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Ultrastructural observations on luminal structures of pleomorphic adenoma of parotid and submandibular salivary glands of man

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Summary. Luminal structures found in salivary pleomorphic adenomas consisted of lumina surrounded by epithelial cells that varied from being packed together to being widely separated except at the luminal margin. Communication between lumina and the surrounding stroma was occasionally seen. Secretory material and cellular debris were seen in lumina, invaginations of the luminal surfaces of periluminal cells, associated vesicles, and vacuoles. Secretory granules, lysosomes and lipofuscin were seen in periluminal cells. Secretory material and debris from necrotic periluminal cells appear to accumulate in lumina, and to be endocytosed and degraded lysosomally by periluminal cells. The finding of communications between lumina and the surrounding stroma suggests that the stromalization of the epithelium includes the luminal structures. The present investigation supports the hypothesis that many of the cellular features of the pleomorphic adenoma relate to the microenvironment.

Key words: Mixed salivary gland tumour – Salivary gland neoplasms – Parotid neoplasms

Introduction

The controversy about whether the salivary pleomorphic adenoma is mixed only in appearance or in both appearance and origin appears to have been settled by morphological investigations that established that the tumour is epithelial and forms regions of a stromal appearance by the secretion of a typically stromal matrix (Harrison and Auger 1982; Dardick et al. 1983a, b; Erlandson et al. 1984; Auger 1985; Lam 1985; Palmer et al. 1985; Caselitz 1986; Seifert et al. 1986). Although the fascination of the stromal development has overshadowed the epithelial luminal structures, some ultrastructural investigations have shown them to be worthy of greater consideration (Cutler et al. 1974; Harrison and Auger 1982; Tandler and Erlandson 1983; Auger 1985), and the present communication describes some features that may help towards our understanding of the development of these structures.

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Materials and methods

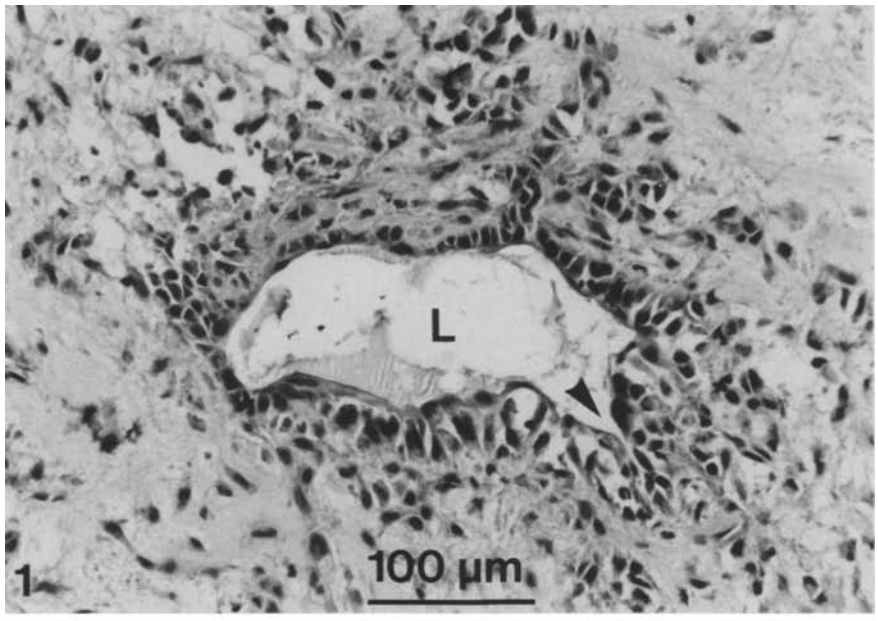
Seven pleomorphic adenomas, 6 of the parotid and 1 of the submandibular salivary gland, were obtained immediately upon surgical removal under general anaesthesia.

For lightmicroscopy, pieces were fixed in 4% formaldehyde with 2% calcium acetate for at least 1 day and were subsequently embedded in wax. Sections were stained with haematoxylin and eosin.

For electronmicroscopy, pieces of less than 2 mm greatest dimension were immersed in a solution (Karnovsky 1965) containing 5% glutaraldehyde, 4% formaldehyde prepared from paraformaldehyde, 0.05% $CaCl_2$ and 0.08 M cacodylate buffer, pH 7.2, at room temperature for 2 h. This was followed by rinsing in 7.5% sucrose in 0.05 M cacodylate buffer, pH 7.2, at 0–4°, osmication, dehydration, and embedding in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

The basic morphology of the luminal structures was as already established (Seifert et al. 1986). They consisted of lumina surrounded by epithelial cells that varied from being packed together to being widely separated except at the luminal margin. The relation of the luminal structures to the surrounding stroma varied from clearly defined to indistinct. Communication between lumina and the surrounding stroma was occasionally seen (Figs. 1, 2).



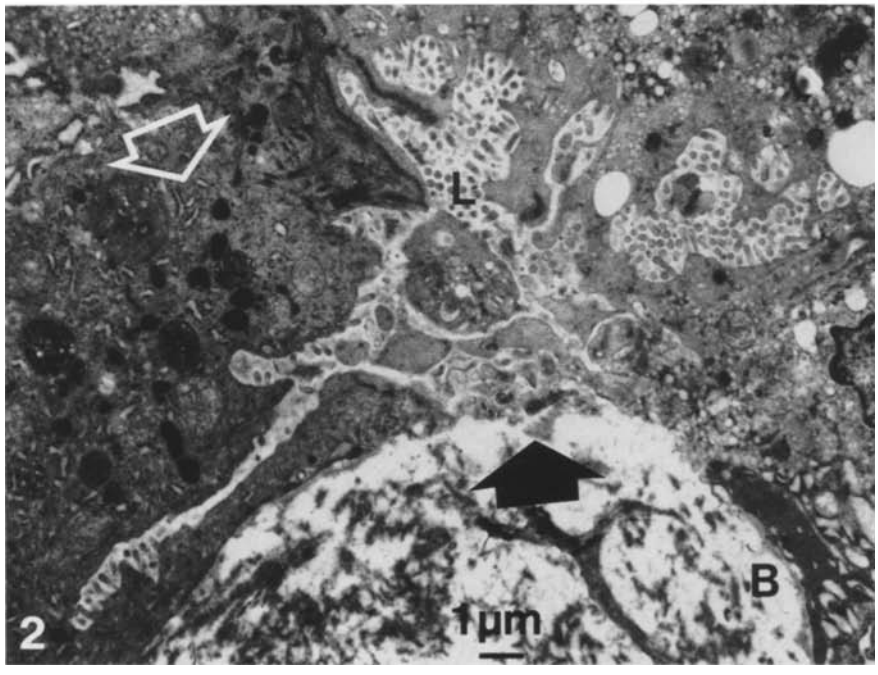


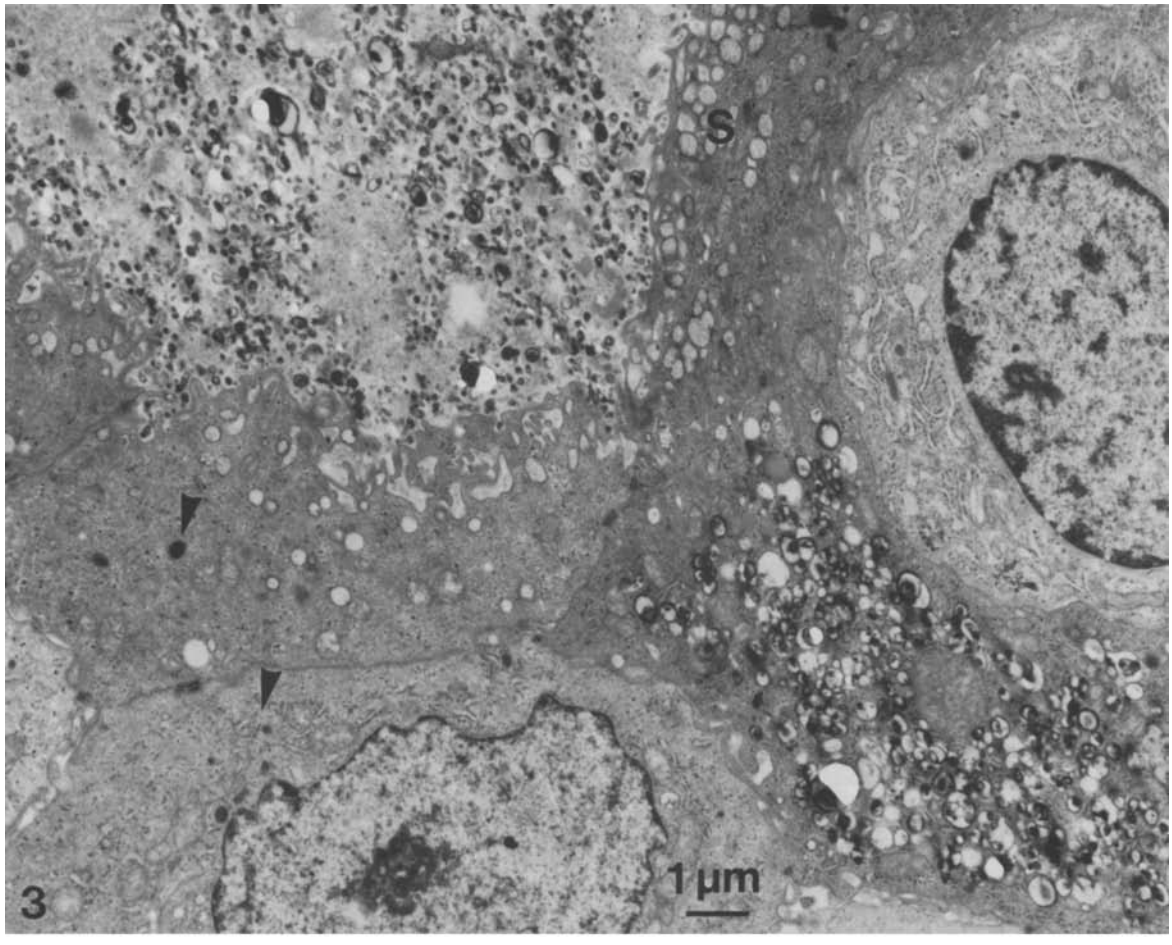
Fig. 1. Lightphotomicrograph of a luminal structure from a pleomorphic adenoma of a parotid gland. The lumen (L) is surrounded by a layer of epithelium that is discontinuous in one region (*arrowhead*) where the lumen communicates with the surrounding myxoid stroma. The epithelium of the luminal structure shows a variable degree of intercellular separation and merges into the myxoid stroma. × 220

Fig. 2. A luminal structure from a pleomorphic adenoma of a parotid gland. The lumen (L) is surrounded by epithelial cells with microvilli and lysosomes (*hollow arrow*). The epithelial cells are joined by tight junctions around the lumen, except in one region (*solid arrow*) where the lumen extends to the stroma. Basement membrane (B) is seen. × 5700

Fig. 3. Part of a lumen and periluminal cells from a pleomorphic adenoma of a parotid gland. The lumen is packed with cellular debris, most of which is membranous. Microvilli are seen. Invaginations of the luminal surfaces and associated vesicles contain debris. The luminal part of one cell also contains many secretory granules (S) that contain secretory material with a reticular pattern. The cell in the lower right corner is packed with membranous debris. Dense lysosomes are seen (*arrowheads*). × 8100

Fig. 4. Part of an epithelial cell in a luminal structure from a pleomorphic adenoma of a parotid gland. Membranous debris is seen within membrane-bound vacuoles. × 26800

Fig. 5. Part of a lumen and periluminal cells from a pleomorphic adenoma of a submandibular gland. The lumen contains dense material that varies from being mottled to homogeneous. Microvilli are seen. Invaginations of the luminal surfaces and associated vesicles and small vacuoles (*arrowheads*) contain similar material to that in the lumen. Four large vacuoles (*asterisks*), one of which is tangentially sectioned, contain mottled material of a similar appearance to that in the lumen. × 6800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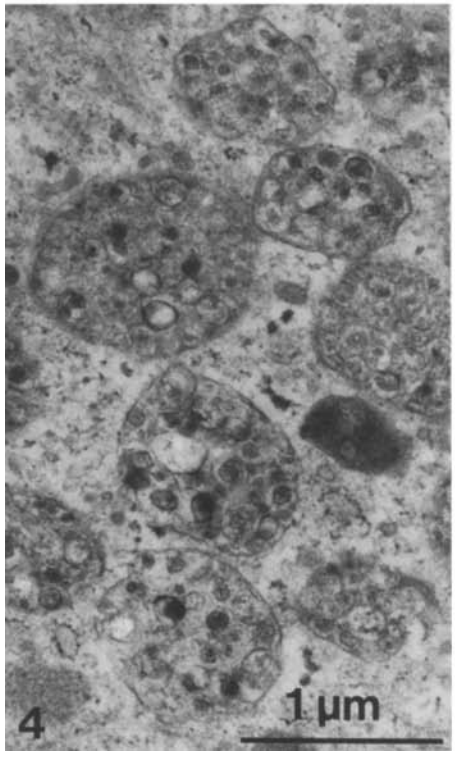
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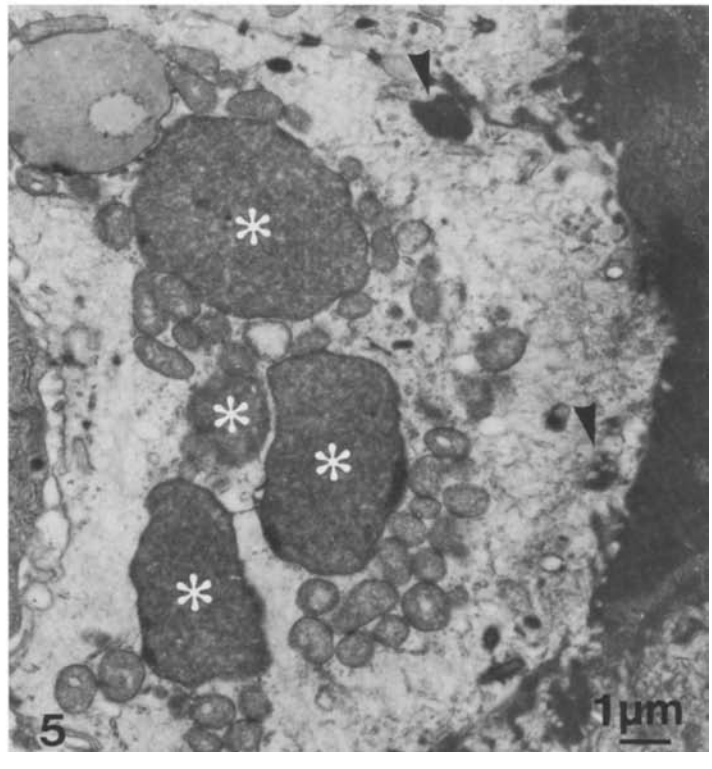
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Much of the luminal contents was relatively amorphous (Fig. 5), of varied electrondensity that sometimes produced a variegated appearance, and resembled the contents of the various components of the secretory granules situated luminally in some of the periluminal cells and reported in detail elsewhere (Harrison and Auger 1989).

A variable amount of cellular debris that was mainly membranous was found in lumina (Fig. 3). Periluminal cells occasionally contained such debris within vacuoles (Figs. 3, 4). Large vacuoles of rounded or oval outline of greatest diameter about 5 μm containing relatively amorphous material of similar appearance to that in lumina were seen occasionally in periluminal cells (Fig. 5). Lysosomes and sometimes lipofuscin were seen in periluminal cells (Figs. 2, 3).

Microvilli were usually seen at the luminal surfaces (Figs. 2, 3, 5). Invaginations of the luminal surfaces and associated vesicles were sometimes seen (Figs. 3, 5), and it could not always be established whether these were secretory granules or endocytotic vesicles.

Discussion

The variation in the arrangement of epithelial cells in the luminal structures from being packed together to being widely separated confirms the investigations that have shown an increasing intercellular accumulation of typically stromal matrix produced by the epithelial cells that eventually isolates them, and the development in the epithelial cells of the morphological characteristics of stromal cells (Harrison and Auger 1982; Dardick et al. 1983a, b; Erlandson et al. 1984; Auger 1985; Lam 1985; Palmer et al. 1985; Caselitz 1986).

The periluminal cells show most ultrastructural similarity to intercalary ductal cells out of the components of the salivary glands, because of the morphology of their secretory granules (Auger and Harrison 1982; Harrison and Auger 1982, 1989; Auger 1985; Harrison et al. 1987), their usually inconspicuous granular endoplasmic reticulum and Golgi apparatuses (Auger 1985; Harrison and Auger 1989), and their distribution of phosphatases (Auger and Harrison 1982; Harrison and Auger 1982; Auger 1985; Harrison et al. 1988).

These morphological and histochemical features indicate that there is a low level of synthesis of secretory granules in the periluminal tumour cells. The morphological similarity between much of the luminal contents and the contents of the secretory granules indicates that the secretory granules are exocytosed into the lumina, possibly without any separate secretion of fluid that would lead to a dilution and decrease of electrondensity. The variegated appearance of some of the luminal contents indicates a lack of mixing of such secretory material. This secretory activity would result in an accumulation of secretory material and an increase of luminal pressure if the lumen were not growing sufficiently rapidly to accommodate it, and the luminal debris, which has been reported previously in pleomorphic adenoma (Tandler and Erlandson 1983) and adenoid cystic carcinoma (Tandler 1971), is possibly the remains of cells damaged by increased luminal pressure.

The situation in the lumina of the pleomorphic adenoma may be comparable to that in the lumina of obstructed salivary glands. An obstructive effect has been produced experimentally by the retrograde infusion of proteins into the parotid gland of rat and was found to result in the endocytosis of the protein by the ducts, and acinar secretory proteins were also found to be endocytosed (Coleman and Hand 1987; Hand et al. 1987). A similar process appears to occur in the luminal structures, in which secretory material is endocytosed from the lumina by means of invaginations and vesicles that fuse to form the large vacuoles, which are similar in appearance to the large endocytotic vacuoles produced in the rat. The luminal debris appears to be similarly endocytosed, although the vacuoles in which it accumulates are smaller. Some of the luminal surfaces and vesicles have been found to contain alkaline-phosphatase activity, which further supports the presence of resorptive processes (Cutler et al. 1974; Harrison and Auger 1982; Auger 1985).

The contents of the endocytotic vacuoles were found to be degraded by lysosomes in the rat (Hand et al. 1987), and this is likely to be the fate of endocytosed material in the periluminal cells. Residues from such lysosomal activity may be responsible for the lipofuscin that is found in the periluminal cells (Buchner and David 1978). Macrophages may also be involved in removing debris and stagnant secretory material from lumina (Harrison 1974).

The fate of lumina in the stromalization of the epithelium of the pleomorphic adenoma does not appear to have been considered previously. The finding of communications between lumina and the surrounding stroma suggests that even luminal structures may succumb to this process.

Tandler and Erlandson (1983) described the presence of giant mitochondria solely in the periluminal cells of a pleomorphic adenoma, and hypothesized that the microenvironment of these cells

was responsible for the cytoplasmic alterations. The present investigation supports this hypothesis and indicates that many features of the periluminal cells relate to the microenvironment.

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