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Immunohistochemical evaluation of p16 expression in cutaneous histiocytic, fibrohistiocytic and undifferentiated lesions

Background: Expression of p16 is frequently evaluated in melanocytic lesions. Expression of p16 in cutaneous histiocytic, fibrohistiocytic and undifferentiated lesions has not been well characterized.
Methods: We evaluated p16 expression in a cohort of histiocytic (reticulohistiocytoma, Langerhans cell histiocytosis, xanthogranuloma, Rosai Dorfman disease and xanthoma), fibrohistiocytic (dermatofibroma, epithelioid fibrous histiocytoma and dermatofibrosarcoma protuberans) and undifferentiated (atypical fibroxanthoma and pleomorphic undifferentiated sarcoma) lesions. A group of melanocytic lesions (Spitz nevus, ordinary nevus, spitzoid melanoma and non-spitzoid melanoma) were also evaluated as reference. Each case was scored by the proportion of p16-positive cells and by staining intensity.
Results: Immunoreactivity for p16 was found in almost all histiocytic (28/30, 93%) and fibrohistiocytic (22/24, 92%) lesions. About half of the undifferentiated lesions also exhibited p16 staining (9/17, 53%). Most of the melanocytic cases examined in this study expressed p16. A wide range of staining intensity and proportion of p16-positive cells was observed in most groups.
Conclusion: Expression of p16 is common, albeit variable in proportion and intensity, amongst a wide variety of cutaneous histiocytic, fibrohistiocytic and undifferentiated lesions. Further studies are required to determine if p16 expression is useful in distinguishing benign from malignant neoplasms of these types.

Keywords: histiocytosis, immunohistochemistry, melanocytic lesions, non-Langerhans cell, p16 protein

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The p16 protein is one of the two major products of the cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene located on chromosome 9p21.[1,2] As a tumor suppressor, p16 halts tumorigenesis by inhibiting the cyclin D-cyclin-dependent kinase 4 complex, which normally phosphorylates the retinoblastoma protein and promotes transition from G1 to S phase.[3] Interestingly, cells harboring oncogenic mutations often respond in a compensatory manner by activating expression of p16 to halt excessive proliferation in a phenomenon known as oncogene-induced senescence. This has been shown to occur in melanocytic nevi with BRAF mutations, neurofibromas from neurofibromatosis type I patients and many other benign and premalignant lesions. To this end, increased expression of p16 generally correlates with clinically indolent behavior, earlier tumor stage and better prognosis.[4,5]

In melanocytes, p16 is essential for triggering cellular senescence and is expressed in benign melanocytic nevi including Spitz nevi,[6,7] whereas decreased or absent p16 expression is frequently observed in melanoma.[8–15] Differential expression of p16 in Spitz nevi and spitzoid melanomas has led to the use of this marker to aid in the distinction of the two;[16,17] however, this must be evaluated in conjunction with clinical and histomorphologic features as loss of p16 has been reported in exceptional Spitz nevi, and many cases of melanoma may retain p16 expression.[18]

Expression of p16 has also been reported in the vast majority of Langerhans cell histiocytosis (LCH) cases involving skin and/or visceral organs.[19,20] Chilosi et al. concluded that oncogene-induced senescence, as evidenced by high p16 expression, distinguishes indolent from aggressive forms of LCH.[21] Literature regarding p16 expression in other forms of cutaneous histiocytoses is sparse. Similarly, little is known about p16 expression in fibrohistiocytic and undifferentiated lesions of the skin.

Index case

We encountered a case of a 24-year-old female with a history of melanoma who presented with a 3-mm pink papule with surrounding tan patch on her buttock. Histologic examination showed a dome-shaped papule composed of large epithelioid to polygonal cells in the superficial dermis with mild fibrosis, increased blood vessels and scattered lymphocytes. The lesional cells contain abundant densely eosinophilic cytoplasm and mildly pleomorphic nuclei without nuclear grooves. Immunohistochemistry revealed these cells to be positive for S100, p16 and CD68, and negative for Melan-A and tyrosinase (Fig. 1). SOX10, CD1a and HMB45 immunostains were also performed; however, the lesional cells were no longer present on these deeper sections. The histologic differential diagnosis rested between an angiomatoid Spitz nevus with non-specific CD68 positivity and a reticulohistiocytoma with S100 and p16 staining. LCH was considered less likely in the absence of characteristic nuclear features.

In working up this case, we performed a literature search and found little data on p16 expression in histiocytic cutaneous lesions. This case prompted us to better characterize p16 expression in a spectrum of non-melanocytic cutaneous lesions of histiocytic, fibrohistiocytic and undifferentiated lineage.

Materials and methods

This study is approved by the Institutional Review Board at our institution. Tissue microarrays were previously constructed from 1-mm cores of formalin-fixed, paraffin-embedded tissue obtained from cutaneous histiocytic, fibrohistiocytic and undifferentiated lesions including reticulohistiocytoma, LCH, xanthogranuloma, Rosai Dorfman disease, xanthoma, dermatofibroma, epithelioid fibrous histiocytoma, dermatofibrosarcoma protuberans (DFSP), pleomorphic undifferentiated sarcoma and atypical fibroxanthoma (AFX). Melanocytic lesions including Spitz nevus, ordinary nevus, spitzoid melanoma and non-spitzoid melanoma, as well as additional cases of reticulohistiocytoma, LCH, xanthogranuloma, Rosai Dorfman disease and xanthoma were obtained from the pathology archive. Sections of 4-μm thickness were deparaffinized and heat-induced epitope retrieval was performed on the Ventana Benchmark Ultra immunostainer using a proprietary Tris-EDTA buffer pH from Ventana Medical Systems (Tucson, AZ, USA; Cell Conditioning solution). After blocking endogenous peroxidase activity, the slides were incubated for 16 min at 37°C with a mouse monoclonal p16 antibody (E6H4, predilute; Ventana Medical Systems) and subsequently detected by using the Ultraview DAB detection system (Ventana medical Systems).

All cases were evaluated by three of the authors (E.H.S., D.R.F. and M.P.C.) and consensus was achieved. Each lesion was scored by the proportion of p16-positive lesional cells (‘proportion

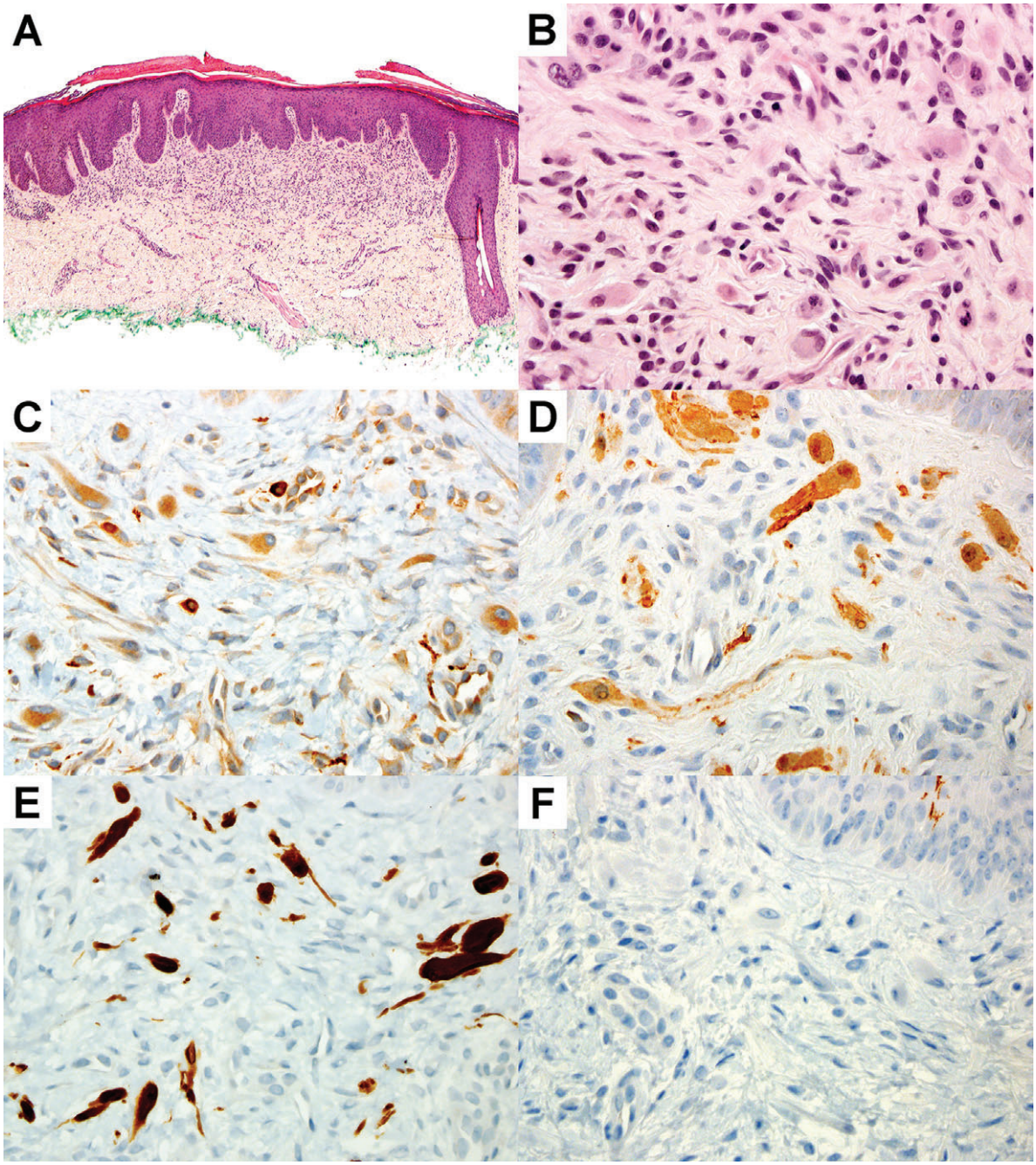

Fig. 1. Index case. A) The lesion is a dome-shaped papule composed of a dermal cellular infiltrate. The overlying epidermis is acanthotic and hyperkeratotic suggestive of irritation (H&E, ×40). B) High magnification reveals scattered epithelioid to polygonal cells with abundant eosinophilic cytoplasm and ovoid nuclei. The background stroma shows an increase in small blood vessels and scattered lymphocytes (H&E, ×400). The epithelioid cells stain positive for (C) CD68, (D) S100 and (E) p16 immunostains (×400). F) These cells are negative for Melan-A (×400).

score': 0 = 0%, 1 = 1–25%, 2 = 26–75% and 3 = 76–100%) and staining intensity ('intensity score': 0 = negative, 1 = mild, 2 = moderate and 3 = strong). Mean proportion score and intensity score were then calculated for each entity. The cellular compartments showing p16 staining (cytoplasmic, nuclear or both) were also documented for each case.

Results

The final cohort consisted of 30 cases of histiocytic, 24 cases of fibrohistiocytic and 17 cases of undifferentiated lesions. Twenty cases of melanocytic lesions were also evaluated. The mean p16 staining scores for all entities are summarized in Table 1. The majority of histiocytic lesions (28/30; 93%) showed p16

Table 1. Expression of p16 in cutaneous histiocytic, fibrohistiocytic, undifferentiated and melanocytic lesions

Lesion	Number (%) of cases with p16 staining	Mean proportion score*(range)	Mean intensity score†(range)
Histiocytic (N = 30)			
Reticulohistiocytoma (n = 9)	9 (100)	2.4 (1–3)	2.5 (1–3)
Xanthogranuloma (n = 7)	7 (100)	2.0 (1–3)	2.1 (1–3)
Rosai Dorfman disease (n = 5)	5 (100)	2.0 (1–3)	1.6 (1–2)
Langerhans cell histiocytosis (n = 6)	5 (83)	2.0 (0–3)	2.2 (0–3)
Xanthoma (n = 3)	2 (66)	1.0 (0–2)	1.0 (0–2)
Fibrohistiocytic (N = 24)			
Dermatofibroma (n = 12)	11 (92)	1.8 (0–3)	1.9 (0–3)
Epithelioid fibrous histiocytoma (n = 7)	7 (100)	1.6 (1–3)	2.0 (1–3)
Dermatofibrosarcoma protuberans (n = 5)	4 (80)	1.0 (0–2)	0.8 (0–2)
Undifferentiated (N = 17)			
Pleomorphic undifferentiated sarcoma (n = 8)	5 (63)	1.3 (0–3)	1.3 (0–3)
Atypical fibroxanthoma (n = 9)	5 (56)	0.7 (0–3)	0.4 (0–1)
Melanocytic (N = 20)			
Spitz nevus (n = 6)	6 (100)	3.0 (3)	3.0 (3)
Ordinary nevus (n = 4)	4 (100)	2.5 (2–3)	3.0 (3)
Spitzoid melanoma (n = 5)	5 (100)	2.6 (1–3)	3.0 (3)
Non-spitzoid melanoma (n = 5)	3 (60)	1.4 (0–3)	1.4 (0–3)

*Proportion score: 0 = 0%, 1 = 1–25%, 2 = 26–75% and 3 = 76–100% positive cells.
†Intensity score: 0 = negative, 1 = mild, 2 = moderate and 3 = strong.

positivity, with the greatest degree of staining observed in reticulohistiocytoma (mean proportion score/mean intensity score of 2.4/2.5), followed by LCH (2.0/2.2), xanthogranuloma (2.0/2.1), Rosai Dorfman disease (2.0/1.6) and xanthoma (1.0/1.0). Expression of p16 was also common in fibrohistiocytic lesions (22/24; 92%) but with generally lower mean proportion and intensity scores (dermatofibroma, 1.8/1.9; epithelioid fibrous histiocytoma, 1.6/2.0; DFSP, 1.0/0.8). Undifferentiated lesions exhibited the least frequent staining of all groups (9/17; 53%), with low mean proportion and intensity scores observed in both pleomorphic undifferentiated sarcoma (1.3/1.3) and AFX (0.7/0.4). Of note, each examined entity displayed a wide range of proportion scores and intensity scores. For reference, p16 was expressed in most of the melanocytic cases (18/20; 90%) including Spitz nevus (3.0/3.0), ordinary nevus (2.5/3.0), spitzoid melanoma (2.6/3.0) and non-spitzoid melanoma (1.4/1.4). All positive cases displayed either a diffuse or 'checkerboard/mosaic' staining pattern as commonly described in melanocytic lesions. Representative cases of each entity are shown in Fig. 2.

No specific trend was identified with respect to the staining cellular compartments. The majority of p16-positive cases showed both nuclear and cytoplasmic staining (70/77; 91%). The remaining cases including AFX (2/77; 3%), pleomorphic undifferentiated sarcoma (2/77; 3%), DFSP (1/77; 1%), xanthogranuloma (1/77; 1%) and non-spitzoid melanoma (1/77; 1%) showed p16 localization to the cytoplasmic compartment exclusively. All of these cases with cytoplasm-only staining had proportion and intensity scores of 1 and 1, respectively.

Discussion

The p16 immunostain has gained widespread use in the evaluation of melanocytic lesions owing to its differential expression in benign nevi and melanomas as shown in various previous studies.[9,16,17,22] Our small reference set of melanocytic lesions supports these previous observations, in which p16 was consistently positive in ordinary and Spitz nevi but variably diminished in melanomas. Our study also shows a high prevalence of p16 expression in a cohort of histiocytic, fibrohistiocytic and undifferentiated proliferations of the skin. These observations reinforce that p16 immunoreactivity should not be regarded as evidence of melanocytic differentiation, especially when faced with a differential diagnosis that includes histiocytic, fibrohistiocytic or undifferentiated proliferations.

As illustrated in our index case and in a previous report,[23] reticulohistiocytoma readily enters the histomorphologic differential diagnosis of Spitz nevus as both entities frequently

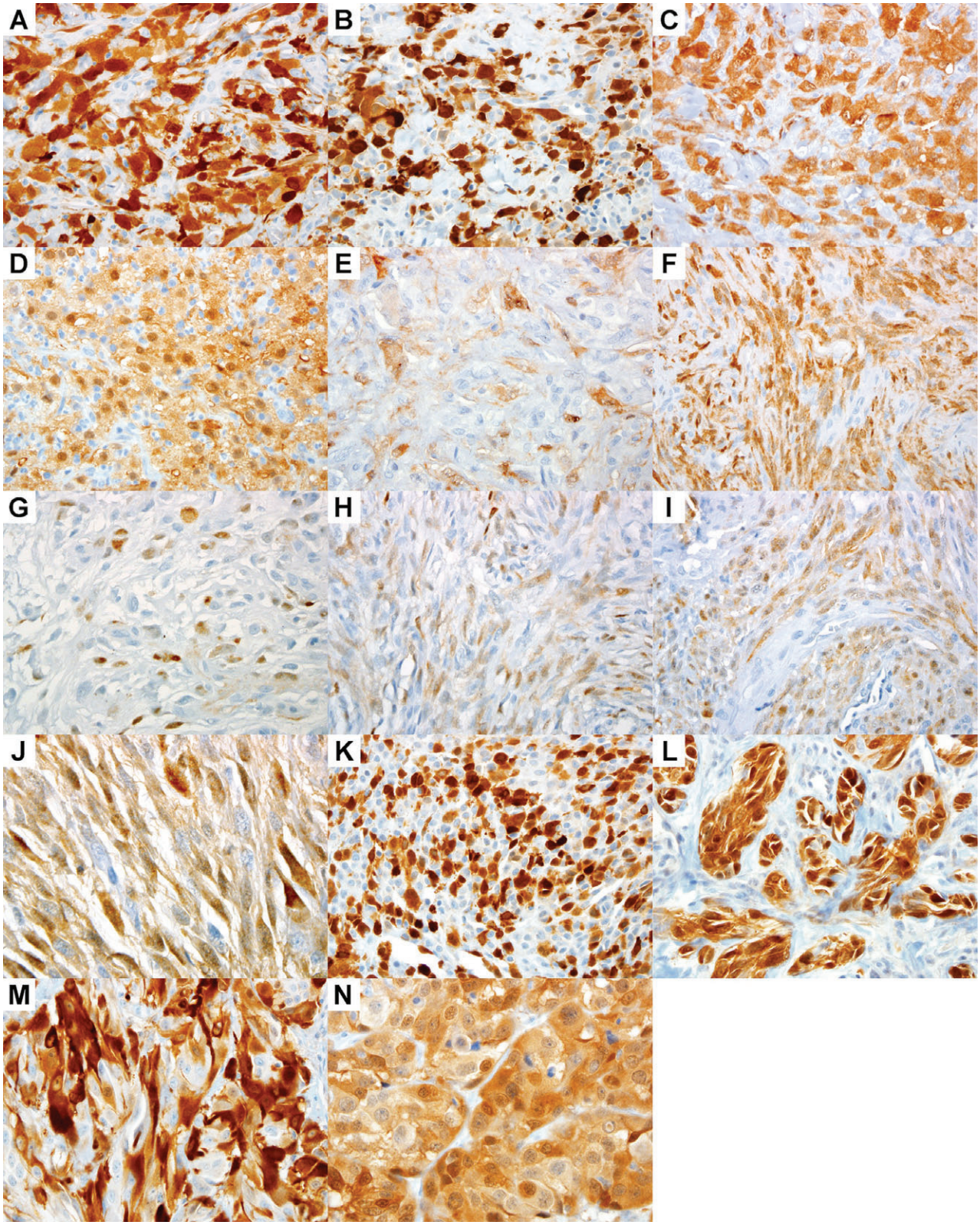

Fig. 2. Expression of p16 in histiocytic, fibrohistiocytic, undifferentiated and melanocytic lesions. A) Reticulohistiocytoma. B) Langerhans cell histiocytosis. C) Xanthogranuloma. D) Rosai Dorfman disease. E) Xanthoma. F) Dermatofibroma. G) Epithelioid fibrous histiocytoma. H) Dermatofibrosarcoma protuberans. I) Atypical fibroxanthoma. J) Pleomorphic undifferentiated sarcoma. K) Ordinary nevus. L) Spitz nevus. M) Spitzoid melanoma. N) Non-spitzoid melanoma (p16 immunostain, ×400).

form a dermal papule consisting of epithelioid cells with abundant eosinophilic cytoplasm. To add to this diagnostic challenge, S100 staining has been reported in a subset of reticulohistiocytoma cases.[24,25] Other melanocytic markers such as Melan-A, HMB45, SOX10 and/or tyrosinase are therefore required to distinguish these lesions in challenging cases. In our index case, unfortunately, additional stains including SOX10, CD1a and HMB45 were inconclusive as the lesional cells were no longer present on deeper sections. Although reticulohistiocytoma was slightly favored based on the negative staining for Melan-A and tyrosinase, the final diagnosis remained uncertain as the additional stains were non-contributory. Our study has proven p16 unhelpful in the differential diagnosis of reticulohistiocytoma and Spitz nevus because of its frequent expression in both. Nevertheless, based on the known function of p16 as a tumor suppressor, its retention perhaps at least provides indirect support to our morphologic impression of a benign lesion.

Concordant with previous reports,[19–21] p16 was found to be frequently expressed in LCH, and the staining was rather diffuse and intense in our cases. As LCH may sometimes simulate an atypical melanocytic lesion given its epithelioid cell morphology and S100 immunoreactivity, additional melanocytic markers and CD1a may be needed to distinguish the two. Given the common finding of p16 expression in both LCH and melanocytic lesions, p16 has no discriminatory role in this differential diagnosis. Interestingly, both LCH and melanocytic nevi share the frequent occurrence of BRAF mutation.[26,27] In melanocytic nevi, it has been shown that the initial growth phase triggered by oncogenic BRAF mutation is typically followed by a near-complete arrest of proliferative activity in conjunction with upregulation of p16 expression.[28] On the other hand, loss of p16 expression is expected to release this senescence barrier and allow for malignant progression. Such p16-dependent oncogene-induced senescence is similarly hypothesized in LCH, in which p16-positive cases have been associated with a more indolent course compared to the p16-negative cases.[21]

Frequent p16 expression was also found in other non-Langerhans cell histiocytoses including xanthogranuloma and Rosai Dorfman disease. As the histiocytic nature of these lesions is usually readily discerned on routine histomorphologic examination, p16 is seldom used in the diagnostic workup. Of all the histiocytoses examined in this study, xanthoma showed the lowest degree of p16 expression which is probably a reflection of its depositional as opposed to proliferative nature.

Occasionally, a spindle cell melanocytic proliferation may mimic a fibrohistiocytic lesion and vice versa. This typically occurs when the proliferation is associated with a sclerotic or desmoplastic stroma, as seen in sclerosing nevus and desmoplastic melanoma. Although sclerosing nevi and desmoplastic melanomas were not evaluated in our cohort, previous studies have shown p16 expression in virtually all desmoplastic Spitz nevi and in up to 72% of desmoplastic melanomas.[22,29] Given the similarly common expression of p16 in both dermatofibroma and DFSP in our study, p16 should not be used to discriminate these entities from desmoplastic melanocytic lesions. Between dermatofibroma and DFSP, we noted a trend toward lower p16 expression in the latter. This is in keeping with our understanding of the effect of p16 loss in promoting malignant transformation. In fact, loss of p16 was noted in over 20% of DFSP in one study and may have heralded fibrosarcomatous progression in one lesion.[30]

Of all the entities examined in this study, AFX was found to have the lowest degree of p16 expression, both in terms of staining intensity and proportion of cells staining. Pleomorphic undifferentiated sarcoma – another undifferentiated lesion characterized by additional adverse histomorphologic features (e.g. deep invasion, lymphovascular invasion, perineural invasion and necrosis) and a higher risk of recurrence compared to AFX[31] – appeared to show a greater degree of p16 expression. While these results may seem counterintuitive and could be a reflection of our small sample size, they remain in concordance with the findings of Knosel et al. where 70% of pleomorphic undifferentiated sarcomas retained p16 expression, and this expression did not correlate with survival.[32] The authors hypothesized that undifferentiated tumors harbored complex karyotypes and deranged cellular signaling pathways upon which cellular senescence via p16 expression was unable to exert its effect. Diagnostically, the potential role of p16 in discriminating melanoma and these undifferentiated lesions remains elusive. Future studies examining sarcomatoid or spindle cell melanoma may be helpful in better defining the utility of p16 in this setting.

As p16 is expressed in both the cytoplasmic and nuclear compartments,[33] it is not surprising that most of the lesions in our cohort

displayed mixed compartmental staining. Only rare cases of AFX, pleomorphic undifferentiated sarcoma, xanthogranuloma, DFSP and non-spitzoid melanoma displayed cytoplasmic staining in the absence of nuclear staining. Cytoplasmic staining alone is considered by some to be non-specific, as it has been shown in formalin-fixed paraffin-embedded pellets of cell lines with negative p16 status.[34] It is therefore possible that cytoplasmic p16 staining alone equates to no expression. In fact, all cases in our cohort with cytoplasm-only staining had proportion and intensity scores of 1 and 1, respectively, further supporting the notion that such staining is non-specific. Although there has been some discussion of variances in the pattern of compartmental expression of p16 amongst melanocytic lesions, we did not observe any specific trend in our cases.

Our study is limited by the small sample size, which precludes comparative analysis of p16 expression between entities. However, to our knowledge, this is the first comprehensive survey of p16 expression in cutaneous histiocytic, fibrohistiocytic and undifferentiated lesions. By showing the rather ubiquitous p16 expression across a variety of benign and malignant non-melanocytic lesions, our study reinforces that p16 has virtually no role in confirming melanocytic differentiation; other melanocytic markers such as S100, Melan-A, HMB45, MiTF, SOX10 and tyrosinase should be utilized for this purpose. Further studies including a larger cohort of cases, including malignant histiocytoses such as Langerhans cell sarcoma and histiocytic sarcoma, are required to determine if p16 expression is useful in distinguishing benign from malignant neoplasms of these types.

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