Keratinized and Calcified Rushton Bodies by Apoptotic Keratinocytes in Dentigerous Cyst

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Rushton bodies are known to be the aberrant keratinization and calcification in the epithelium of odontogenic cyst, which are similar to the features of calcifying odontogenic cyst and pilomatricoma. However, the pathogenetic mechanism of keratinization and calcification of Rushton bodies has not been clearly elucidated. Here, a case of Rushton bodies found in dentigerous cyst was examined by immunohistochemical method using antisera of PCNA, pAKT, HIF, PIM1, and PARP. The globular keratinization in lamellate fashion showed weak birefringency under polarizing microscope, and the Rushton bodies frequently underwent the dystrophic calcification. The polygonal keratinocytes of Rushton bodies were strongly positive for HIF and PARP, and the cyst epithelium was diffusely positive for pAKT and PIM1. Particularly, the cyst epithelium was hyperplastic and focally invaginated into cyst wall with positive reaction of PCNA. These findings may indicate the active response of odontogenic epithelium against the apoptotic stress of the cyst, producing the globular keratinization and irregular calcification in the polygonal keratinocytes. Therefore, it is presumed that the lamellate keratinization and dystrophic calcification of Rushton bodies are aberrant products of retrograding keratinocytes slowly undergoing apoptotic progresses similar to the phenomena of the ghost cells in calcifying odontogenic cyst and pilomatricoma, and also may have a potential for oncogenic proliferation.

Key words: Rushton body, Dentigerous Cyst, Apoptosis

Ⅰ. Introduction

The origin and nature of Rushton bodies have been rather elusive, thus leading to numerous studies through the histologic, histochemical, enzyme histochemical, transmission and scanning electron microscopic, microprobe, microradiographic and immunocytochemical methods. In 1918, Deway 3) described them briefly and suggested that they resulted from hyaline degeneration of new capillaries.

Rushton 2), in 1955, precisely described these structures, which were subsequently named after him. He suggested that these double-contoured structures were a sort of keratin, similar to secondary enamel cuticle of Gottlieb. Wertheimer et al. 3), in 1962, confirmed Rushton's observations on the similarity between hyaline bodies and secondary enamel cuticle, concluding that both represented a form of specialized keratin.

In 1965, Bouyssou and Guilhem 4) found that Rushton's hyaline body demonstrated a histochemical reactivity for...
hemoglobin. They concluded that hyaline bodies are thrombotic varicose venules strangled by epithelial proliferation. Hodson\(^9\), in 1966, used a TPA (tissue polypeptide antigen) reaction and showed that the dental cuticle is hematogenous in origin and composed of denatured hemoglobin, Dent and Wertheimer\(^6\), in 1967, and Sedano and Gorlin\(^7\), in 1968, employed the TPA technique to demonstrate Rushton's hyaline bodies and, while the former doubted the specificity of the technique, the latter concluded that their results suggested a hematogenous origin.

Some investigators\(^8\)-\(^{13}\) suggested that Rushton bodies may be a specialized secretory product of odontogenic epithelium deposited on the surface of particulate matter like cell debris or cholesterol crystals.

Rushton's hyaline bodies are found in odontogenic cysts, in particular in radicular cysts, as amorphous or lamellar concretions\(^{14,16}\). They occur almost exclusively within odontogenic cysts, but not been identified within nasopalatine or fissural cysts which are not of odontogenic origin\(^6,11\). The incidence of Rushton bodies varies between 2.6% and 10% of odontogenic cysts\(^3,14,17\).

It was suggested that the initiating mechanism leading to Rushton body formation was the single-cell necrosis through apoptosis in the epithelium followed by intracellular dystrophic calcification\(^12\). The role of keratinized epithelium in the development of Rushton bodies\(^2,15\) was challenged when no keratin was found in Rushton bodies\(^18,19\). However, the presence of lamellate keratin encircling the periphery of Rushton bodies could affect the epithelial cells located in the center of the Rushton bodies to be subjected to dystrophic calcification\(^12\). The calcification has already been recognized as an important step in the development of Rushton bodies.

In the present study, the nature of Rushton bodies occurred in a dentigerous cyst was explored with histological and immunohistochemical methods, and cellular proliferation apoptosis of Rushton body were compared with those of ghost cells in the calcifying odontogenic cyst and pilomatrixoma.

### II. Case report

A 32 years old male showed a radiolucent lesion associated with the impacted mesiodens in the anterior maxillary area by radiological observation. The lesion was asymptomatic but found during the routine check of oral health. The cystic lesion was removed together with the mesiodens. The specimen was examined pathologically through immunohistochemical stainings using antisera of PCNA (proliferating cell nuclear antigen), pAKT (phosphorylated AKT), PIM1(proto-oncogene serine/threonine-protein kinase), β-catenin, E-cadherin, BCL-2, HIF (hypoxia-inducible factor), and PARP (poly-ADP ribose polymerase), and finally diagnosed as a dentigerous cyst with Rushton bodies.

The Rushton bodies were focally accumulated in the exophytic mass from the thin odontogenic epithelium (Fig. 1A1). They were characterized by globular keratinization and dystrophic calcification dispersed loosely (Fig. 1A2). In the high magnification, the keratinized bodies were in lamellate pattern and the calcification was irregular and granular (Fig. 1A3). Under polarizing microscope, the lamellate keratinized body showed slight birefringency (Fig. 1A4).

Throughout the cyst epithelium the epithelial hyperplasia was evident with the invagination into the cyst wall, but the epithelium itself was not properly organized with the spindle-shaped keratinocytes (Fig. 1A5,A6). A severe acanthosis was also found in the cyst epithelium with a collection of proliferating spindle cells (Fig. 1A7,A8).

In the immunostaining of PCNA, the cyst epithelium was diffusely positive, and in high magnification, the basal cells
Fig. 1. Photomicrographs of Rushton bodies in dentigerous cyst. A: Hematoxylin and eosin stain. B-G: Immunostains. A1: Low magnification, A2: High magnification, multiple round keratinization (arrows) in the epithelial layer, showing dystrophical calcifications (arrow heads), A3: Rushton bodies (*), the keratinized bodies were in lamellate pattern (arrows), and the calcification was irregular and granular (arrow heads), A4: Polarizing microscopic observation of panel A3, showing slight birefringency (arrows), A5, A6: A focal epithelial invagination (arrows) with the loss of epithelial attachment by spindle-shaped keratinocytes (arrows), A7: A severe acanthotic epithelium (arrows), A8: High magnification of panel A7, collection of proliferating spindle cells (arrows), B: PCNA, C: pAKT, diffusely positive in Rushton bodies (arrows), D: PARP, D1-D3, strongly positive in polygonal cells (arrows), E: PIM1, diffusely positive in the epithelium (arrows) around Rushton bodies, F: HIF, strongly positive in the polygonal cells (arrows), G: Negative control, rarely positive,
of thickened epithelium showed frequent positive reaction (Fig. 1 B1,B2). The epithelium around the Rushton bodies was diffusely positive for pAKT (Fig. 1 C1,C2), and PIM1 (Fig. 1 E), and the polygonal keratinocytes in the center of Rushton bodies were strongly positive for HIF (Fig. 1 F1-F3) and PARP (Fig. 1 D1-D3), while they were rarely positive for β-catenin, E-cadherin, BCL-2. On the other hand, the negative control showed almost negative reaction in the same immunostaining (Fig. 1 G). The postoperative healing was uneventful, and the radiolucent lesion was well replaced by the features of osteophyte formation in six months postoperative radiological observation.

III. Discussion

Rushton bodies or hyaline bodies are characteristic products of odontogenic cysts as eosinophilic, straight or curved, irregular or rounded structures. Although the data from published studies suggest an overall incidence approximate to 8%20), the origin and nature of Rushton Bodies have been debated still. The incidence of Rushton bodies is usually high in periapical cysts, and then their incidence is 4.6% in odontogenic keratocyst21), and 3.4% in dentigerous cyst. The present case occurred in anterior maxilla of 32 years old male, at which calcifying odontogenic cyst is frequently involved22). Although it is coincidently agreed that the Rushton’s hyaline body is a rudimentary product of odontogenic cyst, the similarity of Rushton bodies to keratinization and calcification of ghost cells in calcifying odontogenic cyst is still remained to be elucidated.

An alternative hypothesis postulated that Rushton body might be derived from red blood cells or thrombosed capillaries and venules. It was also suggested that the amorphous material in the granular hyaline bodies appeared very similar to the substance of the degenerating red blood cells, and that the extravasated red cells were commonly found in the adjacent connective tissue. The lamellar pattern may result from the segregation of components within the mass rather than by an incremental form of growth15). Although the inadequate oxygenation of the epithelium and the damage to its cells may be caused on occasion by its brittle vascular supply, deposition of hemosiderin-derived iron appears to be a secondary event in Rushton body formation12).

In this study the histological examination disclosed that the cysts involved with Rushton bodies had stratified squamous epithelium variably thickened or frequently ulcerated. Dense lymphoplasmacytic infiltrate was diffusely present in the subepithelial tissues, and a focal area of epithelium had a collection of Rushton bodies. The cyst epithelium was diffusely positive for the immunostain of PCNA, but was sparse for the immunostains of β-catenin, E-cadherin, and BCL-2. The facts of easy fragility and frequent ulceration of cyst epithelium may imply the hematogeneous influence of Rushton body formation in the luminal side of cyst, and the elevated protein expression of PCNA and the reduced protein expression of β-catenin, E-cadherin, and BCL-2 may directly indicate the abnormal genetic regulation of the cystic epithelium, resulted in the retrogressive changes of hyperkeratinization and dystrophic calcification.

The present case showed the well keratinized Rushton bodies in globular and lamellate pattern, and the layer of lamellate keratinization showed a weak birefringency under polarizing microscope. These findings may imply that the keratinization of Rushton body is homogeneous and weakly transparent similar to the crystallization of enamel matrix. As the keratinized Rushton bodies were already known to be negative for periodic acid Schiff reaction but strongly stained with Masson's Trichrome and orcein13). Therefore,
we supposed that the Rushton bodies were relevant to the secretory materials of enamel matrix from the odontogenic cyst epithelium rather than to some hematogenous products. Particularly, this Rushton bodies contained several calcifying foci stained blue by hematoxylin. In the high magnification under light microscope, granular dystrophic calcification occurred in the basophilic cells in Rushton body with gradual loss of their nuclei. In the immunohistochemistry these keratinized cells were strongly positive for HIF and PARP, and the cyst epithelium near the Rushton bodies was diffusely positive for pAKT and PIM1. Therefore, it was presumed that the keratinized cells were under the hypoxia stress in the luminal side of cyst, and they subsequently underwent the apoptotic processes. Nevertheless, the keratinized cells positive for pAKT and PIM1 may have the potential of odontogenic epithelium to be propagated into the ghost cells of calcifying odontogenic cyst.

In conclusion, the present study histologically demonstrated the lamellate keratinization and dystrophic calcification of Rushton bodies in a dentigerous cyst, which were supposed to be aberrant products of retrograding keratinocytes slowly undergoing apoptotic progresses similar phenomena to the ghost cells in calcifying odontogenic cyst and pilomatricoma. Taken together, we suppose that the Rushton body formation could be a unique phenomenon of pathogenetic alterations, which has a potential to be transformed into benign diseases.

IV. References

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