Eosinophilic Intranuclear Inclusion Bodies in a Melanocytic Nevus

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We report a case of eosinophilic or hyaline true intranuclear inclusion bodies in a melanocytic nevus. Although intranuclear pseudoinclusions are frequently found in melanocytic nevi, true intranuclear inclusions are rare. The true intranuclear nature of the inclusions in our case was confirmed with ultrastructural examination. With reverse transcriptase in situ polymerase chain reaction (RT in situ PCR) analysis, eosinophilic bodies stained positive for molluscum-specific primers. This result suggests that such inclusions may be related to molluscum viral infection of melanocytes.

Intranuclear pseudoinclusions, cytoplasmic invaginations within nuclei, are common in melanocytic lesions. True intranuclear inclusion bodies, however, are rare in melanocytes.[1-3] We describe a case of a melanocytic nevus with true intranuclear inclusions that, with reverse transcriptase in situ polymerase chain reaction (RT in situ PCR) analysis, stained positive for molluscum-specific primers.

Case Report

A 28-year-old male prisoner presented with a pigmented verrucous scalp growth (1.0×0.6×0.5 cm) of unclear duration. The patient was not known to be positive for human immunodeficiency virus and had no other known medical conditions. On clinical examination, the growth appeared to be a wart. The lesion was excised and was submitted for histopathologic examination.

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Presented in part at the third joint meeting of the International Society of Dermatopathology, San Francisco, California, March 8–9, 2000.
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The specimen was fixed in 10% buffered formalin and was embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E). Special stain analyses, immunoperoxidase studies, and electron microscopy were performed. In situ hybridization for human papillomavirus (HPV) was done using a published protocol.[4-6] The sample was tested for HPV-1, -2, -3, -5, -6, -8, -11, -13, -16, -18, -26, -27, -30 to -33, -35, -39 to -45, -51, -52, -56, -57, -59, -68, and -70 and for other "novel" types.[4-6]

RT in situ PCR analysis for molluscum virus and Paramyxovirus was performed using an existing protocol.[4,5] Although the molluscum virus is a DNA virus, RT in situ PCR analysis was used because of the availability of primers for molluscum RNA transcripts. Optimal protease digestion time was determined using nonspecific incorporation of reporter nucleotide (10 μmol digoxigenin deoxyuridine 5-triphosphate [dUTP]) as a guide.[4,5] Optimal protease digestion was followed by overnight incubation in ribonuclease-free deoxyribonuclease (10 U/sample; Boehringer Mannheim, Indianapolis, Indiana) and one-step RT in situ PCR analysis using the r-Tth system and digoxigenin dUTP as previously described.[4,5] The primer sequence for the molluscum virus was sense CCGATCTTTGCGAGCGTTCTTAA and antisense TCCCATACAGCGAGGACAGCATA.[7] The Paramyxovirus sequence was also previously described.[5] After 20 cycles, the slides were washed at high stringency (60°C for 10 minutes in 15 mmol salt 2% bovine serum albumin). The digoxigenin-labeled target-specific complementary DNA was detected using the antidigoxigenin-alkaline phosphatase conjugate (1:200 in phosphate-buffered saline for 30 minutes at 37°C; Boehringer Mannheim) followed by chromogens nitroblue tetrazolium and bromochloroindolyl phosphate (Enzo Biochemicals, Farmingdale, New York). Nuclear fast red was used as the counterstain.

A wart with an associated compound nevus was seen in H&E-stained sections (Figure 1A). Many dermal melanocytes contained single or multiple

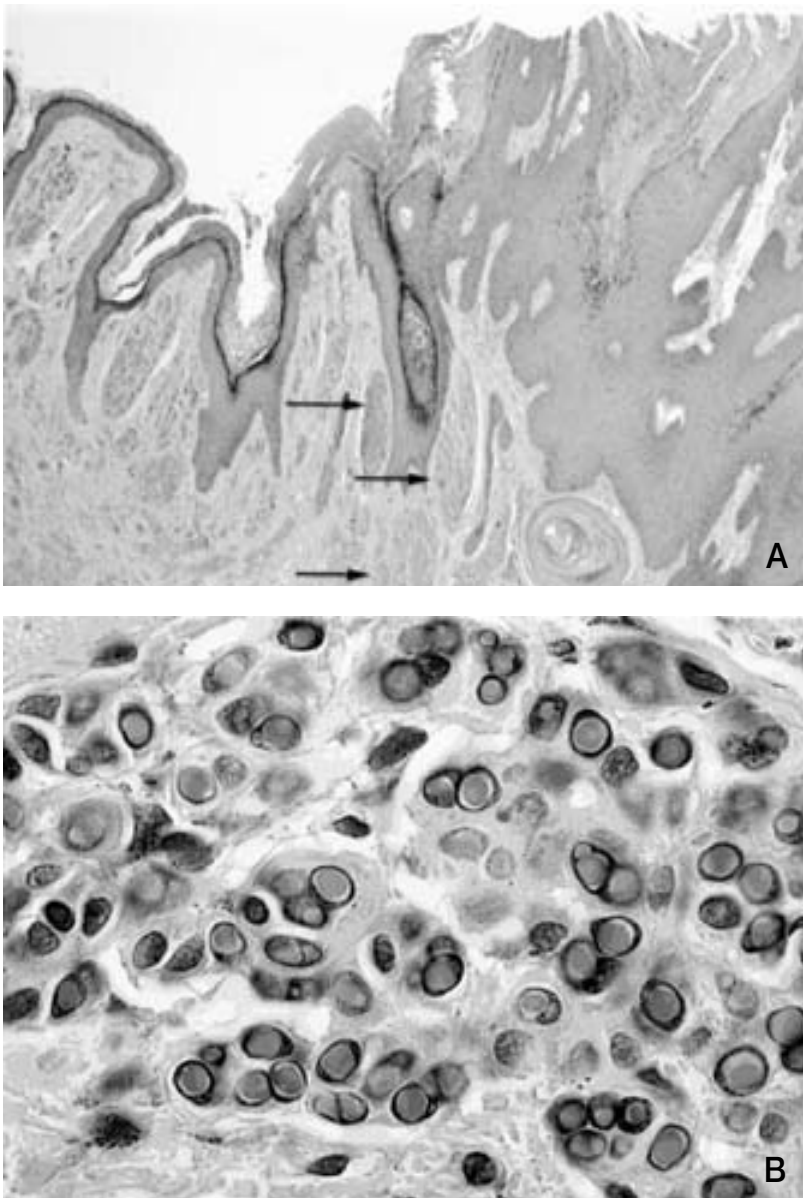

Figure 1. Wart with associated compound nevus (arrows)(A)(H&E, original magnification ×20). Eosinophilic intranuclear inclusion bodies in melanocytes (B)(H&E, original magnification ×1000).

eosinophilic bodies within their nuclei; these bodies were distinct and round to oval (Figure 1B). Periodic acid-Schiff (with and without diastase), trichrome, colloidal iron, iron, Congo red, methyl green-pyronin, and Feulgen stains were negative for eosinophilic bodies. Results of immunohistochemical studies of hyaline bodies were negative for herpes simplex virus-1 and -2, cytomegalovirus, cytokeratin, and κ and λ light chains.

Ultrastructurally, single or multiple inclusions were confined to the nucleus as unenveloped, evenly dispersed, homogenous, electron-dense, granular bodies that blend in with the rest of the nuclear chromatin (Figure 2).

In the nevus cells, in situ hybridization was negative for HPV, although HPV-57 was identified in the epidermal cells in association with the wart. With RT in situ PCR analysis, many hyaline intranuclear inclusion bodies stained positive for molluscum-specific primers (Figure 3); the bodies were negative for Paramyxovirus. Specificity of the RT in situ PCR signal was indicated by localization only to the cells with intranuclear inclusions, signal loss with substitution of molluscum-specific primers either for HPV-57 (a loss detected in the squamous cells in the overlying verruca) or for measles-virus–specific primers, and signal loss after ribonuclease digestion using molluscum-specific primers.

Comment

Intranuclear inclusion bodies have been found in several pathologic entities, including melanocytic

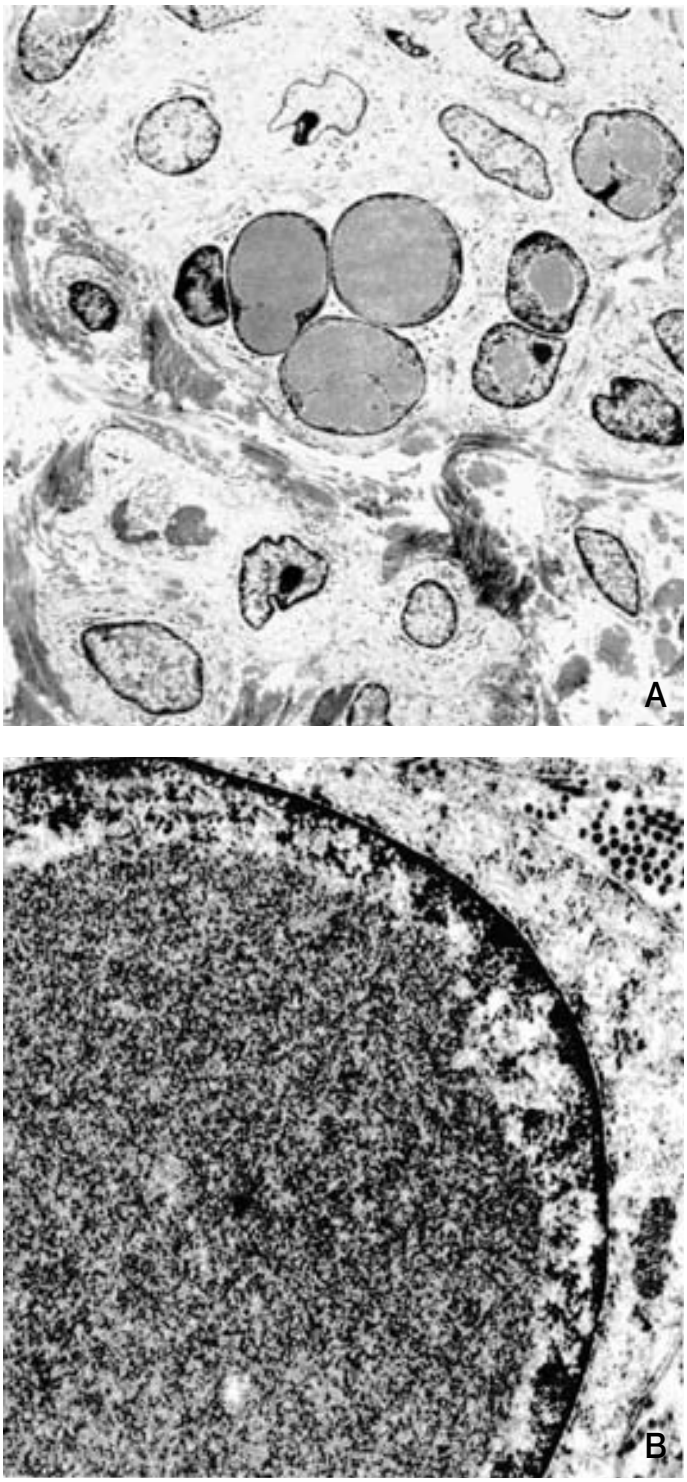

Figure 2. Multiple electron-dense ovoid intranuclear inclusion bodies (A)(electron microscopy, original magnification ×1700). At higher magnification, inclusion bodies appear homogenous and granular (B)(corresponding photomicrograph, ×17,000).

lesions.[1] Pseudoinclusions have cytoplasmic components that only seem to lie within the nucleus; these components are completely demarcated from the nuclear matrix by a nuclear membrane. In contrast, true inclusions lie within the nuclear matrix and lack such a border membrane.[1]

Some cases of intranuclear inclusion bodies in the lung and brain have been particularly well studied. In many of these cases, the inclusions were viral.[8,9] Tavassoli et al[10] described eosinophilic intranuclear inclusion bodies in the breast but were unable to determine their exact nature. Two cases of

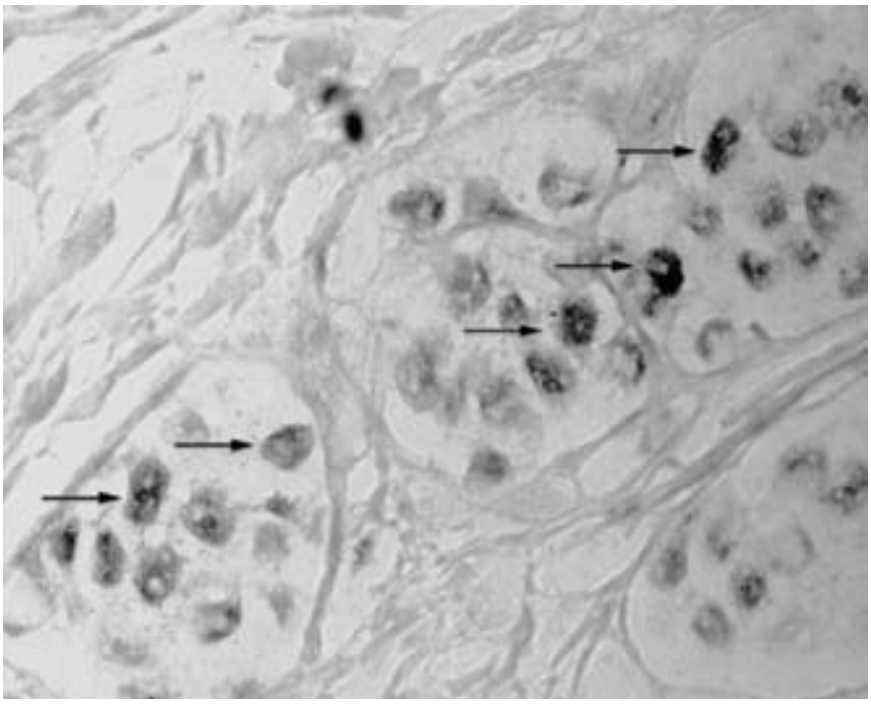

Figure 3. On reverse transcriptase in situ polymerase chain reaction analysis, hyaline bodies stained positive (arrows) for molluscum-specific primers. (Molluscum-specific primer stain, original magnification ×800).

melanocytic lesions with eosinophilic bodies have been reported.[2,3] In both cases, the bodies lie in the nucleus and in the cytoplasm. In one case, results of PCR analysis were positive for measles-virus RNA[3]; in the other case, the investigators speculated that the measles virus may have produced the bodies, but they were unable to prove it.[2]

The eosinophilic inclusions that we describe are unusual in that they were entirely intranuclear and occurred in approximately 80% of melanocytes. The exact nature of these bodies could not be determined from our histochemical and immunohistochemical studies. Using RT in situ PCR analysis, we found that the bodies contained molluscum viral transcript. Under electron microscopy, viral particles produced by the molluscum virus are very characteristic. Ultrastructurally, the inclusions in our case lacked the characteristics of well-formed viral particles. In addition, the negative result of the Feulgen stain suggests that these inclusions were not composed entirely of DNA viral particles. The positive result of the RT in situ PCR analysis, however, suggests that the inclusions may be related to molluscum viral infection of melanocytes.

Conclusion

A rare case of true intranuclear inclusion bodies in a melanocytic nevus has been described. Although their exact nature remains unclear, these inclusions may be related to molluscum viral infection of melanocytes.

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