Conjunctival Melanocytic Nevi With Granular Cell Change

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- Context.—Granular cell change in melanocytic nevus is underrepresented in the literature with only 4 well-documented cases, 1 described in the conjunctiva. Unfamiliarity with the clinical and pathologic features of these lesions contributes to the diagnostic difficulty.

Objective.—To delineate the clinical and histopathologic features of conjunctival nevi with granular cell change.

Design.—In a retrospective observational case series, the medical records of all patients with conjunctival nevi and granular cell change diagnosed between December 2016 and October 2018 were reviewed. Data collected included age, sex, clinical presentation, pathologic findings, and follow-up.

Results.—Twelve patients, 6 males and 6 females, with a median age of 14 years (range, 8–82 years) were identified. The nevus manifested as a pigmented, well-circumscribed nodule (7 of 9; 78%) or patch (2 of 9; 22%) in the bulbar conjunctiva (7 of 9; 78%) or limbal conjunctiva (7 of 9; 78%) or in the plica semilunaris/caruncle (2 of 9; 22%). Cysts were noted in 7 of 9 lesions (78%). Features prompting surgical excision included atypical pigmentation (8 of 9; 89%), growth (7 of 9; 78%), and atypical vascularity (4 of 9; 44%). Microscopically, all lesions comprised a conventional melanocytic nevus with focal granular cell change and immunoreactivity for Melan-A, SOX10, and HMB-45, with Ki-67 proliferative index of less than 2%. Of the 9 lesions with follow-up information, there were no recurrences over mean follow-up of 11.2 months (range, 1–23 months).

Conclusions.—Granular cell change in melanocytic nevi is an underrecognized finding that can simulate melanoma clinically and histopathologically. Young age at diagnosis, lack of associated conjunctival melanosis, bulbar location, cysts, and the absence of mitotic figures with a low Ki-67 proliferative index are helpful clinical and pathologic diagnostic clues.

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Granular cell nevus is a rare benign variant of melanocytic nevus, which can be mistaken for malignant melanoma because of the morphologic and immunohistochemical overlap between the 2 lesions.1–3 To our knowledge, only 4 granular cell nevi have been described in the literature,1–3 of which a single lesion was localized to the conjunctiva.2 Herein, we describe our experience with conjunctival melanocytic nevi with granular cell change to delineate the clinical presentation, morphologic and immunohistochemical features, and biologic behavior of these tumors.

MATERIALS AND METHODS

In this retrospective single-center institutional study, the medical records of all patients diagnosed with conjunctival granular cell nevus at the pathology department between December 2016 and October 2018 were reviewed. The study followed the tenets of the Declaration of Helsinki and was approved by the institutional review board.

Clinical Information

Clinical data collected included patient age, sex, predisposing factors, duration of symptoms, clinical characteristics of the lesion (location, color, shape, size, cysts, feeder vessels, change over time), clinical rationale for resection, rationale for pathology consultation, and when available, postoperative clinical course.

Pathologic Evaluation

Routine sections stained with hematoxylin-eosin were prepared from paraffin-embedded, formalin-fixed tissues. On tissues with sufficient material for immunohistochemical evaluation, the immunostaining was performed with the following primary antibodies: mouse anti-human Melan-A (diluted 1:50; Dako, Carpinteria, California), mouse anti-human SOX10 (prediluted; Biocare Medical, Concord, California), mouse anti-human HMB-45 (diluted 1:40; Thermo Fisher Scientific, Waltham, Massachusetts), and mouse anti-human Ki-67 (prediluted; Dako). Selected lesions were stained with CD163 (diluted 1:50; Leica Biosystems, Lincolnshire, Illinois). Pretreatment was performed according to the Ventana Medical Systems (Oro Valley, Arizona) protocol preprogrammed for antigen retrieval. The immunohistochemical stains were prepared on Ventana Benchmark XT with Enhanced Alkaline Phosphatase Fast Red Detection Kit in accordance with the...
manufacturer’s instructions. The control immunohistochemical stains were performed on the peripheral nerves and sweat glands (SOX10), melanoma (Melan-A and HMB-45), and lymph node tissue (Ki-67, CD163). For negative controls, the primary antibodies were replaced by an antibody diluent (Ventana).

The pathology material was reviewed by 3 observers and a unified interpretation of histopathology and immunohistochemistry was rendered. Morphologic parameters assessed were the nevus type, presence of cysts, the extent, location, and pattern of granular cell change, cytomorphology, presence of lymphocytes and macrophages, mitotic activity, and adequacy of surgical excision. Immunohistochemical stains were interpreted semiquantitatively for staining intensity (0, none; 1+, mild; 2+, moderate; and 3+, strong) and for the percentage of immunoreactive cells (0, none; 1+, mild, 1–25% of cells; 2+, immunoreactivity in 26–50% of cells; 3+, immunoreactivity in 51–75% of cells; and 4+, immunoreactivity in 76–100% of cells).

Literature Search

PubMed (National Center for Biotechnology Information, Bethesda, Maryland) and Medline (Ovid, New York, New York) databases were searched for all documented cases of granular cell nevi with the following key words: granular cell nevus, granular cell naevus, granular cell nevi, granular cell naevi, granular AND nevus/nevox/nevi/navei, and granular cell change AND nevus/nevox/nevi/navei eye.

RESULTS

Review of pathology medical records between December 2016 and October 2018 revealed 210 conjunctival melanocytic nevi. Twelve melanocytic nevi with granular cell change (5.7%) were diagnosed in 6 males and 6 females with a median age of 14 years (mean, 25 years; range, 8–82 years). Two patients (17%) had a family history of cutaneous melanoma. No other systemic relationships were identified. Seven lesions (78%) were excised from patients referred to the institutional oncology service. Five lesions (56%) with accompanying paraffin-embedded tissue blocks were received in consultation from referral pathology centers because of concern for melanoma.

Clinical Features

The patient demographics and clinical features of the lesions are summarized in Table 1. The characteristic clinical appearance of the lesions is documented in Figures 1, A through F; 2A; and 3A. Detailed information on clinical appearance of the lesions was available for 9 of 12 patients (75%). The nevus manifested as a partially or homogenously dark-colored, well-circumscribed flat patch (2 of 9; 22%) or elevated nodule (7 of 9; 78%) in the limbal (2 of 9; 22%) and bulbar (5 of 9; 56%) conjunctiva or in the plica semilunaris/caruncle (2 of 9; 22%). Cysts were noted in 7 of 9 lesions (78%), whereas feeder vessels were observed in 8 of 9 lesions (89%). There was no clinical evidence of primary acquired melanosis associated with the lesions. Features prompting surgical excision included atypical dark coloration (8 of 9; 89%) and stippled pigmentation (5 of 9; 56%); growth over the course of 1 month to 12 years (7 of 9; 78%); atypical location (5 of 9; 56%); including involvement of plica semilunaris/caruncle (2 of 9; 22%); juxta-fornical conjunctiva (2 of 9; 22%); and peripheral cornea (1 of 9; 11%); atypical vascularity (4 of 9; 44%); and large size (2 of 9; 22%). Follow-up information was available on 9 patients (75%). There was no evidence of recurrence or malignant behavior in any lesions with an average follow-up of 11.2 months (range, 1–23 months).

Immunohistochemistry

Immunohistochemical features of the lesions are summarized in Table 2 and illustrated in Figures 2, D through F; and 3, G and H. All nevus cells demonstrated moderate to strong and diffuse staining for Melan-A and SOX10 confirming their melanocytic nature (Figure 2). SOX10 highlighted the nuclear size variation and pleomorphism in the granular cell nevus component (Figure 2). HMB-45 and Ki-67 stains were performed on 11 of 12 lesions (92%) with sufficient tissue. Immunoreactivity for HMB-45 was mostly sufficient tissue. Immunoreactivity for HMB-45 was mostly

Morphologic Features

Morphologic features of the lesions are summarized in Table 2 and illustrated in Figures 2, B and C; and 3, C through F. Microscopic examination demonstrated compound melanocytic nevi in 10 (83%) of the cases, characterized by the presence of both junctional and subepithelial nevus nests. Nevus cells were entirely confined to the substantia propria in 2 of those 10 cases (17%), compatible with subepidermal melanocytic nevus. Intrastromal epithelial cystic rests were seen in 10 of 12 lesions (83%). Maturation toward the base of the nevus was noted either focally or diffusely in 5 of 10 well-oriented specimens (50%; Figure 2). In 8 of 9 lesions (89%) a rounded, “pushing” margin into the underlying stroma was noted (Figure 3). Extension of junctional nevus nests peripheral to the subepithelial component (“shoulder phenomenon”) was observed in 6 of 11 lesions (55%). None of the lesions demonstrated intraepithelial melanocyte migration or pagetoid intraepithelial melanocyte scatter.

Granular cell change was present in 3% to 85% of the nevus cells, manifesting either as a single focus (6 of 12; 50%) or multifocally (6 of 12; 50%), in subepithelial (9 of 12; 75%) or both subepithelial and junctional (3 of 12; 25%) components (Figures 2 and 3). Cytomorphologically, granular cell change was characterized by cells with centrally placed nuclei within an abundant eosinophilic-to-lightly pigmented granular cytoplasm (Figures 2 and 3). Although nucleoli were generally not conspicuous, granular cell nevoid melanocytes demonstrated more-prominent nuclear pleomorphism when compared with the adjacent nevocellular nevus cells, with nuclear size ranging from smaller to 3 times larger (Figures 2 and 3). No mitotic figures were identified. A melanophagic infiltrate was associated with granular cell change in all lesions and ranged from mild (3 of 12; 25%) to severe (6 of 12; 50%) (Figure 2). A lymphocytic infiltrate, either in aggregates or diffuse, was observed in 7 of 12 (58%) of the lesions and was moderate to severe in more than one-half of the inflamed nevi. In well-oriented specimens, surgical margins were free in 6 of 8 cases (75%). The nevus cells extended to the peripheral and/or deep surgical margins in 2 of 8 lesions (25%).

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| Case No. | Age, y/ Sex | Predisposing Factors | Location, Clock h | Location, Area | Size, mm | Shape | Pigment | Cysts | Feeder Vessels | Symptom Duration, mo | Change | Atypical Features | Recurrence/ Follow-up, mo |
|----------|-------------|----------------------|-------------------|----------------|----------|-------|---------|-------|---------------|-------------------------|--------|------------------|--------------------------|
| 1        | 13/M        | N/A                  | N/A               | N/A            | N/A      | 1, 2  | 1       | No    | N/A           | N/A                     | 6      | 1                | N/A                      |
| 2        | 16/M        | Family skin melanoma | 1000              | Bulbar         | 2 × 2 × 1 | 1     | 2       | No    | N/A           | N/A                     | 6      | 1                | N/A                      |
| 3        | 8/M         | N/A                  | N/A               | N/A            | N/A      | 1, 2  | 1       | Yes   | No            | N/A                     | 6      | 1                | N/A                      |
| 4        | 9/M         | No                   | Caruncle          | Caruncle       | 3 × 3 × 1 | 1, 3  | 1, 2    | Yes   | No            | N/A                     | 7      | 1                | 1, 2, 3                  |
| 5        | 29/F        | No                   | 1500              | Bulbar juxta- fornical | 3 × 3 | 1, 3 | 3       | No    | Yes           | 6                       | 1      | 1                | 1, 2, 3, 4               |
| 6        | 14/F        | No                   | 0800–1000         | Limbal, trace corneal | 9 × 6 × 3 | 1, 3 | 1, 2    | Yes   | Yes           | 144                     | 1      | 1                | 1, 2, 3, 5               |
| 7        | 13/F        | N/A                  | N/A               | N/A            | N/A      | 1, 3  | 1       | No    | Yes           | 5                       | 2      | 2                | No/1                     |
| 8        | 11/M        | No                   | 1400–1600         | Limbal         | 8 × 9   | 1, 3  | 2       | Yes   | Yes           | 132                     | 1      | 1                | 1, 2, 4, 5               |
| 9        | 82/F        | No                   | Plica             | Plica          | 3 × 3 × 2 | 1, 3 | 1, 2    | Yes   | Yes           | 1                       | 1      | 1                | 1, 2, 3, 4               |
| 10       | 70/M        | No                   | 1300              | Bulbar juxta- fornical | 5 × 2,5 | 1, 3 | 2       | Yes   | Yes           | N/A                     | 2      | 3                | No/19                    |
| 11       | 14/F        | Family skin melanoma | 0800              | Bulbar         | 1 × 1 × 1 | 1, 2 | 1, 2    | Yes   | Yes           | 5                       | 2      | 2                | No/1                     |
| 12       | 25/F        | No                   | 0900              | Bulbar         | 2 × 1   | 1, 3  | 2       | Yes   | Yes           | >70                     | 1, 2   | 1, 2              | No/2                     |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |
|          |             |                      |                   |                |          |       |         |       |               |                         |        | 1                | 2                       |

Abbreviation: N/A, not available.

* 1, well circumscribed; 2, flat; 3, elevated.
* 1, stippled; 2, variable; 3, diffuse.
* 0, none; 1, size; 2, color.
* 1, growth; 2, color; 3, location; 4, vascularity; 5, size.
* Since adolescence.
Figure 1. The spectrum of clinical features of conjunctival nevi with granular cell change. All lesions are well-circumscribed flat patches (A and B) or elevated nodules (C through F) with either focal (D and F) or diffuse (A through C and E), stippled (A), and feathery (B), intense pigmentation. Prominent vascularity is shown in some lesions (E and F). Cysts are apparent in some nevi (D and F, arrows).
Table 2. Histopathologic and Immunohistochemical Features of Nevi With Granular Cell Change (GCC)

| Case No. | Nevis Type | Cysts | Maturation | Shoulder Phenomenon | Pushing Margin | Lymphs | GCC Location | GCC Focality | GCC Size, | HMB-45 GCC, | Ki-67, | Completely Excised |
|----------|------------|-------|------------|---------------------|----------------|--------|--------------|-------------|----------|-------------|--------|-------------------|
| 1        | C          | Yes   | NA         | No                  | N/A            | 3+     | SE           | Unifocal    | N/A      | 3+/4+, 1+/5             | <1     | UN                |
| 2        | C          | Yes   | No         | Yes                 | Yes            | 2+     | 0            | SE          | Unifocal  | 50          | 1:1    | 2+/4+, 0               | <1     | Yes               |
| 3        | C          | Yes   | No         | Yes                 | Yes            | 2+     | 3+           | SE + J      | Multifocal| 50          | 1:1    | N/A               | <1     | No                |
| 4        | C          | Yes   | No         | Yes                 | No             | 2+     | 0            | SE          | Unifocal  | 20          | 1:1    | 3+/4+, 0               | <1     | Yes               |
| 5        | C          | Yes   | No         | No                  | Yes            | 3+     | 2+           | SE          | Multifocal| 60          | 2:3:1 | 3+/4+, 0               | <1     | Yes               |
| 6        | C          | No    | Focal      | Yes                 | Yes            | 3+     | 1+           | SE + J      | Multifocal| 85          | <1:1  | 2+/4+, 3+/5             | <2     | No                |
| 7        | C          | No    | Focal      | Yes                 | Yes            | 3+     | 1+           | SE          | Unifocal  | 3           | 1:1    | 2+/4+, 0               | <1     | Yes               |
| 8        | C          | Yes   | NA         | NA                  | NA             | 1+     | 3+           | SE + J      | Multifocal| 10          | <1:1  | 3+/4+, 1+/5             | UN     | UN                |
| 9        | SE         | No    | Focal      | No                  | Yes            | 3+     | 0            | SE          | Multifocal| 20          | 2:1    | 2+/4+, 0               | <1     | Yes               |
| 10       | SE         | Yes   | No         | No                  | Yes            | 1+     | 0            | SE          | Multifocal| 10          | 2:1    | 1+/4+, 0               | <1     | UN                |
| 11       | C          | Yes   | Yes        | Yes                 | No             | 3+     | 0            | SE          | Unifocal  | 10          | 1:1    | 1+/4+, 0               | <1     | UN                |
| 12       | C          | Yes   | Yes        | Yes                 | Yes            | 3+     | 1+           | SE          | Unifocal  | 40          | 1:2:1 | 3+/4+, 0               | <1     | Yes               |

Abbreviations: C, compound; J, junctional; lymphs, lymphocytes; MF, melanophages; N, nevocellular nevus; N/A, not available; NA-SN, not applicable-skin nevus; SE, subepithelial; UN, unable to assess.

- a Cellularity scoring: 0, no cells; 1+, mild; 2+, moderate; 3+, severe.
- b Granular cell nevus component in relationship to the entire lesion expressed as the percentage of cells.
- c Nuclear size of granular cell nevus cells compared with nevocellular nevus cells expressed as a ratio.
- d HMB-45 immunohistochemical staining is expressed as intensity: 0, no staining; 1+, mild staining; 2+, moderate staining; 3+, strong staining. The percentage of immunoreactive cells in granular cell change that stained expressed as follows: 0, no staining; 1+, 1%-25%; 2+, 26%-50%; 3+, 51%-75%; 4+, 76%-100%). The pattern of staining in nevocellular nevus was expressed as follows: 0, no staining; S, stratified staining.
- e Ki-67 expressed as a percentage of positively staining nuclei in the lesion.
Figure 2. Case 4. A, Darkly pigmented lesion with peripheral stippled pigmentation and a history of growth that involved the caruncle. B, The lesion is composed of intermixed islands of granular cell change (asterisk) with the characteristic nevocellular nevus cells (arrowhead) that demonstrate...
Ki-67 staining. The melanophagic infiltrate in all lesions lacked expression of Melan-A, SOX10, and HMB-45 and, conversely, expressed macrophage marker CD163.

**Literature Review**

A literature search identified 4 patients with well-documented granular cell melanocytic nevi, 3 in the skin and 1 in the conjunctiva. The average age of the reported patients was 19 years (range, 16–22 years). The granular cell change was noted in the stroma of all lesions in which the nevus cells expressed S100, Melan-A/MART-1, and HMB-45. Ultrastructural evaluation performed on one lesion demonstrated large cells with abundant cytoplasm containing occasional strands of rough endoplasmic reticulum and numerous randomly scattered electron-dense granules of varying sizes suggestive of lysosomes or autophagosomes. Clinical, morphologic, and immunohistochemical characteristics of the previously reported lesions are summarized in Tables 1 and 2.

**DISCUSSION**

Granular cell change in conjunctival melanocytic nevi can raise concern for melanoma both clinically and histopathologically. History of growth, change in color, atypical pigmentation, and prominent vascularity were recurring clinical features of the lesions described in this series. Although the age of our patients varied widely, the nevi were primarily seen in teenagers and young adults. Growth of nevi during puberty, presumably due to hormonally induced changes in pigmentation, inflammation, and enlargement of cysts is a well-recognized phenomenon that can be prominent in juvenile (inflamed) conjunctival nevi and balloon cell nevi, which share clinical and histopathologic characteristics with granular cell nevi. Documented growth since infancy was particularly striking in 2 of our patients. Clinical-pathologic correlation suggests that the granular cell change in nevocellular nevi with an accompanying cell volume expansion and intracytoplasmic pigmentation, melanophagic infiltrate, and lymphocytic infiltrate may all contribute to the apparent change in size of these tumors. Stippled, heterogeneous, feathery, and dense dark pigmentation in the lesions, also seen in primary acquired melanosis and melanoma, raised concern for a malignant process. This pigmentation likely corresponds to the melanophagic infiltrate, and lymphocytic infiltrate may all contribute to the apparent change in size of these tumors. Stippled, heterogeneous, feathery, and dense dark pigmentation in the lesions, also seen in primary acquired melanosis and melanoma, raised concern for a malignant process. This pigmentation likely corresponds to the melanophagic infiltrate, and lymphocytic infiltrate may all contribute to the apparent change in size of these tumors.

In the nevi with granular cell change, architectural features of mitotic figures, and a Ki-67 proliferative index of less than 2% as opposed to the Ki-67 index of greater than 10% in most conjunctival melanomas (Figure 3). Additionally, the “shoulder phenomenon” was limited to the nevi with granular cell change in younger patients, a feature also recognized in the benign juvenile conjunctival nevi and not associated with higher rates of recurrence or malignant transformation.

Various hypotheses have been raised regarding the origin of the granular cell change in nevi, stemming from morphologic and ultrastructural observations. The granular material was thought to represent accumulated activated melanosomes or lysosomes, pathologically processed melanosome-associated proteins, an integration product of lysosomal structures with denser true melanosomes, and mitochondria. The synthesis of lysosomal structures with denser true melanosomes, and mitochondria. The synthesis of lysosomal structures with denser true melanosomes, and mitochondria. The synthesis of lysosomal structures with denser true melanosomes, and mitochondria. The synthesis of lysosomal structures with denser true melanosomes, and mitochondria. The synthesis of lysosomal structures with denser true melanosomes, and mitochondria.
Figure 3. Side-by-side comparison of nevus with granular cell change in case 6 (A, C, E, and G) with melanoma (B, D, F, and H). Documented growth of the nevus since infancy (A, inset). A and B, Both lesions appear as intensely pigmented circumscribed nodules in the limbal-bulbar
negativity for lysosomal markers [lysozyme and CD68]) suggests that accumulation of degenerated or modified melanosomes is most likely responsible for the granular cell change in nevus cells.

Our data indicate that granular cell change is an under-recognized and frequently misinterpreted phenomenon that can raise an undue concern for melanoma. Although we do not have extensive follow-up information on all of our patients, none of the lesions recurred or behaved in a malignant fashion, including the incompletely excised tumors. Awareness of clinical and histopathologic features of granular cell change in the conjunctival melanocytic nevi is important in guiding appropriate management of these lesions.

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