many. However, in the appropriate morphological context, there are some uses for CD10 immunohistochemistry, for example, in the differential diagnosis of renal clear cell carcinoma [20], endometrial stromal sarcoma [21], and cellular neurothekeoma [22]. Demonstration of

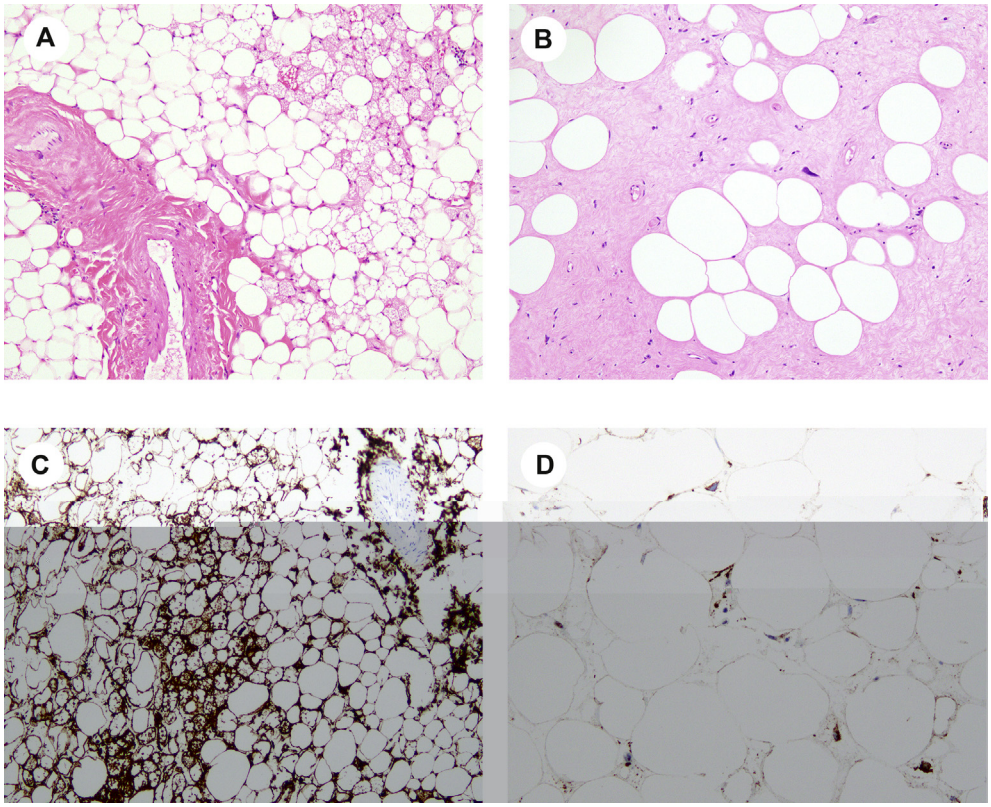

Fig. 4 This unusual example of ALT/WDL showed foci of brown fat differentiation (top right corner) (A) and larger areas with more conventional histology (B). Interestingly, the brown fat component of this tumor was strongly CD10 positive (C), whereas CD10 expression was confined to scattered fibroblastic stromal cells in the rest of the mass (D). ALT/WDL, atypical lipomatous tumor/well-differentiated liposarcoma. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

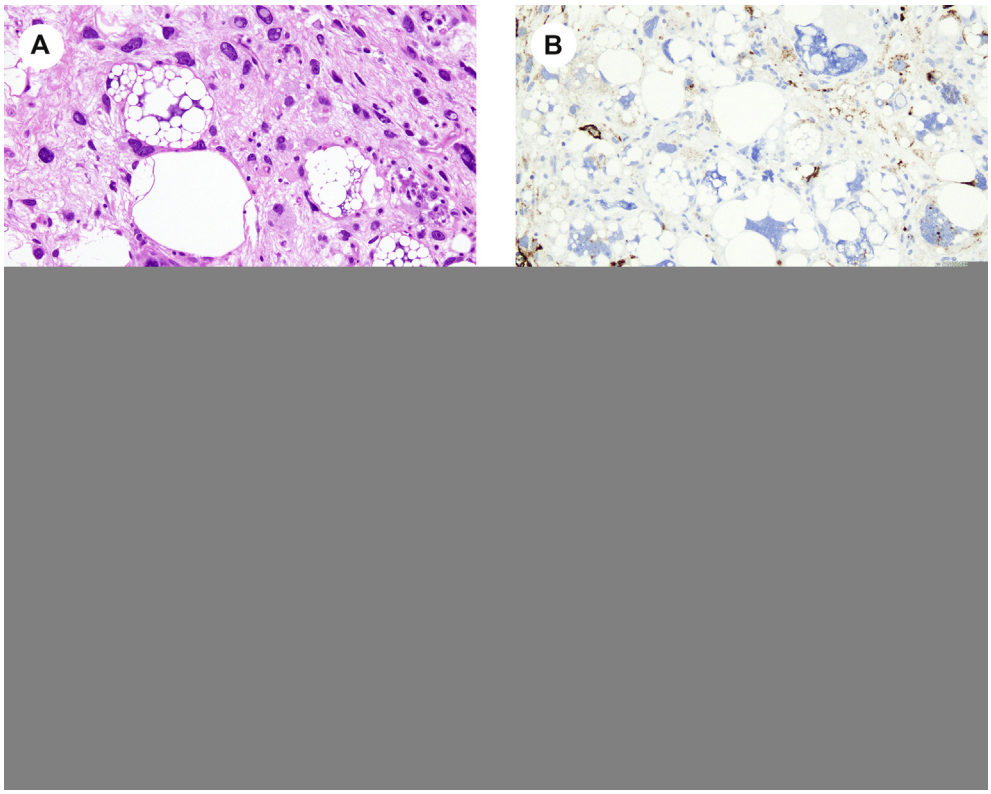

Fig. 5 Pleomorphic liposarcoma (PLPS) (A), negative for CD10 expression in giant, pleomorphic lipoblasts (B). In contrast, this *epithelioid* PLPS (C) was diffusely positive for CD10 (D). Robust expression of CD10 was seen in the lipoblasts of 50% of studied PLPS, raising the possibility that a subset of these tumors may show brown fat differentiation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

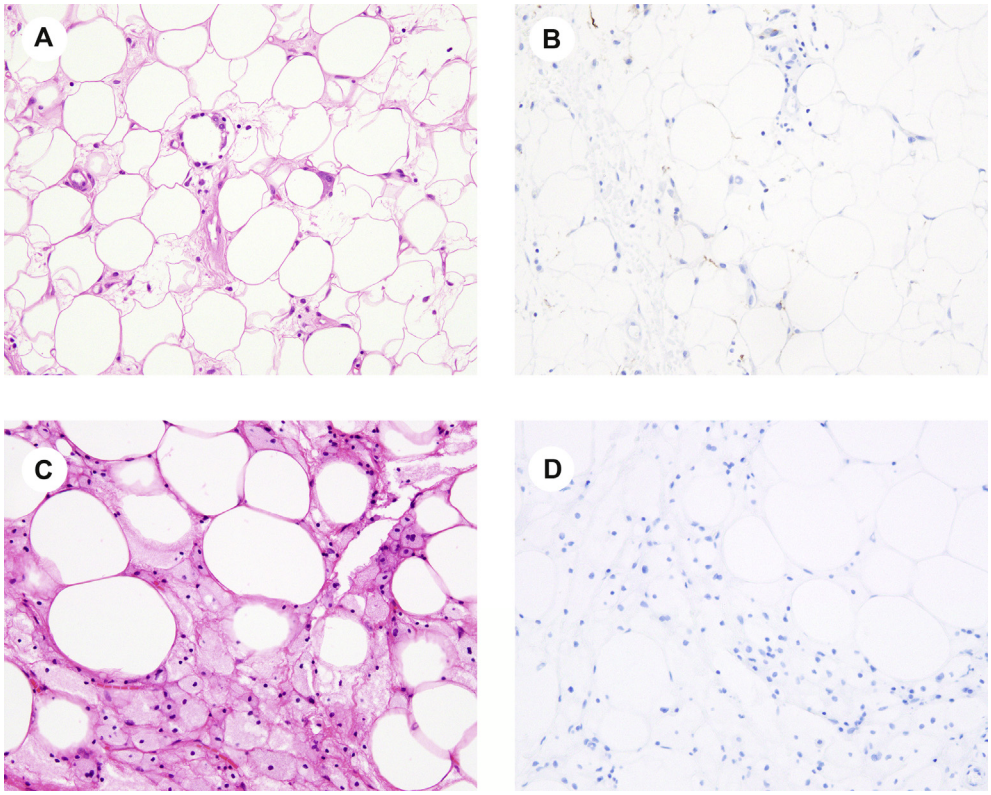

Fig. 6 Fat necrosis with *pseudolipoblastic* lipid-laden histiocytes (A), negative for CD10 (B). This unusual example of fat necrosis with foamy macrophages, somewhat reminiscent of hibernoma (C) was also CD10 negative (D).

CD10 expression is also valuable in the diagnosis of various hematolymphoid neoplasms, in particular acute lymphoblastic leukemia [23].

The results of the present study suggest a potential role for CD10 immunohistochemistry in the differential diagnosis of hibernoma, with strong expression of this marker seen in all hibernomas and almost all examples of non-neoplastic brown fat. In contrast, CD10 expression was uncommon in ALT/WDL and in multivacuolated histiocytes in fat necrosis, and when present was confined to isolated cells, in contrast to the diffuse, robust expression seen in hibernomas. Care must be taken, however, to distinguish CD10 expression in enlarged, hyperchromatic, nonlipogenic fibroblastic stromal cells, a nonspecific finding, from that of brown adipocytes. It must also be kept in mind that very rare WDL may show brown fat differentiation [24] and that the brown fat cells in such cases may express CD10, a phenomenon demonstrated in one case from the present series. Thus, CD10 expression by itself is not necessarily indicative of a benign adipocytic tumor, and the lesion must be evaluated for other salient morphologic features, such as enlarged hyperchromatic stromal cells. Robust expression of CD10 in some PLPSs raises the possibility of brown fat differentiation in a subset of these rare sarcomas, although demonstration of more specific brown fat markers such as UCP1 would be necessary to validate this hypothesis.

Although we do not anticipate CD10 immunohistochemistry replacing immunohistochemistry or fluorescence in situ hybridization (FISH) for *MDM2/CDK4* in the differential diagnosis of hibernoma and ALT/WDL in high-risk areas, such as the retroperitoneum, demonstration of CD10 immunoreactivity may be reassuring in selected cases, in particular those where hibernoma is favored on morphologic grounds. Indeed, as the *ideal* immunohistochemical panel usually includes pertinent positives and negatives, CD10 would likely best be used as part of a panel to include antibodies to MDM2 and CDK4 as well. Certainly, CD10 immunohistochemistry would have the potential to reduce cost and turnaround time in the diagnosis of hibernoma, as compared with FISH, when used judiciously. As CD10 immunohistochemistry is widely available, even to pathologists who see relatively few adipocytic tumors, this simple test also might obviate the need for external consultation in some instances.

In summary, we have shown expression of CD10 (neprilysin) to be a near-ubiquitous feature of hibernoma and non-neoplastic brown fat and one with potential utility in the differential diagnosis of these rare adipocytic tumors from potentially malignant morphologic mimics. When used appropriately and as part of an antibody panel, CD10 immunohistochemistry may serve as a useful adjunct to

MDM2/CDK4 immunohistochemistry and FISH and may save time and expense in selected cases.

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