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HLA antigen expression in melanocytic lesions: Is acquisition of HLA antigen expression a biomarker of atypical (dysplastic) melanocytes?

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Abstract

Background: Although criteria are established for the histologic diagnosis of atypical nevi (AN), consensus about the criteria in the diagnosis of and in the definition of AN is limited. Moreover, intraobserver and interobserver differences in the application of these criteria for the diagnosis of AN have been observed.

Objective: We sought to determine the usefulness of HLA antigen expression as a biomarker of AN.

Methods: The immunoperoxidase reaction was used to mark common nevi and AN with HLA class I heavy chain—, β 2microglobulin (β 2m)—, and HLA class II β chain—specific monoclonal antibodies.

Results: HLA class I heavy chain, β 2m, and HLA class II β chain were expressed in 5 (8.6%) of the 58 common nevi and in 46 (~72%) of the 64 atypical melanocytic lesions. Among common lesions, only halo nevi expressed HLA class I heavy chain, β 2m, and HLA class II β chain. The level of HLA class I heavy chain β 2m and of HLA class II β chain expression correlated with the degree of cytologic atypia and architectural disorder.

Limitations: The number of lesions tested and the subjective nature of the analysis of immunohisto-chemical staining of tissue sections are both limitations.

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Conflicts of interest: None declared.

Conclusions: The data presented suggest that HLA antigen expression is an objective biomarker that correlates well with the degree of cytologic atypia in AN and may: (1) be useful to distinguish common nevi from AN, and (2) represent a more objective measure to determine which AN should be excised.

Keywords

atypical nevus; cancer; classical HLA class I antigen; dysplastic nevus; HLA class II antigen; immune escape; immune surveillance; immunotherapy; melanocyte; melanoma; nevus

The clinical significance of the entity known as the atypical nevus (AN) or dysplastic nevus¹⁻⁴ relates to its association with an increased risk of melanoma and its potential to represent a precursor of melanoma.⁵⁻⁹ Criteria for the histologic evaluation of AN have been established.^{4,7-9} Nevertheless, the establishment of sporadic AN as a specific entity is hampered by the lack of specificity and reproducibility of the criteria used, making the results of studies difficult to compare. Specifically, the histologic diagnosis of AN suffers from: (1) overlapping histologic features found in a variety of common melanocytic lesions,^{8,9} and (2) lack of consensus regarding their histologic definition.^{8,9} Moreover, intraobserver and interobserver differences in the application of these criteria for the diagnosis of AN have been observed.^{5,8,10-17} Lastly, poor interobserver agreement is often observed in the characterization of cytologic atypia in AN,^{5,8,10-17} a parameter regarded as essential for their diagnosis and associated with their potential for malignant transformation.^{6,7}

To date, no reliable bio-markers exist to distinguish between common nevi and AN nor to provide information regarding the degree of atypia among AN. Moreover, it is unknown which histologic features of AN are most useful in predicting melanoma risk. For some time, we have been evaluating HLA antigen expression in malignant lesions, since changes HLA antigen expression, which have been frequently identified in malignant melanocytes, are associated with the clinical course of the disease in patients with melanoma.^{18,19} In the course of the analysis of the expression of HLA antigens in lesions of melanocytic origin we have found that they display a differential expression in common nevi and AN. This finding prompted us to determine the usefulness of HLA antigen expression as a biomarker of AN to help objectify the difficult task of characterizing AN histologically.

METHODS

Tissues

Normal-appearing skin, common nevi, and AN were obtained from Dermatopathology Consultants at the University of Colorado, Denver (Health Sciences Center), Aurora, CO. Specimens were obtained through query of the slide and tissue archive for the diagnosis of common nevi and AN. The diagnosis was confirmed by two independent board-certified dermatopathologists who had no knowledge of the results of immunohistochemical (IHC) staining.^{4,20} Although absolute consensus regarding criteria for the histologic definition of AN is lacking, commonly used characteristics within this study include the presence of: (1) marked junctional proliferation of single and variably shaped nests of melanocytes

mostly along the dermoepidermal junction and often exhibiting bridging between nests; (2) lamellar and concentric fibroplasia within the dermis; (3) melanocytes with enlarged, pleomorphic nuclei, demonstrating variability in size and shape, often with hyperchromasia or an irregular nuclear contour; (4) a variable lymphohistiocytic infiltrate within the dermis; and (5) extension of the junctional (intraepidermal) melanocytic component greater than 3 epidermal rete ridges beyond the dermal melanocytic component to produce a phenomenon known as “shoulders.”^{4,20}

Tissue samples were fixed in 10% neutral buffered formaldehyde and routinely processed within 15 minutes of extirpation. To ensure that formalin-fixed, paraffin-embedded tissue sections did not alter HLA antigen expression as compared with frozen specimens, some samples were frozen in liquid nitrogen and stored at -80°C until use. Then, 4- μm thick cryostat-cut sections were dried and fixed in absolute acetone for 1 minute. Under these fixation conditions, cryostat-cut sections could be stored for at least 3 months at -20°C without loss of reactivity with antibodies.

Monoclonal antibody

The murine monoclonal antibody (mAb) HC-10 that recognizes an epitope expressed on $\beta 2\text{m}$ -free HLA-A3, -A10, -A28, -A29, -A30, -A31, -A32, -A33, -B (excluding -B5702, -B5804, and -B73), and -C heavy chains^{21,22}; the mAb HC-A2 that recognizes an epitope expressed on the alpha-1 domain of HLA-A, -B7301, -E, and -G heavy chains^{21,23}; the $\beta 2\text{m}$ -specific mAb NAMB-1,²⁴ the mAb TP25.99 that recognizes an epitope expressed on $\beta 2\text{m}$ -associated HLA-A, -B, and -C heavy chains and $\beta 2\text{m}$ -free HLA-A1, -A3, -A9, -A11, -A30, -B, and -C heavy chains²⁵; and the mAb LGII-612.14 that recognizes a linear epitope expressed on HLA-DR, -DQ, and -DP β chains²⁶ were developed, characterized, and purified²⁷ as described.

IHC marking

Tissue sections were marked with mAb using the EnVision+ system (Dako Cytomation, Carpinteria, CA); marking was evaluated as described elsewhere.²⁸ The percentage of tumor cells that were marked by the tested mAb in each lesion was enumerated independently by two investigators who had no knowledge of the patients' characteristics and histopathological diagnosis. Variations in the percentage of marked cells enumerated by two investigators were within a 10% range. For this reason, we evaluated the percentage of marked tumor cells at 10% levels. Expression of the epitope recognized by each mAb used in IHC reactions was confirmed via the marking of surrounding normal cells (ie, endothelial cells, keratinocytes, lymphocytes, and peripheral blood mononuclear cells) present in each tissue section. Results were scored as positive, heterogeneous, or negative when the percentage of marked melanocytes in an entire lesion was greater than 75%, 25% to 75%, and less than 25%, respectively, according to the criteria established by the HLA and cancer component of the 12th International Histocompatibility Workshop.²⁸ Because of the difficulty in differentiating between lack and low levels of HLA antigen expression these categories were combined.

Statistical analysis

The differences in HLA expression were analyzed using a χ^2 test. *P* values of less than .05 were considered statistically significant.

RESULTS

Common and atypical melanocytic lesions were obtained from 92 patients. They included 31 male and 55 female patients with an average age of 40.2 years (range: 8–89 years). The 58 common melanocytic lesions analyzed consisted of 16 intradermal, 19 compound, 8 compound lentiginous, 7 junctional lentiginous, 3 blue, and 5 halo nevi. Patient characteristics were unavailable for 4 of the common melanocytic lesions. The 64 atypical melanocytic lesions analyzed consisted of 45 compound, 15 junctional, 2 compound lentiginous, and 1 junctional lentiginous nevi. Among the atypical melanocytic lesions 23, 21, and 20 were diagnosed histopathologically as demonstrating mild, moderate, and severe cytologic atypia and architectural disorder, respectively. Patient characteristics, anatomic site, and type of melanocytic lesion are summarized in Tables I and II.

The results of IHC marking of 58 common and 64 atypical melanocytic lesions with mAb that recognize epitopes of HLA class I heavy chains, β 2m, and HLA class II β chains are summarized in Tables I to III and in Figs 1 and 2. Representative IHC marking patterns are shown in Figs 3 to 6. (Please visit <http://www.jaad.org> to view figures and tables.)

Five (8.6%) of the 58 common melanocytic lesions were marked with a positive score by the HLA class I heavy chain–specific mAb HC-10 and HC-A2, by the β 2m-specific mAb NAMB-1 and by the HLA class II β chain–specific mAb LGII-612.14 (Tables I and III; Figs 1 and 3; available at <http://www.jaad.org>). All of the common melanocytic lesions marked by HLA class I heavy chain–, β 2m–, and HLA class II β chain–specific mAb were diagnosed histologically as halo nevi. Three of the common melanocytic lesions diagnosed as inflamed compound nevi histologically were marked to a certain extent by mAb HC-10, HC-A2, NAMB-1, and LGII-612.14. However, the percentage of marked cells was less than 25%; therefore the lesions were classified as negative. To ensure that formalin fixation and/or paraffin embedding of tissue sections did not alter HLA antigen expression in common melanocytic lesions, frozen sections of two intradermal nevi were used as a substrate in IHC reactions with HLA antigen– and β 2m-specific mAb. No marking of melanocytes was detected in these specimens (data not shown). Notably, in one lesion diagnosed histologically as superficial spreading melanoma arising in an intradermal nevus, atypical cells were marked with mAb HC-10, HC-A2, NAMB-1, and LGII-612.14, whereas common nevus cells were not (Fig 4; available at <http://www.jaad.org>).

In all, 26 (40%) of the 64 atypical melanocytic lesions were marked with a positive score by mAb HC-10, HC-A2, NAMB-1, and LGII-612.14 (Tables II and III; Figs 1, 2, 5, and 6; available at <http://www.jaad.org>). Twenty of these lesions were classified histologically as AN with severe atypia and 6 as AN with moderate atypia (Tables II and III; Figs 1, 2, 5, and 6; available at <http://www.jaad.org>). In all, 22 of the 64 atypical melanocytic lesions were marked with a heterogeneous score by mAb HC-10, HC-A2, NAMB-1, and LGII-612.14 (Tables II and III; Figs 1, 2, 5, and 6; available at <http://www.jaad.org>). Fifteen of these

lesions were classified histologically as AN with moderate atypia and 7 as AN with mild atypia. Sixteen of the 64 atypical melanocytic lesions were marked with a negative score by mAb HC-10, HC-A2, NAMB-1, and LGII-612.14 (Tables II and III; Figs 1, 2, 5, and 6; available at <http://www.jaad.org>). All of these lesions were classified histologically as AN with mild atypia.

To investigate the potential clinical significance of HLA antigen expression in melanocytic lesions, their expression was correlated to the histopathological characteristics of the lesions. The percentage of AN with severe atypia marked with a positive score by HLA class I heavy chain-, $\beta 2m$ -, and HLA class II β chain-specific mAb was significantly ($P < .05$) higher than that of AN with moderate or mild atypia (Fig 2; available at <http://www.jaad.org>). Furthermore, the percentage of AN diagnosed with moderate atypia marked with a positive and a heterogeneous score by HLA class I heavy chain-, $\beta 2m$ -, and HLA class II β chain-specific mAb was significantly ($P < .05$) higher than that of AN with mild atypia (Fig 2; available at <http://www.jaad.org>). Lastly, the level of HLA class I heavy chain, $\beta 2m$, and HLA class II β chain expression was concordant in common, atypical, and malignant melanocytic lesions.

DISCUSSION

This study has demonstrated a differential expression of HLA class I and class II antigens in common nevi and AN. Specifically, in contrast to common nevi, a significant number of AN expressed HLA class I heavy chain, $\beta 2m$, and HLA class II β chain in a coordinated fashion. In addition, acquisition of HLA antigen expression in AN appears to have clinical significance, as in the current study the frequency of HLA class I heavy chain, $\beta 2m$, and HLA class II β chain expression was associated with the degree of cytologic atypia and architectural disorder, markers essential for the diagnosis of AN and thought to be related to their potential for malignant transformation.⁶⁻⁹ After appropriate validation studies have been completed,²⁹ these findings suggest that HLA antigen expression in AN correlates well with the degree of cytologic atypia and may be a useful histologic biomarker to objectify the characterization of AN histologically. These findings have important clinical implications, because the degree of histologic atypia in AN has been associated with melanoma risk,^{6,7} and conservative re-excision is almost universally recommended for patients with AN with positive or narrow margins that demonstrate moderate to severe dysplasia. Future studies should determine whether the degree of HLA antigen expression in an individual patient's AN is useful in predicting melanoma risk.

It is noteworthy that for some time it has been assumed that HLA class I antigens are frequently lost or down-regulated on malignant melanocytes.^{18,19} Nevertheless, in our study, in contrast to common nevi (excluding halo nevi), all of the atypical melanocytic lesions demonstrated coordinated HLA class I heavy chain, $\beta 2m$, and HLA class II β chain expression. In the case of halo nevi, our findings are similar to those described in the literature.^{30,31} Nevertheless, the molecular mechanisms underlying the appearance of HLA antigens on melanocytes in halo nevi remains to be determined. In the case of AN, our findings suggest that the manner in which investigators have depicted the HLA antigen status of malignant melanocytes in the past^{18,19} may not reflect the true biology of HLA

antigen expression. Currently it is not known whether, like malignant melanocytes,^{18,19} common nevi and AN possess structural or functional abnormalities in the genes controlling HLA antigen expression. Future studies should be directed at identifying the molecular mechanisms underlying the appearance or lack of HLA antigen expression on common and atypical melanocytes.

Although the data must be interpreted with caution, particularly because of the relatively small sample size, the frequency of HLA antigen expression we report differs from that of prior studies.^{2,3,19,32–44} In this regard, only about 90 common nevi and 14 AN have been analyzed for HLA antigen expression. HLA antigen expression has been demonstrated in 0% to 20% of common melanocytic lesions. It is unlikely that the use of formalin-fixed, paraffin-embedded tissue sections causes the differences in the frequency of HLA antigen expression, because we were unable to detect these antigens in frozen sections of common nevi. Moreover, we have previously shown that the use of formalin-fixed, paraffin-embedded tissue sections in IHC staining accurately reflects that of melanocytic lesions in vivo.⁴⁵ It is likely that the use of in vitro studies using cultured melanocytes and common nevi,^{2,3,32–44} the varying methodologies and mAb utilized⁴⁶ to assess HLA antigen expression and the subjective evaluation of IHC staining underlie differences in the frequency of HLA antigen expression in common melanocytic lesions. Finally, it should be noted that the level of HLA antigen expression on individual melanocytes located in the basal cell layer of the epidermis could not be determined because of the avid marking of adjacent confluent keratinocytes. This limitation questions whether the findings observed in nested nevomelanocytes can be applied to individual melanocytes.

In conclusion, this study, to our knowledge, evaluated for the first time the frequency and histopathological significance of HLA class I heavy chain, $\beta 2m$, and HLA class II β chain expression in surgically removed common and atypical melanocytic lesions. These findings suggest that malignant transformation of melanocytes, at least those derived from nevi, may be associated with acquisition of HLA antigen expression. Moreover, the level of HLA antigen expression in AN correlates well with the degree of cytologic atypia, and may represent a more objective measure to determine which lesions should be excised. These findings emphasize the need to more appropriately characterize HLA antigen expression in individual melanocytes and common melanocytic lesions.

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Abbreviations used:

AN	atypical nevus
$\beta 2m$	$\beta 2$ microglobulin
IHC	immunohistochemical
mAb	monoclonal antibody

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CAPSULE SUMMARY

- The clinical significance of atypical nevi (AN) relates to their association with an increased risk of melanoma and their potential to represent a precursor of malignant melanoma.
- Although criteria exist for the histologic diagnosis of AN, the consensus regarding the criteria for the diagnosis of AN is limited. Furthermore, both interobserver and intraobserver differences have been observed in the application of these criteria.
- No reliable biomarkers exist to distinguish between common nevi or AN nor to provide useful information regarding the degree of atypia among AN.
- The findings discussed herein suggest that HLA antigen expression may be a useful biomarker to distinguish common nevi from AN and to determine the degree of cytologic atypia in AN.

Type of melanocytic lesion

Fig 1.

Frequency of HLA antigen expression in common, atypical, and malignant melanocytic lesions, as determined by immunohistochemical marking with HLA anti-genespecific monoclonal antibody (mAb). Common, atypical, and malignant melanocytic lesions are scored positive (■), heterogeneous (▒), and negative (□) according to their marking by HLA class I heavy chain-specific mAb HC-10 and HLA class II β chain-specific mAb LGII-612.14 in immunoperoxidase reaction.

Fig 2.

Association of HLA antigen expression with degree of cytologic atypia and architectural disorder in atypical melanocytic lesions. Percentage of lesions with positive (■) or heterogeneous (▒) score as determined by immunohistochemical marking with HLA class I heavy chain-specific monoclonal antibody (mAb) HC-10 and HLA class II β chain-specific mAb LGII-612.14 was significantly higher in lesions demonstrating severe and moderate cytologic atypia and architectural disorder than in those demonstrating mild cytologic atypia and architectural disorder.

Fig 3.

Differential HLA antigen expression in common melanocytic nevi and in surrounding normal cells. Only normal keratinocytes, endothelial cells, and antigen-presenting cells were marked by HLA class I heavy chain-specific monoclonal antibody (mAb) HC-10 and by HLA class II β chain-specific mAb LGII-612.14 in immunoperoxidase reaction in intradermal nevus (**A**) and in halo nevus (**B**). Percentage of tumor cells that were marked by tested mAb in each lesion was enumerated independently by two investigators as described in "Methods" section. (**A** and **B**, Hematoxylin-eosin stain; original magnifications: **A1**, $\times 10$; **B1**, $\times 4$; **B2**, $\times 20$.) (**A** and **B**, mAb HC-10; original magnifications: **A2** and **B3**, $\times 10$; **A3** and **B4**, $\times 20$.) (**A** and **B**, mAb LGII-612.14; original magnifications: **A4** and **B5**, $\times 10$; **A5** and **B6**, $\times 20$.)

Fig 4.

Differential HLA antigen expression in superficial spreading melanoma arising within intradermal nevus. Normal keratinocytes, endothelial cells, and malignant melanocytes but not intradermal nested melanocytes or vertical growth phase melanoma cells were marked by HLA class I heavy chain–specific monoclonal antibody (mAb) HC-10 and by HLA class II β chain–specific LGII-612.14 in immunoperoxidase reaction in superficial spreading melanoma arising within intradermal nevus (1, 2, 5, and 6). 3, 4, 7 to 9, Alternate field of view. Percentage of tumor cells that were marked by tested mAb in each lesion was enumerated independently by two investigators as described in “Methods.” (1 to 4, Hematoxylin-eosin stain; original magnifications: 1 and 4, $\times 4$; 2 and 3, $\times 10$; 3 inset, $\times 20$.) (5, 7, and 8, mAb HC-10; original magnifications: 5, $\times 10$; 7, $\times 20$; 8, $\times 10$.) (6 and 9, mAb LGII-612.14; original magnification: 6, $\times 20$; and 9, $\times 10$.)

Fig 5.

Acquisition of HLA antigen expression in atypical melanocytic nevi. Normal keratinocytes, endothelial cells, antigen-presenting cells, and melanocytes were marked by monoclonal antibody (mAb) HC-10 and LGII-612.14 in immunoperoxidase reaction in mildly (**A**) and moderately (**B**) atypical nevus. Percentage of tumor cells that were marked by tested mAb in each lesion was enumerated independently by two investigators as described in “Methods” section. (**A** and **B**, Hematoxylin-eosin stain; original magnifications: **A1** and **B1**, $\times 10$; **A2** and **B2**, $\times 20$.) (**A** and **B**, mAb HC-10; original magnifications: **A3** and **B3**, $\times 10$; **A4** and **B4**, $\times 20$.) (**A** and **B**, mAb LGII-612.14; original magnifications: **A5** and **B5**, $\times 10$; **A6** and **B6**, $\times 20$.)

Fig 6.

HLA class I heavy chain and HLA class II β chain expression in severely atypical melanocytic nevi. Normal keratinocytes, endothelial cells, antigen-presenting cells, and melanocytes were marked by monoclonal antibody (mAb) HC-10 and LGII-612.14 in immunoperoxidase reaction in severely atypical nevus. (1 to 3, Hematoxylin-eosin stain; original magnifications: 1, $\times 4$; 2, $\times 10$; 3, $\times 20$.) (4 and 5, mAb HC-10; original magnifications: 4, $\times 10$; 5, $\times 20$.) (6, mAb LGII-612.14; original magnification: $\times 20$.)

Table 1.

Characteristics and reactivity pattern of benign melanocytic lesions

Lesion	Sex	Age	Diagnosis	Site	Reactivity pattern
1	F	36	Blue nevus	L ^c Shoulder	N ^e
2	F	58	Blue nevus	R ^d Back	N
3	F	66	Blue nevus	Mid Back	N
4 ^I	M	12	Inflamed CN ^a	L back	N
5 ^I	M	12	Inflamed CN	R back	N
6	M	14	CN	Mid back	N
7 ^{II}	M	23	CN	R arm	N
8	F	24	CN	R breast	N
9	F	26	CN	R abdomen	N
10 ^{III}	F	28	CN	L Flank	N
11 ^{IV}	F	29	CN	L Chest	N
12 ^{IV}	F	29	CN	L Back	N
13 ^V	F	30	CN	R Breast	N
14 ^V	F	30	CN	R Shoulder	N
15	F	32	CN	Nasal Bridge	N
16	F	36	CN	R Chest	N
17 ^{VI}	F	40	CN	L Flank	N
18	F	60	CN	R Abdomen	N
19			CN		N
20			CN		N
21 ^{VII}			CN		N
22 ^{VII}			CN		N
23	F	25	CLN ^b	R Back	N
24 ^{VIII}	M	38	CLN	R Back	N

Lesion	Sex	Age	Diagnosis	Site	Reactivity pattern
25 ^{VIII}	M	38	CLN	L Back	N
26 ^{VIII}	F	39	CLN	R Back	N
27 ^X	F	39	CLN	Arm	N
28 ^{VI}	F	40	CLN	L Forearm	N
29 ^{XI}	M	45	CLN	L Back	N
30	M	9	Halo nevus	Mid Back	p ^m
31	M	10	Halo nevus	R Back	P
32	F	11	Halo nevus	Mid Back	P
33	M	25	Halo nevus	L Back	P
34	M	64	J ^f Halo nevus	L Back	P
35	F	21	IDN ^g	L Sternum	N
36	M	27	IDN	L PA ^k Scalp	N
37 ^{XII}	F	29	IDN	L Back	N
38 ^{XIII}	F	30	IDN	L Frontal Scalp	N
39 ^{XIII}	F	30	IDN	R Frontal Scalp	N
40 ^{XIII}	F	30	IDN	R Forehead	N
41	F	36	IDN	Posterior Neck	N
42	M	37	IDN	Upper Back	N
43	F	40	IDN	L Neck	N
44	M	42	IDN pig ^h	R Back	N
45	F	46	IDN	R Waist	N
46	F	46	IDN	R NLF ^j	N
47	F	51	IDN	R Back	N
48	M	60	IDN	L Posterior Neck	N
49	F	61	IDN	R Arm	N
50	F	66	IDN	Mid Back	N
51	M	45	SSM ⁱ +IDN	R scapula	N/p ⁿ

Lesion	Sex	Age	Diagnosis	Site	Reactivity pattern
52. <i>XIII</i>	M	36	JLN ⁺	R Posterior Neck	N
53. <i>VIII</i>	M	38	JLN	L Thigh	N
54. <i>VI</i>	F	40	JLN	L Back	N
55	M	41	JLN	Mid Back	N
56	M	65	JLN	R Back	N
57	F	76	JLN	L Elbow	N
58	M	76	JLN	L Abdomen	N
59	F	78	JLN	L Scapula	N

^aCN, Compound Nevus;^bCLN, Compound Lentiginous Nevus; $c_{L, \text{Left}}$ $d_{\text{R, Right;}}$ e_N , Negative; $f_{J, \text{ Junction;}}$

σ IDN, Intradermal nevus;

 $h_{\text{Pig, Pigmented}}$ i_j SSM+IDN, superficial melanoma arising in a IDN;^jJLN, Junctional Lentiginous Nevus; $k_{\text{PA, Posterior Auricular;}}$ $I_{\text{NLF, Nasolabial Fold;}}$ m_P , Positive;

n /N/P, nested melanocytes within the IDN were marked as negative while the malignant melanocytes were marked as positive.

Table 2.

Characteristics and reactivity pattern of atypical melanocytic lesions

Lesion	Sex	Age	Diagnos	Degree of Atypia	Site	Reactivity pattern
1	M	8	CN ^a	Mild	Back	N ^g
2	F	20	CN	Mild	R ^e Thigh	N
3	F	23	CN	Mild	Upper Back	N
4. ^{III}	F	28	CN	Mild	R Buttock	N
5. ^{XV}	F	28	CN	Mild	R Back	N
6. ^{XV}	F	28	CN	Mild	L ^f Scapula	N
7. ^{XII}	F	29	CN	Mild	R Abdomen	N
8. ^V	F	30	CN	Mild	Back	N
9	M	34	CN	Mild	R Chest	N
10. ^{XIII}	M	36	CN	Mild	L Calf	N
11. ^X	F	39	CN	Mild	R Chest	N
12. ^X	F	39	CN	Mild	R Abdomen	N
13	F	46	CN	Mild	L Chest	N
14	M	52	CN	Mild	Back	N
15. ^{XVI}	M	53	CN	Mild	Back	N
16	F	58	CLN ^b	Mild	R Abdomen	H ^h
17	F	34	JN ^c	Mild	R Back	H
18. ^{VIII}	F	39	JN	Mild	L Lower Leg	H
19. ^{XVII}	M	55	JN	Mild	L Buttock	N
20. ^{XVII}	M	55	JN	Mild	R Back	H
21	M	79	JN	Mild	L Chest	N
22	M	34	JLN ^d	Mild	R Back	H
23	M	41	JLN	Mild	L Thigh	N

Lesion	Sex	Age	Diagnos	Degree of Atypia	Site	Reactivity pattern
24	F	20	CN	Moderate	L Helix	P ⁷
25	F	23	CN	Moderate	Back	H
26	M	26	CN	Moderate	Scapula	H
27. ^{XV}	F	28	CN	Moderate	R Clavicle	P
28	F	30	CN	Moderate	Upper Back	H
29	F	35	CN	Moderate	R Thigh	H
30	M	36	CN	Moderate	R Thigh	H
31	F	39	CN	Moderate	L Back	H
32. ^X	F	39	CN	Moderate	L Back	H
33	M	41	CN	Moderate	R Buttock	H
34	F	41	CN	Moderate	L Inguinal	H
35. ^{XI}	M	45	CN	Moderate	L Chest	H
36	M	48	CN	Moderate	L Scalp	H
37	F	49	CN	Moderate	R Back	P
38	F	53	CN	Moderate	R Scapula	H
39	M	57	CN	Moderate	L Back	P
40	F	59	CN	Moderate	R Shoulder	H
41	M	69	CN	Moderate	Back	H
42	M	89	CN	Moderate	Abdomen	H
43	F	39	JN	Moderate	R Back	P
44	F	66	JN	Moderate	R Calf	P
45	F	17	CN	Severe	L Chest	P
46. ^{II}	M	23	CN	Severe	R Back	P
47. ^{II}	M	23	CN	Severe	R Back	P
48	F	49	CN	Severe	Sternum	P
49	F	43	CN	Severe	Thigh	P
50. ^{XVI}	M	53	CN	Severe	L Shoulder	P
51	F	52	CN	Severe	L Arm	P
52	F	57	CN	Severe	Vertex Scalp	P

Lesion	Sex	Age	Diagnos	Degree of Atypia	Site	Reactivity pattern
53	M	58	CN	Severe	L Back	P
54	M	63	CN	Severe	Back	P
55	M	85	CN	Severe	L Back	P
56	F	62	CLN	Severe	L Thigh	P
57	F	37	JN	Severe	Nack	P
58	M	42	JN	Severe	R Forearm	P
59	F	46	JN	Severe	L Ear	P
60	F	46	JN	Severe	Abdomen	P
61	F	48	JN	Severe	R Cheek	P
62	M	61	JN	Severe	Back	P
63	F	65	JN	Severe	R Orbit	P
64	M	69	JN	Severe	L Cheek	P

II,III,V,III,X,XI,XII,XIII,XV,XVI,XVII: Indicated lesions were obtained from same patient.

^aCN, Compound Nevus;

^bCLN, Compound Lentiginous Nevus;

^cJN,Junctional Nevus;

^dJLN, Junctionl Lentiginous Nevus;

^eR, Right;

^fL, Left;

^gN, Negative;

^hH, Heterogenous;

ⁱP, Positive.

Table 3.

Reactivity of benign and atypical melanocytic lesions with HLA antigen specific-

Specificity mAb	β_2m free HLA class I heavy chain HC-10	β_2m -free HLA class I heavy chains HC-A2	β_2m NAMB-1	HLA-DR, -DQ, -DP β chains LGII-612.14
<u>Benign nevi</u>				
Positive ^{a,b}	5 ^c	5 ^c	5 ^c	5 ^c
Heterogeneous	0	0	0	0
Negative	54	54	54	54
<u>Atypical nevi</u>				
Positive	26	26	26	26
Heterogeneous	21	21	21	21
Negative	17	17	17	17

^aReactivity pattern;^bAll positive lesions were diagnosed as halo nevi;^cNumber of lesions.