

Ghost cells in compound odontoma: a study of undemineralized material

Adriano PIATTELLI and Paolo TRISI

Dental School, University of Chieti, Chieti, Italy.

ABSTRACT

Calcifications and ghost cells at the enamel surface or in the ameloblastic epithelium were studied in twelve odontomas using undemineralized material.

Calcified material formed focally in the intercellular portion of the enamel epithelium: this material showed a concentric layers arrangement.

Ghost cells were present in most of the odontomas.

These ghost cells were epithelial cells which enlarged, became eosinophilic and underwent an aberrant type of keratinization with the formation of large masses of keratin, that didn't stain as deeply as normal keratin. These cells often showed karyolysis of the nucleus as keratinization progressed. Frequent was the appearance of dystrophic calcifications in individual cells or clusters of cells, characterized by extremely fine basophilic granularity. The outlines of these keratinized cells could often still be discerned, even if with some difficulty.

KEY WORDS:

Ghost cells - Odontogenic tumors - Odontoma - compound.

RESUME

CELLULES FANTOMES DANS L'ODONTOME COMPOSE: ETUDE SUR DU MATERIEL NON DEMINERALISE.

Les calcifications et les cellules fantômes à la surface de l'émail ou dans l'épithélium améloblastique ont été étudiées dans 12 odontomes en utilisant du matériel non décalcifié.

Le matériel calcifié est formé focalement dans les portions intercellulaires de l'épithélium de l'émail: ce matériel montre un agencement en couches concentriques.

Les cellules fantômes sont présentes dans la plupart des odontomes. Ces cellules fantômes sont des cellules épithéliales qui augmentent de taille, deviennent éosinophiles et subissent un type aberrant de kératinisation avec formation de larges masses de kératine qui ne se colorent pas aussi intensément que la kératine normale. Ces cellules montrent souvent une karyolyse du noyau au fur et à mesure de la progression de la kératinisation. Il était fréquent de rencontrer l'apparition de calcifications dystrophiques dans des cellules isolées ou en îlots, caractérisées par des granulations basophiles extrêmement fines. Les contours de ces cellules kératinisées peuvent être encore souvent discernés, quoique avec certaines difficultés.

MOTS-CLES:

Cellules fantômes - Tumeurs odontogènes - Odontome - Composé.

INTRODUCTION

The compound odontoma is composed by a collection of recognizable, although small and misshaped teeth with a high degree of morphodifferentiation.

A peripheral fibrous connective capsule that contains the often large numbers of denticles is present.

The aim of the present investigation was to study the structures associated with the odontogenic epithelium in odontomas, in particular the ghost cells, using thin ground sections.

MATERIAL AND METHODS

Twelve compound odontomas were used for the present study.

The tumors with the attached soft tissues were fixed, immediately upon removal, by immersion in 10% buffered formalin, dehydrated in an ascending series of alcohols and infiltrated and polymerized in Technovit 7200 VLC resin. The final preparation of the histological slides was performed by the cutting-grinding technique (Donath and Breuner, 1982). The non-decalcified thin (10μ) ground sections were stained with toluidine blue, basic fuchsin and methylene blue and were investigated with normal transmitted light.

RESULTS

The compound odontomas presented areas of fibrous connective tissue in which were present groups of cubical or flat epithelial cells: in some areas the epithelial cells formed some structures similar to the enamel organ composed by an outer layer of cubical cells enclosing a central zone of stellate cells.

A flat epithelium 1 or 2 layers deep and cells with long cytoplasmic processes, similar to cells of the stellate reticulum, covered the enamel.

In the intermediate layer the intercellular spaces were wider.

Calcified material was seen to form focally in the intercellular portion of the enamel epithelium. These islands of calcified tissue did not show a characteristic enamel, dentin or cementum structure: they showed a concentric layers organization and different staining aspects. Some of these calcifications adhered to the enamel or dentin surface, others were distinctly separated from neighboring enamel and were situated in the stromal connective tissue of the

tumor, or in the central portion of the intermediate layer.

Areas of so called ghost cells, oval, strongly eosinophilic cells, anuclear but with distinct outlines, could be found located in the tissues of less differentiated odontomas.

These areas were usually found in close proximity to dentin and enamel matrix and were surrounded by a dense basophilic line, indicating calcification. Ghost cells were often intermingled within the stellate reticulum-like areas. These cells showed different degrees of nuclear pyknosis and granular or clear cytoplasm. The nuclei underwent karyolysis as keratinization progressed. Many of the ghost cells underwent calcification or were embedded in an amyloid-like matrix. [Fig. 1]. This matrix, however, blended into calcified globular structures like dysplastic enamel. Single intracytoplasmic calcifications could be observed in some of these cells. [Fig. 2]. The earliest evidence of calcification was the presence of powdery, small, deeply basophilic granules. [Fig. 3, 4]. This granularity tended to increase in size and intensity with the eventual formation of large sheets of calcified material and so in different areas could be seen ghost cells in different stages of calcification. [Fig. 5]. The cells didn't become markedly flattened as in normal keratinization.

The keratin didn't stain as deeply as normal keratin.

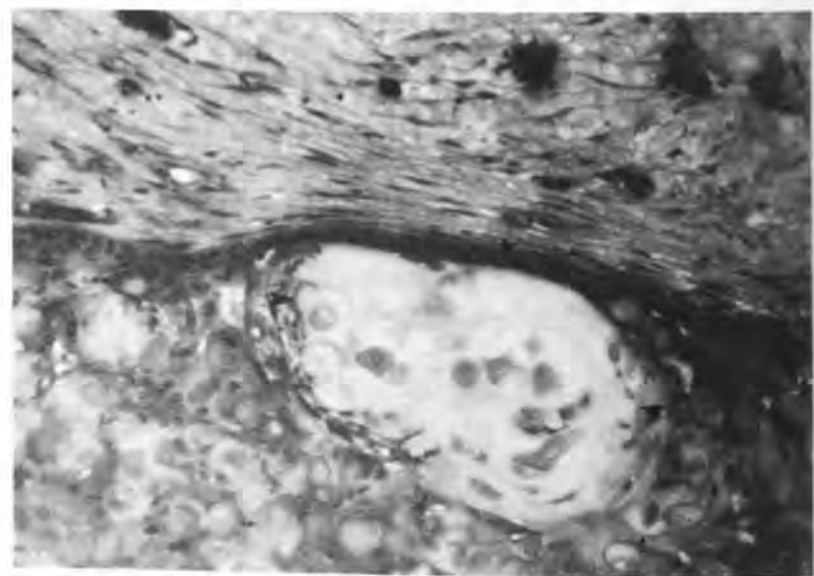


Fig. 1 - Cellular area of a compound odontoma. It is possible to note amyloid like material (large arrows) surrounded by numerous ghost cells (small arrows). (Basic Fuchsin - Methylene blue, 200x).

Fig. 1: Territoire cellulaire d'un odontome composé. Il est possible de noter la présence de matériel d'allure amyloïdique (têtes de flèches) entouré par de nombreuses cellules fantômes (petites flèches). (Fuchsin basique - bleu de méthylène, $\times 200$).



Fig. 2 - Ghost cells with nuclear pyknosis and granular or clear cytoplasm showing basophilic probably calcified intracytoplasmic material (arrow). (Basic Fuchsin - Methylene blue, 1000 \times).

Fig. 2: Cellules fantômes avec des noyaux pycnotiques et un cytoplasme granuleux ou clair, montrant la présence d'un matériel intracytoplasmique probablement calcifié (tête de flèche). (Fuchsine basique - bleu de méthylène, $\times 1000$).

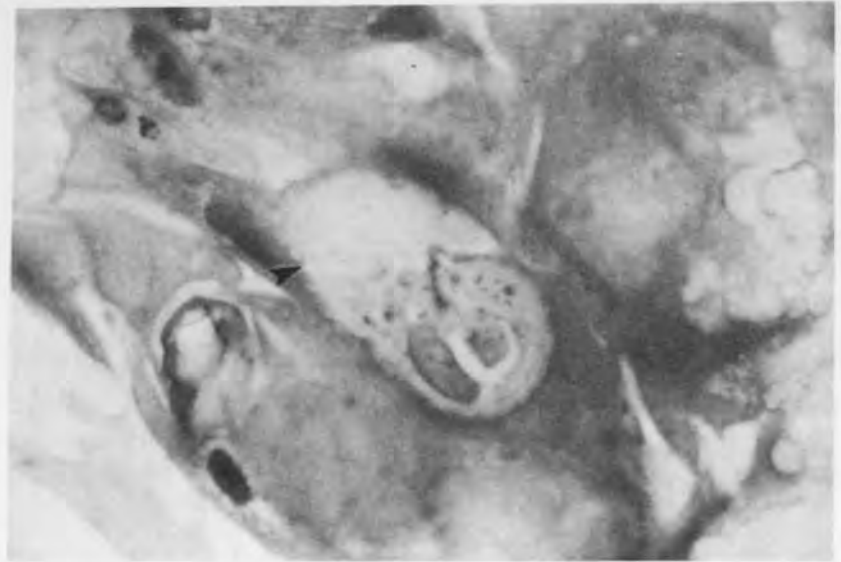


Fig. 4 - Large ghost cell showing characteristic clear granular cytoplasm (arrow) with initial rounded calcification. (Basic Fuchsin - Methylene blue, 1000 \times).

Fig. 4: Large cellule fantôme montrant un cytoplasme clair et granuleux (tête de flèche) avec des calcifications arrondies débutantes. (Fuchsine basique - bleu de méthylène, $\times 1000$).

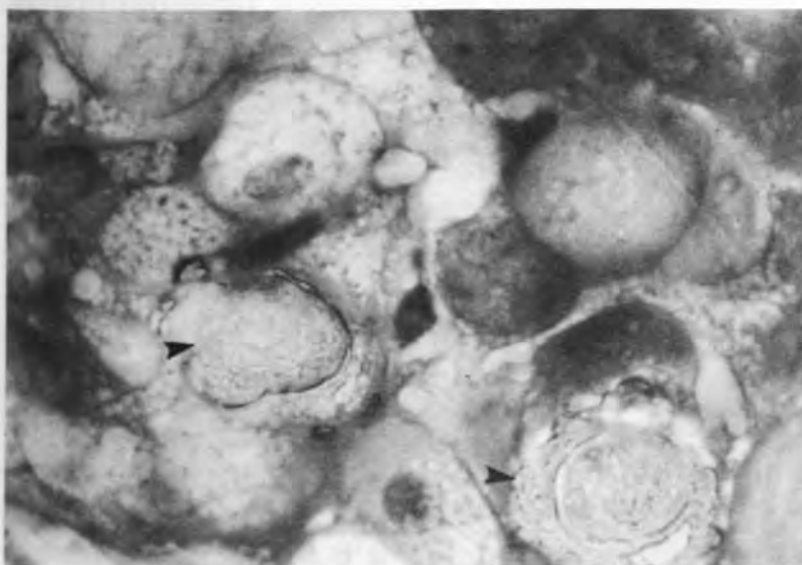


Fig. 3 - Ghost cells displaying intracytoplasmic circular lamellar calcifications similar to Liesegang ring (arrows). (Basic Fuchsin - Methylene blue, 1000 \times).

Fig. 3: Cellules fantômes montrant des calcifications intracytoplasmique lamellaires et circulaires semblables aux stries de Liesegang (têtes de flèches). (Fuchsine basique - bleu de méthylène, $\times 1000$).

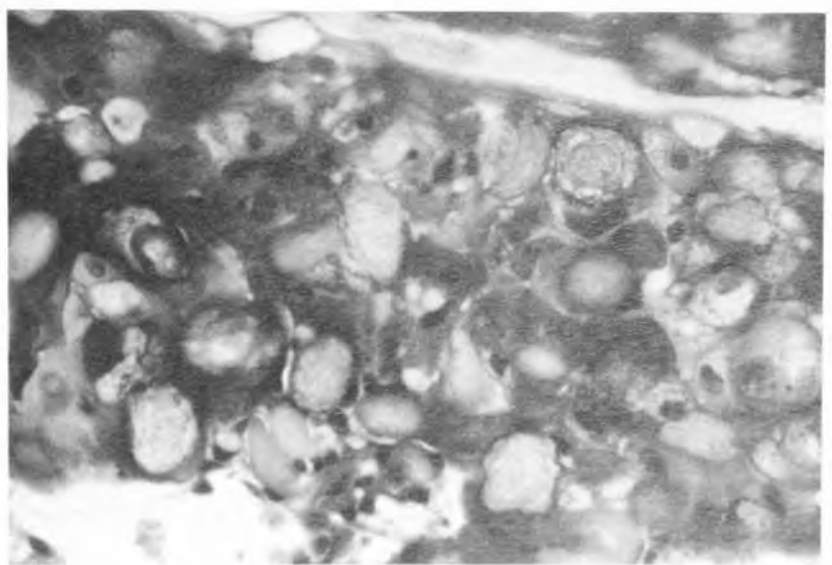


Fig. 5 - Ghost cells in different stages of calcification. (Basic Fuchsin - Methylene blue, 200 \times).

Fig. 5: Cellules fantômes à différents stades de leur calcification. (Fuchsine basique - bleu de méthylène, $\times 200$).

DISCUSSION

Studies on the morphology of the hard and soft tissues constituting a compound odontoma are very few (Kerebel and Kerebel, 1984), probably for the difficulties encountered in their microscopic examination.

The study of the mineralized tissues of teeth has been carried out either in demineralized sections where there is a removal of the mineral salts or in undemineralized (ground) sections.

If demineralized sections of teeth are studied, the inorganic fraction has been removed, the enamel has been lost and only the organic portion remains and it can be readily seen that with such a small part of the total tissues available, misinterpretation of the results is not only possible but probable (Mjör and Pindborg, 1973). Moreover in addition to removing the mineral content, decalcifying agents undoubtedly also have some effect on the organic components, and prolonged exposure to any demineralizing agent may therefore give undesirable results.

On the other hand undemineralized ground sections are usually relatively thick (50-150 μ), produce the destruction of some of the soft tissue components and are usually best examined unstained, because penetration of a dye will depend on the degree of mineralization (Mjör and Pindborg, 1973). All these disadvantages do not allow an accurate assessment of the degree of mineralization of the dental hard tissues and their relationship with the soft tissue components.

The recent introduction of a new technique (Donath and Breuner, 1982) has made possible to obtain very thin (5-15 μ) slides of undemineralized hard dental tissues where it has been possible to maintain a good structural relationship between the organic and inorganic components and with the surrounding soft tissues, to avoid the staining artifacts due to the use of adds, and to obtain a very good microscopic resolution of all the structures. Dysplastic calcified tissues in less differentiated odontomas present different degrees of calcification and organization. In our specimens we could observe two different kind of dysplastic calcified tissue. The first is similar to the rounded concentric calcification formed by Liesegang's rings which have been found by in odontoma, calcifying epithelial odontogenic tumor, odontogenic calcifying cyst. We observed these

concentric formations in contact with the epithelial cells of the intermediate layer and of the stellate reticulum of the enamel organ. The concentric layers of these calcifications could represent a successive stratification of calcifying material.

On the other hand we observed a different type of dysplastic calcified material without the appearance of concentric layers, that seemed to derive from an amyloid-like material produced by the ghost cells. We also observed intracytoplasmatic calcification of these cells indicating that they could calcify completely and be substituted by dysplastic calcified materials. The nature and significance of the ghost cells is yet unclear.

They are present in 10-18% of odontomas, especially of the complex variety (Donath, 1984).

They have been thought to be epithelial cells which undergo a kind of incomplete or abnormal keratinization (Lucas, 1976; Smith et al., 1981, Barnes, 1985). They could have a role in the formation of preenamel or enamel matrix or be just degenerating squamous cells (Barnes, 1985).

Apart from odontomas, ghost cells are an important feature of the calcifying odontogenic cyst and moreover similar structures have also been reported, although much less obviously, in craniopharyngiomas, calcifying epitheliomas of Malherbe, ameloblastomas and ameloblastic fibromas (Lucas, 1976; Barnes, 1985; Regezi and Sciubba 1989). This ghost cell keratinization, which is one of the characteristic features of the calcifying odontogenic cyst, is often associated with the epithelial elements also of odontomas, ameloblastomas, adenomatoid odontogenic tumors, ameloblastic fibroodontomas and ameloblastic fibromas. Whether these represent two unrelated lesions developing simultaneously or a single lesion is uncertain (Regezi and Sciubba, 1989).

In conclusion the ghost cell keratinization that is seen in the epithelial cells of the enamel in some odontomas appears to have no significance for the prognosis and therapy of odontomas, other than indicate the potential of these epithelial cells to keratinize (Donath, 1984; Regezi and Sciubba, 1989).

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Address correspondence to:
Prof. Dr. Adriano Piattelli, Via F. Sciucchi 63
66100 Chieti (Italy).