Advances in salivary gland pathology

W Cheuk & J K C Chan
Department of Pathology, Queen Elizabeth Hospital, Hong Kong

This review summarizes the new findings on salivary gland pathology under the following categories: immunohistochemistry; molecular genetics; newly recognized tumour types; known tumour entities with new findings; and progression of salivary gland tumours. In the application of immunohistochemistry, CD117 can aid in highlighting the luminal cell component of various salivary gland tumours, whereas p63 or maspin can aid in highlighting the abluminal cell component. A high Ki67 index remains the most useful marker to predict adverse outcome in salivary gland carcinoma. Specific chromosomal translocations are recognized in pleomorphic adenoma (with translocation involving PLGA1 or HMGA2 gene) and mucoepidermoid carcinoma (with MECT1–MAML2 gene fusion). Newly recognized entities include: sclerosing polycystic adenosis (with recent molecular evidence supporting its neoplastic nature), sclerosing mucoepidermoid carcinoma with eosinophilia, kerato-cystoma, adenoma with additional stromal component (lymphadenoma, lipoadenoma and adenofibroma), cribriform adenocarcinoma of the tongue and signet ring adenocarcinoma of minor salivary gland. Known tumour entities with new findings include: salivary duct carcinoma (with newly recognized mucinous, micropapillary and sarcomatoid variants), intraductal carcinoma (with controversies in terminology), mucoepidermoid carcinoma (with newly proposed grading parameters and oncocytic variant), epithelial–myoepithelial carcinoma (with newly recognized morphological variants), small cell carcinoma (with most cases being related to Merkel cell carcinoma), extranodal marginal zone B-cell lymphoma (with specific chromosomal translocation) and chronic sclerosing sialadenitis (being a component of IgG4-related sclerosing disease). Progression of salivary gland tumours can take the form of malignant transformation of a benign tumour, progression from low-grade to high-grade carcinoma, dedifferentiation, or stromal invasion of an in situ carcinoma.

Keywords: immunohistochemistry, molecular genetics, salivary gland neoplasm, tumour progression

Abbreviations: AFIP, Armed Forces Institute of Pathology; CK, cytokeratin; EMA, epithelial membrane antigen; MALT, mucosa-associated lymphoid tissue; MAML, mastermind-like gene family; MECT1, mucoepidermoid carcinoma translocated-1; RT-PCR, reverse transcriptase-polymerase chain reaction; WHO, World Health Organization

Introduction

This review aims to take stock of the new information that has accumulated over the past decade on tumours and tumour-like lesions of the salivary gland. Emphasis will be placed on findings of diagnostic importance and new concepts.

What is new in immunohistochemistry?

The entire glandular structure of the salivary gland exhibits a two-tiered organization comprising luminal (acinar and ductal cells) and abluminal cells (myoepithelial and basal cells). The secretory acini and...
Intercalated ducts are wrapped by myoepithelial cells, while the striated ducts and subsequent conducting portion are supported by basal cells. Use of immunohistochemistry to differentiate between luminal and abluminal cells can help in understanding the complex architecture of salivary gland tumours and aid in diagnosis.

**Markers for luminal cells**

Luminal cells are readily highlighted by immunohistochemistry for low-molecular-weight cytokeratin (CK) (such as CAM5.2), carcinoembryonic antigen or epithelial membrane antigen (EMA). Interestingly, although CD117/c-kit is negative in normal salivary gland cells, it is often positive in luminal (glandular) cells of various types of salivary gland tumour, and thus can be utilized to highlight the rudimentary or abortive glands in tumours (Figure 1A). The prior claim that CD117 is relatively specific for adenoid cystic carcinoma among salivary gland tumours cannot be confirmed.1,2 Although CD117 is expressed in ductal cells, clinical trials using a specific tyrosine kinase receptor inhibitor (imatinib) for adenoid cystic carcinoma have shown no beneficial effects.3

**Markers for abluminal cells**

Traditionally, abluminal cells are highlighted by immunohistochemistry for high-molecular-weight CK (such as 34βE12 or CK14) and myoepithelial cells, in addition, are stained with antibodies against myoid proteins (such as muscle-specific actin, smooth muscle actin or calponin). p63 has recently become a popular marker for abluminal cells—both basal cells and myoepithelial cells, as well as their neoplastic counterparts, show nuclear immunoreactivity (Figure 1B).4 However, p63 is not entirely specific for myoepithelial and basal cells. Squamous cells and their tumours are also positive. CD10 can also be used as a myoepithelial marker, but lacks specificity.

Another myoepithelial marker is maspin, a serine protease inhibitor that functions as a tumour suppressor.5 In the normal salivary gland, maspin is selectively expressed in the nuclei and cytoplasm of myoepithelial cells (Figure 2A).6 In salivary gland tumours with dual ductal–myoepithelial cell differentiation (such as pleomorphic adenoma, basal cell adenoma, adenoid cystic carcinoma and epithelial–myoepithelial carcinoma), the myoepithelial or modified myoepithelial cell component generally strongly expresses maspin, whereas the ductal cells are usually negative or show only weak focal immunoreactivity (Figure 2B). While immunohistochemistry for maspin can aid in highlighting the myoepithelial component of salivary gland tumours, it lacks specificity for supporting the myoepithelial nature of myoepithelioma or myoepithelial carcinoma, because this marker is not uncommonly expressed in various types of neoplasm, such as colorectal cancer, lung cancer and oral cancer.7–9

**Markers for prognosis**

The Ki67 proliferative index is the most widely used immunohistochemical marker for prognosis of salivary gland carcinomas. A high Ki67 index has been found to correlate with poor overall survival in mucoepidermoid carcinoma, acinic cell carcinoma and adenoid cystic carcinoma.10 On the other hand, no statistically significant relationship has been found between p53 immunoreactivity and survival.
NM23 protein is a nucleoside-diphosphate kinase which plays a tissue-specific role in relation to tumour metastasis. Both reduced expression and overexpression of NM23 have been demonstrated to correlate with increased metastasis and poorer prognosis in cancers from different sites. Cytoplasmic NM23 staining can be demonstrated in the majority of pleomorphic adenomas, adenoid cystic carcinomas and mucoepidermoid carcinomas, with no significant difference in the frequency of positive cells among these tumours. However, nuclear expression of NM23 is restricted to malignant salivary gland tumours with metastasis. Hence, nuclear NM23 staining may be used for predicting metastasis in salivary gland carcinomas.

Mucoepidermoid carcinomas express, in varying proportions, a variety of membrane-bound mucins, including MUC1, MUC4, MUC5AC and MUC5B. High MUC1 expression is associated with high histological grade, high rate of recurrence and metastasis and short disease-free interval. Conversely, expression of MUC4, a surrogate marker of tumour differentiation, is related to low histological grade, low recurrence rate and a long disease-free interval. Positive staining for MUC5AC is also helpful in distinguishing high-grade mucoepidermoid carcinoma from squamous cell carcinoma.

What is new in molecular genetics?

In recent years, specific chromosomal translocations have been reported in pleomorphic adenomas and mucoepidermoid carcinomas. Further studies are...
required to explore the potential value of these translocations in the diagnosis of these two types of tumour.

### Pleomorphic Adenoma

In approximately 70% of cases of pleomorphic adenoma, cytogenetic aberrations can be demonstrated in one of the following three patterns:\(^1\) (1) rearrangement of 8q12 (39% of cases); (2) rearrangement of 12q13-15 (8% of cases); (3) sporadic, clonal changes not involving 8q21 or 12q13-15 (23% of cases).\(^1\)

The target gene on chromosome 12q13-15 is *PLAG1*, which encodes a zinc finger transcription factor. The partner genes include *CTNNB1* (β-catenin) in t(3;12)(p13;q14) and *SII* (transcription elongation factor SII) in a cryptic translocation. The gene fusion results in dysregulated expression of *PLAG1* due to swapping of the promoter region.\(^2\)–\(^4\) Overexpression of *PLAG1* can also result from cryptic, intrachromosomal 8q rearrangements giving rise to novel fusion products *CHCHD7–PLAG1* or *TCEA1–PLAG1*.\(^\text{24}\)

The target gene on chromosome 12q13-15 is *HMGA2* (previously known as *HMGIC*), which encodes one of the high mobility group proteins that are non-histone chromatin-associated. The partner genes include *FHIT* (fragile histidine triad gene) in t(3;12)(p14;q14) and *NFIB* (nuclear protein involved in transcriptional regulation) in ins(9:12)(p23;q12). The gene fusion results in separation of the DNA-binding domains from potential mRNA-stabilizing motifs, leading to deregulation of *HMGA2* expression.\(^5\)–\(^6\)

Because the *PLAG1* or *HMGA2* gene translocations have so far been identified only in pleomorphic adenomas, their detection by reverse transcriptase-polymerase chain reaction (RT-PCR) or fluorescence *in situ* hybridization may potentially aid in diagnosis.

### Mucoepidermoid Carcinoma

A specific chromosomal translocation has been recognized in mucoepidermoid carcinoma. t(11;19)(q21;p13), which fuses *MECT1* (mucoepidermoid carcinoma translocated-1) at 19p13 with *MAML2* (mastermind-like gene family) at 11q21, is found in up to 70% of cases. The fusion protein is expressed in all different cell types of the breast.\(^\text{32,33}\) This genetic alteration disrupts the Notch signalling pathway.\(^\text{29}\) *MECT1–MAML2* fusion-positive patients have significantly less local recurrences, metastases and tumour-related deaths compared with fusion-negative patients. Median survival for fusion-positive patients exceeds 10 years, whereas that for fusion-negative patients is only 1.6 years.\(^\text{30}\) These findings suggest that *MECT1–MAML2* fusion may be a useful prognostic marker for mucoepidermoid carcinoma. Although there were some prior reports on the presence of t(11;19) in Warthin’s tumour by conventional cytogenetic studies or molecular genetic studies,\(^\text{31–33}\) a recent study using *in situ* hybridization and RT-PCR has failed to demonstrate the presence of *MECT1–MAML2* fusion gene in seven cases of Warthin’s tumour.\(^\text{28}\)

### Adenoid Cystic Carcinoma

The gene expression profile of adenoid cystic carcinoma has been studied by oligonucleotide array. The most overexpressed genes encode for basement membrane and extracellular matrix proteins of myoepithelial differentiation (e.g. laminin-β1, versican, biglycan and type IV collagen-α1). The most underexpressed genes are those encoding for proteins of acinar-type differentiation (e.g. amylase, carboxic anhydrase and salivary proline-rich proteins).\(^\text{34}\) Loss of heterozygosity in chromosome 6q23-25 has been found in 76% of cases of adenoid cystic carcinoma.\(^\text{35}\)

### Newly recognized entities

#### Sclerosing Polycystic Adenosis

**Clinical features and new evidence of its neoplastic nature**

Sclerosing polycystic adenosis was first characterized in 1996 as a rare lesion of uncertain nature with a striking morphological resemblance to fibrocystic changes of the breast.\(^\text{36}\) Despite the presence of additional reports on this entity, it is still under-recognized and frequently misdiagnosed as various types of salivary gland carcinoma, such as acinic cell carcinoma.\(^\text{37–42}\)

It occurs in patients from 9 to 80 years of age (mean 33–44.5 years) with a female: male ratio of 3 : 2.\(^\text{42}\) Most cases arise in the major salivary glands, but rare cases can involve intraoral minor salivary glands.\(^\text{37,42,43}\) The patients present with a slow-growing mass. Recurrence occurs in almost one-third of cases. So far there have been no reports on metastasis or mortality from this lesion.\(^\text{42}\)

Sclerosing polycystic adenosis has been considered a pseudoneoplastic benign entity. A recent molecular study employing the human androgen receptor assay for clonality analysis has demonstrated that this lesion is clonal and, hence, probably neoplastic.\(^\text{44}\)

**Pathological features**

The lesion is well circumscribed and partially encapsulated. There is proliferation of microysts, ducts and...
acinar structures in a sclerotic stroma, which often shows focal lymphocytic infiltration. These glandular units can be widely spaced or crowded, but a lobular pattern is characteristic. There can be variable degrees of epithelial hyperplasia forming solid aggregates and cribriform structures. Strangulated tubules reminiscent of sclerosing adenosis of the breast are common (Figure 4).

The glandular epithelial cells exhibit a spectrum of nondescript, apocrine, foamy, vacuolated and mucinaceous appearance (Figure 5). Characteristically, some cells contain large, brightly eosinophilic granules (Figure 5B). Ductal epithelial atypia ranging from mild dysplasia to carcinoma in situ can be found in 40–75% of cases.37,41

Immunohistochemically, luminal epithelial cells express EMA, BRST-2, oestrogen receptor (focal) and progesterone receptor (focal), but not c-erbB2.41,44 A continuous layer of myoepithelial cells can be demonstrated around the glandular units.

SCLEROSING MUCOEPIDERMOID CARCINOMA WITH EOSINOPHILIA

Clinical features
Sclerosing mucoepidermoid carcinoma with eosinophilia is an uncommon tumour of the thyroid gland that occurs in a setting of sclerosing Hashimoto thyroiditis and is characterized by an indolent clinical course.45,46 Two morphologically similar cases have been reported to occur as primary tumour of the major salivary gland,47 and the tumours apparently behave like a low-grade malignant neoplasm based on the limited data. We have similarly encountered two cases in our consultation practice. Currently, it is unclear whether this represents a distinctive tumour type or merely a variant of conventional mucoepidermoid carcinoma.

Pathological features
The tumour has infiltrative borders. In a sclerotic stroma heavily infiltrated by chronic inflammatory cells and eosinophils, there are islands and trabeculae of carcinoma with low nuclear grade.47 Most tumour cells have a squamoid appearance and focal keratinization can be present. There are admixed mucinous epithelial cells and glandular structures which merge with the squamoid islands or form discrete tubules (Figure 6).

KERATOCYSTOMA

Clinical features
Keratocystoma is a very rare benign tumour, with only three cases having been reported.48,49 All cases have involved the parotid glands of children or young adults (age 8–38 years). There is no recurrence after complete excision. This lesion can potentially be mistaken
histologically for a well-differentiated squamous cell carcinoma.

Pathological features
Grossly, the tumour is a multilocular cystic lesion filled with creamy material. Histologically, there are multiple, randomly disposed cystic structures and solid nests of squamous cells. The former are lined by bland-looking stratified squamous epithelium with ortho- or parakeratosis but lacking a granular layer. The lumens are filled with lamellated keratin. The epithelium is demarcated from the stroma by basement membrane. The stroma is fibrotic and infiltrated by chronic inflammatory cells. There can be foreign-body reaction against keratin extruded from the ruptured cysts.

ADENOMA WITH ADDITIONAL STROMAL COMPONENT
In recent years, several types of salivary gland adenoma characterized by the presence of an additional stromal component, such as lymphoid cells, adipose cells or fibrocellular stroma, have been described. Within this group, the most important tumour to recognize is lymphadenoma, because it can potentially be misdiagnosed as a malignant neoplasm and vice versa.

Lymphadenoma
Lymphadenoma is an adenoma accompanied by a dense lymphoid infiltrate. There is resemblance to sebaceous lymphadenoma except for the absence of a sebaceous component. Lymphadenoma is probably not
a distinctive tumour type, but merely represents a basal cell adenoma or cystadenoma accompanied by a heavy lymphoid infiltrate.\textsuperscript{50–52} All cases have occurred in the parotid glands. Complete surgical excision is curative.

The tumour is well circumscribed and comprises intimately admixed adenomatous and lymphoid components present in variable proportions. The adenomatous component takes the form of anastomosing trabeculae, islands, solid tubules, cystically dilated glands filled with proteinaceous materials, or papillary structures (Figure 7). The cyst or gland-lining cells are cuboidal to columnar without significant cytological atypia. The trabeculae and solid islands are composed of small lymphocytes and plasma cells. The lymphoid component is made up of small lymphocytes and plasma cells, with or without interspersed reactive lymphoid follicles. In some cases, the lymphoid component is so exuberant as to mask the epithelial component, resulting in morphological mimicry of lymphoma. Examination of the lesion at medium magnification often reveals cohesive aggregates of larger cells (epithelial cells) among the lymphoid cells, and periodic acid–Schiff-diastase stain can aid in highlighting the adenomatous component by staining the basement membrane-like material around the epithelial islands.

Lymphadenoma should not be mistaken for lymphoepithelial carcinoma, and vice versa. The latter features invasive growth, definite nuclear atypia, significant mitotic activity and squamous or squamoid rather than glandular differentiation. Epstein–Barr virus can be demonstrated in the tumour cells of lymphoepithelial carcinomas occurring in Orientals and Eskimos. On the other hand, there is at least focal ductal differentiation in lymphadenoma.

Lymphadenoma can be distinguished from lymphoepithelial sialadenitis by the circumscribed borders and presence of a proliferated epithelial component. It can also potentially be mistaken for metastatic adenocarcinoma in lymph node; the distinguishing features are lack of sinuses in the lymphoid tissue and lack of definite nuclear atypia.

**Lipoadenoma (sialolipoma)**

Lipoadenoma, also known as sialolipoma, is a benign tumour consisting of adipose tissue admixed with variable amounts of adenomatous glands. It affects patients of a wide age range, with male predilection. The tumour presents as a slowly growing mass lesion in the major or minor salivary gland. Complete excision is curative.

Histologically, the tumour is thinly encapsulated or circumscribed. It comprises mature adipose cells and proliferated glandular tissue, with the former usually constituting > 90% of the tumour. The glandular component is sharply demarcated from the fat, and comprises duct-acinar units or proliferated glands, which may take the form of sertoliform tubules. Oncocytic change, ductal dilation with fibrosis, sebaceous differentiation or squamous metaplasia can occur in some cases.\textsuperscript{53–57} It is unclear whether the glandular component represents entrapped salivary tissue or an integral part of the tumour.

**Adenofibroma**

Adenofibroma is a very rare neoplasm characterized by an intimate admixture of adenomatous glands and a fibrocellular stroma. The adenomatous glands can show metaplastic changes (such as oncocytic meta-
plasia) or cystic dilation. The stroma comprises delicate spindly cells with uniform nuclei. These spindly cells are CD34+ and lack myoepithelial features (S100-, actin- and p63-negative).

**Cribriform adenocarcinoma of the tongue**

In 1999, Michal et al. described eight cases of an unusual carcinoma of the tongue designated ‘cribriform adenocarcinoma’. Histologically, this is an infiltrative tumour exhibiting diverse growth patterns, including solid, microcystic, follicular, cribriform and papillary. The tumour cells are bland looking and possess uniform, often overlapping, nuclei with vesicular or ‘ground-glass’ chromatin (Figure 8). There is no significant mitotic activity, necrosis or haemorrhage.

It is currently unclear whether this is a distinctive tumour type or merely a morphological variant of polymorphous low-grade adenocarcinoma. However, in contrast to the latter, this tumour occurs exclusively in the base of tongue and the frequency of cervical lymph node metastasis at presentation is much higher (100%).

**Signet-ring cell (mucin-producing) adenocarcinoma of minor salivary gland**

**Clinical features**

An uncommon signet ring cell adenocarcinoma of the minor salivary gland has recently been characterized. The mean age of patients is 56.4 years with a female predilection. All reported cases have occurred in the minor salivary glands of the oral cavity, in the form of an exophytic mass that can be fixed to the underlying tissue. Surprisingly, this behaves as a low-grade malignant neoplasm, with no recurrence or metastasis after excision.

**Pathological features**

The tumour is infiltrative and comprises narrow parallel strands, randomly scattered small nests or isolated cells. Signet ring cells predominate and possess single or several cytoplasmic mucin vacuoles and eccentric indented nuclei. There are admixed minor populations of tumour cells with eosinophilic or clear cytoplasm. The overall cytological atypia is very mild. Mitotic figures are rare or absent, and there is no necrosis. Extracellular mucin pools are seen in only one of seven reported cases. Perineural invasion is not uncommon.

**Known tumour entities with new findings**

**Salivary duct carcinoma**

A number of morphological variants of salivary duct carcinoma have recently been described. These variants will not usually pose problems in diagnosis because a component of classical salivary duct carcinoma is always present at least focally.

**Mucin-rich variant**

The mucin-rich variant features areas of mucinous/colloid carcinoma in which clusters of carcinoma cells, with or without cytoplasmic mucin, float in mucin pools.

**Invasive micropapillary variant**

The micropapillary variant is characterized by morule-like tumour cell clusters without fibrovascular cores, surrounded by a clear space, morphologically similar to the micropapillary variant of breast or urothelial carcinoma (Figure 9A). This variant appears to pursue an even more aggressive course than conventional salivary duct carcinoma.

**Sarcomatoid variant**

In addition to a component of typical salivary duct carcinoma, there is an admixed sarcomatoid component comprising anaplastic spindly cells, bizarre multinucleated giant cells, rhabdoid cells and, rarely, osteosarcomatous cells (Figure 9B). These anaplastic cells frequently demonstrate focal immunohistochemical and ultrastructural evidence of epithelial differentiation. Conceptually, we consider this variant a form of dedifferentiation of salivary duct carcinoma.

---

Figure 8. Cribriform adenocarcinoma of the tongue. The tumour is characterized by invasive cribriform islands comprising cells with uniform pale-staining nuclei and low mitotic activity—cytologically very similar to polymorphous low-grade adenocarcinoma.
Intraductal carcinoma, first described by Chen in 1983, is not a recognized entity in the 2005 World Health Organization (WHO) classification. It is characterized by pure intraductal proliferation of tumour cells, similar to intraductal carcinoma of the breast. The concept of intraductal carcinoma has not gained wide acceptance, probably because some salivary duct carcinomas with apparently pure intraductal-like growth still pursue an aggressive course, and an intraductal-like component can sometimes be found in the metastases. However, these observations can be attributable to the indiscriminate use of the term ‘intraductal-like’—such foci usually represent invasive growth rather than genuine in situ growth.

Strictly defined by the presence of an intact myoepithelial layer around all tumour islands, intraductal carcinoma of the salivary gland represents a tumour of low malignant potential, with behaviour similar to that of the mammary counterpart. This tumour has often been reported in the literature under the designation ‘low-grade salivary duct carcinoma’. In fact, most cases of ‘low-grade salivary duct carcinoma’ are either pure intraductal carcinoma or intraductal carcinoma with microinvasion. The term ‘intraductal carcinoma’ is more appropriate than ‘low-grade salivary duct carcinoma’, because it emphasizes the fundamental nature of the tumour and avoids potential confusion with the vastly more aggressive salivary duct carcinoma. The term ‘low-grade cribriform cystadenocarcinoma’ adopted in the new WHO classification introduces more confusion and we do not recommend adoption of this terminology, a view also shared by Weinreb et al. Whether intraductal carcinoma represents the precursor of conventional salivary duct carcinoma or is biologically a separate entity remains to be clarified.

Clinical features
Similar to salivary duct carcinoma, intraductal carcinoma most frequently affects the parotid gland of the elderly. The minor salivary glands can also be affected. The outcome is excellent after complete excision, with no metastasis or mortality on follow-up, irrespective of nuclear grade. Recurrence can occur as a result of incomplete resection. Given time, an invasive component can supervene.

Pathological features
The tumour is characterized by multiple smooth-contoured ducts expanded by epithelial proliferation forming cribriform, fenestrated, solid-comedo, micropapillary or Roman-bridge patterns, similar to the architectural patterns observed in atypical ductal hyperplasia or intraductal carcinoma of the breast (Figure 10A). The constituent cells usually show low to intermediate grade, but sometimes high-grade, cytological atypia (Figure 10B). They can also show apocrine features. The attenuated layer of myoepithelial cells around the cell islands may or may not be evident on light microscopy. The stroma is sclerotic and may exhibit secondary changes such as haemorrhage, chronic inflammatory infiltrate and dystrophic calcification.

In occasional cases, a microscopic invasive component is present, either at presentation or in recurrence. The clinical significance of microinvasion remains uncertain, but the prognosis appears favourable.
Prerequisites of diagnosis
To render a diagnosis of intraductal carcinoma, the presence of an invasive component must be meticulously ruled out by complete sampling and immunohistochemistry to demonstrate an intact myoepithelial layer around every tumour island (Figure 11).69,73

mucoepidermoid carcinoma
Grading
Many attempts have been made to grade mucoepidermoid carcinoma to stratify patients into groups with different prognoses. Traditionally, mucoepidermoid carcinoma is subdivided into low grade, intermediate grade and high grade based on several morphological parameters, including prominence of cysts, abundance of mucus cells, mitotic activity and cytological atypia.75,76 Evans has proposed a simpler two-tier grading system, assigning a case to low grade or high grade based on the presence of >10% and <10% intracystic spaces, respectively, excluding areas occupied by stroma and extravasated mucus.77

The Armed Forces Institute of Pathology (AFIP) scoring system assigns scores to five features: intracystic component <20% (score 2), neural invasion (score 2), necrosis (score 3), mitoses ≥4/10 high-power fields (score 3) and anaplasia (score 4); and a case is categorized as low grade (score 0–4), intermediate grade (score 5–6) or high grade (score ≥7) based on the total score. This system shows good correlation with outcome for intraoral and parotid mucoepidermoid carcinomas, but does not predict outcome for submandibular mucoepidermoid carcinomas, which have a significant metastatic potential irrespective of histological grade.78,79 However, there is a tendency for the AFIP scoring system to ‘undergrade’ mucoepidermoid carcinomas. A modified grading system adding three parameters (lymphovascular invasion, bone invasion and invasion in the form of small nests and islands) improves the reproducibility and predictability, and furthermore stratifies patients into three fairly uniform groups with different prognoses.80

Oncocytic variant
The rare oncocytic variant of mucoepidermoid carcinoma, characterized by the extensive presence of oncocytic cells, can potentially be misdiagnosed as oncocytoma (Figure 12).81–85 Oncocytoma is usually composed of a pure population of cells, although some cells may show cytoplasmatic clearing. The presence of

Figure 10. Intraductal carcinoma of salivary gland. A, In this parotid gland, there are focal collections of glands and cysts showing micropapillary or cribriform epithelial proliferation, reminiscent of intraductal carcinoma of breast. B, The proliferated epithelial cells show moderately pleomorphic nuclei and eosinophilic cytoplasm.

Figure 11. Intraductal carcinoma of salivary gland. On immunohistochemistry, all tumour islands are surrounded by actin-positive myoepithelial cells, indicating that the neoplastic process is in situ.

Figure 12. Oncocytic variant of mucoepidermoid carcinoma. A, The tumour cells show abundant eosinophilic cytoplasm and round to oval nuclei with fine granular chromatin. B, The tumour cells are negative for actin and smooth muscle actin and show positivity for cytokeratin and pan-cytokeratin.
cytoplasmic mucin and mucin-containing cystic spaces, or the presence of occasional non-oncocytic tumour islands, makes a diagnosis of oncocytoma most unlikely—the possibility of an oncocytic variant of mucoepidermoid carcinoma has to be seriously considered in such circumstances.

**EPITHELIAL—MYOEPIPTHELIAL CARCINOMA**

Recent studies have broadened the morphological spectrum of epithelial–myoepithelial carcinoma. 86

**Oncocytic variant**

Rare cases can exhibit extensive oncocytic change in the luminal cells alone or in both the luminal and abluminal cells. 86,87 Some of these cases may show a prominent papillary growth pattern and there can be sebaceous cell differentiation. 86

**Double clear variant**

The double-clear variant is characterized by clear cell change not only in the abluminal myoepithelial cells, but also in luminal cells. 86 When the epithelial component shows proliferation to form solid or cribriform architecture, it can be difficult to distinguish on morphological grounds between epithelial and myoepithelial cells.

**Ancient change**

The myoepithelial component in epithelial–myoepithelial carcinoma can exhibit ‘ancient change’—occasional nuclei have enlarged hyperchromatic nuclei. 86 In contrast to dedifferentiation or high-grade transformation, the chromatin is smudged, the cytological atypia is random (i.e. occurring in a background of non-bizarre nuclei) and there is no increase in mitotic activity.

**Other morphological features**

Although myoepithelial cells with clear cytoplasm have been emphasized as a hallmark of epithelial–myoepithelial carcinoma, clear cell change may be completely lacking in about 20% of cases. 86 Sebaceous differentiation is described in up to 13.1% of cases. 86 Although it is known that the myoepithelial component can occasionally form spindly cells, exceptionally the nuclei can be palisaded, resulting in resemblance to Verocay bodies. 86

**Progression of epithelial–myoepithelial carcinoma**

There are two forms of progression of epithelial–myoepithelial carcinoma. The first is progression to high-grade myoepithelial carcinoma, characterized by overgrowth of the myoepithelial component with nuclear anaplasia (Figure 13). This phenomenon has also been reported as ‘epithelial–myoepithelial carcinoma with myoepithelial anaplasia’ or defined as nuclear atypia in > 20% of myoepithelial cells. 86,88 The prognosis is worsened.

The second is dedifferentiation to high-grade carcinoma lacking evidence of myoepithelial differentiation. 86,89 The dedifferentiated component shows high mitotic activity, and necrosis is common. The prognosis is greatly worsened. 90

**SMALL CELL CARCINOMA**

Small cell carcinoma of the major salivary glands is an aggressive malignancy, with more than half of patients developing local recurrence or distant metastasis. 91 The overall survival rate is 40–50%, 92–95 comparable to that of cutaneous Merkel cell carcinoma, but much superior to that of conventional pulmonary or non-pulmonary small cell carcinoma. Indeed, 73% of salivary gland small cell carcinomas express CK20, suggesting that the majority of cases are biologically related to Merkel cell carcinoma rather than pulmonary-type small cell carcinoma. 96,97 The Merkel cell type of small cell carcinoma also demonstrates longer overall survival than the pulmonary type (CK20−). 96

**EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF SALIVARY GLAND**

The commonest type of primary salivary gland lymphoma is extranodal marginal zone B-cell lymphoma of
mucosa-associated lymphoid tissue (MALT) type. Four distinctive types of chromosomal translocation are now recognized in this type of lymphoma: t(11;18)(q21;q21): API2/ MALT1; t(1;14)(p22;q32): BCL10/IGH; t(14;18)(q32;q21): IGH/MALT1; t(3;14)(p14.1; q32): FOXP1/IGH.

Although these appear to be diverse types of chromosomal translocation, the final common pathway involves activation of nuclear factor-κB. These chromosomal translocations display organ specificity. For example, t(11;18) is seen predominantly in MALT lymphomas of the gastrointestinal tract and lung, but is very rare in MALT lymphomas at other sites. Among salivary gland MALT lymphomas, 12–22% of cases exhibit t(14;18)(q32;q21), whereas the other three types of chromosomal translocation are practically never found. Interestingly, a study from North America failed to detect t(14;18) in salivary gland MALT lymphomas; the only common abnormality found in that study was trisomy 18. On the other hand, t(14;18)(q32;q21) is not uncommonly found in MALT lymphomas of the eye, skin and liver. The reason for this organ specificity remains elusive.

**CHRONIC SCLerosing Sialadenitis (Kuttner Tumour)**

*New concept of the nature of chronic sclerosing sialadenitis* Chronic sclerosing sialadenitis affects almost exclusively the submandibular glands and is called Kuttner tumour in its advanced stage, as it presents clinically as a hard swelling indistinguishable from a tumour. The disease can be bilateral. Patients are usually middle-aged or elderly and there is slight male predominance. For many years, chronic sclerosing sialadenitis has been considered a chronic inflammatory disease resulting from inspissated secretion, stones or microliths, and perpetuated by ascending infection. Recent studies, however, have raised a different pathogenic mechanism for this disease—chronic sclerosing sialadenitis belongs to the spectrum of IgG4-related sclerosing disease. IgG4-related sclerosing disease is a syndrome characterized by involvement of one or more tissues (most commonly exocrine organs) by a chronic inflammatory cell infiltrate which includes abundant IgG4+ plasma cells, accompanied by atrophy of the normal tissue and sclerosis. Some patients have associated autoimmune disease (such as rheumatoid arthritis) or circulating autoantibodies. The serum IgG4, IgG and IgG4/IgG ratio (normally 3–6%) are typically elevated and there is an excellent response to steroid therapy.

**Pathology**

The histological features of chronic sclerosing sialadenitis are very similar to those of autoimmune pancreatitis, which is a common component of IgG4-related sclerosing disease. The lobular architecture is preserved and the degree of involvement varies from lobule to lobule. In the early stages, lymphoplasmacytic infiltrate commences around the salivary ducts, followed by periductal fibrosis. The ducts may contain inspissated secretion. The lymphocytic infiltrate...
and fibrosis intensify and gradually involve the whole lobule, associated with atrophy of the acini (Figure 14A). Reactive lymphoid follicles are frequently present.

On immunohistochemistry, T cells predominate and show an intimate relationship with ducts and acini. B cells are mostly restricted to the lymphoid follicles. Abundant IgG4+ plasma cells are present (Figure 14B).

We have described a case of chronic sialadenitis featuring a few abnormal lymphoid follicles packed with signet ring cells of the cytoplasmic globular type, suggestive of incipient follicular lymphoma. A case of extranodal marginal zone B-cell lymphoma of MALT type complicating chronic sialadenitis has also been reported. Thus, it appears that lymphoma can rarely emerge in the setting of chronic inflammation in chronic sclerosing sialadenitis.

**Progression in salivary gland tumours**

Tumour development or progression is a multistep process, often involving sequential accumulation of genetic changes. Salivary gland tumours provide a great opportunity to elucidate the mechanisms of tumour progression because of (i) the complexity of cytoarchitecture of salivary gland tumours; and (ii) the existence of many different types of indolent tumours, which provide the ‘soil’ for accumulation of new mutations and hence tumour progression (risk increasing with duration of harbouring the tumour). The various forms of progression of salivary gland tumours are depicted in Figures 15–18.

**Carcinoma ex pleomorphic adenoma: what’s new?**

**Evolution of carcinoma ex pleomorphic adenoma**

Sequential evolution can sometimes be traced in the development of carcinoma ex pleomorphic adenoma, and these different phases have prognostic significance.

1 In the earliest phase, carcinoma cells with large atypical nuclei replace the neoplastic ductal luminal cells while retaining an intact layer of non-atypical myoepithelial cells of the pre-existing pleomorphic adenoma (Figure 19). This can be considered a form of ‘carcinoma in situ’, and there is no metastatic potential.

2 With time, the carcinoma cells break out from the confines of the neoplastic myoepithelial sheath and invade into the surrounding stroma. If this process is still...
confined within the parent pleomorphic adenoma, the carcinoma is considered ‘intracapsular’ (Figure 20). The prognosis is excellent with complete excision. There has been no metastasis,109–111 except for a single case report with cervical lymph node metastasis.112

3 If the invasion extends beyond the fibrous capsule of the parent pleomorphic adenoma, the carcinoma 
ex pleomorphic adenoma is considered ‘invasive’. However, it should be further categorized as being ‘minimally invasive’ or ‘frankly invasive’, although the optimal cut-off point to define minimally invasive carcinoma (tumour with minimal metastatic potential) is currently unsettled. In several series, excellent prognosis has been found in tumours with extracapsular invasion measur-
ing < 8 mm, 5 mm or 1.5 mm beyond the capsule, respectively.\textsuperscript{109,110,113} In the current WHO classification, tumours with invasion of < 1.5 mm from the tumour capsule are considered minimally invasive.\textsuperscript{114} In practice, although it is easy to make the measurements in some cases, it can be extremely difficult to do so in others due to difficulties in defining the boundaries of the parent pleomorphic adenoma.

Cell types that undergo malignant change in pleomorphic adenoma

In most cases (75%), luminal epithelial cells undergo malignant change. In the other cases, the supervening carcinoma shows dual epithelial–myoepithelial differentiation (19% of cases) or pure myoepithelial differentiation (6% of cases).\textsuperscript{115}

**Genetic mechanisms mediating malignant transformation**

Alterations or rearrangements of chromosome 8q21 and 12q13-15 are frequent in carcinoma ex pleomorphic adenoma, similar to its benign counterpart. Loss of heterozygosity at 12q loci may identify a subset of pleomorphic adenomas with a potential for malignant transformation.\textsuperscript{116} Amplification and overexpression of genes in chromosome 12q13-15, including \textit{CDK4}, \textit{HMGA2} and \textit{MDM2}, may represent important genetic events in the malignant transformation.\textsuperscript{117,118}

Alterations of \textit{p53} gene are found in 29–67% and \textit{p53} protein overexpression in 41–75% of cases, suggesting that the gene may play a role in transformation in at least some cases.\textsuperscript{110,119–123}

c-erbB2 overexpression or gene amplification occurs in 21–82% of cases.\textsuperscript{110,124–127} It has been suggested that immunopositivity for c-erbB2 may aid in distinguishing carcinoma ex pleomorphic adenoma from atypical pleomorphic adenoma (Figure 21).\textsuperscript{123,125,127}

DEDIFFERENTIATION OF SALIVARY GLAND CARCINOMAS

'Dedifferentiation' refers to the transformation of a salivary gland carcinoma to a high-grade carcinoma in which the original line of differentiation is no longer evident. The first type of salivary gland carcinoma reported to undergo dedifferentiation is acinic cell carcinoma. The dedifferentiated component takes the form of a high-grade adenocarcinoma, poorly differentiated carcinoma or undifferentiated carcinoma.\textsuperscript{128–130}

In recent years, a wide variety of other types of salivary gland carcinoma have been documented to...
show dedifferentiation, although this is still an uncommon event (Figure 17). Examples are: mucocoeplidermoid carcinoma, adenoid cystic carcinoma, myoepithelial carcinoma, polymorphous low-grade adenocarcinoma, epithelial–myoepithelial carcinoma, hyalinizing clear cell carcinoma and salivary duct carcinoma. All of these tumour types, with the exception of salivary duct carcinoma, are generally indolent tumours to begin with. New genetic alterations probably gradually accumulate in these indolent tumours, eventuating in the emergence of a high-grade aneuploid carcinoma.

Clinical features
Dedifferentiation of salivary gland carcinoma can occur either at initial presentation or at relapse. Clinically, there is usually recent onset of rapid tumour growth in a long-standing tumour, often resulting in bulky disease. Some cases present with a rapidly growing mass on relapse of a low-grade carcinoma. Recurrence and metastasis are common. The prognosis is generally very poor, with an accelerated clinical course compared with the original carcinoma type.

Common pathological features
The dedifferentiated component, which can be in the form of high-grade adenocarcinoma, undifferentiated carcinoma or sarcomatoid carcinoma, usually shows more frankly invasive growth and prominent coagulative necrosis (Figure 22). The neoplastic cells exhibit significant nuclear atypia, pleomorphism and brisk mitotic activity. Usually the original carcinoma and dedifferentiated components are juxtaposed to each other without a transitional zone.

Genetic changes that mediate dedifferentiation
Our knowledge of the molecular mechanisms responsible for dedifferentiation of salivary gland carcinoma is limited. In some cases, involvement of one or several genes has been documented. Examples include p53 mutation (accompanied by strong expression of p53 protein), increased cyclin D1 expression, c-erbB2 protein overexpression or gene amplification, and loss of Rb protein.

References
13. do Nascimento KC, de Faria PR, Dib LL et al. Immunohistochemical localization of the NM23 protein in salivary gland


