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Original

Melan-A/Mart-1- or HMB-45-positive melanocytes are not present in calcifying cystic odontogenic tumors (calcifying odontogenic cysts): a study in 13 Caucasian patients

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Abstract: Melanin pigment and melanocytes may be found in odontogenic cysts and tumors, particularly calcifying cystic odontogenic tumor (CCOT). In the present study we investigated the immunohistochemical expression of the Melan-A/Mart-1 and HMB-45 antigens in 13 Caucasians patients with CCOT. Melan-A/Mart-1- and HMB-45-positive melanocytes were not seen in any of the cases. Our findings are in agreement with the assumption that pigmentation in odontogenic lesions may be a racial phenomenon. (J Oral Sci 54, 33-38, 2012)

Keywords: jaw lesions; odontogenic tumors; calcifying cystic odontogenic tumor; melanocytes.

Introduction

Melanin pigment and melanocytes may be found in odontogenic cysts and tumors (1-3). Melanin may be easily overlooked in routine histological sections, as it resembles hemosiderin and its presence should be confirmed by Masson-Fontana silver impregnation (4). Melanocytes may be present even in the absence of melanin (5,6) and can be recognized by electron microscopy (1,5-8) and immunohistochemistry for S-100 protein (1,2,4,7-12), melanoma-associated antigen (7), or HMB-45 antigen (9,12-14).

The most common pigmented odontogenic lesion is calcifying cystic odontogenic tumor (CCOT, previously referred to as calcifying odontogenic cyst), as among the 47 cases of such lesions reported up to 2007, 20 were CCOTs, that account for less than 2% of all odontogenic lesions (1). Although no pathological significance has been attributed to this phenomenon, it has drawn considerable attention as it may be associated with the pathogenesis of the lesion.

In the present study, we investigated the expression of Melan-A/Mart-1- and HMB-45 antigens in 13 specimens of CCOT from Caucasians patients.

Materials and Methods

Thirteen totally excised intraosseous CCOTs were studied retrospectively. Details of the age, sex, and ethnicity of the patients, as well as the gross size and location of the lesions, were retrieved from the pathology report forms. Foci of calcified material were evident on gross sectioning, but decalcification was not necessary in any of the cases. Two consecutive 5-micrometer-thick formalin-fixed and paraffin-embedded tissue sections were reacted with monoclonal antibodies against Melan-A/Mart-1 (clone A103, dilution 1:25, Dako, Glostrup, Denmark) and HMB-45 (clone MO643, dilution 1:50, Dako), using a standard streptavidin-biotin-peroxidase system and the Dako Envision™ system (Dako).

As positive controls, we used sections of normal gingival mucosa overlying two of the tumors for

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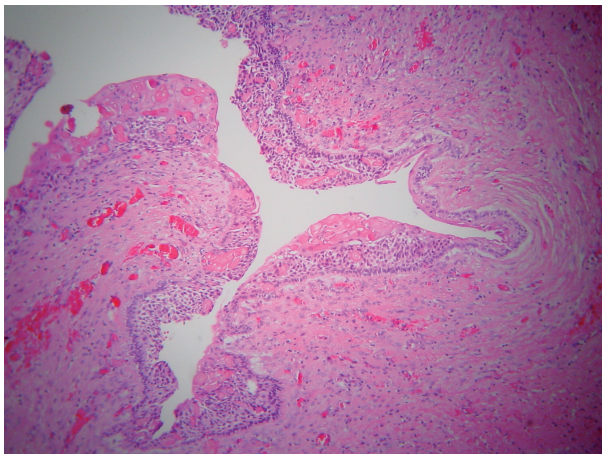
Table 1 Age and sex of the patients, and location and gross size of the lesions, in 13 intraosseous CCOTs

Case No	Sex	Age	Location	Gross size
1	male	25	mandible	0.3 × 0.5
2	female	48	mandible	1.7 × 2
3	n/a*	n/a*	n/a*	0.9 × 1.1
4	male	8	maxilla	0.5 × 1.8
5	female	19	maxilla	1.0 × 1.6
6	male	33	maxilla	0.5 × 0.8
7	female	35	maxilla	0.6 × 1.5
8	male	n/a	maxilla	1.0 × 2.2
9	male	21	mandible	0.6 × 0.6
10	male	26	mandible	0.5 × 0.7
11	male	14	maxilla	0.6 × 1.4
12	male	49	maxilla	0.5 × 0.7
13	female	56	mandible	1.5 × 1.7

*n/a = not available



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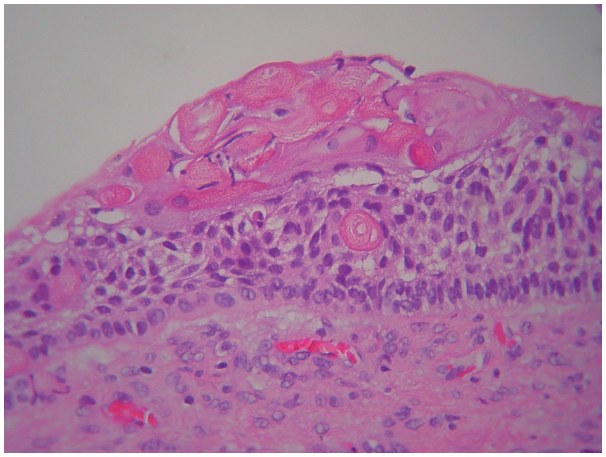

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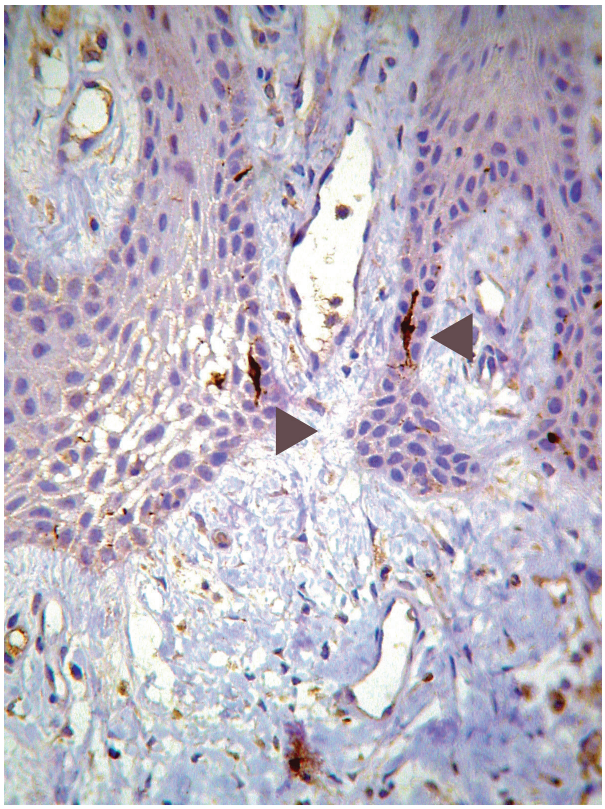
Fig. 1 Photomicrograph of a calcifying cystic odontogenic tumor. (a) The cyst wall consists of vascular fibrous connective tissue lined by ameloblastoma-like epithelium of variable thickness (hematoxylin and eosin stain, original magnification ×200). (b) Note cuboidal or columnar basal cells, stellate reticulum-type cells, and ghost cells (hematoxylin and eosin stain, original magnification ×400).



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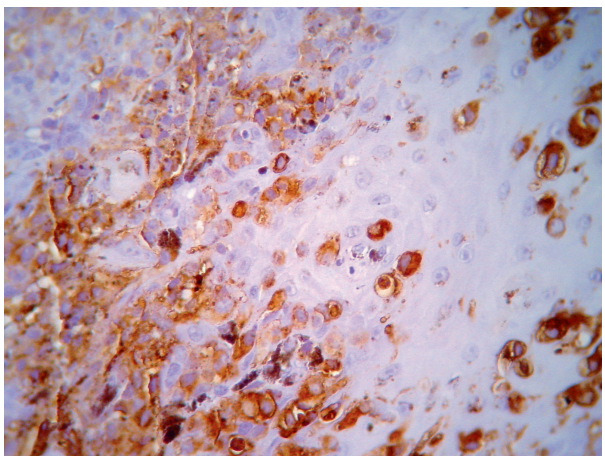

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Fig. 2 (a) Melan-A/Mart-1-positive melanocytes in the basal cell layer of normal gingival mucosa epithelium (arrowheads) and (b) HMB-45-positive melanoma cells in a melanoma (streptavidin-biotin-peroxidase, original magnification ×400).

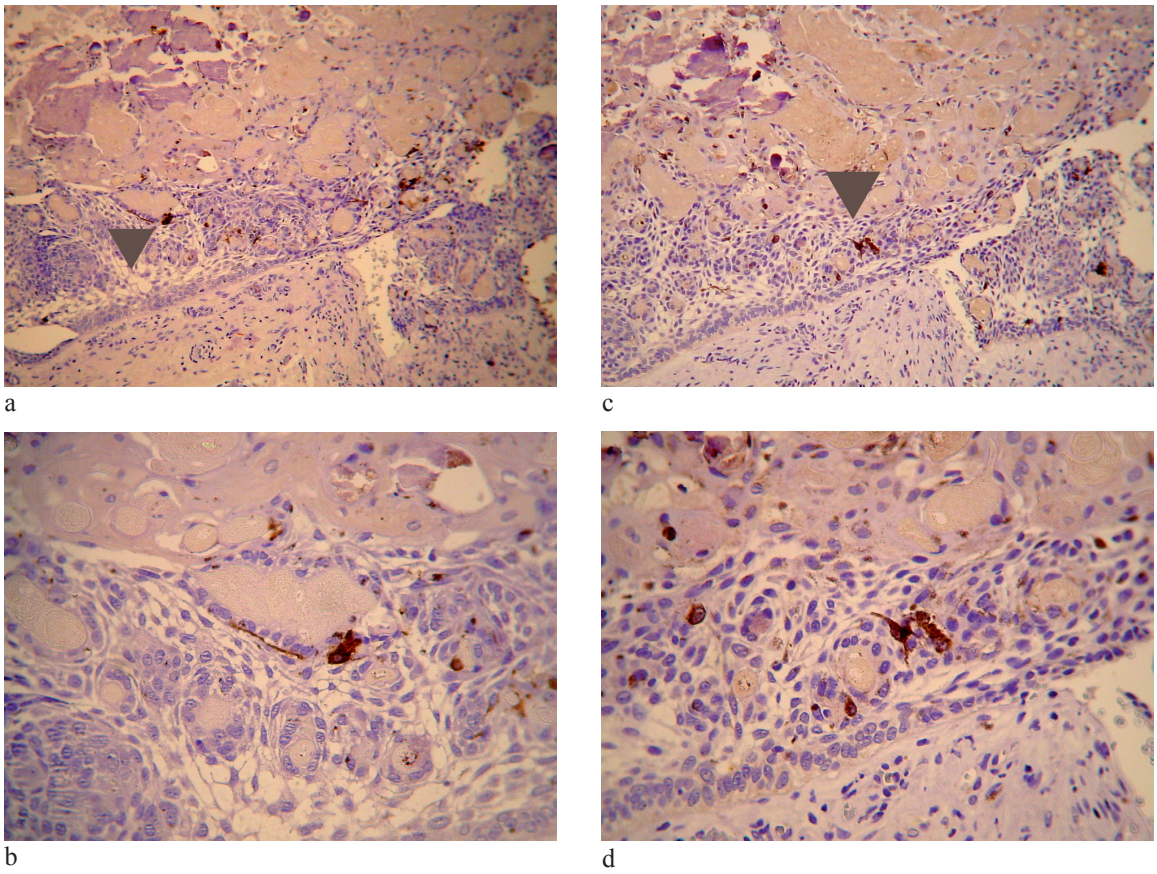

Fig. 3 Dendritic melanocytes (arrowheads) among stellate reticulum-type epithelium of pigmented CCOT: (a) and (b) Melan-A/Mart-1, (c) and (d) HMB-45 (streptavidin-biotin-peroxidase, original magnifications: (a) and (c) ×200, (b) and (d) ×400).

Melan-A/Mart-1; an oral melanoma for HMB-45; and a case of pigmented CCOT from an Asian male, found during selection of the study materials, for Melan-A/Mart-1 and HMB-45. Substitution of the primary antibody with non-immune serum of the same specificity was done as a negative control.

Results

The main clinical features of the 13 cases of CCOT are summarized in Table 1. There were 8 male and 4 female patients, with a mean age at diagnosis 30 ± 15.41 years (age range 8-56 years). Seven cases were located in the mandible and 5 in the maxilla. Grossly, all the lesions were cystic (resected as a simple cystic sac or multiple tissue fragments), with a mean maximum size of 1.27 ± 0.57 cm (size range 0.5-2.0 cm).

Microscopically, the cyst wall consisted of vascular fibrous connective tissue lined by ameloblastoma-like epithelium of variable thickness, featuring cuboidal or columnar basal cells, stellate reticulum-type cells, and variable numbers of ghost cells (Fig. 1). Focal calcifications, in the form of basophilic granules, were commonly seen in the epithelium, and in one case dysplastic dentin (dentinoid) was identified in the connective tissue stroma.

Melan-A/Mart-1 was expressed in scattered dendritic cells, consistent with melanocytes, in the basal cell layer of the normal gingival epithelium (Fig. 2a), while HMB-45 was expressed in melanoma cells infiltrating the oral mucosa in a case of melanoma (Fig. 2b). In the case of pigmented CCOT, a few dendritic melanocytes positive for Melan-A/Mart-1 (Fig. 3a, b) and HMB-45 (Fig. 3c, d) were seen among stellate reticulum-type cells, and melanin pigment was evident among stellate reticulum-type cells and in the cytoplasm of ghost cells. Melan-A/Mart-1- or HMB-45-positive melanocytes were found in none of the 13 cases of CCOT studied (Fig. 4).

Discussion

Neither Melan-A/Mart-1 nor HMB-45 was expressed in melanocytes in the epithelium of 13 CCOTs from Caucasian patients. In previous studies, melanocytes in odontogenic lesions have been described as reactive for

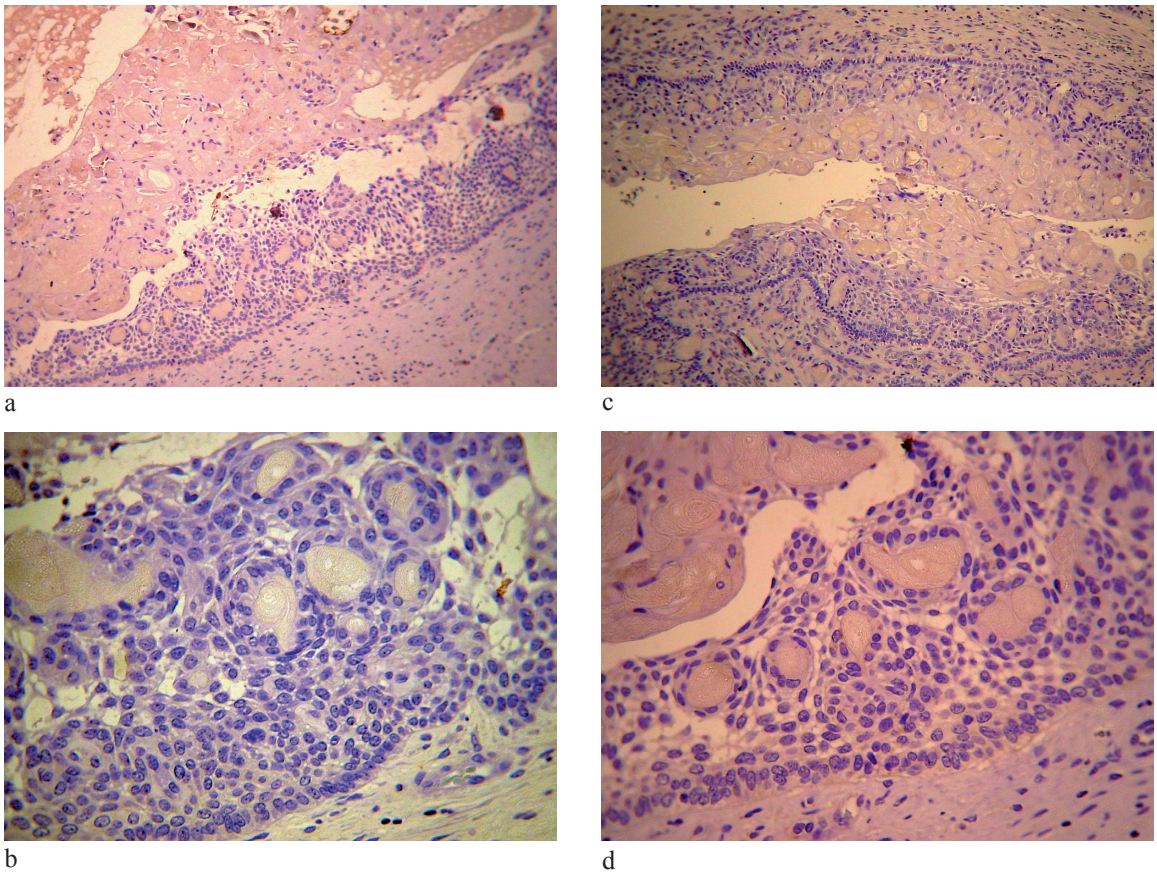

Fig. 4 Absence of positive cells in CCOT. (a) and (b) Melan-A/Mart-1, (c) and (d) HMB-45 (streptavidin-biotin-peroxidase, original magnifications: (a) and (c) ×200, (b) and (d) ×400).

S-100 protein (1,2,4,7-12), non-reactive for melanoma-associated antigen (7), and reactive (12,13) or non-reactive (9,14) for HMB-45 antigen. In the present study, we applied two antibodies that can identify both active and inactive melanocytes. Melan-A/Mart-1 is a cytoplasmic protein considered to be a sensitive and specific marker of melanocytic differentiation (15). HMB-45 is a glycoprotein located in cytoplasmic melanosomes and is used for identification of active melanocytes (16). Although it is normally expressed by fetal and neonatal melanocytes, but not adult resting melanocytes, it is "re-expressed" by reactive or proliferating epidermal melanocytes (16). Thus, in view of the number of cases included in the present study and the technique applied, the lack of melanocytes among the CCOTs examined cannot be considered coincidental.

Although pigmented odontogenic lesions are rare, some authors have suggested that their presence may be underestimated, as melanin pigmentation may be overlooked and melanocytes can be identified only using special techniques (6). It is more common in Asians and Blacks (1,6,11,17) than in Caucasians. In a review, Han et al. (1) described that 4 out of 44 cases of pigmented odontogenic tumors and one out of 17 cases of CCOT were found in the latter racial group. Even in colored persons, however, this phenomenon is rare, as in one study only 1 out of 108 ameloblastomas in Black patients was pigmented (18). In addition, in one fetal study (19), melanocytes were found more commonly in the dental lamina and enamel epithelium of Blacks (6/6 cases) than in Caucasians (3/11 cases). Both the rarity and racial predilection of pigmentation in CCOT, as suggested by other authors (2, 8), is supported by the results of the present study.

Other authors have associated the presence of melanocytes with the origin of odontogenic epithelium from primitive oral epithelium that contains such cells (11), or with the presence in the odontogenic mesenchyme of neural crest cells that have the potential to differentiate into melanocytes (11,20). These melanocytes could remain latent before being activated to produce melanin (9,11). The findings of the present study do not support

the presence of latent melanocytes in CCOT epithelium or mesenchyme, as those cells would be revealed by Melan-A/Mart-1.

Kusama et al. (21) paralleled melanin pigmentation in CCOT with that of cortex cells of normal hair. They suggested that melanin pigmentation, activation of the Wnt-β-catenin-TCF-Lef (T-cell factor/lymphoid enhancer factor) pathway and expression of hard-α keratins in CCOT indicate its differentiation into hair shaft. Lack of melanin and melanocytes from CCOTs in Caucasians cannot refute this theory, as the other two features strongly favor it. Differentiation of CCOT into an ectrotic form of hair shaft that does not present all the phenotypic features of the normal one, is a reasonable explanation.

In conclusion, immunohistochemistry failed to demonstrate active and inactive melanocytes in 13 cases of CCOT in Caucasian patients. The results of our study favor racial pigmentation as the most plausible explanation for the presence of melanocytes in some CCOTs. Studies including more cases, in particular using materials from colored persons, are needed for confirmation of these observations.

Acknowledgments

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