Salivary myoepithelium: distribution, structure, functions and pathologic proliferations

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SUMMARY

Myoepithelial cells are difficult to identify on routine microscopic preparations and before the advent of modern scanning electron microscopic and immunochemical techniques, little was known of their distribution and functions in salivary glands. Acinar myoepithelial cells have been proven to play an important role in the formation and propulsion of saliva, and contraction of intercalated duct associated myoepithelium reduces peripheral resistance by dilating and shortening the ductular portion of the secretory unit. Myoepithelial cells are furthermore responsible for the production of basement membrane proteins and their cilia, which project into invaginations in adjacent secretory cells, may act as salivary chemoreceptors.

The study of myoepithelial participation in pathologic conditions gained momentum by the identification of stem cell populations in mature salivary glands. Myoepithelial differentiation has been proven in neoplasms of intercalated duct origin and although epithelial in nature, they play a central role in the production of mesenchymal stromal deposits in mixed salivary gland tumors.

OPSOMMING

Mioepiteelselle is moeilik aantoonbaar met roetine mikroskopiese tegnieke en voor die era van moderne skandeer elektronmikroskopie en immunositochemiese tegnieke, was baie min bekend oor hulle verspreiding en funksies in speekselkliere. Asinêre mioepiteel speel 'n belangrike rol in die formasie en voortdrywing van speeksel en sametrekking van mioepiteelselle geassosieer met die interkalêre buis, verminder perifere weerstand deur dilatasie en verkorting van die tubulêre gedeelte van die sekretoriese eenheid. Mioepiteelselle is verder verantwoordelik vir die produksie van basaalmembraan proteiene en hulle silia, wat in invaginasies van sekretoriese epiteel projekteer, funksioneer waarskynlik as speeksel chemoreseptore.

Die studie van mioepiteel deelname in patologiese toestande is bevorder deur die identifikasie van stamsel populasies in volwasse speekselkliere. Mioepiteel differensiasie is aangetoon in neoplasms van interkalêre buise oorsprong en alhoewel epiteel van aard, speel hulle 'n sentrale rol in die neerlegging van mesenkiemale stromale weefsel in gemengde speekselklier tumore.

INTRODUCTION

Myoepithelial cells are located beneath the basement membrane of the terminal portion of most exocrine glands. Those surrounding acini of salivary glands are stellate- or star shaped whereas intercalated duct associated myoepithelial cells are spindle shaped and their cytoplasmic processes usually run parallel to the direction of the duct. In view of the presence of prominent cytoplasmic myofilaments, they have long been accepted as contractile in nature and probably play an important role in propulsion of the secretion (Shear, 1966).

Positive identification of salivary gland myoepithelial cells on routine microscopic preparations is unreliable and until recently very little was known of their distribution and functions in salivary glands. Fortunately, modern sophisticated microscopic techniques have resulted in a surge of new information on this cell. Exposure of myoepithelial cells by chemical removal of periacinar connective tissue and basement membrane deposits, a technique which was partially developed in our laboratory (van Niekerk and Raubenheimer, 1986) has made three dimensional scanning electron microscopic examinations possible (Fig. 1).

The difficulty with which myoepithelial cells are identified in salivary glands is compounded by disease processes which disturb glandular architecture. Myoepithelial identification reaches virtually speculative proportions in neoplastic states where all microscopic landmarks are lost and participating cells exhibit various degrees of cytodifferentiation. Fortunately, immunocytochemical identification of myoepithelial cytoplasmic filaments such as cytokeratin, actin (Caselitz *et al*, 1981), myosin (Palmer *et al*, 1985) and S100 protein (Hara *et al*, 1983) have facilitated the study of their participation in pathologic salivary gland conditions. These proteins are unfortunately shared by many other cell types and reliable myoepithelial identifications in diseased salivary

Fig. 1: **Scanning electron micrograph of a periacinar myoepithelial cell (M) of the sublingual salivary gland of a vervet monkey after chemical removal of periacinar connective tissue and basement membrane deposits. (Bar = 10 μm.)**
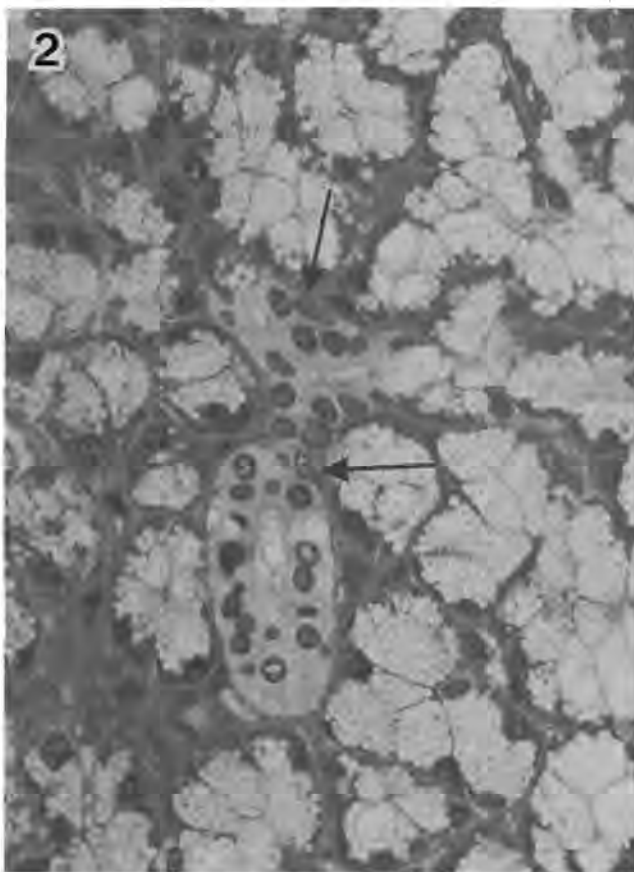

Fig. 2: **High power photomicrograph demonstrating intercalated duct associated myoepithelial cells (arrows) in the parotid salivary gland of the African elephant. Note the absence of myoepithelium on allied acini.**

DISTRIBUTION OF SALIVARY GLAND MYOEPITHELIAL CELLS

Species differences and also differences in the distribution of myoepithelial cells in salivary glands of the same animal exist. The acini of the rat (Redman, Sweney and McLaughlin, 1980), rabbit (Cope, Pratten and Williams, 1976) and African elephant, which secrete a serous (watery) saliva, are devoid of myoepithelial cells and only cytoplasmic processes of intercalated duct associated myoepithelium extend onto the adjacent surfaces of allied acini (Fig. 2). Acinar myoepithelial cells have been identified in the human submandibular gland (Nagai and Nagai, 1985 and Tandler, 1965) and labial salivary gland (Tandler *et al*, 1970) where they cover only a portion of the acinar surface. Myoepithelial cells appear to be more prominent around the acini of palatal mucous glands of humans (Han, Kim and Cho, 1976). The acini of the sublingual glands of the monotreme echnida, *Tachyglossus ucleatus,* which secretes a viscous saliva, are ensheathed by interdigitating myoepithelial processes, almost forming a continuous periacinar muscular coat (Young and van Lennep, 1978). These observations have led to the belief that acinar myoepithelial development is amongst other factors, related to the viscosity of the secretory product. Myoepithelial cells of the retrolingual salivary gland of the hedgehog have an unusual three dimensional configuration, the significance of which is unknown. Many of their cytoplasmic processes are not closely apposed to secretory epithelial cells but pursue a seemingly independant course in the connective tissue (Tandler, 1986).

The processes of intercalated duct associated myoepithelial cells often extend onto the striated duct (Riva *et al*, 1976). Although they are usually fusiform (or spindle shaped) and run parallel to the length of the duct, myoepithelial cells in the parotid of the nine banded armadillo (Ruby, 1978) and retrolingual salivary gland of the European hedgehog (Tandler, 1986) encircle the intercalated duct. In the rat submandibular salivary gland, the intercalated ducts are devoid of myo-

glands is best achieved by a combination of structural, ultrastructural and immunochemical techniques (Raubenheimer, 1986).

Fig. 3: **Transmission electron micrograph of a myoepithelial cell (M) of an elephant submandibular salivary gland. Desmosomal attachments (D) and cytoplasmic myofilaments (F) feature prominent. (Bar = 5 μm.)**

epithelial cells and those identified adjacent to these ducts on transmission electron micrographs are probably an expression of a myoepithelial network extending between adjacent acini (Brocco and Tamarin, 1979).

STRUCTURE OF MYOEPITHELIAL CELLS

Myoepithelial cells appear to be similar in structure, irrespective of the organ or species in which they are studied. Those on secretory endpieces (or acini) have 4-8 primary cytoplasmic branches each with two or more secondary branches. The cytoplasmic extensions of myoepithelial cells located on intercalated ducts usually lie longitudinal and seldom divide.

The stromal (or outer) surface of myoepithelial cells contain caveolar invaginations which are abundant in areas where nerve fibres abut (Young and van Lennep, 1978). The visceral cell surface is smooth, attached by desmosomes to secretory cells (Tandler, 1965) (Fig. 3) and contain isolated cilia which invaginate the basal cytoplasmic membrane and protrude deep into the cytoplasm of the secretory epithelial cell (Tandler *et al*, 1970). Micropinocytotic vesicles are often present on the surface facing secretory cells (Ruby, 1978).

Myoepithelial nuclei are ellipsoidal, surrounded by a few ribosomes and mitochondria are scattered throughout the cytoplasm of the cell body. Other cytoplasmic organelles are in the juxtanuclear position (Tamarin, 1966). Cytoplasmic myofilaments are uniform in diameter, their density varying because of the distance between individual filaments. They run in bundles which often fuse with other bundles, are compact in areas resembling dense elongated bodies characteristic of smooth muscle cells and terminate in attachment devices on the stromal cell membrane (Young and van Lennep, 1978). Intermediate sized cytoplasmic filaments of the cytokeratin type (Caselitz *et al*, 1981) are demonstrable in salivary gland myoepithelium .

Myoepithelial cells are often adjacent to cells which have a similar shape, but possess an extremely electron-lucent cytoplasm that contains almost no organelles. Electron microscopic observations indicate that these cells, termed clear cells, are transformed directly into myoepithelium, since all morphological intergrades between the two cell types have been recognised. These clear cell appear to develop from intercalated duct cells (Tandler, 1965). Myoepithelium therefore probably shares a common ancestry with secreting epithelial cells, which are also of intercalated duct cell origin (Riva *et al*, 1976).

FUNCTIONS OF SALIVARY MYOEPITHELIAL CELLS

Although species differences do exist, it is generally accepted that salivary myoepithelial cells have a dual innervation of parasympathetic as well as sympathetic nerves and impulses from both types cause contraction (Garrett and Emmelin, 1979). The association between nerve axons and salivary gland myoepithelial cells suggests that activation occurs by diffusion of a transmitter substance comparable to that suggested for smooth muscle cells (Tamarin, 1966). Synchronisation of contraction is made possible by gap junctions and overlaps between myoepithelial cell processes (Brocco and Tamarin, 1979).

Acinar myoepithelial contraction (as demonstrated in Fig. 4) facilitates rapid expulsion of the secretion by rupturing 'ripe' mucous cells (Tandler *et al*, 1970), preventing distension of secretory endpieces (Emmelin, Garret and Ohlin, 1968) and reducing lumenal volume (Garrett and Emmelin, 1979). All salivary glands lacking acinar myoepithelial cells (which were referred to previously), notably secrete a watery (serous) saliva. It has recently been shown that the intraductal pressure developed by the rat parotid gland during autonomic nerve stimulation is always lower than that of the rat mandibular gland (Tandler, 1976). The paucity of acinar myoepithelial cells in the rat parotid gland would lead
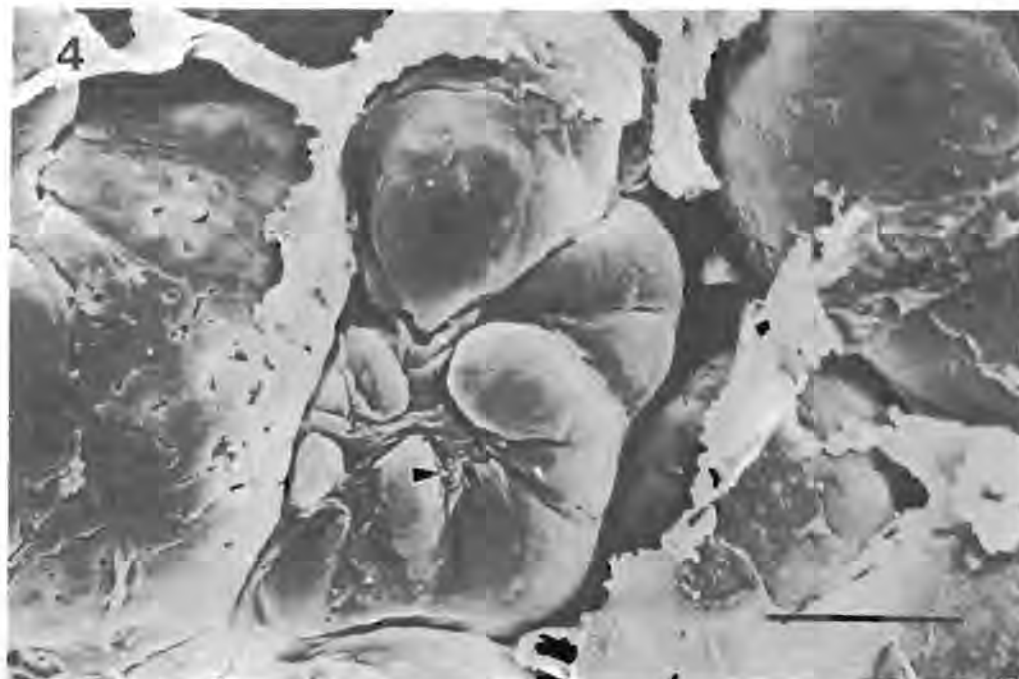
Fig. 4: **Scanning electron micrograph of a contracted myoepithelial cell surrounding the acinus of the submandibular salivary gland of a vervet monkey. Note the contracted appearance of the cytoplasmic processes (arrow). (Bar = 10 μm.)**

one to expect such a result. Furthermore, the pancreas which lacks myoepithelial cells, secretes a watery product and is more prone to irreparable acinar or duct rupture from degrees of obstruction slight enough to produce no ill effects in salivary glands. In contrast, the sublingual salivary gland of the monotreme echnida, *Tachyglossus ucleatus,* which has been referred to, shows extraordinary development of acinar myoepithelial cells. The supporting and contractile functions of acinar myoepithelial cells is therefore likely to be particularly valuable when the viscosity of the saliva is high (Garrett and Emmelin, 1979). However, other factors such as the anatomical location of the gland may also be important. Salivary glands in the floor of the mouth continuously excrete against a vertical pressure gradient and will theoretically require more acinar myoepithelial support than glands located above the orifice of their main excretory duct.

Acinar myoepithelial contraction may also modify the concentration of saliva by decreasing the surface area of the secretory apparatus exposed to interstitial fluid (Tandler *et al*, 1970). Such an effect would diminish the loss of fluid from the secretion and is probably important when viscid saliva has to be forced through narrow channels in a gland (Garrett and Emmelin, 1979). Myoepithelial cilia, which have already been referred to, may act as chemoreceptors in this regard as they have been postulated to have a sensory function (Tandler *et al*, 1970).

Contraction of myoepithelial cells surrounding intercalated ducts reduces lumenal volume and shortens and widens these structures thereby decreasing peripheral resistance (Young and van Lennep, 1977). Extension of their processes onto proximal surfaces of allied acini may serve to align the acinar lumen with that of the duct during contraction, a function which may be particularly important in glands which are continuously distorted during mastication.

The role of the myoepithelial cell in transportation of

metabolites to and from secretory cells is a controversial issue. Basal infoldings of human submandibular salivary gland myoepithelium (Nagai and Nagai, 1985) could serve to increase the surface area exposed to tissue fluid. Pinocytotic vesicles (Tandler *et al*, 1970), positive staining for the iron binding protein ferritin (Toto, 1985) and an increased alkaline phosphatase and magnesium dependant adenosine triphosphatase (ATPase) activity (Yoskihara, Kanda and Kaneko, 1984) are all features supporting this view. It has been pointed out however, that ATPase activity in myoepithelial cells of the palatine glands of rats are rather implicated in cell contraction and, that the vesicle-like structures are in fact invaginations of the plasma membranes and are continuous with the extracellular space (Han *et al*, 1976).

Finally, myoepithelial cells are important in the formation and maintenance of the enveloping basement membrane. Fibronectin, laminin and elastin are major components of basement membranes and are found to be produced by myoepithelial cells (Toto, 1985). Basement membrane proteins are not only an essential scaffold for epithelial multiplication and differentiation (Kleinman, Klebe and Martin, 1981) but also form part of the hypothetic epithelial mesenchymal junction through which all metabolites involved in the synthesis of saliva, pass.

NON-NEOPLASTIC MYOEPITHELIAL PROLIFERATIONS

The bulk of research is focused on the role of myoepithelium in salivary gland neoplasms and very little is known of their participation in non-neoplastic conditions.

After ductal ligation of the parotid and submandibular salivary glands of cats, processes of myoepithelial cells extend into the interstitial spaces and folds of basal lamina aggregate around protruberant parts of the cell thereby increasing the distance between the cell membrane and nerve endings (Emmelin, Garrett and Ohlin, 1974). These alterations may reduce the neuro-effector

efficiency and together with the mechanical disadvantage of rearranged myoepithelial processes, help to explain the altered intraductal pressure responses that occurred after stimulation. Induction of secretion leading to a rise in the intraductal pressure of ligated glands, often causes distention and even disruption of the first part of the striated duct, which lacks myoepithelial support (Garrett and Emmelin, 1979).

Lymphoreticular cell proliferation associated with atrophy of the glandular parenchyma and ductal changes ending in so-called 'epimyoepithelial islands' are characteristics of chronic recurrent (punctate) sialadenitis, sicca syndrome, Sjögrens syndrome and benign lymphoepithelial lesion. The epimyoepithelial islands are formed by metaplastic transformation of ductal epithelial and myoepithelial cells. Some believe myoepithelial cells are few in number and located only around the periphery of the islands (Takeda, 1980) whereas the majority of workers agree that myoepithelium forms an integral part of epimyoepithelial islands (Seifert and Donath, 1976). This controversy underlines the difficulty by which myoepithelial cells are identified microscopically in a state other than normal.

NEOPLASTIC MYOEPITHELIAL PROLIFERATIONS

It is generally known that the exocrine pancreas, which is phylogenetically and structurally related to salivary glands except for the absence of myoepithelial cells, lacks the variety and diversity of salivary gland neoplasms. The wide spectrum of morphologic presentation of salivary gland neoplasms, exceeded only by neoplasms of gonadal origin, is to a great extent a result of the two sided expression of myoepithelium: although epithelial in origin, their cytoplasmic organization is quite similar to mesenchymal smooth muscle. It is therefore not surprising that neoplastic myoepithelial cells have three principal morphologic presentations namely, fibroblastic (or myoid) (Fig. 5a), epithelial-like (or glycogen rich clear cell) (Fig. 5b) and hyaline (or plasmacytoid) (Fig. 5c). Care should be taken not to interpret clear cell formation arising from fixation artefact as myoepithelial differentiation.

As neoplasms develop after oncogenic transformation of mitotically active undifferentiated cells, a study of the reserve (or stem cell) compartments of salivary glands are necessary before attempting to master salivary gland oncogenesis. The basal cells of the excretory duct (the excretory duct reserve cell or EDRC) and the intercalated duct cell (or IDC) act respectively as stem cells for the differentiated ductal and secretory portions of mature salivary glands (Fig. 6). The EDRC has a genetic potential to give rise to columnar and squamous cells (and metaplastic mucus producing cells) lining the excretory duct and the IDC to acinar cells, intercalated duct cells, striated duct cells and myoepithelial cells (Batsakis, 1979).

According to the "bicellular theory" on salivary gland oncogenesis the IDC population is postulated to be the epithelial source for myoepitheliomas, mixed tumours (pleomorphic adenomas), adenoid cystic carcinomas, epithelial myoepithelial carcinomas (or tubular carcino-
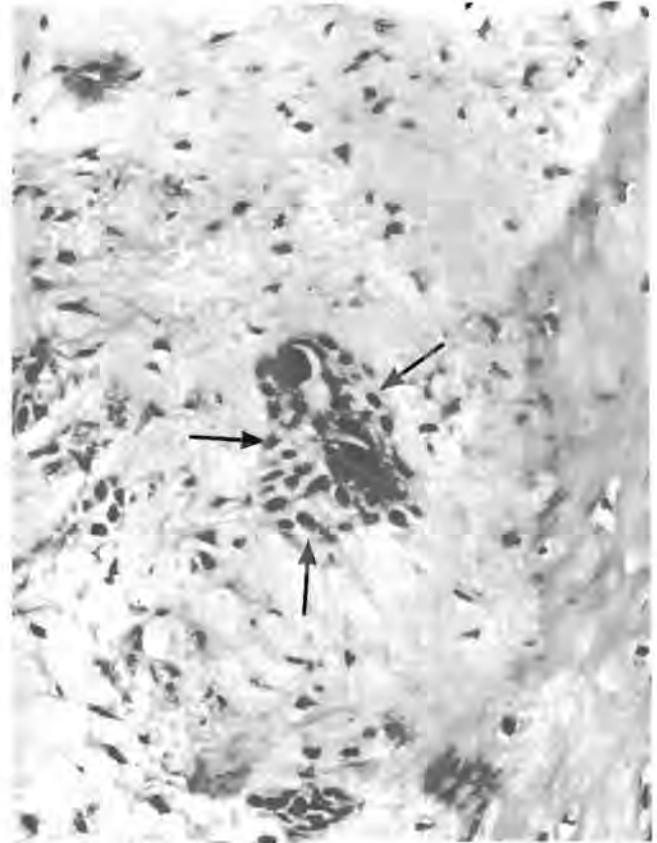

Fig. 5(a): **Fibroblastic (or myoid) myoepithelial differentiation in a myoepithelioma of the palate. Arrows indicate intercalated duct differentiaiton (H & E, X200).**
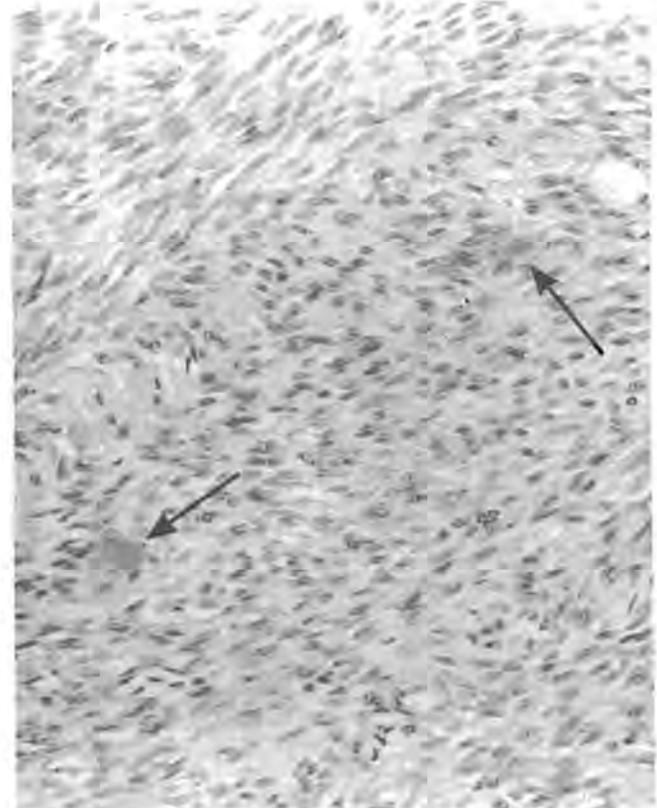

Fig. 5(b): **Epithelial (or clear cell) differentiation (arrows) surrounding an intercalated duct-like structure in a mixed salivary gland tumour of the parotid (H & E, X200).**
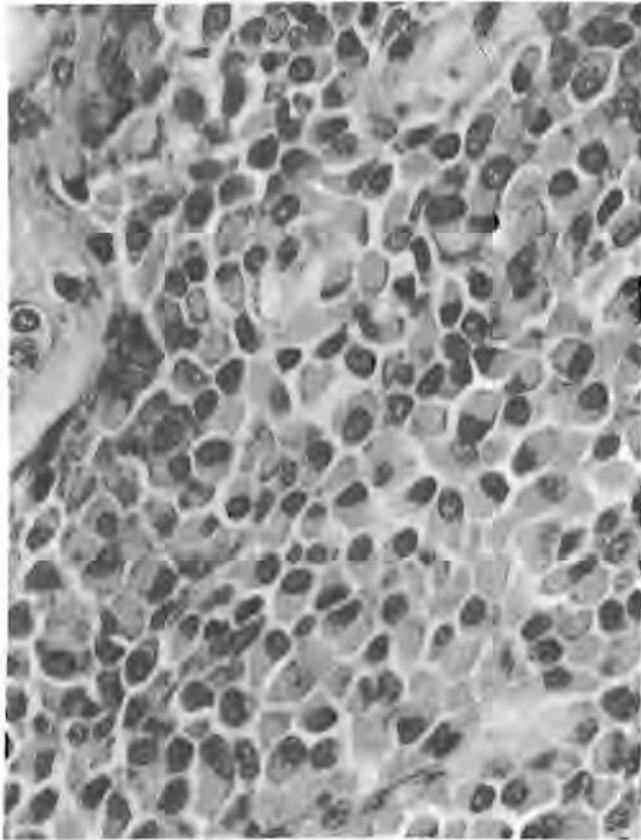
Fig. 5(c): **Hyaline (or plasmacytoid) myoepithelial differentiation in the myoepithelioma of the palate (H & E X300).**

mas) lobular carcinoma (or terminal duct adenocarcinoma) monomorphic adenoma and acinous cell carcinoma (Batsakis, 1980). Theoretically most of these neoplasms and even monomorphic adenomas may, to a greater or lesser extent, exhibit myoepithelial differentiation (Batsakis, Brannon and Scuibba, 1981). The possibility of myoepithelial differentiation, on the other hand should, according to the bicellular theory, be excluded from neoplasms that are postulated to arise from the EDRC (muco-epidermoid carcinoma, papillary mucinous adenocarcinoma and primary salivary squamous carcinoma) (Batsakis, Kraemer and Scuibba, 1983).

Much of the debate on the significance of myoepithelial differentiation in salivary gland neoplasms, have focused around the identity of the characteristic myxoid, chondroid, osteoid, elastic and fibrous interstitial deposits in mixed tumours. After tissue culturing and chemical and ultrastructural investigations, the mesenchymal nature of these deposits has been proven beyond doubt (Azzopardi and Smith, 1959) and evidence of myoepithelial participation in their production has accumulated (Toto and Hsu, 1985; Regezi and Batsakis, 1977; Doyle *et al*, 1968 and Caselitz and Loning, 1981). Some of the spindle shaped cells, often associated with stromal deposits, were found to react positively with immunochemical stains for S100 protein (Nakazato *et al*, 1982) (Fig. 7) and cytoplasmic filaments of the myosin, actin (Caselitz and Loning, 1981) and prekeratin types, (Caselitz *et al*, 1981) characteristics which strongly suggest myoepithelial differentiation. Since myoepithelial cells already exhibit features of smooth muscle, neoplas-
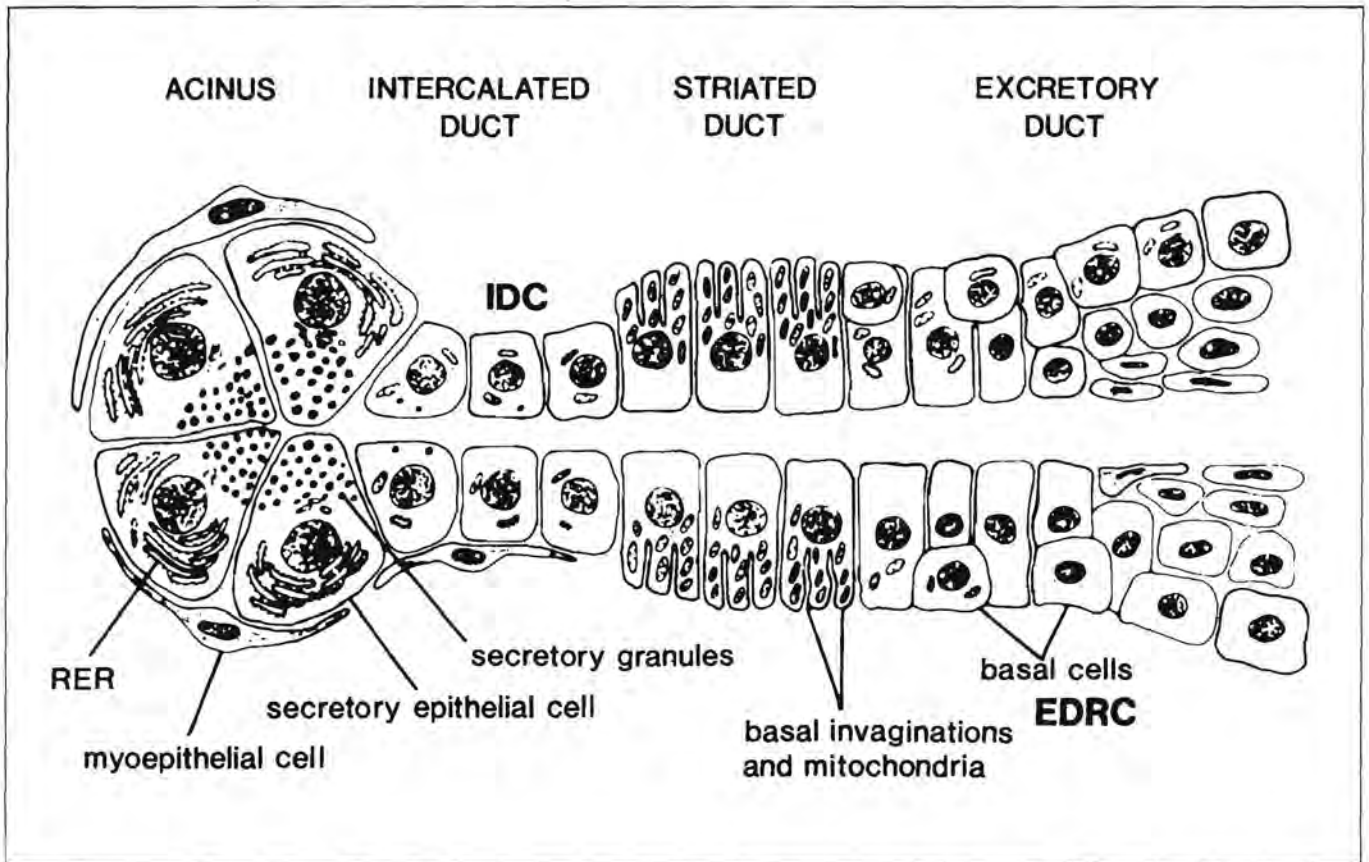

Fig. 6: **Diagrammatic representation of the stem cell compartments of mature salivary glands (IDC = intercalated duct cell and EDRC = excretory duct reserve cell). (Reproduced with permission from Batsakis, 1979, pp.4).**
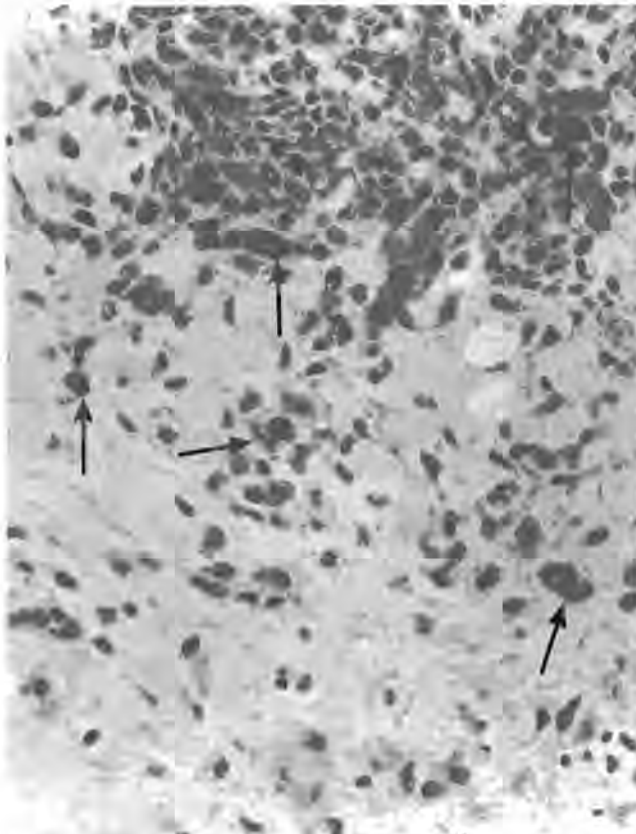
Fig. 7: **Cytoplasmic S100 protein positivity (arrows) indicating myoepithelial differentiation in a myxoid area of a mixed tumour of the parotid salivary gland (Immunoperoxidase stain for S100 protein, X200).**

tic change may expose other mesenchymal characteristics which could lead to the deposition of myxoid, chondroid, fibrous, elastic and even osteoid tissues. These findings not only introduce a new pathologic concept of mesenchymal metaplasia of neoplastic epithelium, but also lessens the rigid classical distinction, based on product definition, made between tissues of ectodermal and mesodermal origin.

ACKNOWLEDGEMENT

REFERENCES

Azzopardi, J.G. & Smith, O.D. (1959) Salivary gland tumors and their mucins. *Journal of Pathology and Bacteriology,* 77, 131-140.

Batsakis, J.G. (1979) *Tumors of the Head and Neck.* Clinical and Pathological Considerations, 2nd ed., Chap. 1, pp.1-75.

Batsakis, J.G. (1980) Salivary gland neoplasia: An outcome of modified morphogenesis and cytodifferentiation. *Oral Surgery, Oral Medicine and Oral Pathology,* 49, 229-232.

Batsakis, J.G., Brannon, R.B. & Scuibba, J.J. (1981) Monomorphic adenomas of major salivary glands: a histologic study of 96 tumours. *Clinical Otolaryngology,* 6, 129-143.

Batsakis, J.G. Kraemer, B. & Scuibba, J.J. (1983) The myoepithelial cell and its participation in salivary gland neoplasia. *Head and Neck Surgery,* 5, 222-233.

Brocco, S.L. & Tamarin, A. (1979) The topography of the rat submandibular gland parenchyma as observed with S.E.M. *Anatomical Record (New York),* 194, 445-459.

Caselitz, J. & Löning, T. (1981) Specific demonstration of actin and keratin filaments in pleomorphic adenomas by means of immunoelectron microscopy. *Virchows Archives (A),* 393, 153-158.

Caselitz, J., Löning, T., Staquet, M.J., Seifert, G. & Thivolet, J. (1981) Immunocytochemical demonstration of filamentous structures in the parotid gland. Occurence of keratin and actin in normal and tumoral parotid gland with special respect to the myoepithelial cells. *Journal of Cancer Research and Clinical Oncology,* 100, 59-68.

Cope, G.H., Pratten, M.K. & Williams, M.A. (1976) Correlative morphological and biochemical study of the effects of isoprenaline on the organelle and membrane content of the rabbit parotid gland. *Histochemical Journal (London),* 8, 403-418.

Doyle, L.E., Lynn, J.A., Panopio, I.T. & Cross, G. (1968) Ultrastructure of chondroid regions — benign mixed tumor of salivary gland. *Cancer,* 22, 225-233.

Emmelin, N., Garrett, J.R. & Ohlin, P. (1968) Neural control of salivary myoepithelial cells. *Journal of Physiology (London),* 196, 381-396.

Emmelin, N., Garrett, J.R. & Ohlin, P. (1974) Secretory activity and the myoepithelial cells of salivary glands after duct ligation in cats. *Archives of Oral Biology,* 19, 275-283.

Garrett, J.R. & Emmelin, N. (1979) Activities of salivary myoepithelial cells. A review. *Medical Biology (Helsinki),* 57, 1-28.

Han, S.S., Kim, S.K. & Cho, M.I. (1976) Cytochemical characterization of the myoepithelial cells in palatine glands. *Journal of Anatomy,* 122, 559-570.

Hara, K., Ito, M., Takeuchi, J., Tjima, S., Endo, T. & Hidaka, H. (1983) Distribution of S100b protein in normal salivary glands and salivary gland tumors. *Virchows Archives (A),* 401, 237-249.

Kleinman, H.K., Klebe, R.J. & Martin, G.R. (1981) The role of collagenous matrices in the adhesion and growth of cells. *Journal of Cellular Biology,* 88, 473-485.

Nagai, T. & Nagai, M. (1985) Scanning electron microscopy of the human submandibular gland. *Archives of Otolaryngology,* 241, 265-266.

Nakazato, Y., Ishizeki, J., Takahashi, O.K., Yamaguchi, H., Kamei, T. & Mori, T. (1982) Localization of S100 protein and glial fibrillary acidic protein-related antigen in pleomorphic adenoma of the salivary gland. *Laboratory Investigation,* 46, 621-626.

Palmer, R.M., Lucas, R.B., Knight, J. & Gusterson, B. (1985) Immunocytochemical identification of cell types in pleomorphic adenoma, with particular reference to myoepithelial cells. *Journal of Pathology,* 146, 213-220.

Raubenheimer, E.J. (1987) The myoepithelial cell: Embryology, Function and Proliferative Aspects. *Critical Reviews in Clinical Laboratory Sciences,* 25, 161-193.

Redman, R.S., Sweney, L.R. & McLaughlin, S.T. (1980) Differentiation of myoepithelial cells in the developing rat parotid gland. *American Journal of Anatomy,* 158, 299-320.

Regezi, J.A. & Batsakis, J.G. (1977) Histogenesis of salivary gland neoplasms. *Otolaryngologic Clinics North America,* 10, 297-307.

Riva, A., Testa-Riva, F., Del Fiacco, M. & Lantini, M.S. (1976) Fine structure and cytochemistry of the intralobular ducts of the human parotid gland, *Journal of Anatomy,* 122, 627-640.

Ruby, J.R. (1978) Ultrastructure of the parotid gland of the nine banded armadillo. *Anatomical Record (New York),* 192, 389-405.

Seifert, G. & Donath, K. (1976) Die morphologie der speicheldrusenerkrangkungen. *Archives of Otorhinolaryngology,* 213, 11-124.

Shear, M. (1966) The structure and function of myoepithelial cells in salivary glands. *Archives of Oral Biology,* 11, 769-781.

Takeda, Y. (1980) Histopathological studies of the labial salivary glands in patients with Sjögrens syndrome. Part II: electron microscopic study. *Bulletin of the Tokyo Medical and Dental University,* 27, 27-42.

Tamarin, A. (1966) Myoepithelial of the rat submaxillary gland. *Journal of Ultrastructural Research,* 16, 320-338.

Tandler, B. (1965) Ultrastructure of the human submaxillary gland. III. Myoepithelium. *Zeitung fur Zellforschung,* 68, 852-863.

Tandler, B. (1986) Unusual myoepithelium in the retrolingual salivary gland of the European hedgehog. *Journal of Submicroscopic Cytology,* 18, 261-270.

Tandler, B., Denning, C.R., Mandel, I.D. & Kutscher, A.H. (1970) Ultrastructure of human labial salivary glands, III. Myoepithelium and ducts. *Journal of Morphology,* 130, 227-245.

Toto, P.D. & Hsu, D.J. (1985) Product definition of pleomorphic adenoma of minor salivary glands. *Journal of Oral Pathology,* 14, 818-832.

Van Niekerk, J. & Raubenheimer, E.J. (1986) Ultrastructure of myoepithelium in salivary glands of African elephant (Loxodonto africana). *Proceedings of the IXth Internation Congress on Electron Microscopy,* 2 869-2 870.

Yoshihara, T., Kanda, T. & Kaneko, T. (1984) A cytochemical study on the salivary gland pleomorphic adenoma (mixed tumor) and the fetal and adult salivary gland. *Archives of Otorhinolaryngology,* 240, 231-238.

Young, J.A. & van Lennep, E.W. (1977) Morphology and physiology of salivary myoepithelial cells. *International Review of Physiology,* 12, 105-125.

Young, J.A. and van Lennep, E.W. (1978) *The Morphology of Salivary Glands,* 1st ed. Ch. 5, pp.93-99. London: Academic Press.

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PAROTID SALIVARY GLAND OF THE AFRICAN ELEPHANT (LOXODONTA AFRICANA): STRUCTURE AND COMPOSITION OF SALIVA

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ABSTRACT

Specimens from parotid salivary glands of full-grown elephant Loxodonta africana a (n=6) and saliva aspirated from their main excretory ducts were examined macroscopically and microscopically and analysed biochemically. The composition of the saliva was compared to that of the blood. The parotids (n=12; $\bar{x}$ = 7,4 kg) are homocrine and of a seromucous nature. Myoepithelial cells are well-developed along intercalated ducts and their processes extend to proximal portions of allied acini. The saliva is hypotonic and contains relatively low concentrations of sodium and glucose and high concentrations of potassium, urea, calcium and phosphorus. Absence of detectable levels of α-amylase negates a digestive role and the voluminous secrete evidently aids swallowing by moisturising and lubricating the large mass of ingested leaves, grass and bark.

Key words: Parotid salivary gland, African elephant, Loxodonta africana, saliva

INTRODUCTION

The bulk of research on salivary glands and most studies on the composition of saliva have been performed in man and laboratory animals. Little is known of the structure of the salivary glands of large mammals and the compositon of their saliva. Investigations which correlate the feeding habits of animals with the structure of their salivary glands and the composition of saliva, could throw more light on many findings which are apparently of a speculative nature.

The functional development of salivary glands is generally adapted to the feeding habits of an animal. Among ...atic mammals, where lubrication of ...od is not necessary, the glands may be absent, as in most Cetacea (dolphins and whales)[8]. At the opposite extreme are ruminants that habitually ingest dry food; their salivary glands are often exceptionally large and well-developed and the volume of saliva secreted is so great that major changes in plasma pH and electrolyte concentrations occur in a tidal fashion with each feeding period[15].

In general terms, salivary glands of mammals can be considered as subservient to various different functions. First, and perhaps most important, they evidently provide lubrication to aid the swallowing of food. The lubricant may take the form of slimy mucus as is usual in lower vertebrates. More serous secretions, as are formed by the parotid glands of most

mammals, clearly achieve the same purpose when the ingested food is very dry. Secondly, by secreting enzymes, salivary glands are able to play a role in digestion. Most mammals have relatively high concentrations of amylase in parotid saliva. Nevertheless, the parotid saliva of animals of the order Carnivora (which includes the dog and cat) and the family Bovidae (which include sheep) contains no amylase. Other possible functions of salivary glands include the secretion of hormones, regulation of body temperature by wetting of the fur and the excretion of toxic substances which play an important role in defence, paralysing and killing of prey[21].

The functional unit of all compound tubulo-acinar salivary glands is composed of a secretory endpiece which may be spheroid (acinar) or tubular in shape, an intercalated duct and a striated duct which leads to the larger interlobular and eventually the main excretory duct[21]. Greatly modified striated ducts, referred to as granular ducts, are found in the salivary glands of certain animals and are probably implicated in the production and secretion of digestive enzymes[14]. Traditionally, homocrine glands are classified according to the acinar secretory cell type as either serous, mucinous or seromucous (mucoid). The term "heterocrine" (or mixed) is used to describe glands with both serous (or seromucous) and mucinous cells in acini. Both acini and intercalated ducts are generally accepted to be surrounded by myoepithelial cells which lie on the inside of the epithelial basement membrane. Their distribution appears to depend, amongst other factors, on the viscosity of the secretion[10]. Saliva is secreted into the acinar lumen and propelled along the

duct system by pressures which develop during the process of secretion and myoepithelial cell contraction. Resorption of water and exchange of electrolytes take place in the striated duct[19].

The African elephant, (Loxodonta africana), belongs to the order Proboscidea and is the largest land animal: an adult animal can weigh close to 7000 kg[1]. It occurs only in Africa south of the Sahara Desert and occupies large areas of semi-arid wooded savannah. According to their feeding habits, elephants are classified as grazers and browsers[5]. Each animal ingests up to 170 kg of forage a day[9] consisting of leaves, dry grass and bark.

As no scientific information is available on the salivary gland system of the African elephant, we undertook this stud to determine the structure of the paroti. gland and the composition of its saliva.

MATERIAL AND METHODS

The parotid salivary glands of fully grown African elephant (n=6) immobilised during a population control programme[6], were removed and the mass determined. Fresh tissue specimens, representing all areas of the glands, were selected, excised and fixed in buffered formalin and 2,5% gluteraldehyde. Specimens were embedded in Epon, serially sectioned (1 micrometer thick) and alternate haematoxylin/eosin and periodic acid Schiff stains performed.

Saliva was aspirated from the main excretory ducts of 2 animals and stored at 4°C before biochemical analyses.

Whole venous blood samples were collected from 20 animals, centrifuged and subjected to biochemical analyses in a batch. The viscosity of the serum and saliva was measured with a Coulter Harkness Viscometer and expressed i centipoise or cp (one hundredth of dyne per second per square centimetre).

RESULTS

The parotid salivary glands of the African elephant are paired organs located caudal to the mandibular ramus and condylar process, rostral to the external auditory meatus and deep to the cervical musculature. Each gland measures 29 x 22 x 19 cm and has an average mass of 7,4 kg (range 6,4 - 8,2 kg). The main excretory duct has a diameter of 9 - 13 mm and leaves the gland at the rostro-ventral pole, follows a rostral and ventral course and opens up on the buccal mucosa in the oral vestibule in relation to the upper caudal teeth. On cut surface, the parotid is seen to have a well-developed fibrous capsule with fibrous septae dividing the gland into many lobes (Fig. 1).

Microscopically the parotid gland is of a complex tubulo-acinar type with slightly elongated homocrine acini, the latter containing secretory cells of the seromucous type (Fig. 2). These cells are intermediate between serous and mucinous cells, contain secretory granules

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which are larger than those of serous cells and no stainable epithelial mucin. The intercalated ducts vary in length (4 - 12 cells) and are lined with flattened, glycogen-rich cells. The transition between intercalated ducts and striated ducts is abrupt (Fig. 2). Striated ducts show extensive branching and extend into the interlobular fibrous septae and are surrounded by a rich capillary network (Fig. 3). The striated ducts are lined by cuboidal to columnar cells with centrally placed nuclei and foldings of the basal cytoplasmic membrane. The larger interlobular ducts are lined by pseudostratified cylindrical epithelium with scattered goblet cells. Stratified squamous epithelium is evident close to the orifice of the main excretory duct.

The composition and viscosity of the parotid saliva in comparison to blood values are reflected in Table 1.

DISCUSSION

The parotid of the African elephant is probably the largest salivary gland in the animal kingdom. It has been shown[19] that stimulated salivary gland tissue produces between 60 and 200 microlitres of saliva min^{-1} gram wet tissue $^{-1}$. Both parotids of elephant theoretically therefore c ld secrete in excess of 50ℓ saliva h^{-1}. During a normal feeding cycle, which extends over the greatest part of 24h[18], this voluminous secretion evidently aids swallowing by moisturising and lubricating the large mass of ingested leaves, bark and grass. The seromucous nature and hypotonicity of the saliva, which, although watery, contains more glycoproteins than serous saliva[17], are best adapted to fulfil these functions.

Structurally, the parotid is less varied among different species than are the other major salivary glands. Although tubular secretory endpieces are occasionally encountered, they usually have an acinar configuration as in the

Fig. 1: Cut surface of the parotid of the African elephant. Note the main excretory duct (arrow) leaving the rostro-ventral surface of the gland
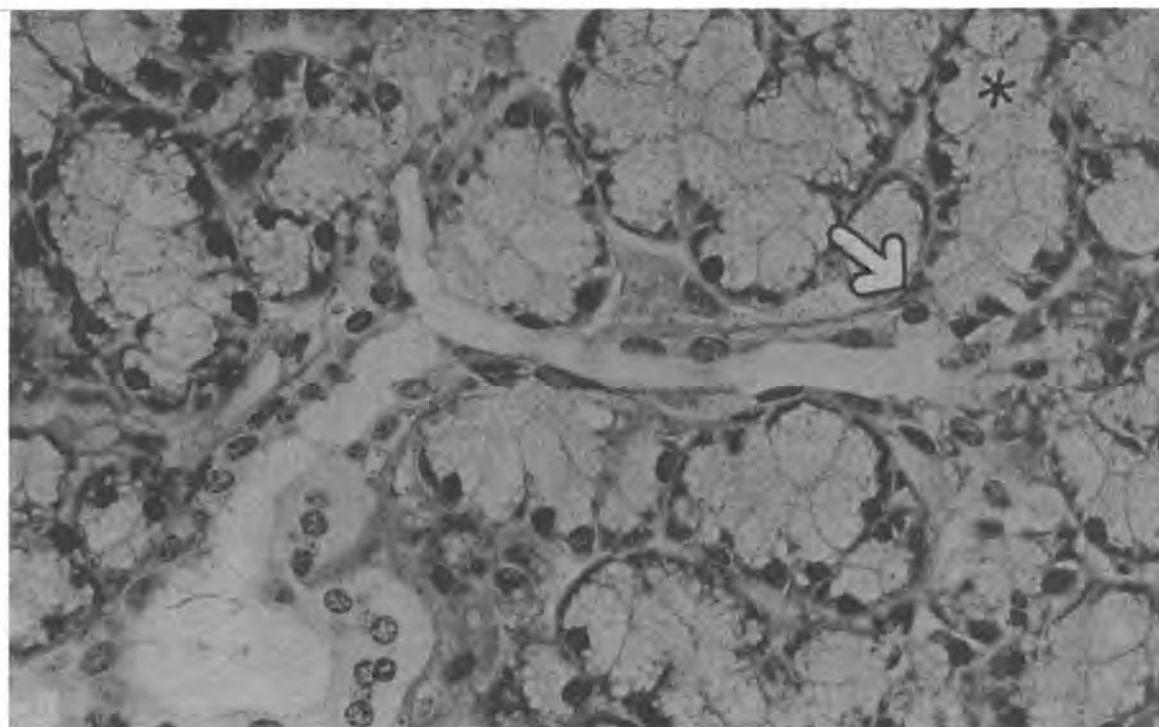

Fig. 2: Homocrine seromucous acinus (asterisk) opening up in an intercalated duct, lined by flattened epithelium and which leads into a striated duct (right upper corner). Note myoepithelial cell body located on interface between acinus and duct (arrow). (H.E. stain, magnification x500)
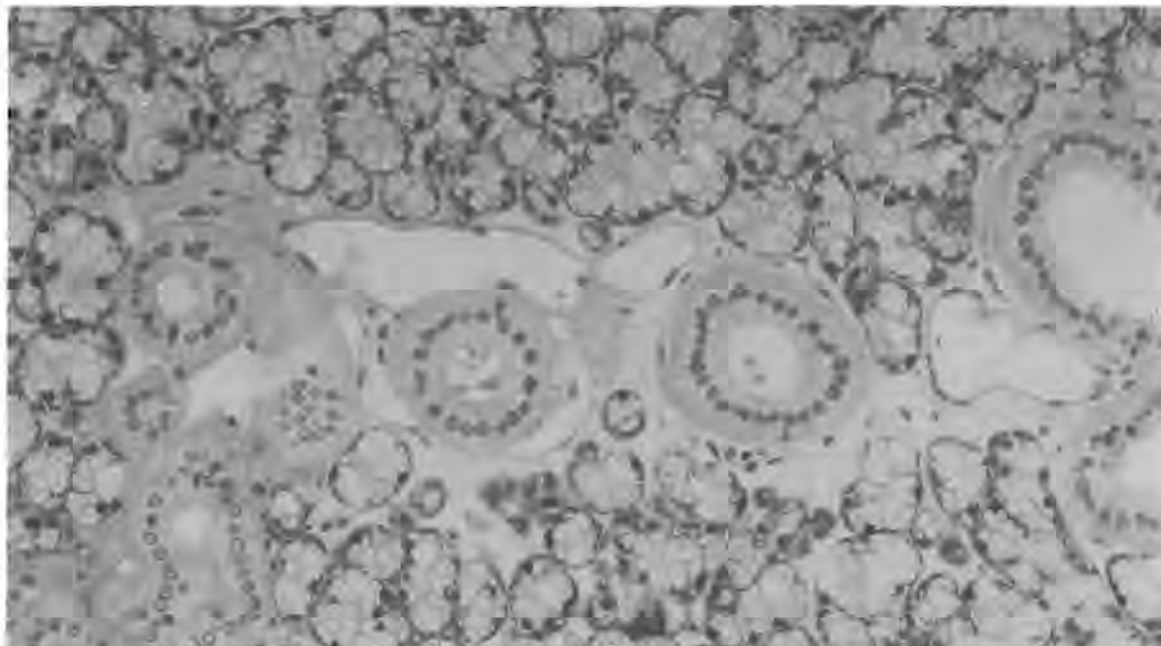
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Fig. 3: Striated ducts in a vascular-rich environment. (H E stain magnification x200)

Table 1: **Biochemical findings in serum and saliva of the African elephant**

	Serum (±SD)	Saliva (Mean values)
Sodium (mmol ℓ^{-1})	127 (2)	Negative
Potassium (mmol ℓ^{-1})	6,6 (0,9)	63
Chloride (mmol ℓ^{-1})	85 (2)	26
Urea (mmol ℓ^{-1})	2,7 (0,5)	6,3
Creatinine (μmol ℓ^{-1})	123 (25)	130
Total protein (g ℓ^{-1})	89 (5)	41
Albumin (g ℓ^{-1})	33 (2)	Negative
Phosphorus (mmol ℓ^{-1})	1,92 (0,26)	15,7
Total calcium (mmol ℓ^{-1})	2,92 (0,13)	5,74
Magnesium (mmol ℓ^{-1})	1,57 (0,23)	4,25
Actual Ca^{++} (mmol ℓ^{-1})	1,48 (0,07)	2,05
pH	7,01 (0,13)	6,53
Ca^{++} at pH 7,4 (mmol ℓ^{-1})	1,17 (0,9)	1,10
Glucose (mmol ℓ^{-1})	7,11 (1,26)	0,4
Viscosity (cp)	1,81 (plasma)	2,01
Amylase (IU ℓ^{-1})	923 (321)	Negative

elephant, but the secretory cells are mostly homocrine and of a serous nature[21]. Seromucous parotid glands, similar to those of the African elephant, were also reported in the domestic cat and dog[13][21], in the brush-tail possum and Trichosurus[21], Djungarian hamster[16] and in man[11][21]. In most animals, the junction of the endpiece and intercalated duct, is usually sharply defined and the intercalated ducts are fairly uniform. The striated ducts, on the other hand, are more varied among species. They are conspicuous in the African elephant and dog and completely absent in some marine carnivora[21]. Myoepithelial development in the gland reflects the relatively low viscosity of the secretion. Unlike the case of mucinous glands which secrete saliva with a high viscosity[21], myoepithelial cells are absent from the peripheral portion of the acini. Intercalated duct-associated myoepithelial cell processes which extend to acini, probably align the latter with allied ducts and ensure patency, especially when the gland is distorted during masticatory movements of the mandible. The bulk of the parotid lies above or on the same level as the orifice of the main duct and the main forces propelling the saliva probably include gravitation, pressures which develop during the process of secretion and contraction of intercalated duct-associated myoepithelial cells. The latter probably not only facilitate propulsion by reducing luminal volume, but also regulate the salivary flow rate by dilating the intercalated ducts, thereby decreasing peripheral resistance.

The absence of α-amylase in the saliva of the elephant probably negates a digestive role. This is in contrast to human parotid saliva which is considerably richer in α-amylase than saliva from the other major human salivary glands. The concentrations of sodium, chloride, total protein, albumin and glucose are significantly less in saliva when compared to serum, whereas the concentrations of potassium, urea, phosphorus, calcium and magnesium are higher. Although the functional significance of many of these alterations are unknown, it supports the proposed active role played by the striated ducts and their vascular environment in rendering the saliva sodium-free and hypotonic[12][20]. Furthermore, it has been proven that the ducts of the rat a rabbit mandibular gland reabsor sodium and chloride and secrete potassium and bicarbonate[19].

The total calcium, ionised calcium (Ca^{++}) and magnesium concentrations of the elephant saliva appear to be higher than serum concentrations, contrary to what is generally found[19]. The high concentration of salivary calcium may, however, be the result of sympathetic nerve activity, as Burgen & Emmelin[2] reported an increase in salivary calcium in the dog mandibular gland after sympathetic stimulation. The phosphorus concentrations are comparable to those of human[4] and sheep[3] parotid saliva. It should be emphasised however, that the electrolyte composition of saliva may vary with the age, hydration state and hormonal status of the animal and the salivary secretory rate[19]. Elephant parotid saliva is low in glucose and hypotonic owing to the absence of sodium. These findings are comparable to those reported in human parotid saliva[7][12]. In contrast to humans however, where the salivary urea concentration is approximately 75 - 90% of the content in blood[7], elephant saliva contains a much higher urea concentration, which is probably indicative of a recycling mechanism similar to that found in cattle where digestive protozoa and bacteria utilise urea for metabolic processes. Future investigations of this nature on other animals may clarify the function of the other components of saliva.

REFERENCES
1. Boolootian R A, Stiles K A 1981 College Zoology 10th edn Macmillan Publishing Company, New York
2. Burgen A S V, Emmelin N G 1961 Physiology of the salivary glands. Arnold, London
3. Compton J S, Nelson J, Wright R, Young J A 1980 A micropuncture investigation of electrolyte transport in the parotid glands of

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0038-2809 Tydskr. S. Afr. vet. Ver. (1988) 59 (4): 184-187

sodium-replete and sodium-depleted sheep. Journal of Physiology, London 309: 429-446

4. Dawes C 1969 The effects of flow rate and duration of stimulation on the concentrations of protein and the main electrolytes in human submandibular saliva. Archives of Oral Biology 14: 277-294

5. Delaney M J, Happold D C D 1979 Ecology of African mammals Longman, London: 118

6. De Vos V 1983 Management of large animals in African conservation areas. In: Owen-Smith R N (ed.) Proceedings of a symposium held in Pretoria 29-30 April 1982 Haum, Pretoria: 213-232

7. Diem K 1982 Documenta Geigy Scientific Tables 6th edn J R Geigy S A, Basle: 517-519

8. Fahrenholz C 1967 Drüsen der mundhöhle. In: Bolk L, Göppert E, Kallius E, Lubosch N (ed.) Handbuch der vergleichenden Anatomie der Wirbeltiere Vol 3 Asher, Amsterdam: 115-210

9. Guy P R 1975 The daily food intake of the African elephant Loxodonta africana Blumenbach, in Rhodesia. Arnoldia Rhodesia 7 (26): 1-8

10. Raubenheimer E J 1987 The myoepithelial cell: embryology, function and proliferative aspects. Critical Reviews in Clinical Laboratory Sciences 25: 161-193

11. Riva A, Riva-Testa F 1973 Fine structure of acinar cells of human parotid gland. Anatomical Record, New York 176: 149-165

12. Schneyer L H, Young J A, Schneyer C A 1972 Salivary secretion of electrolytes. Physiological Review, Bethesda M.D. 52: 720-777

13. Shackleford J M, Klapper C E 1962 Structure and carbohydrate histochemistry of mammalian salivary glands. American Journal of Anatomy 111: 25-47

14. Smith R J, Frommer J 1972 Effects of prepubertal castration on development of granular tubules and amylase activity in the male mouse submandibular gland. Archives of Oral Biology 17: 1561-1571

15. Stacy B D 1969 Augmented renal excretion of calcium and magnesium in sheep after feeding. Quarterly Journal of Experimental Physiology 54: 1-10

16. Suzuki S, Ago A, Mohri S, Nishinakagawa H, Otsuka J 1983 Fine structure of the parotid gland of the Djungarian hamster (Phodopus sungarus). Jikken Dobutsu 32: 175-184

17. Vinning R F, McGinley R A 1985 Hormones in saliva. Critical Reviews in Clinical Laboratory Sciences 23: 95-146

18. Wyatt J R, Eltringham S K 1974 The daily activity of the elephant in the Rwenzori National Park, Uganda. East African Wildlife Journal 12: 273-290

19. Young J A, Schneyer C A 1981 Composition of saliva in mammalia. Australian Journal of Experimental Biology and Medical Science 59: 1-53

20. Young, J A, Van Lennep E W 1978 Transport in salivary and salt glands. In: Glebisch G, Tosteson D C, Ussing H H (ed.) Membrane Transport in Biology Vol 4 Springer, New York

21. Young J A, Van Lennep E W 1978 The morphology of salivary glands Academic Press, London: 1-48, 99

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ULTRASTRUCTURE OF MYOEPITHELIUM IN SALIVARY GLANDS OF AFRICAN ELEPHANT (LOXODONTA AFRICANA)

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The great potential of scanning electron microscopy (SEM) in salivary gland research has not yet been realized.   This technique has however, been utilized in describing the three dimensional features of acinar myoepithelial cells of the submandibular gland of the male mouse (1) and rat (2), sublingual gland of the rat (2,3) and human submandibular gland (4).   The acinus of the rat parotid gland appears to be devoid of myoepithelium (2,5), with only the processes of the intercalated duct myoepithelial cells extending onto the basal region of the acinus (6). A similar distribution has also been observed in the parotid of the rabbit (7).

The purpose of this study was to establish the surface morphology of myoepithelial cells of the parotid and submandibular salivary glands of the largest terestrial animal, the African elephant (Loxodonta africana). Fresh surgical specimens of the parotid and mandibular salivary glands of three full grown elephant bulls were fixed in 2,5% glutaraldehyde and buffered in 0,1M sodium cacodylate.   After fixation the samples were treated in 8N HCl and ultrasonically cleaned.

With stromal tissue and basement membranes removed, myoepithelial cell bodies or processes could not be identified on the parenchymal aspect of the acini of the parotid glands (Fig. 1).   The absence of acinar myoepithelial cells was confirmed with transmission electron microscopy (Fig. 2).   The submandibular glands on the other hand, showed prominent interdigitating myoepithelial processes forming an uninterrupted layer around the acini (Fig. 3).   The cell bodies appeared to be situated eccentrically towards the junction of the acinus and intercalated duct.

The prominence of myoepithelial cells in the submandibular glands of the animals under study, is reminiscent of myoepithelial development in the sublingual gland of the monotreme echnida, Tachyglossus aculeatus, which is reported to secrete an extremely viscous saliva (8).   This phenomenon lead some investigators to postulate that the supporting function of salivary myoepithelial cells is likely to be particularly valuable when the content of mucin in the secretory product is high (9).   This observation is also supported by other independent studies (2). Although we agree that the viscosity of saliva could parallel acinar myoepithelial development, other factors probably have an equal bearing on the prominence of these cells as both the submandibular and parotid salivary glands in elephants secrete a serous (watery) saliva.   Much of the parotid gland of the elephant is located on the level of or above the orifice of the main excretory duct.   Propulsive forces created by the secretory process and contraction of myoepithelial cells surrounding

intercalated ducts should therefore be sufficient for flow of saliva in this gland. The submandibular gland, however, which is situated much lower than its orifice, excretes against a higher pressure gradient. Acinar myoepithelial cells therefore not only play an important supportive role against acinar distention potentiated by this increased luminal pressure, but also add a supplementary propulsive force through their well established contractile function.

REFERENCES

1. Taugner, B., Schiller, A. Cell Tissue Res. 206:65-72 (1980).
2. Nagato, T., Yoshida, A. and Uehara,Y. Cell Tissue Res. 209:1-10 (1980).
3. Nagato, T. J. Electron Microsc. (Tokyo) 27(3):235-236 (1978).
4. Nagai, T. and Nagai, M. Arch. Otolaryngol. 241:265-266 (1985).
5. Mark, M.R., Sharawy, M., Pennington, C. Scan. Elec. Micro. III:137-145 (1981).
6. Garrett, J.R. and Parsons, P.A. Histochem. J. 5:463-471 (1973).
7. Cope, G.H., Prattern, M.K. and Williams, M.A. Histochem. J. 8:403-418 (1976).
8. Young, J.A. and van Lennep, E.W. The morphology of salivary glands. Academic Press Inc. (London) (1978).
9. Garrett, J.R., Emmelin, N. Med. Biol. 57:1-28 (1979).

Fig 1: SEM of acini of parotid gland

Fig 2: TEM of parotid gland

Fig 3: SEM of acinus of submandibular gland illustrating interdigitating myoepithelial processes
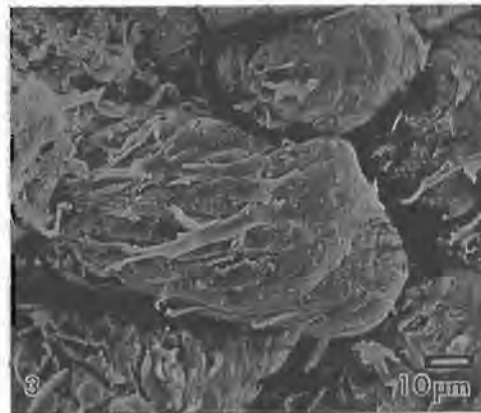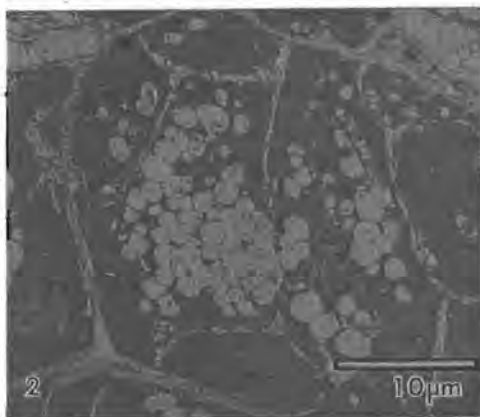
Tyrosine-rich crystalloids in a polymorphous low-grade adenocarcinoma

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A polymorphous low-grade adenocarcinoma with tyrosine-rich crystalloid deposits is reported. The literature is reviewed, and diagnostic and histogenetic implications of this finding are discussed.
(ORAL SURG ORAL MED ORAL PATHOL 1990;70:480-2)

Tyrosine-rich crystalloids occur mainly in salivary gland mixed tumors where they are reported in between 1.5% and 21% of cases.[1-4] The incidence of these deposits appears higher in mixed tumors involving black patients than white patients.[2,5] Salivary gland carcinomas that have been reported to contain tyrosine-rich crystalloids include one terminal duct adenocarcinoma,[6] an adenoid cystic carcinoma,[7] and a malignant mixed tumor.[8]

The origin of tyrosine-rich crystalloids in salivary gland neoplasms is speculative. The principal tumor cell associated with these deposits is the modified neoplastic myoepithelial cell,[6] which is also believed to be the source of the stromal matrix deposits in mixed tumors.[9]

CASE REPORT

A 36-year-old black female patient had a 3 × 2 cm, firm midline swelling at the junction of the hard and soft palate. No ulceration was present. A clinical diagnosis of benign mixed tumor was made and an incisional biopsy taken. Although tyrosine crystals were observed, perineural invasion prompted a provisional diagnosis of a polymorphous low-grade adenocarcinoma and wide excision was recommended. Microscopic examination of the surgical specimen showed an infiltrative neoplastic growth with a lobular architecture. Solid masses of epithelial cells, areas exhibiting ductlike differentiation, and cells arranged in long, single-layered strands were observed. The neoplasm was further characterized by a low mitotic activity, histologic diversity with

cylindric, clear cell, and mucus cell differentiation, and a lack of pleomorphism. Evidence of perineural invasion was present (Fig. 1). Extensive crystalloid deposits were present in the connective tissue stroma and between the cells in the solid epithelial masses (Fig. 2). These crystals showed distinct brown staining with the Millon reaction and were nonbirefringent under polarized light. A diagnosis of polymorphous low-grade adenocarcinoma of minor salivary gland origin with tyrosine-rich crystalloid deposits was made.

DISCUSSION

Polymorphous low-grade adenocarcinoma, also referred to as *terminal duct adenocarcinoma* or *lobular carcinoma of minor salivary gland origin*,[9,10] is a recently described entity occurring most commonly in the palate and is characterized by a favorable prognosis. Histologically, the lesion is distinguished from other malignant tumors of salivary gland origin by its frequent lobular growth pattern, low mitotic rate, and cytologic uniformity. Although extensive nerve invasion and a cribriform growth pattern may resemble adenoid cystic carcinoma, polymorphous low-grade adenocarcinomas are characterized by histologic diversity, with cells exhibiting cuboidal to low columnar differentiation and an eosinophilic cytoplasm. The stroma, furthermore, often exhibits mucohyaline change in contrast to the bland basement membrane–like deposits of adenoid cystic carcinoma.[10-12] If these criteria are to be applied, it appears as if the adenoid cystic carcinoma containing tyrosine-rich crystalloids reported by Gould and coworkers[7] might possibly have been a polymorphous low-grade adenocarcinoma. If this case were to be accepted as a polymorphous low-grade adenocarcinoma, it would bring the total

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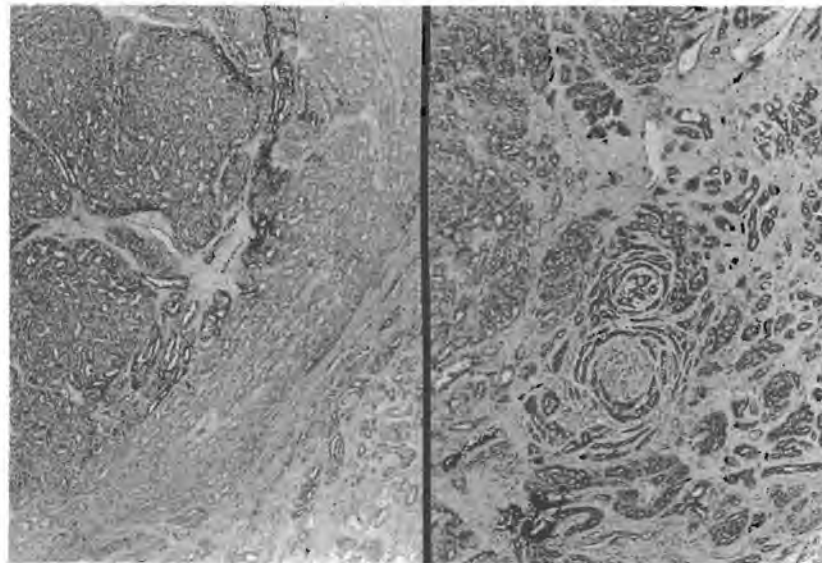7/14/20170

Fig. 1. Distinctly lobular arrangement and infiltrative growth of tumor *(left)* with perineural infiltration *(right)*. (Hematoxylin-eosin stain; original magnification, ×40.)
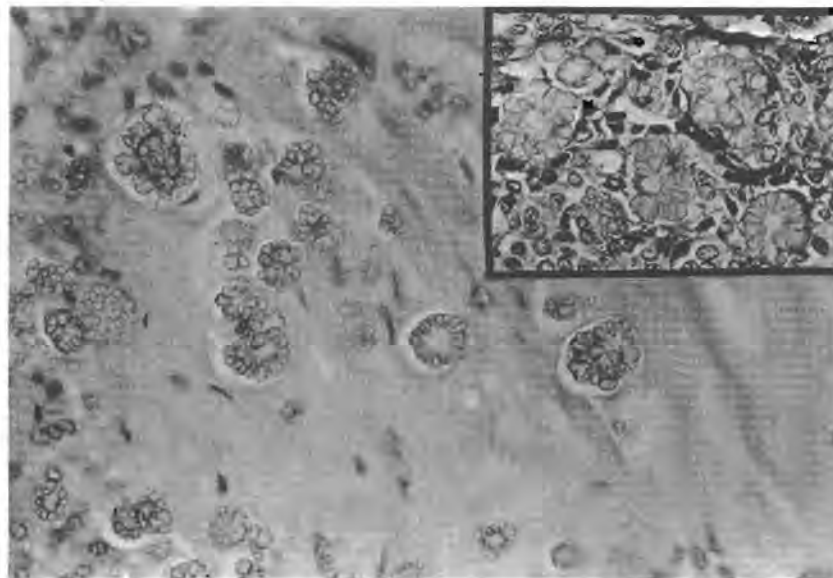

Fig. 2. Stromal and interepithelial *(inset)* deposits of tyrosine-rich crystalloids. (Hematoxylin-eosin stain; original magnification, ×200.)

number with tyrosine-rich crystalloids to three, including the case described by Harris and Shipkey[6] as a "terminal duct adenocarcinoma."

The identification of tyrosine-rich crystalloids in a neoplasm other than benign mixed tumor has important diagnostic implications, as many recent publications regard these crystalloids as a unique microscopic feature of salivary gland mixed tumors.[13-15] It is speculated that the formation of tyrosine-rich crystalloids in polymorphous low-grade adenocarcinomas may place these lesions on a level of cytodifferentiation closer to that of benign mixed tumors than to the other more malignant tumors of salivary gland origin.

REFERENCES

1. Humphrey PA, Ingram P, Tucker A, Shelburne JD. Crystalloids in salivary gland pleomorphic adenomas. Arch Pathol Lab Med 1989;113:390-3.
2. Thomas K, Hutt MSR. Tyrosine crystals in salivary gland tumours. J Clin Pathol 1981;34:1003-5.
3. Campbell WG, Priest RE, Weathers DR. Characterization of two types of crystalloids in pleomorphic adenomas of minor salivary glands. A light microscopic, electron-microscopic, and histochemical study. Am J Pathol 1985;118:194-202.
4. Chaplin AJ, Darke P, Patel S. Tyrosine-rich crystals in pleomorphic adenomas of parotid glands. J Oral Pathol 1983; 12:342-6.
5. Thackray AC, Lucas RB, eds. Tumors of the major salivary glands. Washington, D.C.: Armed Forces Institute of Pathology, 1974:32.
6. Harris BR, Shipkey F. Tyrosine-rich crystalloids in neoplasms and tissues of the head and neck. Arch Pathol Lab Med 1986;110:709-12.
7. Gould AR, Van Arsdall LR, Hinkle SJ, Harris WR. Tyrosine-rich crystalloids in adenoid cystic carcinoma: histochemical and ultrastructural observations. J Oral Pathol 1983;12:478-90.
8. Gerughty RM, Scofield HH, Brown FM, Hennigar GR. Malignant mixed tumors of salivary gland origin. Cancer 1969; 24:471-86.
9. Raubenheimer EJ. The myoepithelial cell: embryology, function, and proliferative aspects. CRC Crit Rev Clin Lab Sci 1987;25:161-93.
10. Aberle AM, Abrams AM, Bowe R, Melrose RJ, Handlers JP. Lobular (polymorphous low-grade) carcinoma of minor salivary glands. A clinicopathologic study of twenty cases. ORAL SURG ORAL MED ORAL PATHOL 1985;60:387-95.
11. Evans HL, Batsakis JG. Polymorphous low-grade adenocarcinoma of minor salivary glands. A study of 14 cases of a distinctive neoplasm. Cancer 1984;53:935-42.
12. Freedman PD, Lumerman H. Lobular carcinoma of intraoral minor salivary gland origin. ORAL SURG ORAL MED ORAL PATHOL 1983;56:157-65.
13. Nochomovitz LE, Kahn LB. Tyrosine crystals in pleomorphic adenomas of the salivary gland. Arch Pathol 1974;97:141-5.
14. Thomas KM, Hutt MSR, Borgstein J. Salivary gland tumors in Malawi. Cancer 1980;46:2328-34.
15. Bottles K, Ferrell LD, Miller TR. Tyrosine crystals in fine needle aspirates of a pleomorphic adenoma of the parotid gland. Acta Cytol 1984;28:490-2.

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Evaluation of the nucleolar organizer region associated proteins in minor salivary gland tumors

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Van Heerden WFP, Raubenheimer EJ: Evaluation of the nucleolar organizer region associated proteins in minor salivary gland tumors. J Oral Pathol Med 1991; 20: 291–5.

Forty-three intraoral salivary gland tumors were studied to determine the value of the AgNOR technique in the assessment of these neoplasms. Well defined black dots were visible in the nucleii of all the specimens studied. The mean AgNOR count per nucleus for each tumor was calculated as follows: pleomorphic adenoma ($n=15$) 1.52; Polymorphous low-grade adenocarcinoma ($n=12$) 1.90; adenoid cystic carcinoma ($n=6$) 2.92; mucoepidermoid carcinoma ($n=4$) 1.93; carcinoma ex mixed tumor ($n=4$) 2.05; undifferentiated carcinoma ($n=1$) 3.13 and epithelial-myoepithelial carcinoma ($n=1$) 2.23. The difference between the means of benign and malignant tumors ($P<0.01$) and polymorphous low-grade adenocarcinoma and adenoid cystic carcinoma ($P<0.01$) were highly significant. The overlapping of the AgNOR count betwen various tumors prohibited the use of this technique as an absolute criterion in establishing a final diagnosis. It could however be used as a diagnostic aid in differentiating between salivary gland neoplasms.

Nucleolar organizer regions (NORs) are loops of ribosomal DNA that transcribe to ribosomal RNA and thus ultimately to protein (1). NORs have been utilized by cytogeneticists for the evaluation of certain genetic disorders, notably trisomies and are located on the short arms of the five acrosentric chromosomes 13, 14, 15, 21, and 22 (2). The NORs can be demonstrated by means of a silver staining technique (AgNORs) that is performed at room temperature on paraffin embedded tissues (3). This technique is based on the argyrophylia of the NOR-associated proteins. The known NOR associated proteins are RNA polymerase I, C23 (nucleolin), B23, 100K and 80K protein (1). Their function is uncertain although a role in rDNA transcription is postulated (1).

AgNOR counts appear to relate to cell ploidy (4) as well as the rate of cellular proliferation of individual cells (5). Quantification of NORs by means of the AgNOR technique has been used to distinguish between high and low grade lymphomas (5) and between benign and malignant counterparts of various origins (6–9).

Small biopsy specimens from salivary gland tumors is often difficult to interpret and additional microscopic criteria can only benefit the diagnostic process. MORGAN et al. (10) and MATSUMURA et al. (11) have found a statistically significant difference between the numbers of AgNORs in the nuclei of benign versus malignant salivary gland neoplasms. For this technique to have an application in diagnostic histopathology, it's ease of interpretation and reproducibility between laboratories is important. This study was undertaken to evaluate the AgNOR staining technique as a diagnostic aid for salivary gland neoplasms.

Material and methods

Forty-three intraoral salivary gland tumors were retrieved from the files of the Department of Oral Pathology, Medical University of Southern Africa. Fifteen were diagnosed as pleomorphic adenomas (PA) twelve as polymorphous low-grade adenocarcinomas (PLA), six as adenoid cystic carcinomas (ACC), four carcinomas ex pleomorphic adenoma, four as mucoepidermoid carcinomas (MEC), one as an undifferentiated carcinoma and one as an epithelial-myoepi-

thelial carcinoma. The tissue samples had all been fixed in 10% formalin and processed to paraffin wax. Two 3 μm paraffin sections of each specimen were cut. One was stained with hematoxylin-eosin and the other with the AgNOR method as described by PLOTON et al. (3). The H&E sections were all reassessed and revised with regards to histologic classification. The AgNOR stained sections were examined under a 100X oil immersion lens by the two authors and intranuclear dots were counted in 200 randomly selected nuclei using an eyepiece graticule to prevent recounting. Nuclei of overlapping tumor cells were not included. Nucleolar clusters were counted as a single AgNOR and no attempt was made to resolve the clusters into their discernible number of discrete dots. The mean number of AgNOR dots per nucleus was determined for each specimen. The resulting data were analyzed by means of student's t-test for uncorrelated data.

Results

The NOR associated proteins were visible as well defined black dots inside and

Table 1. Mean number of AgNORs in the nuclei of salivary gland neoplasms

Specimen	PA (Type)	PLA	ACC (Growth Pattern)	MEC (Grade)	(Ca ex PA)	Undiff ca	EPI
1	1.11 (I)	1.30	3.06 (T)	1.19 (IG)	2.37	3.13	2.23
2	1.37 (I)	1.96	2.00 (T)	1.80 (IG)	1.40		
3	1.23 (III)	1.53	3.01 (T)	2.34 (HG)	2.63		
4	1.75 (I)	1.84	4.29 (C)	2.36 (HG)	1.79		
5	1.73 (I)	1.86	2.84 (C)				
6	1.71 (I)	1.88	1.78 (C)				
7	0.98 (I)	2.02					
8	1.52 (II)	1.93					
9	1.93 (I)	2.47					
10	1.41 (I)	2.09					
11	1.60 (I)	2.24					
12	1.48 (I)	1.64					
13	1.30 (I)						
14	2.25 (I)						
15	1.37 (III)						
Mean	1.52	1.90	2.83	1.93	2.05	3.13	2.23
SD	0.32	0.31	0.89	0.55	0.55		

PA = pleomorphic ademona; PLA = polymorphous low-grade adenocarcinoma; ACC = adenoid cystic carcinoma; MEC = mucoepidermoid carcinoma; CA ex PA = carcinoma ex pleomorphic adenoma; Undiff Ca = Undifferentiated carcinoma; EPI = epithelial-myoepithelial carcinoma; T = tubular/trabecular; S = solid; C = cribriform; IG = intermediate grade; HG = high grade

outside of the nucleolus of the tumor cells studied. Careful focussing was essential to clearly identify all the dots. The results were summarized in Table 1. The lowest mean of AgNOR dots per nucleus was found in PA (Fig. 1) and the highest in an ACC (Fig. 2). The dots in the malignant neoplasms had a greater variability in size and shape compared to those in the PA. The difference in the mean number of dots per nucleus between PA and PLA (Fig. 3) and between PLA and ACC were statistically highly significant ($P < 0.01$). The correlation coefficient between the two observers was 0.97.

Discussion

The reason for the varying quantities of AgNORs in nuclei of different tumors is uncertain. The NORs are located on the 5 acrocentric chromosomes resulting in 10 NOR bearing chromosomes during metaphase. These individual NORs are usually not discernible because they are tightly aggregated in the one or two nucleoli normally present in a cell (12). Active cell proliferation may be accompanied by nucleolar dissociation, resulting in dispersed AgNORs throughout the nucleus. This as well as an increase in transcriptional activity
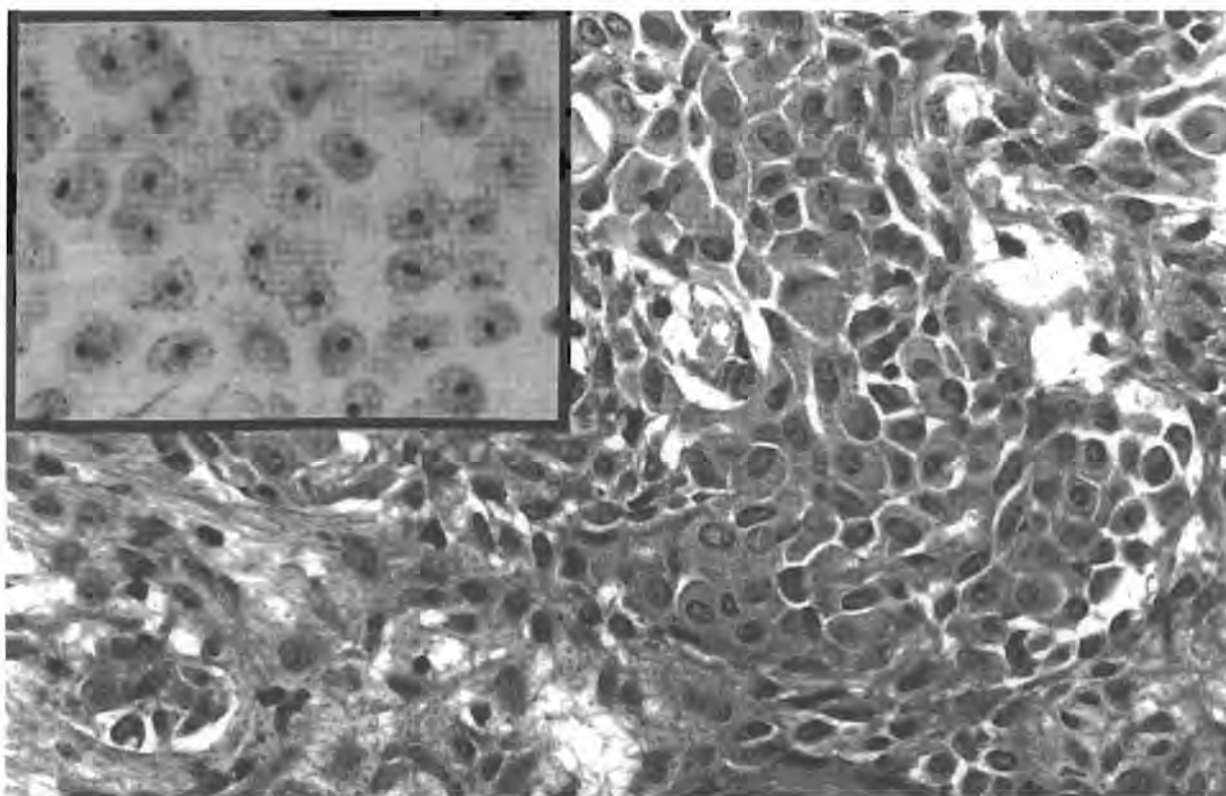

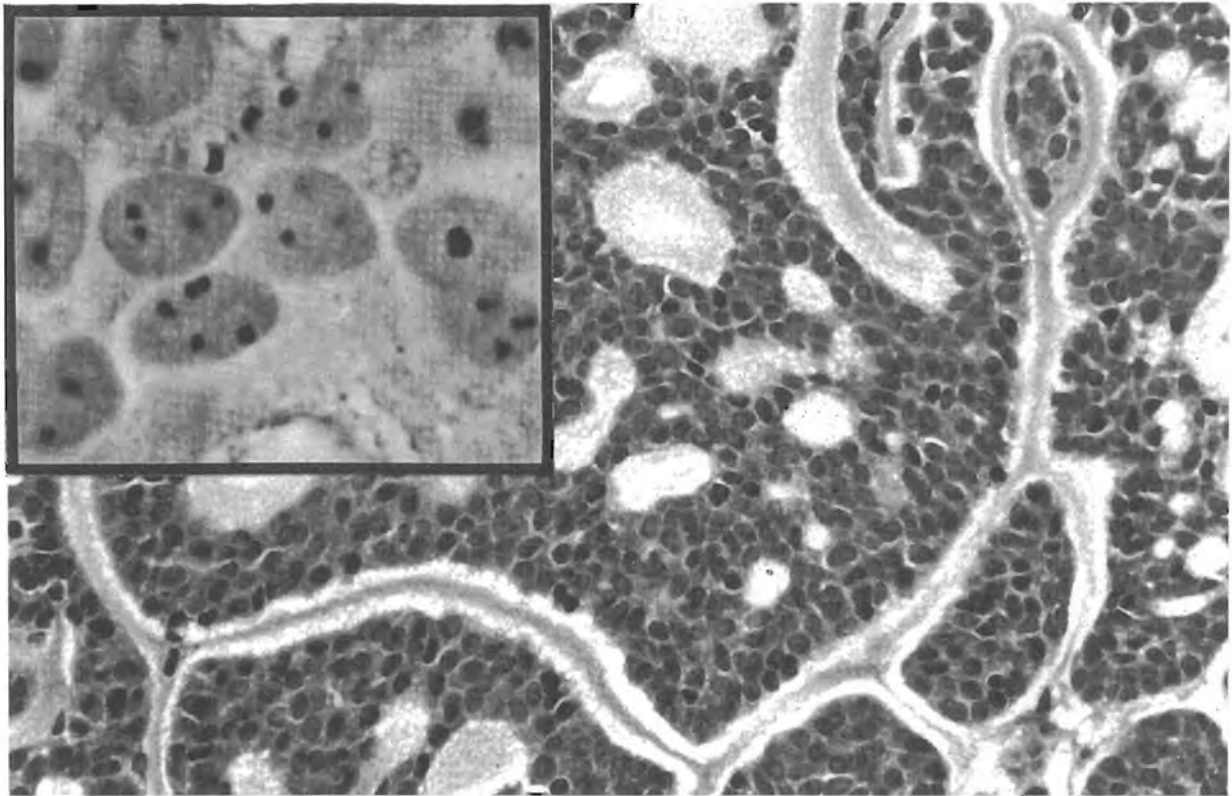Fig. 1. Pleomorphic adenoma with plasmacytoid tumor cells. × 200. Inset: most cells contained one AgNOR dot per nucleus. × 400.

Fig. 2. Adenoid cystic carcinoma with cribriform growth pattern. × 200. Inset: multiple small AgNOR dots were present in nuclei. × 1000.
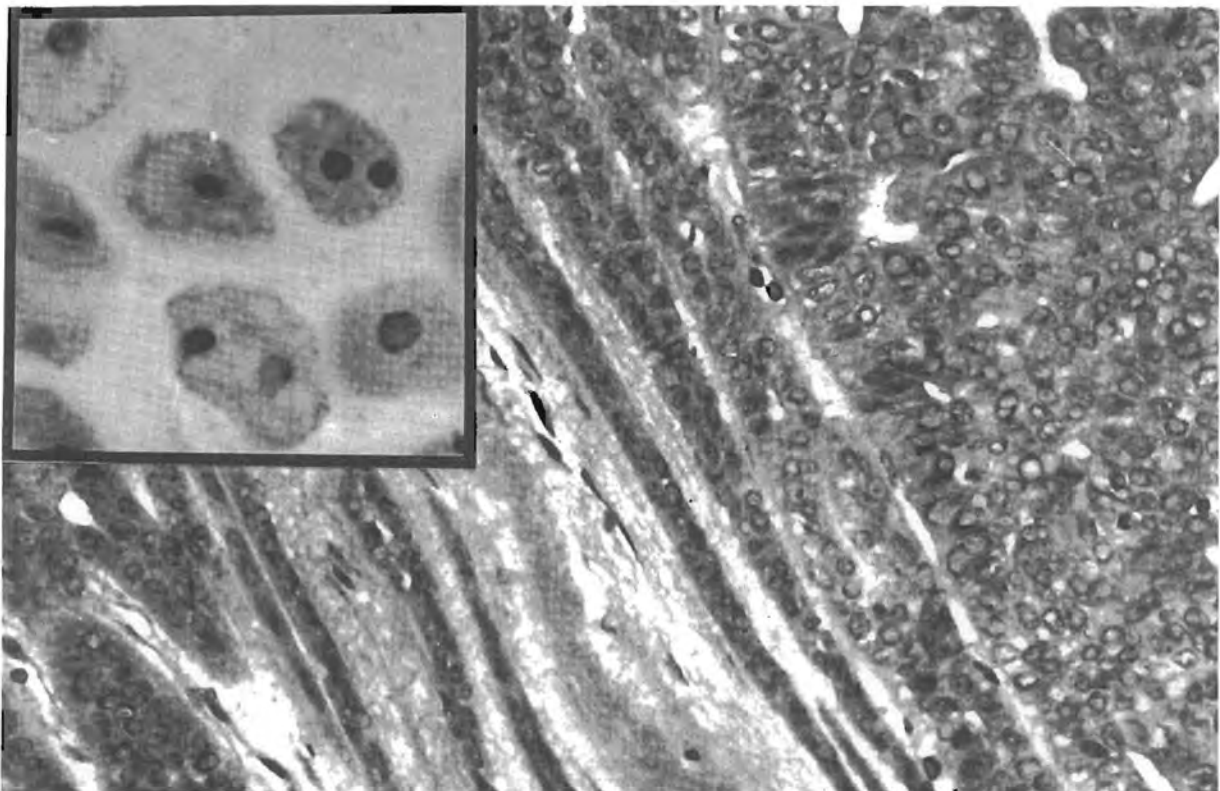

Fig. 3. Polymorphous low-grade adenocarcinoma. × 200. Inset: nuclei contained one or two large AgNOR dots. × 1000.

Table 2. Reported mean AgNOR counts in salivary gland neoplasms

Neoplasm	Present study	MORGAN et al.(10)	MATSUMURA et al. (11)
PA	1.52	1.47	1.62–1.68*
ACC	2.83	3.92	2.78
MEC	1.93	4.25	2.59

PA = pleomorphic adenoma; ACC = adenoid cystic carcinoma; MEC = mucoepidermoid carcinoma; * = different cell types in the same tumor were separately counted

will result in an increase in the mean AgNOR count of a cell population. In malignancy, the AgNORs tend to become more dispersed through the nucleus and thus more readibly discernable (12). HALL et al. (13) have shown that there is a significant correlation between the AgNOR count and presence of positive Ki-67 immunostaining in cells. Ki-67 is a monoclonal antibody that recognizes a nuclear antigen present only in proliferating cells (14). The possibility that the AgNOR count is related to cellular activity is also suggested by PLOTON et al. (3). SURESH et al. (4) have shown that AgNOR counts in non neoplastic trophoblastic tissue are a reflection of ploidy rather than cell proliferation. They suggested that the relationship between cell ploidy and AgNOR counts can be obscured in neoplastic lesions because of excessive proliferative activity of tumor cells.

Both ACC and PLA have an infiltrative growth pattern with an affinity for perineural spread. Cribriform, tubular and solid tumor cell arrangements can be found in ACC and PLA (15). Histologically, ACC differs from PLA in that the tumor cells have very little cytoplasm and contains hyperchromatic nucleii. Mitotic activity can be found in both tumors, although none of the PLA in our collection had a mitotic index of more than 5 mitotic figures per 10 high power fields (× 400). Pleomorphism is absent in PLA whereas polymorphism is seldom seen in ACC. Despite these differences, it can be very difficult to distinguish between PLA and ACC, especially when only a small tissue fragment is submitted for histologic examination. ACC and PLA are thought to develop from the same precursor cell line (16) with the result that special staining techniques used as diagnostic procedures must be able to distinguish between cellular differentiation or activity of the two lesions. Various immunohistochemical techniques have shown potential with regards to the pathogenesis and differentiation of salivary gland tumors, although their reliability and diagnostic value is often unclear (10). A

statistically significant difference between the mean AgNOR count in PLA and ACC was found in the present study. The higher count in ACC could probably be related to the more aggressive behaviour of this neoplasm when compared to PLA. The mean count in ACC did not correlate with the histologic growth pattern, a prognostic factor for tumor behavior in ACC. The highest count was present in a tumor with a predominantly cribriform growth pattern. In a study to evaluate the prognostic factors for ACC, HAMPER et al. (17) inter alia assessed DNA contents of the tumor cells using single cell scanning cytophotometry. They concluded that the shortest survival time was found in patients with tumors showing atypical histograms of nuclear contents of which 42% had a cribriform growth pattern. Previous studies (10, 11) evaluating the AgNOR technique in salivary gland neoplasms did not identify PLA as a separate entity, making comparison with the present study regarding PLA impossible. The overlapping of the AgNOR count between PLA and ACC prohibited the use of this technique as an absolute criterion to establish a final diagnosis but it could be used as a diagnostic aid to differentiate between these two neoplasms.

The mean value for MEC in the present study was lower than the value determined by MORGAN et al. (10) and MATSUMURA et al. (11) (Table 2). They do not specity the histologic grade of MEC included in their study. Although only four MEC's were examined in the present study, a substantial higher AgNOR count was found in the two high grade MEC. No significant difference in the AgNOR counts was found between the different types of BMT as classified using the criteria of SEIFERT et al. (18). This is supported by the findings of CHAU & RADDEN, (19) that there is no difference in the recurrence rate and frequency of capsular infiltration between the different subtypes of BMT.

The overlap between the AgNOR ranges in different tumors can be ex-

pected, since the absolute numbers of AgNORs in nucleii are not counted in 3 μm sections. Some AgNORs may have been missed in the 3 μm sections, especially in the malignant and high grade tumors where the nuclei were large and multiple small AgNORs were present.

The argyrophilic staining of AgNOR is not a method for demonstrating the nucleolus, but rather a technique to demonstrate its substructures in such a way as to allow study of their shape and number. Although the evaluation of AgNOR stains are time consuming, it appears to be of value in differentiating between salivary gland neoplasms.

References

1. CROCKER J. Nucleolar organiser regions. In: UNDERWOOD J C E, ed. *Current topics in pathology.* New York: Springer-Verlag, 1990: 92–143.
2. ANONYMOUS. NORs – a new method for the pathologist. *Lancet* 1987; 1: 1413–4.
3. PLOTON D, MENAGER M, JEANNESSON P, HIMBERG G, PIGEON F, ADNET J J. Improvement in the staining and in the visualization of the argyrophilic proteins of the nucleolar organizer region at the optical level. *Histochem J* 1986; 18: 5–14.
4. SURESH U R, CHAWNER J, BUCKLEY C H, FOX H. Do Agnor counts reflect cellular ploidy or cellular proliferation? A Study of trophoblastic tissue. *J Pathol* 1990; 160: 213–5.
5. CROCKER J, NAR P. Nucleolar organizer regions in lymphomas. *J Pathol* 1987; 151: 111–8.
6. MACKIE R M, WHITE S I, SEYWRIGHT M M, YOUNG H. An assessment of the value of AgNOR staining in the identification of dysplastic and other borderline melanocytic naevi. *Br J Dermatol* 1989; 120: 511–6.
7. SMITH R, CROCKER J. Evaluation of nucleolar organizer region-associated proteins in breast malignancy. *Histopathology* 1988; 12: 221–3.
8. ROSA J, MEHTA A, FILIPE M I. Nucleolar organizer regions in gastric carcinoma and its precursor stages. *Histopathology* 1990; 16: 265–9.
9. CROCKER J, SKILBECK N. Nucleolar organizer region associated proteins in cutaneous melanotic lesions: a quantitive study. *J Clin Pathol* 1987; 40: 885–9.
10. MORGAN D W, CROCKER J, WATTS A, SHENOI P M. Salivary gland tumours studied by means of the AgNOR technique. *Histopathology* 1988; 13: 553–9.

11. MATSUMURA K, SASAKI K, TSUJI T, SHI-NOZAKI F. The nucleolar organizer regions associated protein (AgNORs) in salivary gland tumors. *Int J Oral Maxillofac Surg* 1989; **18**: 76–8.

12. UNDERWOOD J C R, GIRI D D. Nucleolar organizer regions as diagnostic discriminants for malignancy. *J Pathol* 1988; **155**: 95–6.

13. HALL P A, CROCKER J, WATTS A, STANSFIELD A G. A comparison of nucleolar organizer region staining and Ki-67 immunostaining in non-Hodgkin's lymphoma. *Histopathology* 1988; **12**: 373–81.

14. GERDES J, SCHWAB U, LEMKE H, STEIN H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983; **31**: 13–20.

15. EVANS H L, BATSAKIS J G. Polymorphous low-grade adenocarcinoma of minor salivary glands. *Cancer* 1984; **53**: 935–42.

16. ABERLE A M, ABRAMS A M, BOWE R, MELROSE R J, HANDLERS J P. Lobular (polymorphous low-grade) carcinoma of minor salivary glands. *Oral Surg Oral Med Oral Pathol* 1985; **60**: 387–94.

17. HAMPER K, LAZARF, DIETELM *et al.* Prognostic factors for adenoid cystic carcinoma of the head and neck: a retrospective evaluation of 96 cases. *J Oral Pathol Med* 1990; **19**: 101–7.

18. SEIFERT G, MIEHLKE A, HAUBRICH J, CHILLA R, eds. *Diseases of the salivary glands.* Stuttgart: Georg Thieme Verlag, 1986: 182–94.

19. CHAU M N Y, RADDEN B G. A clinico-pathological study of 53 intra-oral pleomorphic adenomas. *Int J Oral Maxillofac Surg* 1989; **18**: 158–62.

oral pathology

Intraoral salivary gland neoplasms: A retrospective study of seventy cases in an African population

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Intraoral salivary gland neoplasms diagnosed in the Department of Oral Pathology, Medical University of Southern Africa, Medunsa, were reassessed and revised with regard to histologic diagnosis. New entities and subclassifications that have been described in recent years were taken into account. Seventy cases were diagnosed during an 8-year period, and the sample consisted of black patients only. Benign mixed tumor was the most common entity and accounted for 48% of all tumors. Polymorphous low-grade adenocarcinoma comprised 15.7% of the sample and was the most frequent malignant tumor. The mean age of patients with benign and malignant tumors were 36.5 and 49.8 years, respectively ($p < 0.05$), and the palate was the most common site involved. Geographic differences do exist in the pattern and pathology of intraoral salivary gland neoplasms when compared with findings in other studies. (ORAL SURG ORAL MED ORAL PATHOL 1991;71:579-82)

T he distribution and frequency of intraoral salivary gland neoplasms has been discussed in several published series,[1-5] in the majority of which the World Health Organization (WHO) classification[6] was used. However, various new entities and subclassifications that are not included in these articles have been described in recent years.[7, 8] This study was undertaken to determine the relative frequency and distribution of intraoral salivary gland neoplasms in a predominantly rural black African population and to provide data for comparison with findings in other geographic locations.

MATERIAL AND METHODS

All the intraoral salivary gland neoplasms diagnosed during the last 8 years were retrieved from the files of the Department of Oral Pathology, Medical University of Southern Africa, Medunsa. Most patients seen at the hospitals served by the department are black and of rural southern African origin. Representative slides stained with hematoxylin and eosin were available for review, and, where necessary, appropriate special stains were used to establish a diagnosis. All cases were reassessed and revised with regard to histologic classification. Diagnosis was made with the WHO classification[6] as the basis. New entities such as polymorphous low-grade adenocarcinoma, and subclassifications that have been described in recent years, were taken into account. This includes the subclassification of mixed salivary gland tumors into types I to IV according to the proportion of the stroma in the tumor mass.[8] The polymorphous low-grade adenocarcinomas were divided into the terminal duct type and the papillary type according to the criteria of Slootweg and Müller.[9] The working classification used in this study is shown in Table I. Age, sex, and site were noted from the clinical records.

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Table I. Working classification

Benign
Mixed tumor
Type I (stroma 30%-50%)
Type II (stroma >80%)
Type III (stroma <20%)
Type IV (myoepithelioma)
Monomorphic adenoma
Malignant
Mucoepidermoid carcinoma
Low grade
Intermediate grade
High grade
Adenoid cystic carcinoma
Cribriform
Tubular/trabecular
Solid
Polymorphous low-grade adenocarcinoma
Terminal duct
Papillary
Acinic cell carcinoma
Carcinoma ex mixed tumor
Epidermoid carcinoma
Adenocarcinoma
Epithelial-myoepithelial carcinoma
Undifferentiated carcinoma

Table II. Location and subclassification of 34 mixed tumors

Type	Palate	Upper lip	Total (%)
I	25	1	26 (76)
II	2	1	2 (6)
III	5	1	6 (18)
IV			0
Total (%)	31 (91)	3 (9)	34 (100)

RESULTS

The sample consisted of a total of 70 cases of intraoral salivary gland neoplasms. Forty-three (62%) of the patients were female and 27 (38%) were male, yielding a female/male ratio of 1.6:1. The patients ranged in age from 10 to 85 years. Thirty-four cases (48%) were classified as benign; all these were mixed tumors in patients ranging in age from 10 to 64 years, with a mean age (±SD) of 36.5 ± 14.7 years. The female/male ratio was 2.4:1, with the mean age for females 34.9 ± 14.9 years and 40.3 ± 13.1 years for males. The palate was most commonly affected, accounting for 31 tumors (91%). The remaining mixed tumors were found on the upper lip. The location and subclassification of mixed tumors according to the criteria of Seifert et al.[8] are shown in Table II.

Thirty-six cases (52%) were classified as malignant. The patients ranged in age from 22 to 85 years, with a mean age of 49.8 ± 16.3 years. The difference in the mean age of patients with benign tumors and that of those with malignant tumors was statistically significant ($p < 0.05$). The female/male ratio was 1.1:1 for patients with malignant neoplasms. The distribution and location of the malignant tumors are shown in Table III.

Eleven tumors were diagnosed as polymorphous low-grade adenocarcinoma, accounting for 30% of the malignant neoplasms and 15.7% of all neoplasms. The patients' ages were between 32 and 70 years, with a mean age of 53 ± 12.6 years, and the female/male ratio was 1.2:1. Nine lesions (82%) occurred on the palate, and one each in the buccal mucosa and upper lip, respectively. Two tumors had a papillary-type growth pattern, and nine were either lobular or tubular in appearance. Nerve infiltration was present in one papillary-type and in two terminal duct–type tumors.

Nine cases of adenoid cystic carcinoma accounted for 12.8% of all tumors and 25% of the malignant tumors. The patients had a age range of 33 to 85 years with a mean of 54 ± 15.5 years, and the male/female ratio was 1.2:1. Seven patients had lesions on the palate, and one lesion each was located in the floor of the mouth and on the upper lip. Five tumors had a predominantly cribriform growth pattern, and two each had solid and tubular or trabecular growth, respectively.

Six patients with mucoepidermoid carcinoma accounted for 8.6% of all tumors and 16.7% of malignant tumors. The youngest patient was 22 years and the oldest 52 years of age, and the mean age at time of consultation was 39.8 ± 10.1 years. Sex distribution was equal, and the most common site of occurrence was the palate, with four tumors. One tumor was located on the buccal mucosa and mandibular gingiva, respectively. One mucoepidermoid carcinoma was classified microscopically as low grade, three as intermediate grade, and two as high grade.

Five carcinomas ex mixed tumor were diagnosed. Three of the patients were women and two were men; they ranged in age from 31 to 70 years, with a mean age of 48.2 ± 19.3 years. Four tumors occurred on the palate, and one in the retromolar area. The carcinomatous component in all five was classified as undifferentiated.

Three cases were diagnosed as adenocarcinomas, not otherwise specified. Two tumors were located in the buccal mucosa and one on the palate. Two cases occurred in females, and the mean age of this group was 57.3 ± 13.2 years. One tumor that occurred on the palate of a 49-year-old woman was diagnosed as an undifferentiated carcinoma.

One patient, a 65-year-old woman, had a epithelial-myoepithelial carcinoma of the palate. No case of monomorphic adenoma, acinic cell carcinoma, or

Table III. Distribution and location of malignant tumors

Tumor	Palate	Upper lip	Buccal mucosa	Mouth floor	Retromolar	Mandibular gingiva	Total	% of total	% of malignant
PLA	9	1	1				11	15.7	30
ACC	7	1		1			9	12.8	25
Mucoepidermoid CA	4		1			1	6	8.6	16.7
CA ex mixed tumor	4				1		5	7.1	14
Adenocarcinoma	1		2				3	4.3	8.3
Undifferentiated CA	1						1	1.4	2.8
E-M CA	1						1	1.4	2.8
Total (%)	27 (75)	2 (5.5)	4 (11)	1 (2.8)	1 (2.8)	1 (2.8)	36		

ACC, Adenoid cystic adenocarcinoma; *CA,* carcinoma; *E-M,* Epithelial-myoepithelial; *PLA,* polymorphous low-grade adenocarcinoma.

epidermoid carcinoma of the minor salivary glands occurred in this series.

DISCUSSION

In most studies benign mixed tumors constitute the jority of minor salivary gland neoplasms.[1-5] The frequency of benign mixed tumors is reported as 43% in the study of Eveson and Cawson,[4] 41% by Waldron et al.,[1] and 54% by Chau and Radden.[5] In Isacsson and Shear's series[2] 70% of the tumors were classified as benign mixed tumors. They postulated that the high frequency in their series was the result of the relative higher number of black than white patients, although 60% of their white patients had mixed tumors diagnosed. Schulenburg[10] reported that intraoral benign mixed tumors in his South African sample were 3.5 times more common in black than in white patients. In the present series, where the sample consisted of black patients only, 34 (48%) of the tumors were classified as benign mixed tumors, a frequency comparable to that reported in population samples in the United States and Europe.[1,4]

The majority of tumors (52%) in the present study were malignant, a finding that does not support the of benign to malignant tumors in recent reports. The proportion of benign tumors varied from 53%[11] to 72%[2] in recent studies. However, 80% of the cases reported by Spiro et al.[12] were classified as malignant. This high percentage of malignant tumors can be explained by the fact that their institution is a major cancer referring center.

The palate was the most common site of involvement of both malignant and benign tumors. The proportion of benign tumors occurring on the palate was larger than in the malignant group, although the difference is not statistically significant. Eighty-one percent of benign mixed tumors reported by Isacsson and Shear[2] occurred on the palate. This high frequency of palatal involvement might be due to the presence of black patients in both samples. The distribution of palatal tumors from several large series compared with our findings is reflected in Table IV.

Table IV. Reported frequency of intraoral salivary gland tumors of palate

Author	Frequency (%) Mixed tumor	Frequency (%) Malignant tumor
Present study	91	75
Thomas et al.[14]	65	63
Isacsson and Shear[2]	81	60
Eveson and Cawson[4]	60	55
Waldron et al.[1]	54	42
Regezi et al.[3]	55	49
Chau and Radden[5]	70	54
Chaudhry et al.[11]	65	35

The benign mixed tumors occurred at a significantly younger age than did the malignant tumors ($p < 0.05$), and a high percentage of the benign tumors affected female patients. These observations support the proposal by Isacsson and Shear[2] that in an African population a salivary gland tumor of the palate occurring in a relatively young patient is more likely to be benign than malignant. This appears to be especially true in women.

Seifert et al.[8] divided benign mixed tumors into four types according to the volume and properties of the stroma and the differentiation of the epithelial cells. Although types III and IV constituted 35% of minor salivary gland mixed tumors in their series, almost 50% of the carcinomas ex mixed tumor arose from tumors with these growth patterns.[8] We are unable to comment on the rate of malignant transformation of types III and IV because only small fragments of benign mixed tumor were present in the carcinomas ex mixed tumor in our series. The finding of Seifert et al.[8] could be related to the more common occurrence of mitotic activity in the solid areas. The majority of benign mixed tumors in the present series were classified as type I. Although mitotic activity, when present, was usually restricted to the solid parts of the tumor, the subclassification depended on the

amount of sections taken, because the growth pattern varied through the tumor.

The absence of monomorphic adenomas in the present study may be due to the fact that our sample consisted of black patients only. Isacsson and Shear[2] found three monomorphic adenomas (2.2%) in their sample of 136 black patients. Davies et al.,[13] in a study of salivary gland tumors in Uganda, found no monomorphic adenomas in 33 intraoral tumors. Thomas et al.,[14] who analyzed salivary gland tumors in Malawi, found one monomorphic adenoma (2%) in their total of 57 minor tumors. These frequencies are in contrast with the 10.7% reported by Waldron et al.,[1] 11% by Evenson and Cawson,[4] and 10% by Regezi et al.[3]

Polymorphous low-grade adenocarcinoma was the most common malignant tumor in the present series. Comparison of the frequency of polymorphous low-grade adenocarcinoma with that reported in other studies is difficult because the majority employed the WHO classification,[6] which does not recognize polymorphous low-grade adenocarcinomas as a separate entity. Polymorphous low-grade adenocarcinoma constituted 30% of the malignant tumors in the present study. Freedman and Lumerman[15] found polymorphous low-grade adenocarcinoma to constitute 7% of the 150 malignant intraoral tumors they examined. Aberle et al.[16] reviewed 109 cases of adenocarcinoma not otherwise specified, malignant mixed tumor, and adenoid cystic carcinoma, and found that 17% of their cases met the criteria of polymorphous low-grade adenocarcinoma. In the study of Waldron et al.[1] 26% of the malignant tumors were diagnosed as polymorphous low-grade adenocarcinoma. The differences among these findings are probably related to the criteria used for diagnosis of polymorphous low-grade adenocarcinoma, because overlapping histologic features with adenoid cystic carcinoma do exist.

The frequency of adenoid cystic carcinoma (12.8%) in our series is similar to that reported in the literature, for example, 13.1% by Eveson and Cawson,[4] 10.9% by Regezi et al.,[3] and 10.4% by Isacsson and Shear.[2] Adenoid cystic carcinoma accounted for 25% of the malignant tumors in our series, a figure lower than the 38% reported by Isacsson and Shear[2] and the 31% of Regezi et al.[3] Polymorphous low-grade adenocarcinoma was not classified as a separate entity in the previously mentioned series, and the reported frequencies of adenoid cystic carcinoma are probably too high.

In the majority of studies mucoepidermoid carcinoma was the most frequent type of malignant tumor, accounting for 15%[1] to 34%[11] of all intraoral salivary gland tumors. Mucoepidermoid carcinoma accounted

for 8.6% of all tumors in the present series. This figure compares with the 6.5% reported by Isacsson and Shear,[2] also in a South African population. This corroborates the suggestion by Everson and Cawson[17] that a geographic variation in the frequency of mucoepidermoid carcinoma exists.

REFERENCES

1. Waldron CA, El-Mofty SK, Gnepp DR. Tumors of the intraoral minor salivary glands: a demographic and histologic study of 426 cases. ORAL SURG ORAL MED ORAL PATHOL 1988;66:323-33.
2. Isacsson G, Shear M. Intraoral salivary gland tumors: a retrospective study of 201 cases. J Oral Pathol 1983;12:57-62.
3. Regezi JA, Lloyd RV, Zarbo RJ, McClatchey KD. Minor salivary gland tumors: a histologic and immunohistochemical study. Cancer 1985;55:108-15.
4. Eveson JW, Cawson RA. Tumours of the minor (oropharyngeal) salivary glands: a demographic study of 336 cases. J Oral Pathol 1985;14:500-9.
5. Chau MNY, Radden BG. Intra-oral salivary gland neoplasms: a retrospective study of 98 cases. J Oral Pathol 1986;15:339-42.
6. Thackray AC, Sobin LH. Histological typing of salivary gland tumours. Geneva: World Health Organization, 1972.
7. Evans HL, Batsakis JG. Polymorphous low-grade adenocarcinoma of minor salivary glands. Cancer 1984;53:935-42.
8. Seifert G, Miehlke A, Haubrich J, Chilla R. Diseases of the salivary glands. New York: Georg Thieme, 1986:184-7.
9. Slootweg PJ, Müller H. Low-grade adenocarcinoma of the oral cavity: a comparison between the terminal duct and the papillary type. J Craniomaxillofac Surg 1987;15:359-64.
10. Schulenburg CAR. Salivary gland tumors: a report on 105 cases. S Afr Med J 1954;23:910-4.
11. Chaudhry AP, Labay GR, Yamane GM, Jacobs MS, Cutler LS, Watkins KV. Clinico-pathologic and histogenetic study of 189 intraoral minor salivary gland tumors. J Oral Med 1984;39:58-78.
12. Spiro RH, Koss LG, Hajdu SI, Strong EW. Tumors of minor salivary origin: a clinicopathologic study of 492 cases. Cancer 1973;31:117-29.
13. Davies JNP, Dodge OG, Burkitt DP. Salivary gland tumors in Uganda. Cancer 1964;17:1310-22.
14. Thomas KM, Hutt MSR, Borgstein J. Salivary gland tumors in Malawi. Cancer 1980;46:2328-34.
15. Freedman PD, Lumerman H. Lobular carcinoma of intraoral minor salivary gland origin. ORAL SURG ORAL MED ORAL PATHOL 1983;56:157-65.
16. Aberle AM, Abrams AM, Bowe R, Melrose RJ, Handlers JP. Lobular (polymorphous low-grade) carcinoma of minor salivary glands: a clinicopathologic study of twenty cases. ORAL SURG ORAL MED ORAL PATHOL 1985;60:387-95.
17. Eveson JW, Cawson RA. Salivary gland tumours: a review of 2410 cases with particular reference to histologic types, site, age and sex distribution. J Pathol 1985;146:51-8.

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The relationship between Nucleolar Organiser Regions and DNA content in Salivary Gland Neoplasms

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Abstract

Thirty-three intraoral salivary gland neoplasms were evaluated to determine the proliferative index ($+G_2M$ fraction) and ploidy status and to correlate these findings with the nucleolar organiser regions (NOR) counts. Formalin fixed, paraffin-embedded tissue was used in all the cases. The mean proliferative index for each tumour was calculated as follows: pleomorphic adenoma (n = 11) 4.1; polymorphous low grade adenocarcinoma (n = 8) 6.8; adenoid cystic carcinoma (n = 3) 6.7; mucoepidermoid carcinoma (n = 4) 7.3; carcinoma ex pleomorphic adenoma (n = 3) 5.1; undifferentiated carcinoma (n = 1) 4.5 and epithelial-myoepithelial carcinoma (n = 1) 8.2. Three tumours, two adenoid cystic ircinomas and one carcinoma ex pleomorphic adenoma showed aneuploid stemlines.

Although a positive correlation between the AgNOR count and proliferative index of the salivary gland neoplasms was found, it was statistically not significant.

Introduction

Nucleolar organiser regions (NORs) are collections of nucleolar proteins associated with ribosomal genes that can be demonstrated in histologic sections using a silver staining technique (AgNOR)[1]. This technique is based on the argyrophylia of the NOR-associated proteins[2]. NORs are located on the short arms of the five acrocentric chromosomes 13, 14, 15, 21 and 22. The known NORs are RNA polymerase I, nucleolin, B23, 100K and 80K protein[1]. Their function is uncertain but a role in rDNA transcription is postulated. The quantification of AgNORs in histologic sections has been used as a diagnostic aid in distinguishing between benign and malignant tumours of various origins[3-6]

DNA content can be determined by flow cytometry by sing fluorescent dyes that bind stoichiometrically to DNA[7]. The fluorescence intensity emitted by each nucleus through laser excitation is directly proportional to the DNA content of the cell[7]. The cell cycle is divided according to the amount of DNA in the nucleus at a particular time. Nuclei of cycling cells in the pre synthesis or G1 phase has a diploid or 2N amount of DNA. When the cells start to duplicate their DNA they have an intermediate amount of DNA between 2N and 4N. This phase is referred to as the synthesis phase (S-phase) and is of variable duration. After completion of the S-phase the cells enter the post synthesis phase (G2 phase) in which they have a 4N amount of DNA. The cells finally enter the mitotic phase (M-phase) and divide, whereafter they return to the G1 phase or enter a resting (G0 phase). In flow cytometry, cells in the G0 and G1 phases cannot be distinguished from each other, as they all have 2N DNA content. The same implies to cells in the G2 and M phases with a 4N DNA content (Figure 1).

The association between aneuploidy and aggressive tumour behaviour has been established for neoplasms from various sites[8-10]. The proliferation rate as defined by the S-phase fraction has also been used as a prognostic factor in adenoid cystic carcinomas of the head and neck[11].
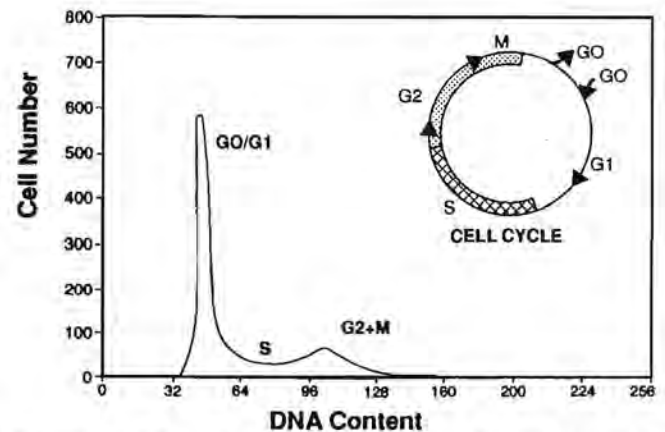

Figure 1: *Schematic representation of the relationship between DNA changes during the cell cycle and DNA histogram by flow cytometry. G0 represents the resting cells not taking part in the cell cycle.*

The reason for different quantities of AgNORs in nuclei is uncertain. The relationship between the AgNOR count and cellular activity has been demonstrated by Crocker and Nar[3]. Suresh *et al* however, have shown that AgNOR counts in non-neoplastic trophoblastic tissue are a reflection of ploidy rather than cell proliferation[12]. The purpose of this study was to determine the proliferative index and the ploidy status of minor salivary gland neoplasms and to correlate these findings with the AgNOR counts previously evaluated in the same tumours.

Materials and Method

Formalin fixed, paraffin embedded tissue from thirty-three intraoral salivary gland neoplasms, all included in a previous study where the AgNOR counts were evaluated[13] were retrieved. The tissue samples had all been fixed in 10% formalin and processed to paraffin wax. Sections were cut at 3cm thickness and dewaxed. The AgNOR solution comprised 2% gelatin in 1% formic acid that was mixed in a proportion of 1:2 volumes with 50% aqueous silver nitrate. This was immediately poured over the tissue sections and left for 30 min at room temperature. Counter staining was not performed. The AgNOR stained sections were examined under a 100x oil immersion lens and intranucleur dots were counted in 200 randomly selected nuclei using an eyepiece graticule to prevent recounting. Nuclei of overlapping tumour cells were not included. Nucleolar clusters were counted as a single AgNOR and no attempt was made to resolve the clusters into their discernible number of descrete dots. The mean number of AgNOR dots per nucleus was determined for each specimen. Eleven were diagnosed as pleomorphic adenomas (PA), eight as polymorphous low grade adenocarcinomas (PLA), five as adenoid cystic carcinomas (ACC), four as mucoepidermoid carcinomas (MEC), three as carcinoma ex pleomorphic adenoma, one as an undifferentiated carcinoma and one as an epithelial-myoepithelial carcinoma. Four 50μm sections from each

paraffin embedded block were cut and prepared for flow cytometry according to the Hedley method using a 0,5% pepsin solution[14]. The final cell suspension was passed through a 35mm mesh and the cell concentration established by means of a Coulter counter (Model FZ, Coulter Electronics, Hialeah, F1). The cell concentration was adjusted to ± 2.0x10⁶ cells/ml. The nuclei were stained with Propidium Iodide using a Coulter DNA Prep system, according to the manufacturers instructions. The cells were then analysed on an Epics Elite flow cytometer (Coulter Electronics, Hialeah, F1) which had been calibrated with chicken red blood cells and DNA check beads. The Elite was operated at 15 mW and emitted an Argon ion laser at 488nm. The date rate varied between 20 – 200 events/second and 10 000 – 20 000 events were collected on a single parameter histogram. All data was collected in listmode fashion and the DNA histograms were analysed using Multi-cycle DNA analysis software program (Phoenix Flow Systems, San Diego, CA).

By convention, when using paraffin embedded tissue, the first peak was considered to be the normal DNA diploid peak representing the G0G1 phase of the cycle. DNA aneuploidy was reported when at least 2 separate G0/G1 peaks could be demonstrated. The coefficient of variation (CV) was calculated using the width of the peak (number of channels) at 61% of the maximum peak height divided by the peak height channel number, multiplied by a factor of 2.

The proliferative index (PI) was defined as the percentage of cells in the S + G2M phases combined. The correlation between the AgNOR count and PI were analysed using the Pearson's method while the Mann-Whitney Test was used to compare the PI between benign and malignant salivary gland neoplasms.

Results

Three tumours had aneuploid stemlines. Two were adenoid cystic carcinomas and the other was a carcinoma ex pleomorphic adenoma (**Figure 2**). The predominant growth pattern in the ACC were cribriform and tubular/trabecular respectively. Their AgNOR counts were 4.29 and 3.01 (**Figure 3**). The AgNOR count for the carcinoma ex PA was 2.37. Diploid stemlines were present in the remaining 30 neoplasms (**Figure 4**). The mean CV of the flow cytometry

results were 3.96 ± 3.1 (SD). The proliferative index and AgNOR counts of the diploid tumours are summarised in Table 1.

A positive correlation between the mean PI and mean AgNOR counts in the various neoplasms were found. This
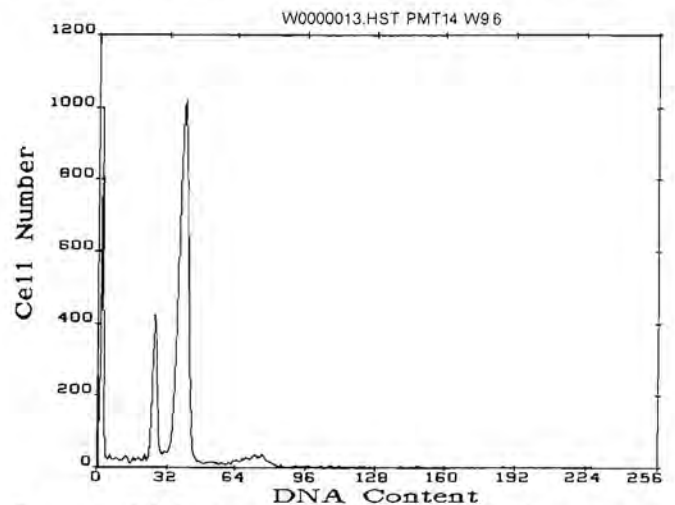
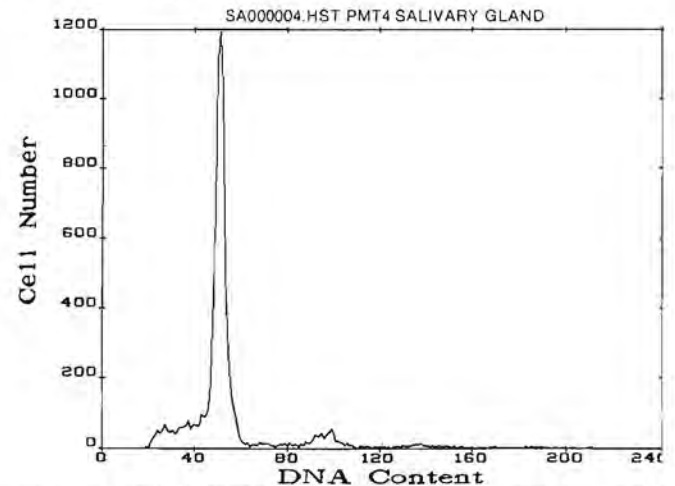**Figure 2**: *DNA histogram of an adenoid cystic carcinoma showing aneuploidy.*

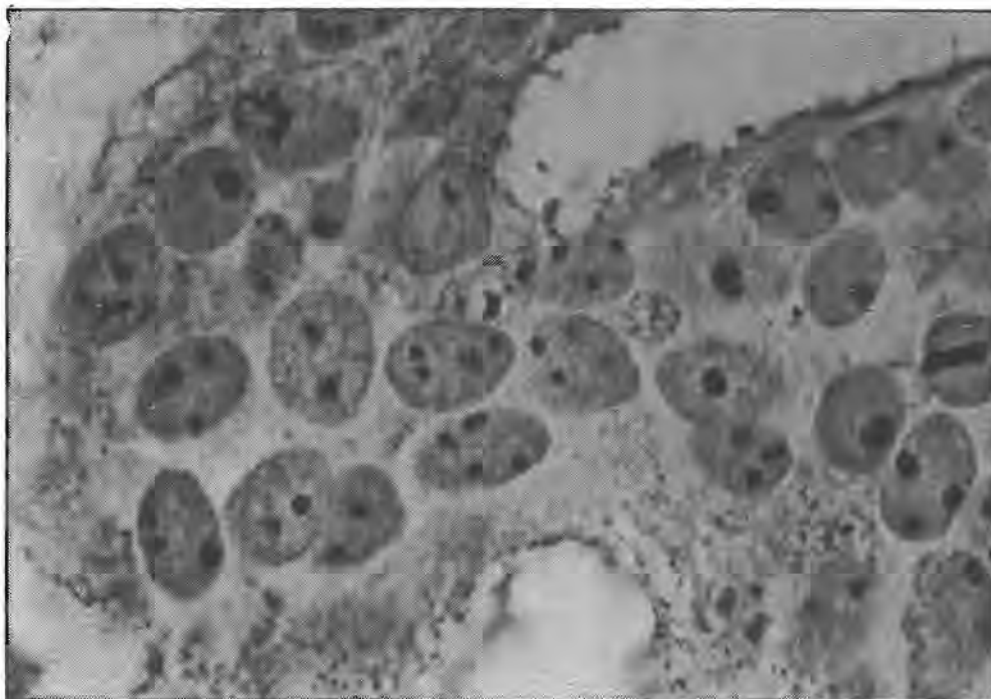**Figure 4**: *Diploid DNA histogram from a pleomorphic adenoma.*

Figure 3: *AgNOR stain of a cribriform adenoid cystic carcinoma with an aneuploid DNA content. Original magnification, × 400.*

	PA	PLA	ACC	MEC	Ca ex PA	Undiff Ca	EPI
PI	4.1 ± 1.5	6.8 ± 5.3	6.7 ± 5.55	7.3 ± 2.3	5.1 ± 5.6	4.5	8.2
AgNOR	1.48 ± 0.3	1.85 ± 0.3	2.28 ± 0.7	1.93 ± 0.5	1.60 ± 0.3	3.13	2.23
n	11	8	3	4	2	1	1

PA = pleomorphic adenoma; PLA = polymorphous low grade adenocarcinoma;
ACC = adenoids cystic carcinoma; MEC = mucoepidermoid carcinoma;
Ca ex PA = carcinoma ex pleomorphic adenoma; Undiff Ca = undifferentiated carcinoma;
EPI = epithelial-myoepithelial carcinoma; PI = proliferative index

correlation however, was statistically not significant (P = 0.45). The difference between the mean PI of the benign salivary gland neoplasms and the diploid malignant salivary gland neoplasms was also not significant.

Discussion

The cell cycle distribution as determined by flow cytometry is usually calculated using commercially available mathematical software programs. Corrections are made to subtract background debri which intervene with the various phases of the cell cycle. Creation of debri by means of tissue preparation is a problem especially when using paraffin-embedded tissue for flow cytometric analysis. Although these corrections have shown to enhance the prognostic value of particularly the S-phase[15], it must always be borne in mind that neoplastic cells might be eliminated as debri. Expression of exact percentages of cells in the various stages of cell is therefore proliferation, a questionable practice. It is much more reliable to use the PI as a rough indicator of proliferative activity, especially when evaluating paraffin embedded tissues.

The number of aneuploid tumours (n = 3) in the present study was too small to make definite comments regarding its correlation with the AgNOR counts. It is interesting to note however, that the two AgNOR counts of the aneuploid ACC were the first and third highest count among the salivary gland neoplasms. In a study to evaluate the prognostic factors for ACC, Hamper et al concluded that the shortest survival time was found in patients with tumours showing aneuploid DNA contents[16]. Forty-two percent of these tumours had a predominant cribriform growth pattern. This fact correlates with our finding that the mean AgNOR count in ACC did not correspond with the histologic growth pattern, a prognostic factor for tumour behaviour in ACC. The highest count was present in a tumour with a predominant cribriform growth pattern. The same applied for the flow cytometric analysis. The two aneuploid ACC had more favourable cribriform and tubular growth patterns respectively. Luna et al however, found that aneuploidy is more frequently present in the solid pattern[17]. This is an indication that the growth pattern in ACC alone is not solely responsible for tumour behaviour.

The fact that only 3 tumours of the sample that included 22 malignant neoplasms were aneuploid is probably related to the phenomenon that malignant salivary gland neoplasms generally have a less aggressive behaviour compared to other malignancies.

A high AgNOR count in neoplasms may be related to an increase in cell ploidy due to a real increase in the number of chromosomes. Since the NORs are present only on the 5 acrocentric chromosomes, it may be possible that these chromosomes are not affected in a neoplastic transformation that is accompanied with hyperploidy. The NORs are usually tightly aggregated in one or two nucleoli in a cell[18]. Proliferative activity may be associated with nucleolar dissociation resulting in spreading of AgNORs through the nucleus. This, together with the transcriptional activity may result in an increase in the mean AgNOR count[18].

A variety of techniques are available to determine cellular proliferation in histological material. Visualisation of the NORs by means of a silver staining technique is frequently used. The percentage of cells in the S + G2M phases of the cell cycle can be determined with flow cytometry and immunohistochemical techniques using antibodies against proliferating cell nuclear antigen (PCNA) as well as Ki-67, a monoclonal antibody that recognises a nuclear antigen present only in proliferating cells, are some of the more advanced methods used.

This study failed to show a significant relationship between the mean PI and mean AgNOR count of the various neoplasms although they were positively correlated. Crocker et al found a significant linear correlation between the mean AgNOR count and S-phase fraction of high and low grade non Hodgkin's lymphomas, but not between the AgNOR count and ploidy status[19]. The difference between the mean PI of the benign and malignant tumours was not significant. This is in contrast to the highly significant difference between the same tumours when evaluating the mean AgNOR counts[13]. From this study it would appear that the AgNOR technique, which is fast and inexpensive, may be more suitable to accurately determine the proliferative activity when using paraffin embedded tissues

References

1. Crocker J., Nucleolar organiser regions. In: Underwood JCE, ed. Current topics in pathology New York: Springer-verlag, 1990: 92 – 143.
2. Ploton D., Menager M., Jeannesson P., Himberg G., Pigeon F., Adnet J.J., Improvement in the staining and in the visualisation of the agyrophilic proteins of the nucleolar organiser region at the optical level Histochem J 1986 18: 5 – 14.
3. Crocker J., Nar P., Nucleolar organiser regions in lymphomas J Pathol 1987 151: 111 – 118.
4. Mackie R.M., White S.I., Seywright M.M., Young H., An assessment of the value of AgNOR staining in the identification of dysplastic and other borderline melanocytic naevi Br J Mermatol 1989 120: 511 – 516.
5. Smith R., Crocker J., Evaluation of nucleolar organiser region-associated proteins in breast malignancy Histopathology 1988 12: 221 – 223.
6. Rosa J., Mehta A., Filipe M.I., Nucleolar organiser regions in gastric carcinoma and its precursor stages Histopathology 1990 16: 265 – 269.
7. Shapiro H.M., Flow cytometry of DNA content and other indicators of proliferative activity Arch Pathol Lad Med 1989 113: 591 – 597.
8. Visakorpi T., Proliferative activity determined by DNA flow cytometry and proliferating cell nuclear antigen (PCNA) immunohistochemistry as a prognostic factor in prostatic carcinoma J Pathol 1992 168: 7 – 13.
9. Sigurdson H., Baldetorp B., Borg A., et al., Indicators of prognosis in node-negative breast cancer N Engl J Med 1990 322: 1045 – 1053.
10. Merkel D.E., McGuire W.L., Ploidy, proliferative activity and prognosis. DNA flow cytometry of solid tumors Cancer 1990 65: 1194 – 1205.
11. Greiner T.C., Robinson R.A., Maves M.D., Adenoid cystic carcinoma. A clinicopathologic study with flow cytometric analysis Am J Clin Pathol 1989 92: 711 – 720.
12. Suresh U.R., Chawner J., Buckley C.H., Fox H., Do Agnor counts reflect cellular ploidy or cellular proliferation? A study of trophoblastic tissue J. Pathol 1990 160: 213 – 215.
13. Van Heerden W.F.P., Raubenheimer E.J., Evaluation of the nucleolar organiser region associated proteins in minor salivary gland tumours J. Oral Pathol Med 1991 20: 291 – 295.
14. Hedley D.W., Friedlander M.L., Taylor I.W., Rugg C.A., Musgrave E.A., Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry J Histochem Cytochem 1983 31: 1333 – 1335.
15. Kallioniemi O.P., Visakorpi T., Holli K. Heikkinen A., Isola J., Koivula T., Improved prognostic impact of S-phase values from paraffin-embedded breast and prostate carcinomas after correcting for nuclear slicing Cytometry 1991 12: 413 – 421.
16. Hamper K., Lazar F., Dietel M., et al., Prognostic factors for adenoid cystic carcinoma of the head and neck: a retrospective evaluation of 96 cases J Oral Pathol Med 1990 19: 101 – 107.
17. Luna M.A., El-Nagger A., Batsakis J.G., Weber R.S., Gernser L.A., Geopfert H., Flow cytometric DNA content of adenoid cystic carcinoma of submandibular gland. Correlation of histologic features and prognosis Arch Otolaryngol Head Neck Surg 1990 116: 1291 – 1296.
18. Underwood J.C.R., Giri D.D., Nucleolar organiser regions as diagnostic discriminants for malignancy J. Pathol 1988 155: 95 – 96.
19. Crocker J., McCartney J.C., Smith P.J., Correlation between DNA flow cytometric and nucleolar organiser region data in non-Hodgkin's lymphoma J. Pathol 1988 154: 151 – 156.

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A review of recent developments in the diagnosis of epithelial neoplasms of salivary gland origin

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Abstract The adoption by the World Health Organization of a revised classification for salivary gland neoplasms has introduced a new chapter in the diagnosis of these diverse growths. Universal acceptance of this proposal will contribute significantly to diagnostic uniformity. The introduction of an outline for the grading of malignant salivary gland neoplasms benefit preoperative prognostication and rationalize therapeutic regimes. The utilization of fine needle aspiration and frozen section for the establishment of a diagnosis are discouraged. Despite recent developments in histochemistry, immunohistochemistry and DNA content analyses of salivary gland neoplasms, the diagnosis still relies mainly on the growth pattern and cytologic features of a tumor. *(Eur J Lab Med 1995;1:107-112).*

Introduction

Although salivary glands share similar cellular phenotypes with sweat glands, mammary glands and the exocrine pancreas, neoplastic proliferations in the former are infinitely more complex and, from a cellular viewpoint, represent the most heterogenous group of proliferations in the human body. Despite recent developments in the understanding of the histogenesis of salivary gland neoplasms, the diagnostic process still relies mainly upon growth characteristics and cellular morphology. Special laboratory investigations like electron microscopy and cellular markers form a minor part of the diagnostic process and often only subtle microscopic differences distinguish neoplasms with diverse clinical outcomes. The subjectivity involved in the diagnosis of salivary gland neoplasms is highlighted in a recent study where 101 salivary gland neoplasms were reevaluated by a panel of senior pathologists. In a third there were minor disagreements, mostly related to subclassification, whereas major diagreements relating to benign versus malignant occurred in 7.9% of cases[1].

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The purpose of this paper is to give an overview of recent developments in the diagnosis of salivary gland neoplasms.

Classification

For universal acceptance, a classification of pathologic proliferations should be based on patterns of differentiation that reflect the cell types of the parental tissue and simultaneously group neoplasms in prognostic categories. The most likable classification of salivary gland neoplasms is the morphologic working classification initially proposed by the Armed Forces Institute of Pathology[2] (Table I) and later adopted by the World Health Organization's Committee on salivary gland tumors. Although the patterns of differentiation of salivary gland neoplasms is not addressed systematically in this classification, malignant growths are now for the first time prognostically grouped. The diagnostic refinement introduced by this new approach is clearly evident in a study which revised salivary gland neoplasms originally diagnosed according to the 1972 World Health Organizations classification[3]. In 29 cases the original diagnosis was changed and in 7 it resulted in a change from benign to malignant or vice versa[4]. Although the new approach to the classification has valuable clinical implications, it is by no means complete. Entities like the salivary gland anlage tumor[5], sialoblastoma[6,7] and hyalizing clear cell carcinoma[8] lack suffi-

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Table I. AFIP classification of primary epithelial neoplasms of salivary gland origin

Benign

Mixed tumor (pleomorphic adenoma)
Papillary cystadenoma lymphomatosum (Warthin's tumor)
Oncocytoma
Cystadenoma
Basal cell adenoma
Ductal papillomas
   – Sialadenoma papilliferum
   – Inverted ductal papilloma
   – Intraductal papilloma
Myoepithelioma
Sebaceous adenomas
   – sebaceous adenoma
   – sebaceous lymphadenoma
Adenoma NOS

Malignant

Low-Grade

Mucoepidermoid carcinoma (low grade)
Acinic cell adenocarcinoma
Polymorphous low grade adenocarcinoma
Basal cell adenocarcinoma
Adenocarcinoma (NOS) low grade
Metastasizing mixed tumor

Intermediate-Grade

Mucoepidermoid carcinoma (intermediate grade)
Adenoid cystic carcinoma (cribriform-tubular types)
Epithelial-myoepithelial carcinoma
Adenocarcinoma NOS (intermediate grade)
Clear cell carcinoma
Cystadenocarcinoma
   – papillary
   – non papillary
Sebaceous carcinomas
   – sebaceous carcinoma
   – sebaceous lymphadenocarcinoma
Mucinous adenocarcinoma

High-Grade

Mucoepidermoid carcinoma (high grade)
Adenoid cystic carcinoma (solid)
Malignant mixed tumor
   – carcinoma ex mixed tumor
   – carcinosarcoma
Adenocarcinoma NOS (high grade)
Squamous cell carcinoma
Undifferentiated carcinoma
Oncocytic carcinoma
Adenosquamous carcinoma
Salivary duct carcinoma
Myoepithelial carcinoma

cient numbers for the establishment of behavioral patterns and may only find their way into future reappraisals of this classification.

Terminology

Arguments on the preferentiability of the designation "mixed tumor" or the term "pleomorphic adenoma" are unproductive and both are now accepted. "Adenolymphoma" as a synonym for papillary cystadenoma lymphomatosum should fall in disuse because any implication that this benign tumor is linked to lymphoma is misleading. Oxyphil cell adenoma and oncocytoma are used interchangebly. The ambiguous term "monomorphic adenoma" has fallen into disuse and all unclassifiable adenomas are now proposed to be designated as "adenoma, not otherwise specified". Although myoepithelioma is classified as a separate entity, no counter argument exists that this neoplasm is in fact an extreme differentiation on the diverse spectrum of pleomorphic adenoma. Separate categorization of myoepithelioma may however, prevent confusion with benign mesenchymal neoplasms, many of which resemble myoepitheliomas microscopically.

The term malignant mixed tumor (or malignant pleomorphic adenoma) should not be used as a specific diagnosis as it includes three different entities: carcinoma ex mixed tumor (or a carcinoma arising in a mixed tumor), carcinosarcoma (true malignant mixed tumor) and metastasizing mixed tumor. The suffix-tumor is now replaced by "carcinoma" in two neoplasms which are now known to be malignant: acinic cell carcinoma and mucoepidermoid carcinoma. As refinements in classifications proceed, the utilization of terms like "adenocarcinoma not otherwise specified" decrease. Although new clinico-pathological entities such as salivary duct carcinoma, terminal duct carcinoma and epithelial-myoepithelial carcinoma reduce the frequency by which this category is used, there still remain those adenocarcinomas which cannot be accommodated in other categories.

Frozen sections and fine needle aspirations

Frozen sections (FS) and fine needle aspirations (FNA) are increasingly accepted as cost effective and time saving techniques for the diagnosis of abnormal body masses. The cellular diversity which may be experienced within a salivary gland neoplasm decreases the potential accuracy of all techniques which suffer the disadvantage of not representing all cell types in a neoplastic proliferation. The status of invasion is one of the most important parameters in predicting the biologic behavior of salivary gland neoplasms[2]. The small sample obtained through FNA precludes the disclosure of this important parameter. Studies investigating the sensitivity and specificity of FNA frequently compare its diagnostic accuracy with histological diagnoses

based on dated classification systems, most of which do not recognize modern refinements in the diagnosis of salivary gland neoplasms. FNA appears to have a high success rate in distinguishing between benign and malignant salivary gland neoplasms[9-11]. The distinction between benign and malignant in a diagnosis on which the therapeutic approach is decided, is probably equally important to the grading of a specific malignant growth. In this respect, the limited sample obtained through FNA is often inadequate and its results cannot be compared with those obtained through incision biopsy. The cytological atypia frequently present in benign salivary gland neoplasms[9,12,13] and potential confusion with non-epithelial stromal neoplasms[14] are further pitfalls in the interpretation of FNA. Although there are unquestionable clinical indications for FNA, none merit its inclusion as part of the systematic evaluation on which the therapeutic approach is based[15].

In a series of 310 patients subjected to FS, the correct type of malignancy was diagnosed in only 51% of cases and in four patients, a false positive diagnosis of malignancy was made. The authors of this study conclude that FS is no more accurate in the evaluation of salivary gland tumors than FNA[16]. Although there are no indications against the utilization of FS for determining clear margins during excision, a primary diagnosis should not be established on FS alone.

Grading of salivary gland malignancies

This aspect of the diagnosis of malignant neoplasms is important particularly in the case of mucoepidermoid carcinomas, adenoid cystic carcinoma and adenocarcinoma which may be classified in more than one grade of malignant behavior. The microscopic criteria applied for grading are controversial and often highly subjective. Auclair, Goode and Ellis[17] proposed a point scoring system for the objective grading of muco-epidermoid carcinomas. The histopathologic features that indicate high grade behavior are an intracystic component of less than 20%, four or more mitoses per 10 high-power fields, neural invasion, necrosis and cellular anaplasia. Most differences of opinion involve the distinction between low and intermediate grades and their proposed point system may provide a basis for an objective solution. Factors that indicate a poor prognosis in adenoid cystic carcinomas encompass failure of local disease control at the initial surgical procedure, a solid pattern histologically, recurrent disease and distant metastases[2]. Despite the description of new clinicopathological entities like the salivary duct carcinoma and epithelial myoepithelial carcinoma which were formerly grouped in the adenocarcinoma "not otherwise specified" category, there still remain a group of adenocarcinomas that cannot be accommodated in conventional classifications. These malignancies are divided into low-, intermediate- and high grade categories on growth patterns and cytologic features[2]. Although histopathologic grading of acinic cell adenocarcinomas is possible, the influence of the different grades on the prognosis is debateable[18,19]. The limited malignant potential and excellent survival of patients with polymorphous low-grade adenocarcinoma is little affected by patterns of differentiation[20].

Histochemistry and immunohistochemistry

Although histochemical and immunohistochemical techniques have played an important role in investigations of the histogenesis of salivary gland neoplasms, their diagnostic applications are limited. This is mainly due to the wide spectrum of differentiation which may occur within a single salivary gland neoplasm, with each growth pattern exhibiting its own immunohistochemical characteristics[21-23]. Salivary gland neoplasms furthermore often share immunohistochemical staining characteristics with other neoplasms. Positive staining for prostate-specific antigen and prostate-specific acid phosphatase are frequently found in benign and malignant salivary neoplasms[24], a pitfall in the microscopic distinction between salivary gland carcinomas and metastatic deposits of prostatic carcinoma. Alpha 1-antitrypsin is a useful marker of basement membrane-like material[25] and can be helpful in distinguishing this product from myxoid interstitial deposits. A potential distraction to the diagnosis of myoepithelial tumors of salivary glands (i.e. myoepithelioma and myoepithelial carcinoma) is confusion with spindle cell mesenchymal proliferations. Demonstration of myoepithelial differentiation requires careful evaluation of immunohistochemical stains. The identification of S100 protein, actin and keratin either focally or diffusely, is helpful in confirming myoepithelial differentiation[26].

Microscopically, myoepitheliomas differentiate into three distinct cellular patterns: a spindle cell-, plasmacytoid- or a combination of plasmacytoid and spindle shaped cellular patterns[2]. If immunohistochemical criteria had to be applied rigorously, it is debateable whether the plasmacytoid variety, which is reported to stain negative for muscle specific actin, does represent true myoepithelial differentiation[27].

Confusion between the microscopic appearances of polymorphous low grade adenocarcinoma and benign pleomorphic adenoma may be avoided by employing stains for glial fibrillary acidic protein (GFAP). The former does not stain for this antigen

whereas its positivity is common in pleomorphic adenomas[28]. A greater diagnostic dilemma is the distinction between polymorphous low grade adenocarcinoma and adenoid cystic carcinoma. The immunochemical reactions of these two tumors are not sufficiently dissimilar to be of any practical value[29] and differences are mainly cytological and to a lesser extent morphological in nature. The presence of both sex steroids and the receptor for progesterone in adenoid cystic carcinomas[30] suggests a good possibility that some tumors in this group may respond to endocrine therapy.

Various reports propose a useful place for the counting of nucleolar organizer regions (NOR's) in order to predict the proliferative activity and prognosis of malignant salivary gland neoplasms[31-33] and distinguish between benign and malignant growths[34]. Our experience with this technique[35] as well as those of other researchers[36] were less rewarding and we believe this technique provides nothing but redundant information.

Research into the use of cellular markers to predict the behavior of salivary gland tumors is in its infancy. An association is reported between the expression of erbb2 oncoprotein and aggressiveness of malignant salivary gland tumours[37,38]. Loss of cellular differentiation appears to be linked with under expression of the c-fos oncogene[39] and evaluation of Ki-67 expression[40], immunoreactivity for PCNA[41] and c-myc, ras p21 and p53 expression[42] may become important determinants for malignant behavior.

DNA content analysis

The positive correlation between prognosis and ploidy status of malignant neoplasms is well established. Despite the presence of atypical cells in benign pleomorphic adenomas, all benign salivary gland tumours have diploid DNA contents and the malignant ones frequently display an aneuploid pattern[43,44]. A statistically significant correlation was found between DNA content and tumor size, histological grade, lymph node metastasis and lethality of 55 salivary gland carcinomas[45]. Flow cytometry was however, unable to predict the development of metastasis in cases of proven metastasizing mixed tumor[46]. DNA ploidy was shown to correlate with the prognosis of epithelial-myoepithelial carcinoma[47] myoepithelioma[48] and muco-epidermoid carcinoma[49]. The value of this technique in prognosticating adenoid cystic carcinomas is debateable[50,51] whereas no prognostic correlation could be found between DNA ploidy and the course of acinic cell adenocarcinomas[52,53]. Larger series will shed more light on the usefulness of DNA content analysis in the prediction of the behavior of salivary gland tumors.

References

1. Van der Wal JE, Carter RL, Klijanienko J, Micheau C, Rilke F, Seifert G, et al. Histological reevaluation of 101 intraoral salivary gland tumours by an EORTC-study group. J Oral Pathol Med 1993;22: 21-2.
2. Ellis GL, Auclair PL, Gnepp DR. Surgical Pathology of the Salivary Glands. W.B. Saunders Company, Philadelphia. 1991.
3. Thackray AC, Sobin LH. Histological Typing of Salivary Gland Tumours. Geneva. World Health Organization, 1972.
4. Van der Wal JE, Snow GB, van der Wal I. Histological reclassification of 101 intraoral salivary gland tumour (new WHO classification). J Clin Pathol 1992;45:834-5.
5. Dehner LP, Valbuena L, Perez-Atayde A, Reddick RL, Askin FB, Rosai J. Salivary gland anlage tumor (congenital pleomorphic adenoma). A clinicopathologic, immunohistochemical and ultrastructural study of nine cases. Am J Surg Pathol 1994;18: 25-36.
6. Hsueh C, Gonzalez-Crussi F. Sialoblastoma: a case report and review of the literature on congenital epithelial tumors of salivary gland origin. Pediatr Pathol 1992; 12:205-14.
7. Batsakis JG, Frankenthaler R. Embrioma (sailoblastoma) of salivary glands. Ann Otol Laryngol 1992;101:958-60.
8. Milchgrub S, Gnepp DR, Vuitch F, Delgado R, Albores-Saavedia J. Hyalinizing clear cell carcinoma of salivary gland. Am J Surg Pathol 1994;18:74-82.
9. Chan MKM, McGuire LJ, King W, Li AKC. Cytodiagnosis of 112 salivary gland lesions. Correlation with istologic and frozen section diagnosis. Acta Cytologica 1992;36:353-63.
10. Barnard NA, Paterson AW, Irvine GH, Mackenzie EDF, White H. Fine needle aspiration in maxillofacial surgery - experience in a district general hospital. Br J Oral Maxfac Surg 1993;31:223-6.
11. Pitts DB, Hilsinger RL, Karanday E, Ross JC, Caro JE. Fine-Needle Aspiration in the diagnosis of salivary gland disorders in the community hospital setting. Acta Otolaryngol Head Neck Surg 1992;118 479-82.
12. Laucirica R, Farnum JB; Leopold SK, Kalin GB, Youngberg GA. False positive diagnosis in fine-needle aspiration of an atypical Warthin tumor: histochemical differential stains for cytodiagnosis. Diagn Cytopathol 1989;5:412-5.
13. Thunnissen FB, Peterse LJ, Bucholtz R, van der Beek JM, Bosman FT. Polypoidy in pleomorphic adenomas with cytological atypia. Cytopathology 1992;3:101-9.
14. Mair S, Leiman G. Benign neurilemmoma (Schwan-

noma) masquerading as a pleomorphic adenoma of the submandibular salivary gland. Acta Cytol 1989;33:907-10.

15. Batsakis JG, Sneige N, el-Naggar A. Fine needle aspiration of salivary glands: its utility and tissue effects. Ann Otol Rhinol Laryngol 1992;101:185-8.

16. Heller KS, Attie JN, Dubner S. Accuracy of frozen section in the evaluation of salivary tumors. Am J Surg 1993;166:424-7.

17. Auclair PL, Goode RK, Ellis GL. Mucoepidermoid carcinoma of intraoral salivary glands. Evaluation of application of grading criteria in 143 cases. Cancer 1992;69:2021-30.

18. Batsakis JG, Luna MA, el-Naggar A. Histopathologic grading of salivary gland neoplasms: II. Acinic cell carcinomas. Ann Otol Rhinol Laryngol 1990; 99:929-33.

19. Oliveira P, Fonseca I, Soares J. Acinic cell carcinoma of the salivary glands. A long term follow-up study of 15 cases. Eur J Surg Oncol 1992;18:7-15.

20. Norberg LE, Burford-Mason AP, Dardick I. Cellular differentiation and morphologic heterogeneity in polymorphous low grade adenocarcinoma of minor salivary gland. J Oral Pathol Med 1991;20:373-9.

21. Takahashi H, Tsuda N, Fujita S, Tezuka F, Okabe H. Immunohistochemical investigation of vimentin, neuron-specific enolase, alpha 4 1-antichymotrypsin and alpha 1-antitrypsin in adenoid cystic carcinoma of the salivary gland. Acta Pathol Jpn 1990;40: 655-64.

22. Huang JW, Mori M, Yamada K, Isono K, Ueno K, Shinohara M, et al. Mucoepidermoid carcinoma of the salivary glands: immunohistochemical distribution of intermediate filament proteins, involucrin and secretory proteins. Anticancer Res 1992;12:811-20.

23. Mori M, Kasai T, Yuba R, Chomette G, Auriol M, Vaillant JM. Immunohistochemical studies of S100 protein alpha and beta subunits in adenoid cystic carcinoma of salivary glands. Virchows Arch B Cell Pathol 1990;59:115-23.

24. Van Krieken JH. Prostate marker immunoreactivity in salivary gland neoplasms. A rare pitfall in immunohistochemistry. Am J Surg Pathol 1993;17:410-4.

25. Takahashi H, Fujita S, Okabe H, Tsuda N, Tezuka F. Distribution of tissue markers in acinic cell carcinomas of salivary gland. Pathol Res Pract 1992; 188:692-700.

26. Herrera GA. Light microscopic, ultrastructural and immunochemical spectrum of malignant lacrimal and salivary gland tumors, including malignant mixed tumours. Pathobiology 1990;58:312-22.

27. Franquemont DW, Mills SE. Plasmacytoid monomorphic adenoma of salivary glands. Absence of myogenous differentiation and comparison to spindle cell myoepithelioma. Am J Surg Pathol 1993;17: 146-53.

28. Anderson C, Krutchoff D, Pederson C, Cartun R, Barman M. Polymorphous low grade adenocarcinoma of minor salivary gland: a clinicopathologic and comparative immunohistochemical study. Mod Pathol 1990;3:76-82.

29. Simpson RH, Clarke TJ, Sarsfield PT, Gluckman PG,

Babajeus AV. Polymorphous low-grade adenocarcinoma of the salivary glands: a clinicopathological comparison with adenoid cystic carcinoma. Histopathology 1991;19:121-9.

30. Ozoho S, Onozuka M, Sato K, Ito Y. Immunohistochemical localization of estradiol, progesterone and progesterone receptor in human salivary glands and salivary adenoid cystic carcinomas. Cell Struct Funct 1992;17:169-75.

31. Freitas RA, de Araujo VC, Araujo NS. Argyrophilia in nuclear organizer regions (AgNOR) in adenoid cystic carcinoma and polymorphous low grade adenocarcinoma of the salivary glands. Eur Arch Otorhinolaryngol 1993;250:213-7.

32. Chomette G, Auriol M, Wann A, Guilbert F. Acinic cell carcinomas of salivary glands histoprognosis. Value of NOR's stained with AgNOR-technique and examined with semi-automatic image analysis. J Biol Buccale 1991;19:205-10.

33. Landini G. Nuclear organizing regions (NOR's) in pleomorphic adenomas of the salivary glands. J Oral Pathol Med 1990;19:257-60.

34. Cardillo MR. AgNOR technique in fine needle aspiration cytology of salivary gland masses. Acta Cytol 1992;36:147-51.

35. Van Heerden WFP, Raubenheimer EJ. Evaluation of the nuclear organizer region associated proteins in minor salivary gland tumors. J Oral Pathol Med 1991;20:291-5.

36. Cardillo R, el-Naggar A, Luna MA, Roderiques-Peratto JL, Batsakis JG. Nuclear organized (NOR's) and myoepitheliomas: a comparison with DNA content and clinical course. J Laryngol Otol 1992; 106:616-20.

37. Stenman G, Sandros J, Nordkuist A, Mark J, Sahlin P. Expression of the ERBB2 protein in benign and malignant salivary gland tumors. Genes Chromosom Cancer 1991;3:128-35.

38. Sugano S, Mukai K, Tsuda H, Hirohashi S, Furuya S, Shimosato Y, et al. Immunochrotochemical study of c-erB-2 oncoprotein overexpression in human major salivary gland carcinoma: an indicator of aggressiveness. Laryngoscope 1992;102:923-7.

39. Birek C, Lui E, Dardick I. C-Fos oncogene underexpression in salivary gland tumors as measured by in situ hybridization. Am J Pathol 1993;142:917-23.

40. Murakami M, Ohtani I, Hojo H, Wakasa H. Immunohistochemical evaluation with Ki-67: an application to salivary gland tumours. J Laryngol Otol 1992;106:35-8.

41. Yang L, Hashimura K, Quin C, Shrestha P, Sumitomo S, Mori M. Immunoreactivity of proliferating cell nuclear antigen in salivary gland tumours: an assessment of growth potential. Virchows Arch A Pathol Anat Histopathol 1993;422:481-6.

42. Deguchi H, Hamano H, Hayashi Y. c-myc, ras p21 and p53 expression in pleomorphic adenoma and its malignant form of the human salivary glands. Acta Pathol Jpn 1993;43:413-22.

43. Tytor M, Gemryd P, Wingren S, Grenko RT, Lundgren J, Lundquist PG. Heterogeneity of salivary gland tumors studied by flow cytometry. Head Neck 1993;15:514-21.

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44. Felix A, Fonseca I, Soares J. Oncocytic tumors of salivary gland type: a study with emphasis on nuclear DNA ploidy. J Surg Oncol 1993;52:217-22.

45. Carillo R, Batsakis JG, Weber R, Luna MA, el-Naggar A. Salivary neoplasms of the palate: a flow cytometric and clinicopathological analysis. J Laryngol Otol 1993;107:858-61.

46. Wenig BM, Hitchcock CL, Ellis GL, Gnepp DR. Metastasizing mixed tumor of salivary glands. A clinicopathologic and flow cytometric analysis. Am J Surg Pathol 1992;16:845-58.

47. Fonseca I, Soares J. Epithelial-myoepithelial carcinoma of the salivary glands. A study of 22 cases. Virchows Arch A Pathol Anat Histopathol 1993; 422:389-96.

48. el-Naggar A, Batsakis JG, Luna MA, Goepfert H, Tortoledo ME. DNA content and proliferative activity of myoepitheliomas. J Laryngol Otol 1989;103: 1192-7.

49. Hamper K, Caselitz J, Arps H, Askensten U, Auer G, Seifert G. The relationship between DNA content in

salivary gland tumors and prognosis. Comparison of mucoepidermoid tumors and acinic cell tumors. Arch Otorhinolaryngol 1989;246:328-32.

50. Greiner TC, Robinson RA, Maves MD. Adenoid cystic carcinoma. A clinicopathologic study with flow cytometric analysis. Am J Clin Pathol 1989;92: 711-20.

51. Eibling DE, Johnson JT, McCoy TP, Barnes EL, Syms CA, Wagner RL, et al. Flow cytometric evaluation of adenoid cystic carcinoma: correlation with histologic subtype and survival. Am J Surg 1991;162:307-172.

52. el-Naggar A, Batsakis JG, Luna MA, McLemore D, Byers Rm. DNA flour cytometry of acinic cell carcinomas of major salivary glands. J Laryngol Otol 1990;104:410-6.

53. Hamper K, Mausch HE, Caselitz J, Arps H, Berger J, Askensten U, et al. Acinic cell carcinoma of the salivary glands: the prognostic relevance of DNA cytomorphometry in a retrospective study of long duration (1965-1987). Oral Surg Oral Med Oral Pathol 1990;69:68-75.

Intraoral Salivary Duct Carcinoma: A Report of 5 Cases

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Salivary duct carcinoma (SDC) is a high-grade malignant epithelial tumor of salivary glands first described by Kleinsasser et al.[1] It has also been termed cribriform salivary carcinoma of excretory ducts,[2] infiltrating salivary duct carcinoma,[3] and intraductal carcinoma.[4] SDC has a poor prognosis,[2,5,6] although patients with prolonged disease-free survival have been reported.[2,5] Low-grade variants of SDC have also been described.[7,8]

The peak incidence of SDCs is in the sixth and seventh decades of life, and it has a male predominance.[6,9] This neoplasm has a striking resemblance to ductal breast carcinoma and is characterized by the presence of intraductal, circumscribed tumor islands with a papillary, cribriform, or solid growth pattern associated with an infiltrative component. Comedonecrosis is frequently present.

SDCs occur almost exclusively in the major salivary glands with the parotid gland predominantly affected.[10] Only isolated cases involving minor salivary glands have been reported.[1,8,11-18] In this study, we report the clinicopathologic and immunohistochemical features of 5 cases of intraoral SDC. The DNA ploidy status of these tumors was also studied.

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Materials and Methods

Malignant intraoral salivary gland tumors diagnosed at the Departments of Oral Pathology at the University of Pretoria and Medical University of Southern Africa were reviewed. Four cases of SDC were included while an additional case was reclassified as an SDC according to the criteria of the World Health Organization.[19] Clinical and follow-up information was obtained from the patients' files and supplemented by communication with the referring practitioners or clinics and immediate family members. All the specimens were fixed in 10% buffered formalin, and the histologic features were evaluated by reviewing all the sections stained with hematoxylin and eosin. Additional slides of paraffin blocks were prepared for immunohistochemical analysis using the standard avidin-biotin peroxidase method. A panel of commercially available antibodies with appropriate controls was used (Table 1). The extent of immunohistochemical staining was evaluated and scored as + (1% to 9% of tumor cells), ++ (10% to 50% of tumor cells), and +++ (>50% of tumor cells). Staining intensity was not evaluated.

Flow cytometry was performed on 50 μm sections of the formalin-fixed paraffin-embedded tumor blocks. Tissue was processed according to the modified method described by Heiden et al.[20] The sections were enfolded with 50-μm nylon mesh and deparaffinized in xylene, hydrated in graded alcohols, and digested with Carlsberg solution. The nuclei were stained with DAPI solution (4',6-diamidino-phenylindole) containing 0.2 M trisodium citrate dihydrate, and at least 10,000 events from each case were analyzed using a PAS III flow cytometer equipped with a high-pressure 100-W mercury lamp (Partec, Münster, Germany).

Results

The patients ranged in age from 47 to 71 years (mean age, 58.2 years). Two were female and 3 were male. All of the tumors were located in the palate and ranged in size from 5 to 14 cm. The tumors presented

Table 1. SPECIFICATIONS OF ANTIBODIES USED

Antibody	Source	Dilution	Antigen Retrieval	Detection
High–molecular-weight cytokeratin	DAKO (34BE12)	Prediluted	*	DAKO LSAB2
α-Smooth muscle actin	DAKO (1A4)	Prediluted	None	DAKO LSAB2
Vimentin	DAKO (V9)	Prediluted	None	DAKO LSAB2
Anti–S-100A	DAKO	Prediluted	*	DAKO LSAB2

*Microwave pressure cooker in citric buffer, pH 6.0.

as painful masses (Figs 1 through 3). No association with the parotid gland could be shown in any case using computed tomography investigation (Fig 4). The possibility of metastasis from an intraductal breast carcinoma was also excluded. Clinical evidence of regional lymph node metastasis was present in 2 cases. Three patients refused any form of treatment and were subsequently lost to follow-up. No information could be obtained from the referring clinics. One patient was treated with radical resection and is currently receiving postoperative radiotherapy. The clinicopathologic findings are summarized in Table 2.

Microscopically, all tumors consisted of an intraductal component with a predominantly cribriform pattern and central comedonecrosis (Fig 5). Infiltrating tumor islands, in a trabecular and cribriform pattern, in a stroma that varied from cellular to regions of hyalinization were also present in all 4 cases (Fig 6). The tumor cells had well-defined cell borders with eosinophilic cytoplasm and vesicular nuclei. Mitotic activity varied from moderate to high (Fig 7). The immunohistochemical results are shown in Table 3.

Flow cytometry analysis showed 4 tumors to be aneuploid (Fig 8) and one diploid (case 2) (Fig 9). The coefficient of variance (CV) of all the measurements was less than 3%.

Discussion

The possibility of metastases should be eliminated before a final diagnosis of SDC is made. Metastatic ductal carcinoma from the breast could be excluded with careful clinical examination and mammography. The histologic features of these tumors are very similar, although the presence of estrogen receptor protein and absence of carcinoembryonic antigen in breast carcinomas have been used to differentiate between SDC and ductal carcinoma of the breast.[21] Metastatic prostatic carcinoma can in the majority of cases be excluded by the absence of both prostate-specific antigen and prostate-specific acid phosphatase in the tumor cells.

The histologic differential diagnosis of SDC includes high-grade mucoepidermoid carcinoma, undifferentiated carcinoma, adenocarcinoma (not otherwise specified), dedifferentiated acinic cell carcinoma, and adenoid cystic carcinoma. High-grade mucoepidermoid carcinomas have epidermoid and intermediate basaloid cells as well as cells with mucicarmine demonstrable intracellular mucin, whereas only luminal mucin is found in SDC. Cribriform and papillary-cystic growth patterns are not found in mucoepidermoid carcinomas. Undifferentiated carcinoma lacks the eosinophilic cytoplasm of SDC and does not form glandular structures. Adenocarcinoma,
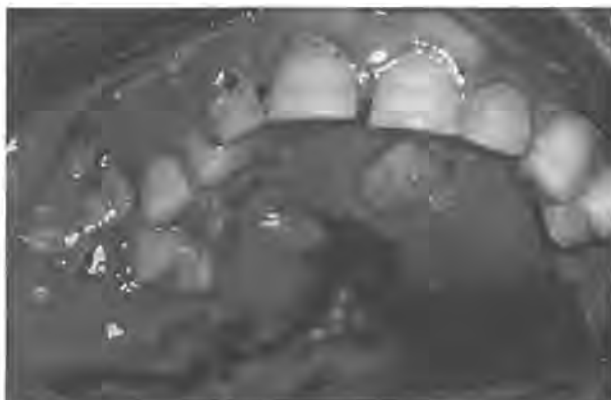

FIGURE 1. Intraoral view of case 1 showing a massive tumor destroying the right maxilla, extending across the midline.
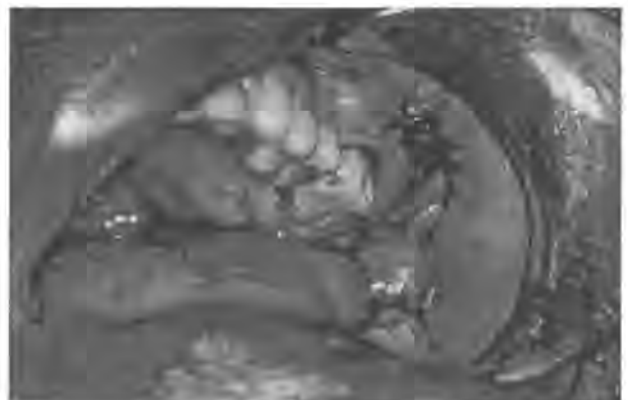

FIGURE 2. A tumor located in the left maxilla with buccal and palatal expansion from case 2.
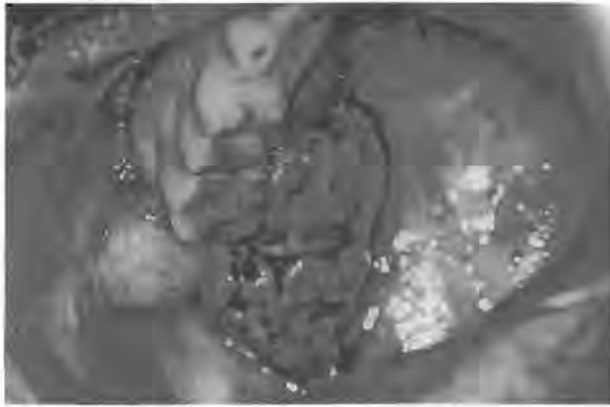
FIGURE 3. Case 3 presenting with an ulcerated tumor in the right palate involving the alveolar ridge.

not otherwise specified, shows glandular and ductal differentiation but lacks the distinctive features of SDC and is basically a diagnosis of exclusion.[10] Dedifferentiated acinic cell carcinoma may present as a poorly differentiated adenocarcinoma or undifferentiated adenocarcinoma but always in association with a usual-type low-grade acinic cell carcinoma.[22] Adenoid cystic carcinoma cells usually contain little cytoplasm and have angulated, basophilic nuclei. Comedonecrosis is also not a feature of adenoid cystic carcinoma.

The diagnosis of primary intraoral SDC necessitates exclusion of direct spread from one of the major salivary glands, especially the parotid, where most SDC arise. Computed tomography scans and other imaging techniques should not show any association

FIGURE 4. Computed tomography-scan of patient described in case 2 showing a tumor in the left maxilla and palate with no parotid involvement.

with the parotid or any other major salivary gland. This is especially true when SDC of the cheek is diagnosed, which is not a common site for minor salivary gland tumors.[23]

Table 2. CLINICOPATHOGIC FEATURES OF 5 PATIENTS WITH INTRAORAL SDCs

| Patient	Age (yr)	Gender	Site	Clinical Presentation	Tumor Size (cm)	Treatment	Follow-Up
1	53	F	Right palate and right alveolar ridge	Fungating mass; difficulty in breathing and eating; lymphadenopathy	±14	Biopsy	Patient refused treatment, lost to follow-up
2	71	M	Left palate and buccal sulcus	Pain, nerve fallout of II, III, V_b, and VII; lymphadenopathy	±5	Biopsy	Patient refused treatment, lost to follow-up
3	57	M	Right palate	Ulcerated tumor	±6	Biopsy	Patient refused treatment, lost to follow-up
4	63	F	Left palate	Painful, ulcerated tumor	±7	Maxillectomy, left neck dissection, postoperative radiotherapy	No recurrences after 10 months
5	47	M	Left palate	Fungating, nonulcerated tumor	±5	Biopsy	Patient still considering surgical treatment
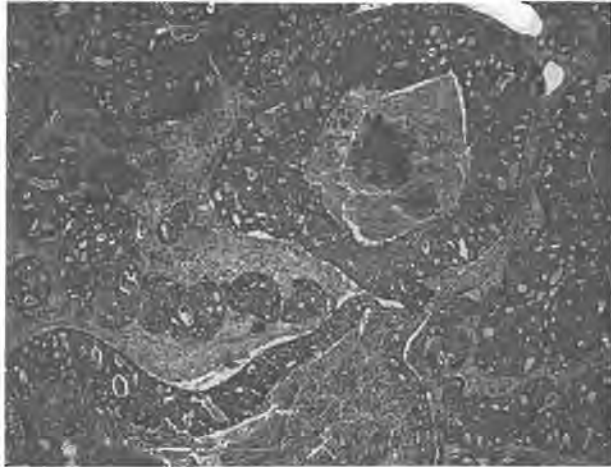
FIGURE 5. Photomicrograph of an SDC showing intraductal growth pattern with a cribriform appearance and comedonecrosis in the larger tumor islands (hematoxylin-eosin stain, original magnification × 25).
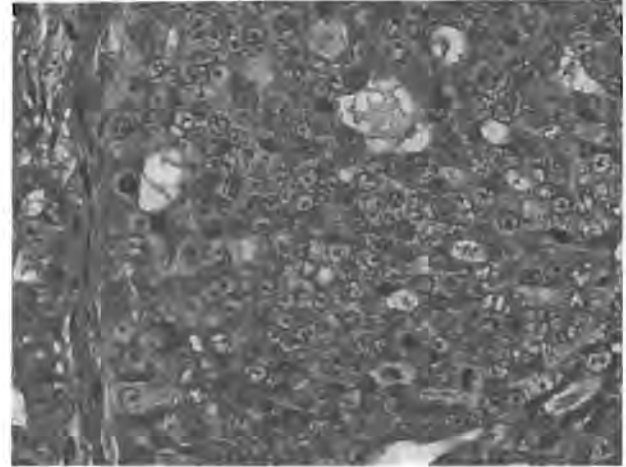

FIGURE 7. The tumor cells had vesicular nuclei with prominent nucleoli. Mitotic figures were prominent (hematoxylin-eosin stain, original magnification ×200).

Carcinoma ex pleomorphic adenoma is not uncommon in minor salivary glands[23] and SDC have been reported as the malignant component of these malignancies.[6,15,24] None of our cases had any histologic evidence of a preexisting pleomorphic adenoma, nor did a longstanding history suggest such an association. SDC has also been reported as a hybrid carcinoma of the minor salivary glands combined with an adenoid cystic carcinoma[25] and Warthin's tumor.[13]

SDC of the major salivary glands is an aggressive, high-grade malignancy. Comparison of the behavioral quality of SDC originating from minor salivary glands with that of the major glands is difficult, as only isolated cases of SDC have been reported. The clinical characteristics of our cases are similar to other studies reported for SDC of the major glands in that predominantly older male patients were involved. It was not possible to determine the clinical behavior of our 5

FIGURE 6. Infiltrating tumor islands in a cellular stroma (hematoxylin-eosin stain, original magnification ×100).

cases due to lack of follow-up information. However, the clinical appearance (large size and ulceration) of these tumors together with detectable peripheral neuropathy (ie, paresthesia, paralysis) and presence of fixed lymph nodes suggesting metastatic spread was supportive of an aggressive clinical behavior. The size of primary SDC was found to correlate with malignant potential. Hui et al[26] reported that tumors smaller than 3 cm correlate with a lower malignant potential, whereas Delgado et al[15] found a similar correlation with tumors smaller than 2 cm.

Immunohistochemical evaluation of some intermediate filaments in the tumor cells indicated that SDC is composed of predominantly ductal cells with little or no myoepithelial cell involvement. The strong expression of cytokeratin in the tumor cells is supported by the majority of studies on SDCs.[2,6,15,27] The tumor cells were negative for vimentin and smooth muscle actin, whereas positive staining with S-100 antibody was found in less than 10% of tumor cells in all 5 cases. Diffuse positive staining of S-100 was found by Brandwein et al[2] in 7 of 9 cases as well as in a single case of SDC reported in the palate.[25] Most studies, however, reported no immunoreactivity with S-100
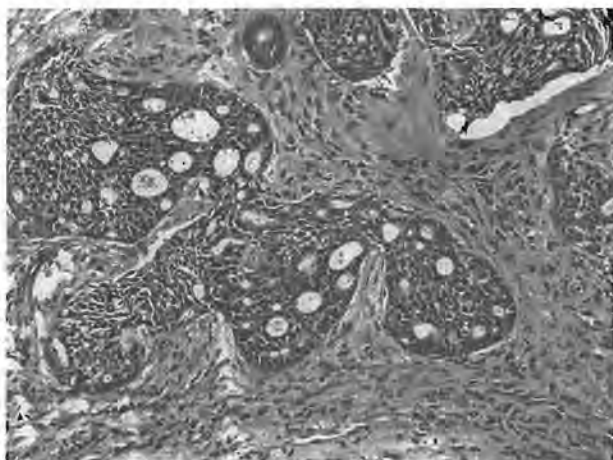
Table 3. IMMUNOHISTOCHEMICAL FINDINGS IN THE 5 INTRAORAL SDCs

Antibody	Patient			
	1	2	3	4
Keratin	+++	++	+++	++
α-Smooth muscle actin	−	−	−	−
Vimentin	−	−	−	−
S100	+	+	+	+

NOTE. −, Negative; +, 1% to 9% of tumor cells; ++, 10% to 50% of tumor cells; +++, more than 50% of tumor cells.

antibody.[15,17,27] The isolated (1% to 10%) positive staining of S-100 in our 4 cases may be due to the presence of Langerhans cells between the tumor cells, although tumor cells with a ductal differentiation may express S-100 protein.[28] Ultrastructurally, SDCs are composed of cuboidal to polygonal cells with interdigitations and cells forming ductlike structures with microvilli and apical vesicles; myoepithelial cells are absent. These findings support the ductal origin of SDC.[29]

Four of the SDCs in the present study displayed DNA aneuploidy. Several studies have measured the DNA content of SDC using flow cytometry with varied results. The majority found no correlation between the ploidy status and prognosis,[6,24,30] whereas Martinez-Barba et al[27] found a positive correlation between aneuploidy and the presence of distant metastases and fatal clinical outcome. Nuclear suspensions for flow cytometry analyses were obtained from paraffin-embedded sections in all of the above-mentioned studies, but none of these studies mentioned the CV obtained for the flow cytometry measurements. The CV of DNA measurements using paraffin-embedded tissue will invariably be higher than when using fresh tissue from the same tumor. It is possible that the reported diploid cases were in fact false diploid as tumor cells with a near diploid peak, implying small deviations of their DNA content from normal diploid cells, could not be distinguished due to the relatively high CV.

SDC is a distinct tumor that can originate from minor salivary glands. The histologic features are similar to those of tumors originating in the major salivary glands. The palate appeared to be the most common intraoral site for SDC. Although clinical features were suggestive of an aggressive behavior, more reported cases are required to determine the behavior in SDCs of minor salivary glands.
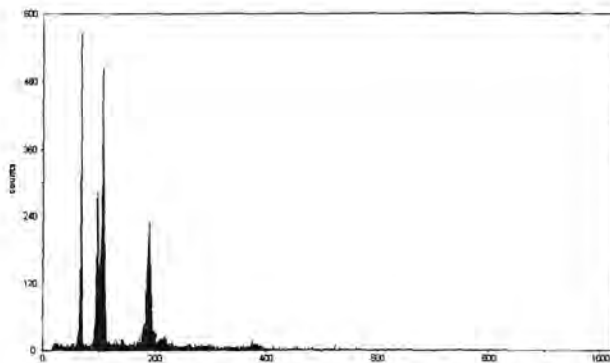

FIGURE 8. DNA histogram of case 1 showing the normal diploid peak at channel 100. Hypodiploid and hyperdiploid tumor cells were present.
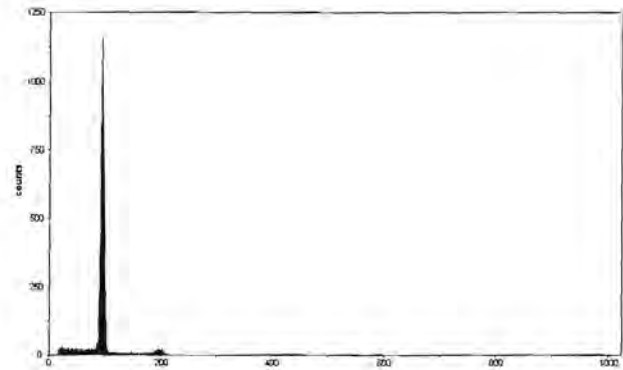

FIGURE 9. Diploid DNA histogram with diploid cells at channel 100 and the small peak at channel 200 representing the cells at G_2M phase.

References

1. Kleinsasser O, Klein HJ, Hubner G: [Salivary duct carcinoma. A group of salivary gland tumors analogous to mammary duct carcinoma]. Arch Klin Exp Ohr Nasen Kehlkopfheilkunde 192:100, 1968
2. Brandwein MS, Jagirdar J, Patil J, et al: Salivary duct carcinoma (cribriform salivary carcinoma of excretory ducts). A clinicopathologic and immunohistochemical study of 12 cases. Cancer 65:2307, 1990
3. Chen KT, Hafez GR: Infiltrating salivary duct carcinoma. A clinicopathologic study of five cases. Arch Otolaryngol 107:37, 1981
4. Anderson C, Muller R, Piorkowski R, et al: Intraductal carcinoma of major salivary glands. Cancer 69:609, 1992
5. Afzelius LE, Cameron WR, Svensson C: Salivary duct carcinoma: A clinicopathologic study of 12 cases. Head Neck Surg 9:151, 1987
6. Lewis JE, McKinney BC, Weiland LH, et al: Salivary duct carcinoma. Clinicopathologic and immunohistochemical review of 26 cases. Cancer 77:223, 1996
7. Delgado R, Klimstra D, Albores-Saavedra J: Low grade salivary duct carcinoma. A distinctive variant with a low grade histology and a predominant intraductal growth pattern. Cancer 78:958, 1996
8. Tatemoto Y, Ohno A, Osaki T: Low malignant intraductal carcinoma on the hard palate: A variant of salivary duct carcinoma? Eur J Cancer Oral Oncol 32B:275, 1996
9. Barnes L, Rao U, Krause J, et al: Salivary duct carcinoma. Part I. A clinicopathologic evaluation and DNA image analysis of 13 cases with review of the literature. Oral Surg Oral Med Oral Pathol 78:64, 1994
10. Ellis GL, Auclair PL: Tumors of the salivary glands, in Ellis GL, Auclair PL (eds): Atlas of Tumor Pathology (3rd Series, Fascicle 17). Washington, DC, Armed Forces Institute of Pathology, 1996, p 324
11. Chen KT: Intraductal carcinoma of the minor salivary gland. J Laryngol Otol 97:189, 1983
12. Watatani K, Shirasuna K, Aikawa T, et al: Intraductal carcinoma of the tongue: Report of a case. Int J Oral Maxillofac Surg 20:175, 1991
13. Yoshimura Y, Tawara K, Yoshigi J, et al: Concomitant salivary duct carcinoma of a minor buccal salivary gland and papillary cystadenoma lymphomatosum of a cervical lymph node: Report of a case and review of the literature. J Oral Maxillofac Surg 53:448, 1995
14. Pesce C, Colacino R, Buffa P: Duct carcinoma of the minor salivary glands: A case report. J Laryngol Otol 100:611, 1986
15. Delgado R, Vuitch F, Albores-Saavedra J: Salivary duct carcinoma. Cancer 72:1503, 1993
16. Guzzo M, Di Palma S, Grandi C, et al: Salivary duct carcinoma: Clinical characteristics and treatment strategies. Head Neck 19:126, 1997

17. Suzuki H, Hashimoto K: Salivary duct carcinoma in the mandible: Report of a case with immunohistochemical studies. Br J Oral Maxillofac Surg 37:67, 1999

18. Kumar RV, Kini L, Bhargava AK, et al: Salivary duct carcinoma. J Surg Oncol 54:193, 1993

19. Seifert G, Sobin LH: Histological typing of salivary gland tumours, in Seifert G, Sobin LH (eds): World Health Organization International Histological Classification of Tumours (ed 2). New York, NY, Springer-Verlag, 1991

20. Heiden T, Wang N, Tribukait B: An improved Hedley method for preparation of paraffin-embedded tissues for flow cytometric analysis of ploidy and S-phase. Cytometry 12:614, 1991

21. Wick MR, Ockner DM, Mills SE, et al: Homologous carcinomas of the breasts, skin, and salivary glands. A histologic and immunohistochemical comparison of ductal mammary carcinoma, ductal sweat gland carcinoma, and salivary duct carcinoma. Am J Clin Pathol 109:75, 1998

22. Henley JD, Geary WA, Jackson CL, et al: Dedifferentiated acinic cell carcinoma of the parotid gland: A distinct rarely described entity. Hum Pathol 28:869, 1997

23. van Heerden WF, Raubenheimer EJ: Intraoral salivary gland neoplasms: A retrospective study of seventy cases in an African population. Oral Surg Oral Med Oral Pathol 71:579, 1991

24. Grenko RT, Gemryd P, Tytor M, et al: Salivary duct carcinoma. Histopathology 26:261, 1995

25. Kamio N, Tanaka Y, Mukai M, et al: A hybrid carcinoma: Adenoid cystic carcinoma and salivary duct carcinoma of the salivary gland. An immunohistochemical study. Virchows Arch 430:495, 1997

26. Hui KK, Batsakis JG, Luna MA, et al: Salivary duct adenocarcinoma: A high grade malignancy. J Laryngol Otol 100:105, 1986

27. Martinez-Barba E, Cortes-Guardiola JA, Minguela-Puras A, et al: Salivary duct carcinoma: Clinicopathological and immunohistochemical studies. J Cran Maxillofac Surg 25:328, 1997

28. Chen J-C, Gnepp DR, Bedrossian CWM: Adenoid cystic carcinoma of the salivary glands: An immunohistochemical study. Oral Surg Oral Med Oral Pathol 65:316, 1988

29. de Araujo VC, de Souza SO, Sesso A, et al: Salivary duct carcinoma: Ultrastructural and histogenetic considerations. Oral Surg Oral Med Oral Pathol 63:592, 1987

30. Felix A, El-Naggar AK, Press MF, et al: Prognostic significance of biomarkers (c-erbB-2, p53, proliferating cell nuclear antigen, and DNA content) in salivary duct carcinoma. Hum Pathol 27:561, 1996