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Fatty acid synthase and acetyl-CoA carboxylase are expressed in nodal metastatic melanoma but not benign intracapsular nodal nevi

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Abstract

Background: Melanoma is a potentially lethal form of skin cancer for which the current standard therapy is complete surgical removal of the primary tumor followed by sentinel lymph node biopsy when indicated. Histologic identification of metastatic melanoma in a sentinel node has significant prognostic and therapeutic implications, routinely guiding further surgical management with regional lymphadenectomy. While melanocytes in a lymph node can be identified by routine histopathologic and immunohistochemical examination, the distinction between nodal nevus cells and melanoma can be morphologically problematic. Previous studies have shown that malignant melanoma can over-express metabolic genes such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). This immunohistochemical study aims to compare the utility of FASN and ACC in differentiating sentinel lymph nodes with metastatic melanomas from those with benign nodal nevi in patients with cutaneous melanoma.

Materials and Methods: Using antibodies against FASN and ACC, 13 sentinel lymph nodes from 13 patients with metastatic melanoma and 14 lymph nodes harboring benign intracapsular nevi from 14 patients with cutaneous malignant melanoma were examined. A diagnosis of nodal melanoma was based on cytologic atypia and histologic comparison with the primary melanoma. All nodal nevi were intracapsular and not trabecular. Immunohistochemistry for Melan-A, S100, HMB45, FASN and ACC were performed. The percentage of melanocytes staining with HMB45, FASN and ACC was determined and graded in 25% increments; staining intensity was graded as weak, moderate or strong.

Results: All metastatic melanomas tested had at least 25% tumor cell staining for both FASN and ACC. Greater than 75% of the tumor cells stained with FAS in 7/13 cases and for ACC in 5/12 cases. Intensity of staining was variable; strong staining for FASN and ACC was observed in 69%

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and 50% of metastatic melanoma, respectively. HMB45 was negative in 40% of nodal melanoma cases all of which stained with FASN and ACC. Capsular nevi were uniformly negative for FASN, ACC and HMB45 immunoreactivity.

Conclusions: All metastatic melanoma cases involving sentinel lymph nodes were positive for FASN and ACC while no staining was observed in intracapsular nevi. These findings suggest that FASN and ACC could be utilized as valuable ancillary stains in the distinction between nodal nevi and metastatic melanoma.

Introduction

Melanoma has witnessed a fast rise in incidence in the United States and is currently responsible for more deaths than any other form of cutaneous malignancy. (1) The current standard therapy is complete surgical removal of the primary tumor when possible followed by sentinel lymph node biopsy in select patients. Histologic characteristics of the primary lesion imparting critical prognostic information include tumor thickness, mitotic rate and ulceration. (2–4) In addition, identification of metastatic melanoma in a sentinel node portends significantly worse prognosis, routinely guiding further surgical staging with regional lymphadenectomy. (5–7)

Benign nevus cells are commonly identified in lymph nodes of patients with cutaneous melanoma and must be differentiated from metastatic melanoma. The morphologic features of nodal nevi have been described in detail. (8) Nevocytes have been reported in up to 22% of lymphadenectomy specimens and while most nevocytes are restricted to a single lymph node, approximately 13% and 3% of lymphadenectomy specimens contain benign nevocytes in two and three lymph nodes, respectively. While most nodal nevi are identified on H&E stained sections, about 20% are missed and highlighted upon staining with S100. More than 90% of nodal nevi occur in the peripheral capsule with the remaining being intratrabecular. Nodal nevus cells are identified from less than 1% up to 22% of lymphadenectomy specimens and are significantly more likely to be seen in sentinel lymph nodes compared to non-sentinel nodes. Moreover, nevus cells are more frequently encountered in staging lymph nodes removed after a diagnosis of cutaneous melanoma. (8–12) It is hypothesized that a growing cutaneous melanoma can disrupt adjacent nevi, displacing benign nevus cells to draining lymph nodes. Alternatively, nodal nevi may arise during embryogenesis secondary to abnormal migration of neural crest cells. (13) Nodal nevi can be identified by routine histopathologic and immunohistochemical examination; however, distinguishing benign nodal nevus cells from melanoma is not always straightforward and can be morphologically challenging.

Several morphologic features have been evaluated and utilized in distinguishing benign nodal nevi from metastatic melanoma. Benign nodal nevi are characterized by melanocytes that are limited to the peripheral capsule or less commonly located within internal trabecula. Nevus cells form compact aggregates of small bland cells with little cytoplasm, round nuclei, small nucleoli, absent mitotic activity and minimal to no melanin pigment. In contrast, metastatic melanoma generally localizes to the subcapsular sinus or within the lymphoid tissue, shows cytologic atypia and mitoses and is morphologically similar to its

cutaneous counterpart. (8, 14) Nevertheless, distinguishing benign and malignant melanocytic cells can be challenging. Nodal nevi have been reported in a subcapsular location or within the lymph node parenchyma, can be pigmented, may exhibit features of cellular blue nevus and can percolate throughout the fibrous tissue of a lymph node. (14) In addition, lymph nodes harboring both benign nevus cells and melanoma have been encountered. (8, 15)

Several immunohistochemical stains including S100, Melan-A, HMB45, Ki-67, p16, 5-hydroxymethylcytosine, SOX2, IMP3 and nestin have been used in an effort to distinguish benign nodal nevomelanocytes from metastatic melanoma. (8, 14, 16–21) Fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC), two metabolic genes involved in fatty acid synthesis, have been shown to be over-expressed in malignant melanoma. Both genes are thought to promote tumorigenesis in melanoma as well as in other malignancies. (22–26) Herein, we evaluate the utility of FASN and ACC expression in distinguishing benign nodal nevi from metastatic melanoma in sentinel lymph node biopsies of patients with cutaneous melanoma.

Material and Methods

A total of 27 staging sentinel lymph nodes from 27 patients with cutaneous melanoma were included in the study. All melanomas were diagnosed on biopsy or excision and sentinel lymph node biopsies were performed for tumors with a Breslow thickness ≤ 1 mm. At our institution, all sentinel lymph nodes were first bisected at the hilum along the longest axis of the node then sectioned at 2 mm intervals. Three hematoxylin and eosin (H&E) stained sections are prepared per tissue block. If metastatic melanoma was not identified on initial H&E then additional levels, Melan-A and S100 immunostains were ordered.

Thirteen lymph nodes contained metastatic melanoma and 14 lymph nodes harbored benign intracapsular nevi. A diagnosis of melanoma was based on cytologic atypia and a histology that was comparable to the patient's primary melanoma. None of the lymph nodes contained both melanoma and nevus cells. Immunohistochemical stains for FASN (A301-323A, Bethyl), ACC (Ser79, Cell Signaling Technology), S100 (polyclonal, Dako), Melan-A (A103, Ventana), and HMB45 (HMB45, Dako) were performed on all lymph nodes with capsular nevi and in the majority of lymph nodes with metastatic melanoma. S100 staining was interpreted as positive when localized to both the nucleus and the cytoplasm. Only cytoplasmic staining with Melan-A, HMB45, ACC and FASN was interpreted as positive. Appropriate positive and negative controls were obtained for each case.

Melanocyte staining with HMB45, FASN and ACC was evaluated following a scoring system similar to that used by Innocenzi et al. (24) The percentage of staining was graded as: (1) 1-25%, (2) 26-50%, (3) 51-75% or (4) >75%. Staining intensity was graded as (1) weak, (2) moderate, or (3) strong by comparing the staining with that of appropriate internal control tissue as described by Uchiyama et al. (27) An immunohistochemical (IHC) score was obtained by multiplying the percentage and intensity of staining.

The study was approved by the Institutional Review Board.

Results

Nodal nevi were all intracapsular in location and consisted of small clusters of nevic cells with uniform round to oval nuclei, smooth regular nuclear membranes, fine chromatin, inconspicuous nucleoli and moderate eosinophilic cytoplasm. None of the lymph nodes contained trabecular collections of nevomelanocytes. Nevus cells stained with S100 and Melan-A in all cases and were uniformly negative for HMB45, FASN and ACC; Figure 1, Table 1.

Thirteen lymph nodes contained metastatic melanoma with most harboring a single metastatic focus. The tumor was cytomorphologically similar to that of the cutaneous primary in all cases (data not shown) and frequently featured pleomorphic large epithelioid cells with amphophilic to eosinophilic cytoplasm, conspicuous nucleoli and intranuclear inclusions. S100, Melan-A, FASN and ACC were positive in all tested cases. Staining in more than 25% of tumor cells was seen for FASN in all cases and in 9 of 12 cases for ACC; Figure 2, Table 1. Greater than 75% tumor cell staining was seen in seven cases with FAS and in five cases with ACC. The intensity of staining was variable; metastatic melanoma cells showed strong staining intensity for FASN in 69% of cases and strong staining for ACC in 50% of cases. Immunohistochemical staining for both FASN and ACC was performed on twelve lymph nodes and showed identical IHC scores in 42% of cases; FASN had a higher IHC score in six of the seven remaining cases. The percentage of positive cells paralleled the staining intensity for both FASN and ACC. Moderate to intense staining with FASN in >51% of tumor cells was seen in 10 of the 13 cases; two cases showed intense staining in 26-50% tumor cells and one case showed weak staining in 26-50% of tumor cells. Moderate to intense staining with ACC in >51% of tumor cells was seen in 7 of the 12 tested cases; two cases demonstrated weak staining in 51-75% of tumor cells and three cases showed weak staining in 1-25% of tumor cells. HMB45 failed to stain metastatic melanoma in 4 of the 10 tested cases; Figure 3.

Overall, FASN and ACC were 100% sensitive and specific at identifying metastatic melanoma and distinguishing it from capsular nevi. HMB45 demonstrated a sensitivity and specificity of 60% and 100%, respectively. S100 and Melan-A highlighted both melanoma and nevus cells in all cases but were of no utility in distinguishing the two entities.

Discussion

In our study, we evaluated the utility of FASN and ACC immunohistochemistry in distinguishing benign nodal nevi from metastatic melanoma in sentinel lymph node biopsies of patients with cutaneous melanoma. All cases of nodal metastatic melanoma were positive for FASN and ACC in contrast to intracapsular nodal nevi which were uniformly negative. Moreover, FASN and ACC were more sensitive than HMB45 in highlighting metastatic melanoma. These findings suggest that FASN and ACC could be utilized as valuable ancillary stains in the distinction between nodal nevi and metastatic melanoma.

The predominant source of fatty acids available to tumor cells is generated through the process of de novo synthesis within tumor cells even in the presence of an adequate extrinsic

fatty acid supply (28). ACC and FASN are key enzymes in the endogenous pathway of fatty acid synthesis. ACC is the rate-limiting enzyme that converts acetyl-CoA to malonyl-CoA. FASN generates long chain fatty acids from both acetyl-CoA and malonyl-CoA. (29, 30) Both enzymes are over-expressed in malignancy. FASN appears to be constitutively expressed in a variety of malignancies including lung, endometrial, ovarian, prostate, breast and colon cancers, may herald malignant transformation in preneoplastic lesions and is an independent predictor of worse clinical outcome. (31–42). FASN expression may be upregulated in tumor cells subjected to hypoxia, acidosis or nutritional deprivation, conferring a selective growth advantage. (43,44)

Innocenzi et al. compared cutaneous melanoma and benign nevi for differences in the expression of FASN. All nevi showed absent to low level expression of FASN while 44% of melanoma had higher immunohistochemical staining scores. All cutaneous and nodal metastasis also showed strongly positive FASN staining. Higher FASN positivity and staining intensity correlated with Breslow thickness and predicted a worse outcome suggesting that FASN may be an important diagnostic and prognostic biomarker in cutaneous melanoma. (24) Similar results were reported by Kapur et al. showing stronger expression of FASN in metastatic melanomas compared to conventional and Spitz nevi. FASN scores were also significantly greater for Clark levels IV and V melanomas and with increasing Breslow thickness. (23)

The Ten-Eleven Translocation (TET) family of 5-mC hydroxylases plays a critical role in the epigenetic regulation of DNA methylation by converting 5-methylcytosine to 5-hydroxymethylcytosine. Loss of 5-hydroxymethylcytosine reflects an underlying dysfunction in TET and consequently a state of active DNA demethylation. (45,46) 5-hydroxymethylcytosine is retained in benign cutaneous nevi but shows partial or complete loss in dysplastic nevi and melanoma. (47) Lee et al. examined the utility of 5-hydroxymethylcytosine in differentiating metastatic melanoma from nodal nevi by performing dual labeling with MART-1. Loss of 5-hydroxymethylcytosine nuclear staining with retained MART-1 labeling was seen in all metastatic melanoma cases. In contrast, all nodal nevi demonstrated retained 5-hydroxymethylcytosine in MART-1-positive cells. (16)

Insulin-like growth factor-II messenger RNA-binding protein-3 (IMP3), a member of oncofetal family of proteins, plays several important roles during early embryogenesis, regulates cell growth, cell migration, and RNA trafficking and stabilization. (48) IMP3 is normally expressed in the ovary, testis, brain, placenta, intestine and lymph node germinal centers. (49–51) In neoplastic conditions, IMP3 enhances the expression of insulin-like growth factor-II protein thereby promoting tumor cell proliferation. (52) It is differentially expressed in cutaneous melanoma but not in benign nevi. (53) Mentrikoski et al. assessed the utility of IMP3 immunohistochemistry in separating nodal melanoma from nodal nevi. IMP3 was expressed in 70% of nodal melanoma and in none of the nodal nevi tested. (20)

The p16 protein, a product of the *CDKN2* tumour suppressor gene negatively regulates the cell cycle through inhibition of G1 cyclin-dependent kinases, preventing phosphorylation of *RB* gene thereby decreasing transition from G1 to S phase. (54,55) Loss of p16 is associated with tumor progression of melanoma. (56) Mihic-Probst et al. attempted to differentiate

nodal nevi and metastatic melanoma using p16 immunohistochemistry. Strong nuclear and cytoplasmic staining was seen in all nevus cells whereas all cases of metastatic nodal melanoma showed loss of nuclear staining. (19)

Zubovits et al. studied the staining patterns of S100, NK1/C3, HMB45, and MART-1 in nodal metastatic melanoma and found a heterogeneous pattern of staining with S100, NK1/C3, MART-1 and HMB45 staining 98%, 93%, 82% and 76% of cases, respectively. Despite demonstrating superior sensitivity, S100 and NK1/C3 stained non-neoplastic cells as well. MART-1 and HMB45 were more specific and overall, MART-1 stained more diffusely and intensely than HMB45. (57) Our study also showed a low sensitivity for HMB45 with only 60% staining in cases of metastatic melanoma.

The proliferation marker Ki-67 is more likely to be positive and in a greater percentage of cells in nodal melanoma compared to nodal nevi. This observation was made by Lohmann et al. who examined nodal nevi and both nodal and cutaneous melanoma metastases for the expression of S100 protein, HMB45, MART-1, tyrosinase and Ki-67. Ninety-three percent of nodal nevi were completely negative for Ki-67 and one cellular nodal nevus showed <0.2% melanocyte nuclear positivity. In contrast, all metastatic melanoma cases showed staining in 2% to 80% of tumor cell nuclei. (21) Biddle et al. studied Ki-67, S100, MART-1 and HMB45 in 13 patients with nodal nevus cell aggregates. Nevus cells stained with S100 and MART-1 but did not label with HMB45; less than 1% of nevus cells labeled with Ki-67. (58)

Several immunohistochemical stains have been studied in an effort to distinguish nodal nevi from metastatic melanoma. SOX2, also known as sex determining region Y-box 2 (SRY) is expressed by neural progenitor cells of the central nervous system and acts as a transcription factor by binding to and regulating nestin core enhancer. (59) Nestin, a marker of neural stem cells, is a type VI intermediate filament which is expressed in migrating and proliferating neuroectodermal cells. (60) Both markers have been shown to be expressed in primary cutaneous and metastatic melanoma but not in benign cutaneous nevi. (61–64) Chen et al. studied the combined use of SOX2 and nestin in differentiating nodal nevi from nodal melanoma. (17) Nestin and SOX2 were positive in 100% and 57% of metastatic melanoma; however, weak/focal staining for both markers was identified in a minority of nodal nevi.

Among the multitude of biomarkers used to distinguish nodal nevi from metastatic melanoma, we have shown that FASN and ACC immunohistochemistry stain all metastatic melanomas and are more sensitive than HMB45. However, among the melanomas tested, difficulty was encountered in distinguishing focal weak tumor staining from non-specific background staining in a minority of cases. Nonetheless, these findings expand on the repertoire of available immunohistochemical stains that can be used in distinguishing benign nodal nevi from melanoma.

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Figure 1.

Sentinel lymph node with capsular nevus in a patient with cutaneous melanoma. The nevus cells appear bland and uniform (indicated by the arrow) and are highlighted with Melan-A immunostain. HMB45, FASN and ACC are negative; (H&E, 200X; immunohistochemistry, 100X).

Figure 2.

Metastatic melanoma to sentinel lymph node. Tumor cells are diffusely positive for Melan-A, HMB45 and S100 and show cytoplasmic staining with ACC and FASN; (H&E, Melan-A, HMB45 and S100, 100X; ACC and FASN, 200X).

Figure 3.

Sentinel lymph node with a single subcapsular focus of melanocytes that are highlighted with S100 and Melan-A but are negative for HMB45. FASN demonstrates weak cytoplasmic staining while ACC shows faint cytoplasmic staining. Overall the findings are those of metastatic melanoma; (H&E and immunohistochemistry, 400X).

Table 1.

Immunohistochemical Index of Nodal Melanoma and Benign Nodal Nevi

Nodal Melanoma Cases	Total IHC score (0-12)			Nodal Nevi Cases	Total IHC score (0-12)		
	HMB45	FASN	ACC		HMB45	FASN	ACC
1	9	9	9	1	0	0	0
2	0	6	3	2	0	0	0
3	9	12	12	3	0	0	0
4	9	6	1	4	0	0	0
5	0	8	8	5	0	0	0
6	0	2	1	6	0	0	0
7	3	6	3	7	0	0	0
8	12	12	1	8	0	0	0
9	NP	6	12	9	0	0	0
10	0	12	9	10	0	0	0
11	NP	12	12	11	0	0	0
12	NP	12	12	12	0	0	0
13	12	12	NP	13	0	0	0
-	-	-	-	14	0	0	0

Abbreviations: FASN, fatty acid synthase; HMB45, human melanoma black 45; IHC, immunohistochemical; NP, not performed/insufficient material for further testing; ACC, acetyl-CoA carboxylase. The IHC score was obtained by multiplying the percentage of cells staining and the intensity of staining with a maximum score of 12 as described in the materials and methods section.