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ORIGINAL ARTICLE

Secretory cells in adenomatoid odontogenic tumour: tissue induction or metaplastic mineralisation?

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OBJECTIVE: To undertake a detailed histological investigation of a large series of adenomatoid odontogenic tumours (AOT) to document the frequency and histomorphology of secretory cells which might indicate an inductive capacity.

MATERIALS AND METHODS: Haematoxylin and eosin stained sections of 51 cases of AOT were reviewed. Selected cases were stained with periodic acid-schiff (PAS) and Congo red.

RESULTS: In five cases, secretory structures with a circular arrangement of tall columnar cells secreting enameloid-like matrix material were identified. Such structures have only very rarely been identified in AOT and their frequency, distribution and morphology have not been adequately documented.

CONCLUSIONS: We have documented the presence of tall secretory columnar cells, arranged in a circular configuration actively secreting enameloid-like material and believe that such an ordered arrangement of secretory cells is more likely a result of tissue induction rather than metaplasia. The origin of these secretory structures from the pseudo-ductular component is unlikely but cannot be ruled out.

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Introduction

The adenomatoid odontogenic tumour (AOT) has long been the subject of controversy with regard to its nature and classification. In the second edition of the WHO Histological Typing of Odontogenic Tumours (Kramer *et al*, 1992), the AOT was classified under the subsection of tumours showing varying degrees of inductive change. Recently, however, in a revision of the WHO classification, it was stated that the presence of dental hard tissue within the AOT was not due to induction but was rather a metaplastically produced mineralisation (Philipsen and Reichart, 2002) and it was recommended that the AOT be re-classified with those odontogenic tumours that arise from 'odontogenic epithelium with mature fibrous stroma, odontogenic ectomesenchyme not present'. The authors argued that the AOT was not characterised by having a stroma composed of odontogenic ectomesenchyme, but that the rather scant stroma was that of a mature fibrous variety and thus was not of a type that could lead to inductive phenomena (Philipsen and Reichart, 2002; Philipsen and Nikai, 2005). Their recommendation was partly based on the work of Gao *et al* (1997), who in an immunohistochemical study had found no evidence of bone morphogenetic proteins (BMPs) in the AOT while tumours such as ameloblastic fibrodentinoma and compound odontoma, amongst others, which show inductive phenomena, were positive for BMPs. In contrast, the expression of BMPs in odontogenic tumours has also been studied by Kumamoto and Ooya (2006) using RT-PCR and immunohistochemistry. They demonstrated reactivity for BMPs, bone morphogenetic protein receptors and core-binding factor-α1 in both epithelial and mesenchymal cells in tooth germs and epithelial odontogenic tumours including AOT and concluded that BMPs and their associated molecules might play a role in the cytodifferentiation of normal and neoplastic odontogenic epithelium via epithelial – mesenchymal interactions. This re-classification of the AOT has once again kindled the debate as to the nature of the cellular events, which might lead to dental matrix material deposition in this tumour. The aim of this study was to undertake a detailed histological investigation of a large series of AOTs to document the frequency, distribution and morphology of secretory cells, which might indicate an inductive capacity.

Materials and methods

Fifty-one cases of AOT were retrieved from the archives of the Division of Oral Pathology, University of the

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Witwatersrand. Representative haematoxylin and eosin (HE) stained sections were reviewed to identify cases with dental matrix material. Those cases in which dental matrix material other than hyaline droplets was identified were also stained with PAS and Congo red to facilitate histological evaluation. The frequency, nature, distribution and morphology of secretory cells were studied and recorded.

Results

The histological structure of the AOT is very well described and it is not our intention to repeat this information. Suffice to say that there is little, if any, variation in the epithelial configuration with a nodular arrangement of sheets of epithelial cells, layered rosettes, pseudo-ductular structures of varying sizes and thin cords of epithelial cells arranged in a lace-like pattern surrounding a loose, oedematous and haemorrhagic stroma. In a few cases, islands of squamoid appearing epithelial cells are present resulting in a calcifying epithelial odontogenic tumour (CEOT)-like appearance (Miyake *et al*, 1996). The stroma is extremely scanty. Variations do however occur in the extracellular deposits. These either take the form of small widely scattered uncalcified hyaline droplets, small calcified foci embedded in cellular nests, larger eosinophilic amorphous globular masses, dystrophic calcification and round secretory structures in which dental matrix material is surrounded by a single layer of tall secretory columnar cells arranged in a circular or duct-like configuration (Figure 1a, c, d, e).

The hyaline droplets were numerous, small and irregular in shape and were present in nearly all of the 51 cases, being widely scattered throughout the epithelium except in the lace-like areas. They stained positively with the PAS stain. The smaller foci of calcified dental matrix material were present within solid nests of tumour cells in only 15 cases. In these instances, no secretory activity of the adjacent cells could be identified nor were there any columnar cells adjacent to this material.

The large eosinophilic amorphous globular masses were also present in the same 15 cases and consisted of numerous extracellular deposits of varying size and shape. Small clusters of epithelial cells were intimately associated with these deposits, surrounding or within the globular masses, which stained intensely with the PAS stain and showed focal Congo red positivity with green birefringence when viewed with polarised light. Again, no secretory activity of the epithelial cells was evident. There were no columnar cells adjacent to or in close association with the globular masses. In five of these cases, circular structures were identified which consisted of a single row of tall columnar cells with abundant eosinophilic cytoplasm and basally located nuclei, which were arranged in a circular or duct-like configuration (Figure 1a, c, d, e). These circular structures were abundant in one case and scanty in four cases. The columnar cells resembled secretory ameloblasts and were clearly secreting enameloid-like matrix material, which also stained intensely with the PAS stain (Figure 1b, e, f). The secretory product was Congo-red negative. The layer of columnar cells was not always complete, being continuous with smaller cuboidal cells, which had still to differentiate (Figure 1a). Enameloid-like matrix material was present in the middle of these secretory structures.

Discussion

The presence of round or circular secretory structures surrounded by tall columnar cells showing secretory activity has only rarely been identified in AOTs and their frequency, distribution and morphology have not been adequately described. In their review, Philipsen and Reichart (1998) mention the presence of tall columnar cells resembling ameloblasts adjacent to small and large masses of calcified bodies or globules but do not provide any further details. The presence of tall columnar cells in AOTs is referred to by Philipsen *et al* (1991), in their review of 499 cases and is illustrated by Philipsen and Nikai (2005) in the new WHO publication but these cells are not associated with secretion of dental matrix material. Reference to tall columnar cells that are associated with dentinoid and sometimes also enameloid deposition in lesions that show histological features suggestive of AOT, has been made by several other authors (Dunlap and Fritzlen, 1972; Orlowski *et al*, 1991; Nomura *et al*, 1992; Tajima *et al*, 1992; Takeda, 1995; Allen *et al*, 1998; Vargas *et al*, 2006). However, these cases generally lack the classic clinico-pathological characteristics of AOT, occurring in very young, or in much older patients, often in the mandibular third molar region, are often not associated with unerupted teeth and some have attained a very large size; some exhibit aggressive behaviour. In our opinion, considerable doubt exists as to the true nature of some of these lesions.

Allen *et al* (1998) described their cases as adenomatoid dentinomas, a previously uncharacterised entity, while Vargas *et al* (2006) proposed the term adenomatoid odontogenic hamartoma. They point out that the cases reported by Dunlap and Fritzlen (1972), Orlowski *et al* (1991), Tajima *et al* (1992) and Allen *et al* (1998) in fact represent examples of this entity. The case reported by Nomura *et al* (1992) appears to represent an adenoid-ameloblastoma with dentinoid or odontome formation and was treated by jaw resection, while the case reported by Takeda (1995) does have the features of an AOT unusually showing both induction of osteodentine and abortive enamel. We were only able to find one publication, which illustrates secretory activity by tall columnar cells resembling ameloblasts identical to those, which we have described (Takata *et al*, 2000a). These authors showed that the secretory product stains positively for enamelysin, as do the surrounding columnar ameloblast-like cells, although with decreased intensity.

Enamelysin is a tooth specific protease, which is secreted by differentiating ameloblasts into the enamel matrix where *in vivo* experiments have shown that it will

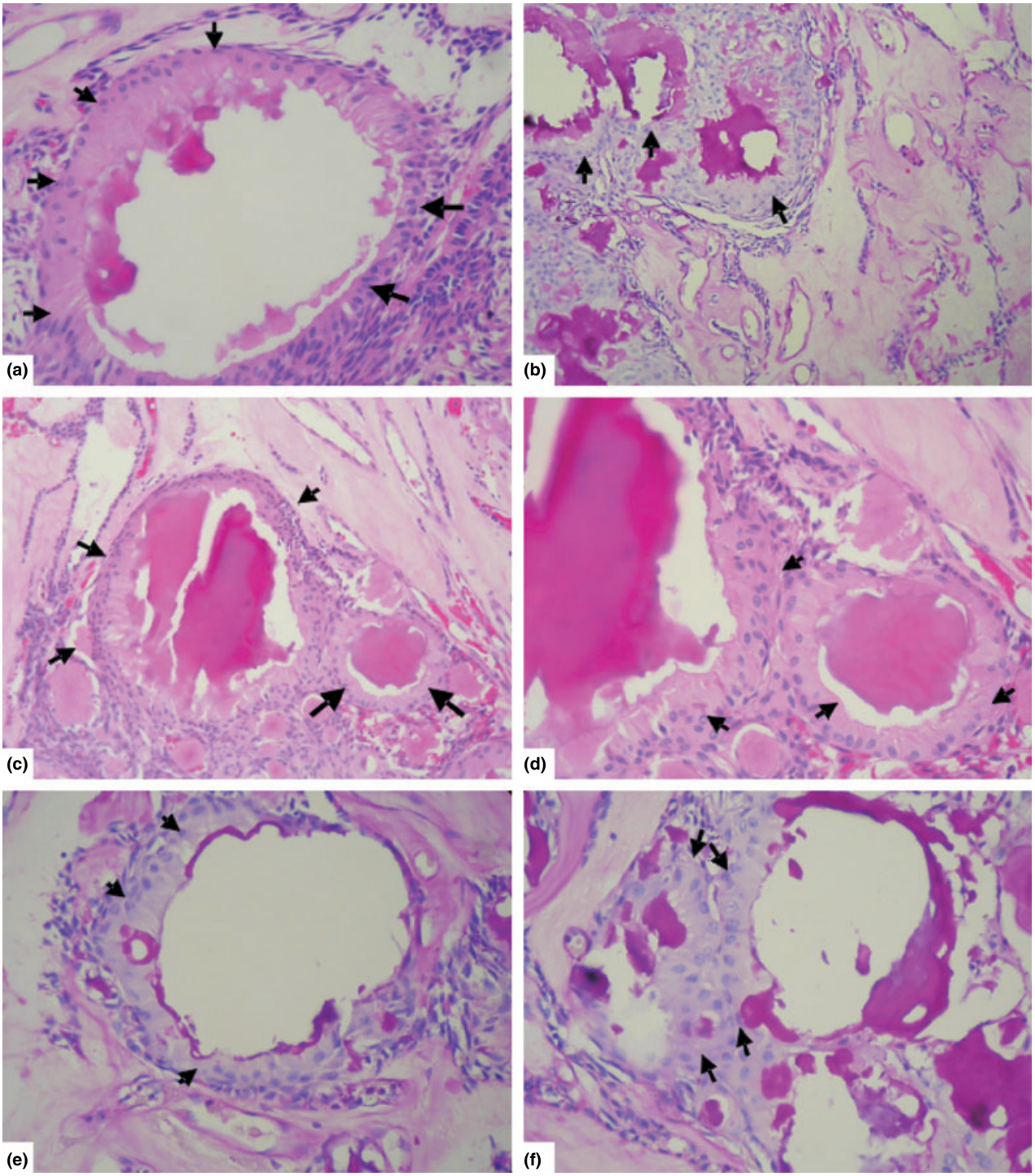

Figure 1 (**a**) The cells on one side of the round secretory structures are fully differentiated and actively secreting (arrowed) while on the other side, they are less differentiated (bold arrows) [haematoxylin and eosin (HE), original magnification ×40]. (**b**) Several circular structures (arrowed) consisting of a single layer of peripherally palisaded tall columnar cells surrounding a central lumen and secreting PAS positive enameloid-like matrix material (PAS, original magnification ×20). (**c**) In the larger secretory structure (arrowed) the row of columnar cells is incomplete while in the smaller structure (bold arrows) the tall columnar cells completely surround the newly formed enameloid (HE, original magnification ×20). (**d**) Higher power view of previous case showing enameloid, basally placed nuclei, eosinophilic homogenous cytoplasm and resemblance of these cells (arrowed) to actively secreting ameloblasts (HE, original magnification ×40). (**e**) Another case showing a single large secretory unit. Note the secretory activity of the tall columnar cells (arrowed) (PAS, original magnification ×40). (**f**) Several round secretory structures lined in part by tall columnar cells showing secretory activity (arrowed) (PAS, original magnification ×40)

cleave amelogenin. No positive staining reaction has been seen in other mineralised tissues such as dentin, cementum or bone. Odontogenic tumours show increased immunostaining of enamelysin over that observed in normal tissue (Takata *et al*, 2000a).

It is interesting to speculate on whether there is any relationship between the eosinophilic globular masses and round secretory structures, which we have identified as they were present in the same cases. It is possible that the tall columnar cells having completed their function disappear leaving behind only the globular masses. Further studies comparing the structure and composition of the dental matrix material in the globular masses and round secretory structures are necessary. On one hand, enamelysin has been identified at both the periphery of the globular masses and in the secretory product of the round secretory structures supporting the possibility of a common origin, but on the other hand, the globular masses contain an admixed amyloid-like material and possibly dentinoid (Lee, 1974; Hatakeyama and Suzuki, 1978; El-Labban, 1992; Philipsen and Reichart, 1996; El-Labban and Lee, 1998) while the secretory product in the secretory structures consists only of enameloid. The AOT is characterised histologically by numerous pseudo-ductular structures of varying size, which consist of a single layer of palisaded columnar non-secreting cells surrounding a central lumen or microcystic space. The round secretory structures which we have described should not be confused with these pseudo-ductular structures in which the cells are usually not as tall, have a more basophilic cytoplasm and are not associated with dental matrix material deposition. The question must however be asked as to whether these secretory structures might be derived from the pseudo-ductular component. While on one hand, the circular or duct-like arrangement around a central lumen is virtually identical for the two structures, on the other hand, the cells are morphologically quite different with the pseudo-ductular lining cells being shorter and having a more centrally placed nucleus and a more basophilic cytoplasm without showing secretory activity. In the secretory structures, the cells are taller with a more basally placed nucleus and homogenous eosinophilic cytoplasm and demonstrate secretion of enamel matrix. It could however be argued that as a result of induction, the 'duct' lining cells could elongate and differentiate into tall secretory columnar cells but there is no evidence to support such a possibility.

In considering the histogenesis of the pseudo-ductular structures, Shear (1962) wrote that the so called 'ducts' represented invaginations of odontogenic epithelium that carried with them ectomesenchymal stroma and that much of the stroma cut off from its blood supply underwent atrophy or necrosis while the surviving stroma, mostly at the periphery of the pseudo-ductular structures, retained its inductive capacity and induced the columnar cells to lay down predentine matrix. Ultrastructurally, it has been shown that the inner surface of the duct-like structures contains basal lamina-like material and a granular deposit, which has been regarded as a product of secretory activity by the cells lining the ducts (Schlosnagle and Someren, 1981; Poulson and Greer, 1983). Further support for a secretory function for these cells has been the immunohistochemical finding of amelogenin strongly localised in the cytoplasm of the tall columnar cells and enamelin, which is localised within the luminal space of the duct-like structures (Murata *et al*, 2000). In addition to these enamel proteins' extracellular matrix (ECM) components such as laminin, heparin sulphate, proteoglycan, fibronectin and collagen lV and V have been demonstrated within the luminal space and along the inner rim of the duct-like structures (Murata *et al*, 2000). This colocalisation of enamel proteins and ECM components suggests that the columnar lining cells at one time differentiated towards ameloblasts but failed to mature further because of increased production of ECM molecules.

Various forms of extracellular deposits characterise the AOT and there is substantial evidence to show that most represent dental matrix material or more specifically enameloid. The evidence has been obtained from both immunohistochemical and electron-microscopic studies. Enamel proteins such as enamelin, amelogenin, sheathlin and enamelysin have been identified immunohistochemically in the hyaline droplets, in the smaller calcifications within the solid nests of cells, at the periphery of the larger amorphous calcifications and sometimes in the tumour cells associated with these deposits (Mori *et al*, 1991; Saku *et al*, 1992; Takata *et al*, 2000a,b), while in the round secretory structures enamelysin has been identified in the periphery of the secretory material and in the adjacent tall columnar cells (Takata *et al*, 2000a). The round secretory structures were not represented in the previously mentioned studies, so it is not known whether other enamel proteins are also present, but this is likely to be the case.

In conclusion, we have documented the presence of tall secretory columnar cells, arranged in a circular configuration actively secreting enameloid-like material and believe that such an ordered arrangement of secretory cells is more likely a result of tissue induction rather than metaplasia. This postulate is supported by the recent demonstration of BMPs, and their associated molecules in AOTs thus pointing to an induction capacity (Kumamoto and Ooya, 2006). Further studies will be necessary to confirm the nature and origin of these secretory cells and of the product they are producing.

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