Ultrastructure of ameloblastoma and its variants

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Abstract
Ameloblastoma is a benign, locally aggressive, slow-growing neoplasm derived from the epithelial odontogenic tissues, which are part of the tooth-forming apparatus. It exhibits microscopic diversity which occurs either in a single pattern or in multiple patterns. There are very few reports available on ultramicroscopy regarding ameloblastoma. This paper aims at discussing and correlating the ultrastructure of ameloblastoma with the histological types for a proper understanding of its pathogenetic mechanism and thereby helping us to diagnose for a better and appropriate patient management.

Introduction
Odontogenic tumors (OT) are a group of lesions arising from the tooth-producing tissues or its remnants.[1] Ameloblastoma is a benign, locally aggressive, slow-growing neoplasm of odontogenic origin involving predominantly mandible (80%) compared to maxilla.[2-4] It is an epithelial OT of the jaw which exhibits microscopic diversity.[5] It was first discussed in 1868 by Broca and reported about 1-2% of all tumors of the jaws.[6]

The World Health Organization (WHO) defines it as locally invasive polymorphic neoplasm consisting of proliferating odontogenic epithelium, which usually has a follicular or plexiform pattern lying in a fibrous stroma.[6] Ameloblastic epithelium has been hypothesized to arise from (1) cells from the rests of enamel organ, but also from, (2) cells of the sheet of Hertwig’s or epithelial cell rests of Malassez, (3) epithelium of an odontogenic cyst, particularly a dentigerous cyst, (4) basal cells of the oral mucosa, and (5) heterotopic epithelial from other parts of the body.[5]

Clinically, it presents as a painless swelling, ulceration, and periodontal disease.[6] Usually, radiographs appear as a well demarcated unilocular or multilocular radiolucency that may or may not be associated with unerupted tooth.

Histopathologically, islands of tumor consist of a central mass of loosely connected polyhedral cells mimicking stellate reticulum surrounded by a layer of cuboidal or columnar cells.[9]

Since ameloblastoma shows histologic patterns which vary greatly, a number of subtypes can be distinguished.[10] Among these are follicular, plexiform, acanthomatous, pseudoglandular, cystic, desmoplastic, basal cell, granular cell, mucous cell, hemangiomatous, extragnathic and keratoameloblastoma and papilliferous ameloblastomas.[11,12]

The wide variation in histologic findings associated with the ameloblastoma is reflected in its ultrastructure and thus aid in treatment planning.[13,14] There are very few reports available on ultramicroscopy regarding ameloblastoma. This paper aims at discussing the ultrastructure of ameloblastoma as well as its differences between the variants, which could be correlated to the histological differences thereby helping us in better understanding of its pathogenesis.
Moe et al. were the first to acknowledge that the peripheral cells of the solid, follicular ameloblastoma (FA) were ultrastructurally similar to inner enamel epithelium. This viewpoint was subsequently supported by several studies.\(^\text{[16]}\)

Kim et al. found that, in addition to the strong resemblance of the columnar cells of the tumor to the cells of the inner enamel epithelium at an early stage of differentiation, the star-shaped cells of the tumor epithelium mimics the stellate epithelium of the normal enamel organ in many aspects.\(^\text{[19]}\)

In a transmission electron microscopy study of 12 plexiform and 9 FA, Nasu and Ishikawa found that the follicular variant composed of two cell populations, one resembling the stellate reticulum, and the other resembling the inner enamel epithelium of the normal enamel organ. The plexiform variant, on the other hand, mimics squamous epithelium.\(^\text{[15]}\)

Lee et al. described the ultrastructure of a “simple” ameloblastoma (SMA), the occurrence of cells possessing single cilia which arose from a basal body and occasional cells containing langerhans granules. In addition, the tumor stroma contained oxytalan fibers.\(^\text{[14]}\)

Mucin-producing cells were reported by Mincer and McGinnis to occur in a multicystic ameloblastoma.\(^\text{[10]}\) The discovery of these cells had earlier led Hodson to propose a subclass of mucoepidermoid ameloblastoma.\(^\text{[12]}\)

The occurrence of intracytoplasmic desmosomes in a maxillary ameloblastoma was reported by Cutler.\(^\text{[20]}\)

The occurrence of so-called hyaline bodies, ultrastructurally similar to those found in odontogenic cyst epithelium and cyst walls, have been demonstrated. The stroma has described as containing fibroblasts and collagen fibers but Rothouse et al. also demonstrated the occurrence of myofibroblasts that showed the formation of plaque-like structures on extended cell processes, which the authors identified as intracellular septate junctions.\(^\text{[21]}\)

Smith and Bartov confirmed the finding of abundant myofibroblasts in a case of recurrent SMA.\(^\text{[22]}\)

**Discussion**

The ameloblastoma is a rare OT composed of enamel organ-like tissue which does not differentiate to the level of enamel production.\(^\text{[23]}\)

The wide microscopic variation associated with ameloblastoma is in accordance with its ultrastructure, and these variations are indicative of its degree of differentiation mimicking the odontogenic apparatus.\(^\text{[12]}\)

**Follicular and Plexiform Ameloblastoma**

According to the 1992 WHO definition, an SMA is “a polymorphic neoplasm consisting of proliferating odontogenic epithelium, which usually has a follicular or plexiform pattern, lying in a fibrous stroma.”

The definition used by present authors is as follows:

“A neoplasm consisting of proliferating odontogenic epithelium, usually occurring in two main patterns. In the follicular type of growth, the tumor consists of enamel organ like islands or follicles of epithelial cells, while in the plexiform type the epithelium forms continuous anastomosing strands. In both types of tumor, islands or follicles of odontogenic epithelium were seen in a connective tissue stroma. In general, a tumor shows one or the other patterns throughout. However, not infrequently both patterns are present in the same tumor.”

**Light microscopy**

The islands of tumor consist of a peripheral layer of cuboidal or columnar cells resembling ameloblasts or preameloblasts enclosing a central mass of loosely connected polyhedral cells resembling stellate reticulum [Figure 1a and b]. Cystic degeneration commonly observed within the epithelial odontogenic islands.\(^\text{[24]}\)

In the plexiform pattern, the tumor cells are arranged as a network bound by a layer of cuboidal to columnar cells and between these layers may be found cells mimicking stellate reticulum [Figure 1c]. Cyst formation is also common.\(^\text{[24]}\)

**Electron microscopy**

Electron microscopy discusses epithelial elements, connective tissue elements, and its junction.

Studies done by Chawla et al., have observed that ultramicroscopically, the FA consist of two cell types, the peripheral cells and central cells, whereas the plexiform ameloblastoma contains a single cell population.\(^\text{[25]}\)

The peripheral cells were tall columnar with irregular nuclei containing prominent nucleoli. Mitochondria were found swollen. Tonoilaments, rough endoplasmic reticulum (RER), and ribosomes were seen throughout the cytoplasm. A large number of secretory granules and coated vesicles along with Golgi complex were noticed indicating that the cells were in early secretory ameloblast stage [Figure 2a-c].

Spindle to stellate shaped central cells was joined with adjacent cells by desmosomes. The cells consisted of irregularly contoured nuclei containing patches of chromatin.

![Figure 1: (a and b) Follicular ameloblastoma (H & E 100×). (c) plexiform ameloblastoma](image-url)
and nucleoli. RER, randomly distributed mitochondria and tonofilaments were seen in cytoplasm. Numerous number of secretory granules and coated vesicles were also present [Figure 2d and e].

Follicular and plexiform ameloblastomas showed marked difference in ultrastructure. Epithelial cells of FA were more tightly adhered compared to plexiform type. The cells of the follicular type contained many lysosomes, whereas plexiform cells consisted of numerous vacuoles and tonofilament bundles joined by desmosomal attachment which mimics squamous epithelium [Figure 2f and g].

The connective tissue stroma constitutes of stellate cells and fibers. The cytoplasm showed RER, Golgi apparatus, and swollen mitochondria [Figure 2h]. Many transverse and longitudinal sections of collagen fibers were also seen [Figure 2i].

Epithelial cells adjacent to the interface contained numerous mitochondria. Dentinal or enamel matrix secretion was not seen indicating that the tumor cells were in early developmental stage [Figure 2j and k].

The histological variation between the types represents the differentiation tendency of dental lamina remnants during the transformation of neoplasm.

**Granular Cell Ameloblastoma**

The term granular cell is applied when the tumor, most often of follicular type, shows an extensive granular transformation of stellate reticulum like cells in the center so that it takes on a granular appearance. These cytoplasmic granules represent lysosomal aggregation. It was found that this variant accounted for 5% of all ameloblastomas.

In OTs, these granular cells have been discussed in granular cell ameloblastoma and granular cell ameloblastic fibroma. The granular cells in normal and neoplastic tissues include oxyphil (parathyroid), oncocyes (salivary glands), Hurthle or Askanazy cells (thyroid) and granular cell myoblastoma, granular cell ameloblastoma, granular cell ameloblastic fibroma and congenital epulis. Studies have proved that the granules seen in these cells are

![Figure 2:](image-url)
mitochondria. Ara et al. reported that there is increased synthesis of β-catenin and Wnt-5 in GCA and their secretion is impaired resulting in accumulation as autophagosomes.[26]

**Light microscopy**

The tumor was composed of numerous neoplastic epithelial odontogenic islands composed of granular transformation of central stellate reticulum like cells bounded by peripheral columnar or cuboidal cells [Figure 3]. The cytoplasmic granules vary from fine to coarse in nature and in some cells eosinophilic rounded, homogenous masses which resembles inclusion bodies larger than the coarse granules. The nuclei of granular cells vary in position and are often crescent shaped. Cell outlines are usually clear with no intercellular bridges.[27]

**Electron microscopy**

The peripheral cells of tumor islands are separated from the stroma by a basement membrane, 620-930 Å thick. The tall columnar peripheral cells are in close apposition with each other with microvilli projecting into the intercellular spaces. The nuclei of the cells showed an irregular invaginated contour, uniformly distributed with one or two nucleoli. The most characteristic cytoplasmic feature is the presence of numerous homogenous amorphous osmiophilic granules. Some contained concentric laminated membranes (“myelin figures”); others composed of bundles of fine parallel membranes running in different direction resembling fingerprints. Apart from the granules, the cytoplasm contains mitochondria, Golgi complexes and pleomorphic vacuoles limited by a single membrane. Electron microscopic studies have proved that the cytoplasmic eosinophilic granularity in all these cells is mitochondria. On the contrary, the granularity in myoblastoma is not due to abundant mitochondria but due to numerous vacuoles and pleomorphic osmiophilic granules, interpreted by some researchers as lysosomes.[27] In histochemical and ultrastructural studies of eosinophilic cells, when stained with enzyme stains to demonstrate mitochondria, the granular cells of ameloblastoma showed only rare positive cytoplasmic granules suggesting that the granules were not mitochondria. Based on the polymorphism of these granules, their limitation by a single membrane, their positive reaction with Periodic acid–Schiff stain and histochemically, high demonstrable levels of acid phosphatase activity, it strongly proved that they are lysosomes which accumulated due to impairment of an enzyme or protein.[26] The strong expression of laminins 1, 5 and fibronectin reported in granular cells of ameloblastoma provided some insight into this rare tumor.

**Unicystic Ameloblastoma**

It is a special type of ameloblastoma described in 1977 by Robinson and Martinez.

**Light microscopic findings**

Histologic UA classification (Ackermann et al.)

1. Luminal UA
2. Luminal and intraluminal
3. Luminal, intraluminal and intramural
4. Luminal and intramural.[28]

The criterion for UA is the presence of a single cystic sac with an odontogenic epithelium often accompanied by an innocuous epithelium of varying histologic appearance that may mimic dentigerous or radicular cystic lining.

**Luminal**

The epithelial lining consists of cuboidal or columnar basal cells with polarized nuclear palisading, hyperchromatic nuclei, cytoplasmic vacuolization with intercellular spacing and subepithelial hyalinization [Figure 4a].

**Intramural**

A thick fibrous connective tissue encloses the tumor island giving it a nodular appearance. A pronounced cystic degeneration is also observed in this subgroup [Figure 4b and c].

**Figure 3:** Granular ameloblastoma (hematoxylin and eosin)[27]

**Figure 4:** (a-c) Cystic ameloblastoma. EM showing a cyst lumen (H & E, ×110), (d) cystic ameloblastoma. EM of mucus-like cells showing intracytoplasmic “lumen” formation (~22,000)[29]
Electron microscopy
Studies done by Mincer and McGinnis\(^{(19)}\) had described cystic lumen which was lined by squamatoid epithelial cells having a foamy, basophilic cytoplasm which contained relatively few organelles. These individual cells among the lining epithelium which at the light microscopic level resembled mucin-producing cells. Such kind of cells were noticed in previous microscopic studies of odontogenic cysts and tumors. Discovery of these cells led Hodson\(^{(12)}\) to propose a subclass - mucoepidermoid ameloblastoma. Cytoplasmic vacuoles were haphazardly dispersed, the origin of which could be extrapolated to dilated endoplasmic reticulum, secretory vacuoles or invaginations of the plasma membrane. These membrane-bound vacuoles contained flocculent granules arranged in a reticular pattern. These differed from mucus secreting granules in that there was a manifestation of nonspecific cellular degeneration. These cystic spaces are bounded by short villi and are surrounded by a periluminal rim of tonofilaments. These cells also contained multivesiculated lysosomes along with dilated Golgi apparatus [Figure 4d].

Pseudoglandular Ameloblastoma
Highly cellular tumor composed of interlacing strands, nests and trabeculae of epithelium within which was enmeshed a scant connective tissue stroma.

Ultrastructurally, Mincer and McGinnis\(^{(19)}\) had studied a case where the cells were in close approximation with each other and showing minimum intercellular spaces. They also demonstrated numerous osmiophilic autophagolysosomes (cytosegrosomes). The nucleoplasm was less dense as compared to other variants with a more regular nuclear outline. One or two nucleoli were commonly seen. Irregular masses of heterochromatin were scattered throughout the nucleus, with occasional occurrence of electron dense inclusions (1-10) within the peripheral layer. These were virion-like structures of approximately 450Å in diameter and with a clear zone of approximately 125Å thickness. Subepithelial hyalinization was ultrastructurally seen as a dense band of collagen fibrils beneath the basal lamina. This hyaline band which Gorlin et al. characterized after histologic study as possibly an abortive attempt at dentin formation apparently represents tumor desmoplasia [Figure 5a-d].

Peripheral Ameloblastoma (PA)
PA is also known as the extraosseous ameloblastoma. It is firm, sessile, painless exophytic growth, color varies from normal or pink and red or dark red. It is generally described as an exceedingly rare lesion.

Light microscopic findings
The tumor follicles of epithelium exhibit palisading of columnar cells, but a stellate reticulum is seldom conspicuous. A basaloid lesion without the classical follicular component but often exhibit acanthomatous areas. Some follicles contain stellate reticulum like cells centrally or show cystic/acanthomatous change. There was a marked mixed inflammatory infiltrate in lamina propria and submucosa beneath the zone of altered covering epithelium [Figure 6].

Ultrastructural findings
Tumor follicles: The peripheral cells of the follicles are columnar in shape with ovoid nucleus. The cells are in close apposition to each other. Cytoplasm contained abundant of tonofilaments and mitochondria scattered throughout. The basal surface of the peripheral cells is firmly attached to a bilamellar basal lamina by hemidesmosomes. The stellate cells had few demonstrable organelles in their cytoplasm, and their nuclei showed more convolutions.\(^{(19)}\)

Conclusion
Ameloblastoma is a rare OT with histomorphologic diversity. There are very few reports available on ultramicroscopy regarding
ameloblastoma. Thus, this article is an attempt to compile all the limited literature available for a proper understanding of its pathogenetic mechanism and thereby helping us to diagnose for a better and appropriate patient management.

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