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Intralymphatic nevus cells in benign nevi☆

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ABSTRACT

The histogenesis of nevus cell aggregates in lymph nodes lesion is controversial, and various hypotheses have been used to explain their origin. One of them is the transport of cells from cutaneous nevi or lesions to lymph nodes, called mechanical transport theory. We investigated in our cases of benign nevi to obtain evidence to substantiate this theory. A total of 369 benign cutaneous nevi were prospectively evaluated in excisional biopsy samples. Immunohistochemical stainings for CD31 and podoplanin (D2-40) were performed in the cases with intralymphatic nevus cell aggregate (ILNA), suspected for ILNA, and/or intralymphatic nevus cell protrusion. A total of 13 ILNAs were found in 10 patients. Six ILNA were verified with their histology as well as immunohistochemically with D2-40 and CD31. Protrusions of nevus cells inside the lymphatics (intralymphatic nevus cell protrusion) were seen in all cases of ILNA and also in 27 nevi where an ILNA was not observed. In most nevi, the perilymphatic orientation of nevus cells and their affinity to the lymphatics were observed. We suggested that ILNAs can be dislodged with local minor trauma and be carried inside the lymphatic vessel to the draining lymph node. Besides, whether ILNA or not, nevus cells could also move toward lymphatic spaces with mechanical effects due to their affinity to lymphatics and their localizations that are very close to the endothelium. Our findings might support the mechanical transport theory.

1. Introduction

Since the first description in 1931 by Stewart and Copeland [1], there have been a number of cases of benign nevus cells within lymph nodes reported in the literature [2,3]. At first, this entity was considered to be infrequent, but subsequent studies have indicated that nevus cell aggregates in lymph nodes (NALNs) can no longer be considered a rare phenomenon [4]. Although an NALN rate of 0.12% to 0.54% was observed in full lymph node dissections in melanoma patients [5,6], this rate can increase up to 3.9% to 13% in sentinel lymph nodes [3,7]. Besides, NALNs were found to accompany skin adnexal carcinoma and squamous cell carcinoma of the tonsil and were reported even in individuals without any malignancy [4]. Nevertheless, the origin of these cells remains unclear and has been the subject of academic interest for a long time.

Various hypotheses have been used to explain their origin. Lymphatic nevus cell aggregates (ILNAs) in benign nevi indicate that nevus cells are probably transferred from a cutaneous nevus to the draining lymph node via the lymphatics [4]. However, there are only 8 reported cutaneous nevus cases with intralymphatic nevus cells as far as we are aware. We evaluated our benign nevi cases to look for evidence supporting this possibility.

2. Materials and methods

2.1. Case selection

Benign cutaneous compound and intradermal nevi were prospectively evaluated in excisional biopsy samples by 2 separate pathologists during the routine reporting procedure at our pathology laboratory between February and September 2015. Junctional nevi were excluded as they do not contain a dermal component. Four to 16 sections on 1 or 2 hematoxylin and eosin (H&E)–stained slides were screened with the objective ×20 for each nevus. The histologic criteria specified below were created based on lymphovascular invasion descriptions previously used in the literature for the detection of malignant tumor emboli within lymphatics [8-10]. According to these criteria, immunohistochemical stainings for CD31 and podoplanin (D2-40) were performed in the following conditions: (i) cases with ILNA, (ii) cases with intralymphatic nevus cell protrusion (ILNP), and (iii) if differentiation was not possible morphologically between retraction artifact and ILNA. Immunohistochemical analyses were performed on 5-μm-thick formalin-fixed, paraffin-embedded sections. We used clone JC70 (mouse monoclonal, 1:50 dilution; Cell Marque, Rocklin, CA) for CD31 and clone D2-40 (mouse monoclonal, 1:50 dilution; Cell Marque) for podoplanin.

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The VENTANA NexES Automated immunostainer (Ventana Medical Systems, Inc, Tucson, AZ) was used for immunohistochemical staining.

Thickness and maximum diameter of the nevus, nevus depth, the dermal level where the ILNA was found, and the region of the nevus where the ILNA was located were recorded in the cases with ILNA.

Demographic data were obtained from the referral letter and patient files and if necessary by obtaining information from the patients by a telephone call.

2.2. Histologic criteria for ILNA

Cell groups found inside a space were accepted as ILNAs if they met all the following conditions: (i) There should be an endothelial cell layer lining the space containing the aggregate and the periphery of the aggregate. (ii) The cell aggregate inside the space should be morphologically similar to the surrounding nevus cells. (iii) There should be no relationship of the intraluminal nevus cell aggregate with a vessel wall or it should be focally attached to the vessel wall. (iv) All or almost all the aggregate cells should be observed intraluminally. (v) There should be no smooth muscle or elastic support at the wall of the vessel containing the nevus aggregate.

Spaces not lined with endothelium around the nevus cell aggregate or spaces lined with cells with similar morphology to the nevus cell were thought to be secondary to retraction artifact or discohesion, respectively. Nevus cell aggregates where all or almost all of the aggregate was not in the lumen were accepted as ILNP.

2.3. Immunohistochemical criteria for ILNA

The lesions that met all of the following conditions were immunohistochemically accepted as intralymphatic nevus cells: (i) meeting all the above histologic criteria, (ii) all cells surrounding the space being stained with CD31 and/or podoplanin, and (iii) the periphery of the intravascular cell aggregate being partially or completely stained with CD31 and/or podoplanin.

3. Results

A total of 369 nevi excised from 206 patients consisting of 61 males and 145 females were evaluated. Although 278 were intradermal, 76 were common acquired nevi of the compound type, and only 15 were congenital nevi (5 intradermal, others compound). The location was the head and neck in 295, the trunk in 54, the upper extremity in 9, and the lower extremity in 11. The age range was 6 to 73 years (median, 36).

Intralymphatic nevus cell aggregate was seen in 9 and ILNP in 29 nevi investigated histopathologically using the criteria explained in the material and method section. We were unable to definitely differentiate 21 cases histologically from a retraction artifact and could not decide whether the lesion was ILNA. Thus, CD31 and podoplanin (D2-40) staining procedures were performed immunohistochemically in a total of 59 cases (9 cases with ILNA +29 cases with ILNP +21 cases histopathologically suspicious for ILNA).

When histologic and immunohistochemical findings were evaluated together, a total of 13 ILNA instances were found in 10 cases (Table 1; Fig. 1). Six aggregates were verified as ILNA with their histology as well as with both immunohistochemical markers. Because tissue loss due to shaving was present in new sections prepared from paraffin blocks for immunohistochemistry, some ILNAs detected in H&E sections decreased in size in immunohistochemical sections (aggregates 4, 5, and 7). In 1 ILNA, the area attached to the vessel wall became apparent (aggregate 3). Because 4 ILNAs disappeared in subsequent tissue sections, they could not be verified immunohistochemically (aggregates 2, 8, 9, and 10). In 1 case, ILNAs were not seen in the relevant areas on H&E sections, but 3 ILNAs were found on immunohistochemical sections (aggregates 11, 12, and 13).

We found that 11 of the 13 ILNAs were inside superficial dermal lymphatics. One was in the mid-dermis and the other in the deep dermis. Although 10 ILNAs were observed in the peripheral/lateral parts of the nevus, 3 were in the central parts of the nevus.

Clinicopathologic findings of the cases are summarized in Table 2. Three cases were on the trunk, whereas the remaining cases were located on the head and neck. Three of the cases were congenital nevi, and the patients were younger than 21 years. Three cases were compound nevi, and 7 cases were intradermal nevi. The biggest diameter of the nevi varied between 3 and 16 mm. Although the nevi were limited to the superficial reticular dermis in 2 cases, nevi cells were seen deeper in the others. Subcutaneous fat tissue involvement was seen only in 1 congenital nevus. Two cases presented as a polypoid mass in the clinic. Four cases were accompanied by lymphangiectasia. Multiple ILNAs were found in 2 cases. One of them was a congenital nevus.

Whether ILNA or not, a close relationship between nevus cells and lymphatics was noticed in most of the nevi investigated in this study. Protrusions of nevus cells inside the lymphatics (ie, ILNP) were seen in all cases of ILNA (10/10; 100%) and also in 27 nevi where an ILNA was not observed (27/369; 7.3%).

Some aggregates inside lymphatics were thought to be protrusions, as they were located on the vessel wall with a wide base, despite being suspicious in terms of an ILNA.

4. Discussion

Lymphatic invasion by nevus cells is not uncommon finding in Spitz nevi. In 1 study, it was detected in 7 of 49 childhood Spitz nevi [11]. However, lymphatic invasion from a banal nevus is rare. The characteristics of 8 cases reported in the literature so far [12-15] are shown in Table 3. Our study contributes to the literature with 10 new cases.

Before deciding on an ILNA in the present study, we had to rule out the possibility that pseudovascular spaces were misidentified as a lymphatic vessel. Irregular clefts or slits resembling lymphatic or

Table 1
The morphological and immunohistochemical features of ILNAs found in this study

Cases	No. of ILNA	Determined method of ILNA	Dermal level of ILNA	Location of ILNA within the nevus
Case 1	1	H&E, CD31, D2-40	Superficial dermis	Peripheral/lateral part of the nevus
	2	H&E	Deep reticular dermis	Central part of the nevus
Case 2	3	H&E, CD31, D2-40	Superficial dermis	Peripheral/lateral part of the nevus
Case 3	4	H&E, CD31, D2-40	Superficial dermis	Peripheral/lateral part of the nevus
Case 4	5	H&E, CD31, D2-40	Superficial dermis	Peripheral/lateral part of the nevus
Case 5	6	H&E, CD31, D2-40	Superficial dermis	Peripheral/lateral part of the nevus
Case 6	7	H&E, CD31, D2-40	Mid-dermis	Central part of the nevus
Case 7	8	H&E	Superficial dermis	Peripheral/lateral part of the nevus
Case 8	9	H&E	Superficial dermis	Peripheral/lateral part of the nevus
Case 9	10	H&E	Superficial dermis	Peripheral/lateral part of the nevus
Case 10	11	D2-40	Superficial dermis	Central part of the nevus
	12	D2-40	Superficial dermis	Peripheral/lateral part of the nevus
	13	CD31	Superficial dermis	Peripheral/lateral part of the nevus