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Anetodermic Pilomatricoma: Molecular Characteristics and Trauma in the Development of Its Bullous Appearance

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Abstract

Pilomatricoma is a common benign neoplasm of the skin characterized by a solid cutaneous nodule of hair matrix origin. The anetodermal or lymphangiectatic variant of pilomatricoma is rare, and its bullous appearance is often associated with attenuated collagen and elastic fibrils and dilated lymphatic vessels in the overlying dermis. However, the tumors of anetodermic pilomatricoma have never been characterized at the molecular level and the exact mechanism for their development is unknown. In this study, we evaluated histological and molecular features of a bullous pilomatricoma along with five control tumors, and determined that tumors of both anetodermic and control pilomatricoma comprise similar molecular features such as nuclear lymphoid enhancer binding factor 1 (LEF1) localization and the expression of keratins. In addition, we associated the development of the anetodermic pilomatricoma with mechanical trauma, scar tissue formation and increased numbers of blood and lymphatic vessels. This study suggests that the development of the anetodermic form of pilomatricoma is unlikely to be associated with the intrinsic properties of the tumor but with the mechanical trauma which disrupts the dermal integrity and vascular microenvironment.

Keywords

Pilomatricoma; Hair follicle; Tumor; Bullous; Anetodermic

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INTRODUCTION

Pilomatricoma, also known as pilomatrixoma or calcifying epithelioma, is a hair follicle tumor, ^{1,2} frequently observed in children and young adults with extremely rare incidence of malignant transformation. ^{1,3,4} Pilomatricoma is characterized by a solid and slow-growing nodule usually located in lower dermis but may extend into subcutaneous tissue. ^{1,3} Histologically, pilomatricoma exhibits features of hair follicle matrix cell differentiation, such as the presence of basoloid hair matrix cells, transitional cells and eosinophilic amorphous shadow (ghost) cells. ^{3,5,6}

The anetodermic pilomatricoma, which often displays a bullous appearance, is rare (Supplemental Table 1). Lymphatic vessel congestion and subsequent dilation were characteristic for this variant of pilomatricoma, hence also designated as lymphangiectatic pilomatricoma. ⁷⁻¹¹

The etiology of the bullous appearance of anetodermic pilomatricoma is unclear. While trauma has been postulated as a cause of the anetodermic pilomatricoma, ¹² no cause-effect relationship was established. Tumors of anetodermic pilomatricoma resemble those of classic pilomatricoma at both clinical and histological levels. However, molecular features of these tumors have not been compared directly. Whether the former contains intrinsic properties underlying the development of its bullous appearance is unknown.

In this study, we demonstrated for the first time that the tumor of anetodermic pilomatricoma contains similar molecular features as classic pilomatricoma, such as nuclear localization of lymphoid enhancer binding factor 1 (LEF1) and the expression of keratins. By linking a documented trauma with scar tissue formation and abnormal dermal vasculature, this study further demonstrated that mechanical trauma might have played an important role in the development of anetodermic pilomatricoma.

MATERIALS AND METHODS

Tumor was removed in whole from the shoulder of the patient, immediately fixed in 4% formalin, embedded in paraffin and processed for routine hematoxylin and eosin (H&E) staining. Control pilomatricoma were obtained from the tissue bank of the Department of Pathology of Peking Union Medical College Hospital. All specimens were fixed in formalin before embedding and sectioning.

Immunohistochemistry was carried out by a NovoLink™ Polymer Detection System according to the manufacturer's instructions (Leica Microsystems, Bannockburn, IL, USA). Antibodies used in immunohistochemistry include a lymphatic endothelium-specific monoclonal antibody D2-40 (MAB-0567, Maixin Bio, Fuzhou, China), and CD34 (QBEnd/10, Leica Microsystems, Buffalo Grove, IL, USA). Verhoeff-van Gieson staining was used to stain elastic fibers. Images were acquired by NanoZoomer 2.0-RS slide scanner (Hamamatsu Photonics, Hamamatsu, Japan) and processed with Adobe Photoshop CS3.

Immunofluorescence labeling was performed as described previously. ¹³ Primary antibodies used in this study are listed in Supplemental Table 2. Photographs were taken with a Nikon Eclipse 90i microscope in conjunction with the NIS-Elements AR 3.0 imaging software (Nikon, Melville, NY, USA).

Blood or lymphatic vessels were counted on images taken from three representative areas (0.15 mm²) of stained (ACTA2 or D2-40) sections of the anetodermic and control pilomatricoma (n = 5) and used for analysis. The student's T-test was used to calculate statistical significance. P < 0.05 was considered statistically significant.

CASE REPORT

A 20-year-old female presented to the dermatology clinic with a bulla forming on her shoulder. The patient had a 4-month history of a slow-growing cutaneous mass about the size of a pea and 1-month progressive bulla formation after surgical incision into the mass in an effort to discharge the lump. Dermatological examination revealed a semi-translucent and hemorrhagic bulla, 35 mm × 25 mm in size, with a bluish discoloration (Fig. 1a). On palpation, a firm and mobile nodule was detected below the bulla. Clinical features of this and control tumors were summarized in Table 1.

The tumor of anetodermic pilomatricoma was well-circumscribed, consisted primarily of amorphous eosinophilic enucleated shadow (ghost) cells (Fig. 1b), and displayed characteristic matrix to shadow cell transition (Fig. 1d). Hemorrhages and infiltrating inflammatory cells were also evident (Fig. 1c and inset). In focal areas of the dermis, interstitial space was dramatically increased (Fig. 1b), collagen fibrils attenuated (Fig. 1e), and elastic fibers reduced (Fig. 1f and Fig. S1a).

Matrix cells of all tumors expressed LEF1 in the nuclei, suggesting activation of the canonical Wnt signaling pathway as a consequence of *CTNNB1* mutation in all cases (Fig. 1g and Fig. S1b).¹⁴ We performed the first analysis of a type II hair keratin, KRT81, on pilomatricoma and localized cells expressing KRT81 to transitional cells in all tumors (Fig. 1g and Fig. S1b). KRT81-positive cells localized interiorly to LEF1-positive cells without overlapping (Fig. 1g and Fig. S1b), resembling a differentiating hair follicle.^{15,16} KRT17 positive cells were observed sporadically at the periphery of all tumors and at the core of the tumor mass ((Fig. 1h and Fig. S1c)), whereas, KRT14 positive cells were rarely detected (data not shown).

The dermis of anetodermic pilomatricoma contained a significantly ($p = 0.05$) higher number of blood vessels (20.7 ± 6.8 per microscopic field) than that of control pilomatricoma (7.0 ± 2.2) (Fig. 2a, b, c, f). These blood vessels were often seen vertically orientated between the retia, suggesting their association with scar tissue formation (Fig. 2a, b). Examination with other vascular markers, such as FLT1 and CD34 resulted in similar findings (see below and data not shown).

Lymphatic vessels were detected by D2-40, a lymphatic endothelium-specific antibody (Fig. 2d, e and Fig. S2). Lymphatic vessels were not only abnormally dilated (Fig. S2a) but their number also significantly ($p < 0.05$) increased, averaging at 8.3 ± 2.3 in anetodermic pilomatricoma (3.5 ± 2.6 in control pilomatricoma) per microscopic field (Fig. 2g).

DISCUSSION

In this study, we performed the first molecular characterization of anetodermic pilomatricoma and confirmed that a tumor of this variant contains similar molecular features to that of classic pilomatricoma, suggesting that the intrinsic properties of anetodermic pilomatricoma are unlikely contributors to the development of its bullous appearance.

In addition to previously reported lymphatic vessel dilation, disruption of collagen fibrils and the absence of elastic fibers, in this study, we employed immunofluorescence labeling and observed an increase in the numbers of small blood vessels and lymphatic vessels. Thus, this study suggests that increased vessel number is also associated with the pathological development of anetodermic pilomatricoma.

Mechanical irritation and trauma were postulated to contribute to the development of anetodermic pilomatricoma, however, no prior studies were able to establish a cause-effect

relationship. The anetodermic pilomatricoma reported herein not only had an incision prior to the acute (1 month) development of skin bulla, it also contained histological and immunohistological evidence of dermal scar tissue formation. These findings suggest that mechanical trauma might have played a critical role during the evolution of anetodermic pilomatricoma by not only directly disrupting elastic fibers that confer dermal integrity, but also by disrupting lymphatic drainage and inducing blood vessel proliferation. This study also offered an explanation to the often rapid development of skin bulla in a number of anetodermic pilomatricoma,^{9,11,17-19} in which external force might have accelerated traumatic damages to collagen and elastic fibers caused by the solid tumor mass. Whether the recruitment of macrophages to the site of injury and the subsequent release of elastases and collagenases further escalated the disruption of dermal integrity remains to be further determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Clinical, histological and molecular characteristics of anetodermic (bullous) pilomatricoma. (a) A 3.5 cm × 2.5 cm, translucent, hemorrhagic bulla with a bluish discoloration on the right shoulder of a patient. (b - e) The tumor is circumscribed with fibrous tissue (arrows) (b). Basoloid cells were restricted to the boundary of the tumour (b). Enlargement of boxed area revealed characteristic organization of basoloid matrix cells (MC), transitional cells (TC) and amorphous shadow cells (SC) (d); extra-vascular red blood cells (asterics) and inflammatory cells (inlet) (c); and increased interstitial space (arrow heads) in the dermis above the tumor (e). (f) Verhoeff-van Gieson staining revealed dramatic disruption and reduction of elastic fibers. (g) LEF1 (red) is restricted to the nuclei. Note that LEF1 (red) overlapped with DAPI (blue), therefore, appeared pink. KRT81 (green) is expressed in transitional cells. (h) KRT17 (green) is expressed in cells at the periphery of the tumour. MC, matrix cells; TC, transitional cell; SC, shadow cell. Scale bar = 2.5 mm in (b); 100 μm in (c and d), 250 μm in (e and f) and 50 μm in (g and h).

Fig.2.

Blood and lymphatic vessels in the dermis of pilomatricoma. (a) Histological examination revealed a fibrous scar and vessels (arrows) in anetodermic pilomatricoma. Some vessels are dilated (arrow heads). (b - c) Blood vessels labeled by smooth muscle actin (ACTA2, red) revealed dramatically increased number in anetodermic (b) but not in Control (c) pilomatricoma. Dotted lines indicate the location of the basement membrane. (d - e) Lymphatic vessels labeled with D2-40 revealed increased numbers of lymphatic vessels (arrow heads) in the dermis of anetodermic (d) but not control (e) pilomatricoma. (f - g) Statistical analysis of results demonstrated a significantly increased number of blood (f) and lymphatic vessels (g) in anetodermic (Bullous) than classic (Control) pilomatricoma. $n = 5$ for Control, $p < 0.05$. Scale bar = 100 μm .

Table 1
Clinical features of pilomatricoma

Patient ID	Sex	Age (years)	Duration (months)	Location
N1	M	21	6	Back
N2	F	17	5	Back
N3	M	35	6	Back
N4	M	20	5	Arm
N5	M	47	6	Head
B1	F	20	4	Shoulder

N1-N5 are nonbullous pilomatricoma; B1 is a bullous pilomatricoma