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Epithelial-myoepithelial carcinoma of the salivary glands. A study of 22 cases

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Abstract. Twenty-two cases of epithelial-myoepithelial carcinoma of major and minor salivary glands were studied retrospectively to define the clinico-pathological profile and to assess the value of DNA ploidy as a prognostic tool. Fifty-nine percent of the cases occurred in the major salivary glands, the patients being mostly females in their 5th to 8th decades. The clinical course was characterized by a high number of recurrences (in 50% of cases). Death due to the neoplastic disease was found in 40% of the patients. The only morphological feature found to be correlated to prognosis was the presence of nuclear atypia in more than 20% of the tumour cells. In 18 cases, cytophotometric DNA analysis was performed; 15 cases had a diploid DNA histogram and 3 an aneuploid one. All the cases that were DNA aneuploid were of the solid, predominantly clear-cell type and were associated with fatal outcome.

Key words: Salivary gland neoplasms – Epithelial-myoepithelial carcinoma – Prognosis – DNA ploidy

Introduction

Epithelial-myoepithelial carcinoma of the salivary glands (EMC) is a rare neoplasm first described by Donath et al. (1972). Its designation stresses the cellular composition of the neoplasm, as "a carcinoma made by duct epithelial cells and myoepithelial cells" (Corio et al. 1982; Donath et al. 1972). The recognition of this neoplasm as a distinct variant among the adenocarcinomas of the salivary glands was proposed by Corio et al. (1982) a few years later. However, previous reports of tumours with similar morphology had received various designations and, interestingly, most of them supported the benign nature of the neoplasm: adenomyoepithelioma (Bauer and Fox 1945) and clear cell adenoma (Corridan 1956; Goldman and Klein 1972; Saksela et al. 1972).

The histological hallmark of EMC is a peculiar double cell arrangement, formed by an internal layer of duct-like cells and an external layer of myoepithelial-like cells (Corio et al. 1982; Luna et al. 1987). The double-cell layered neoplastic structures can either form tubules, papillae, cords and trabecula or be arranged in solid nests (Corio et al. 1982; Luna et al. 1987). Infiltrating margins and perineural invasion are characteristically present in most cases of EMC.

This retrospective study was undertaken to investigate the clinico-pathological profile of the neoplasm using a series of 22 cases. Image cytometric analysis of the tumour cells DNA content was performed in 18 of them, and was correlated with the disease evolution (morbidity and mortality) to evaluate its value as a prognostic marker.

Materials and methods

From a series of 954 consecutive cases of salivary gland tumours, treated at the Instituto Português de Oncologia de Francisco Gentil, Lisbon, Portugal, we retrieved 22 cases of EMC, that account for 2% of the whole series.

In all cases the tumour was excised along with the affected gland. The neoplasms were fixed in 10% unbuffered formalin and paraffin-embedded.

In each case, the clinical chart was re-evaluated, and H & E and PAS sections were reviewed.

Immunohistochemical study was performed to confirm the presence of a dual differentiated cell population (Luna et al. 1987) using the avidin-biotin-complex method with antisera to low molecular weight keratin (CAM 5.2; 1:5) and S100 protein (Dako Z311; 1:3000) and muscle specific actin (Dako M851; 1:500).

The following microscopic characteristics were assessed as present or absent. Nuclear atypia was represented by hyperchromasia, irregularity of the nucleus and the existence of abnormal forms. The large and irregular nuclei were considered atypical if they were also hyperchromatic and this change was considered present, when it was found in more than 20% of the tumour cells. Necrosis, neural invasion and lymphatic permeation were other characteristics. The number of mitoses per 10 HPF were counted in each case.

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Table 1. Clinico-pathological and cytophotometric data of the tumour collective

Age	Sex	Local	Arch	Atyp	Mit	Necr	Neural inv	Lymph inv	Ploidy	Rec	Met	Follow-up
54	f	sp	sol	+	+	−	+	−	d	+	−	174/rec
74	f	sp	sol	+	−	−	in	−	−	+	−	158/dod
56	m	lp	tub	−	−	−	in	−	d	−	−	36/a & w
75	f	lp	sol	+	−	−	in	+	d	+	−	24/rec
69	f	sp	sol	+	−	−	+	+	d	−	−	−
76	f	lp	tub	−	−	−	+	−	−	−	−	12/a & w
77	m	sl	crib	+	+	+	+	+	d	−	+	24/dod
45	f	lp	crib	+	+	−	in	+	d	+	−	36/rec
74	f	sp	sol	+	+	−	in	in	−	−	−	−
49	f	lp	tub	+	−	−	+	−	d	+	+	162/dod
42	m	lp	sol	−	−	+	+	+	a	+	−	72/dod
74	f	ms	pap	+	+	−	+	−	d	+	−	252/dod
74	f	lp	sol	+	+	+	+	−	a	+	+	154/dod
45	f	lp	sol	−	−	−	+	−	−	−	−	120/a & w
61	m	lp	sol	+	−	+	in	in	d	+	+	72/rm
47	m	lp	sol	+	+	−	+	in	d	+	−	84/rec
56	f	lp	tub	−	−	+	−	−	d	−	−	72/a & w
70	m	lp	tub	−	−	+	−	−	d	+	−	60/dod
42	f	sp	tub	+	−	−	+	−	d	−	+	12/met
77	f	rp	sol	+	+	+	+	+	a	+	−	2/dod
63	m	om	sol	+	−	−	+	−	d	−	+	94/met
51	f	sp	sol	+	−	+	+	−	d	−	+	3/met

Arch, architecture; atyp, atypia; mit, mitosis; necr, necrosis; lymph, lymphatic; inv, invasion; sp, soft palate; lp, left parotid; sl, sublingual; ms, maxillary sinus; rp, right parotid; om, oral mucosa; sol, solid; tub, tubular; crib, cribriform; pap, papillary; in, inappreciable; d, diploid; a, aneuploid; dod, dead of disease; a & w, alive and well; rec, recurrence; met, metastases; rm, recurrence and metastases

In 18 cases, 4 μm paraffin sections were Feulgen stained after acid hydrolysis (hydrochloric acid 5 M, 1 h, at room temperature) and DNA ploidy was evaluated using an image analyser based on a CCD-TV camera (Ahrens ACAS System, Germany). At least 100 nuclei were measured as well as 10–20 lymphocytes and neutrophils that served as 2c standard. The histograms were classified as: DNA diploid in type, if they presented a single major peak at the 1.8–2.2 c region and DNA aneuploid either when there was a dispersion of values, or there were distinct peaks outside the 2 c and 4c regions.

To estimate the prognostic value of the variables examined, the 20 cases with follow-up information were subdivided into two groups according to disease evolution: one with favourable outcome included patients without any evidence of recurrent neoplastic disease until the end of the follow-up period and the other with an unfavourable oucome included patients with recurrence and/or metastases and those that died of their tumours.

The significance of differences between groups was evaluated with the Fisher test. The survival curves were performed using the Kaplan-Meier method, and the statistical significance determined by the log-rank test.

Results

Age of the patients ranged between 42 and 77 years (mean age: 61.4 years). Fifteen patients were females and 7 males. The tumours were located at the parotid gland in 13 cases (12 in the left and 1 in the right), at the sublingual gland in 1 case and at the minor salivary glands of the oral cavity in 7 cases (6 at the soft palate and 1 at the oral mucosa NOS) and 1 at the maxillary sinus (Table 1).

The tumours ranged between 1.5 and 12 cm in their largest dimension. All neoplasms had infiltrating margins and the cases affecting minor glands invaded surrounding soft tissues. The two cell types that composed every tumour were present in variable proportions. The duct-like cells were small, with scant eosinophilic cytoplasm and a central, round and dark nucleus and they stained positively for CAM 5.2 antiserum. The myoepithelial-like cells had vast, mostly vacuolated, clear cytoplasm, and an eccentric irregular nucleus. In some areas they were spindle-shaped. Basement membrane-like hyaline material surrounded either solid, clear cell groups or tubular double-layered structures. Nuclear and cytoplasmic immunoreactivity with S-100 protein

Table 2. Morphological characteristics related to tumour behaviour

		Favourable outcome	Unfavourable outcome	*p* value
Atypia	No	4	2	$p=0.002$
	Yes	0	16	
Necrosis	No	3	11	$p=0.53$
	Yes	1	7	
Neural[a] invasion	No	1	1	$p=0.36$
	Yes	3	15	
Vascular[b] invasion	No	4	9	$p=0.18$
	Yes	0	6	
Mitosis	<1	4	10	$p=0.13$
	>1	0	8	
DNA	Diploid	4	11	$p=0.44$
	Aneuploid	0	3	

[a] Unappreciated in 2 cases; [b] unappreciated in 3 cases

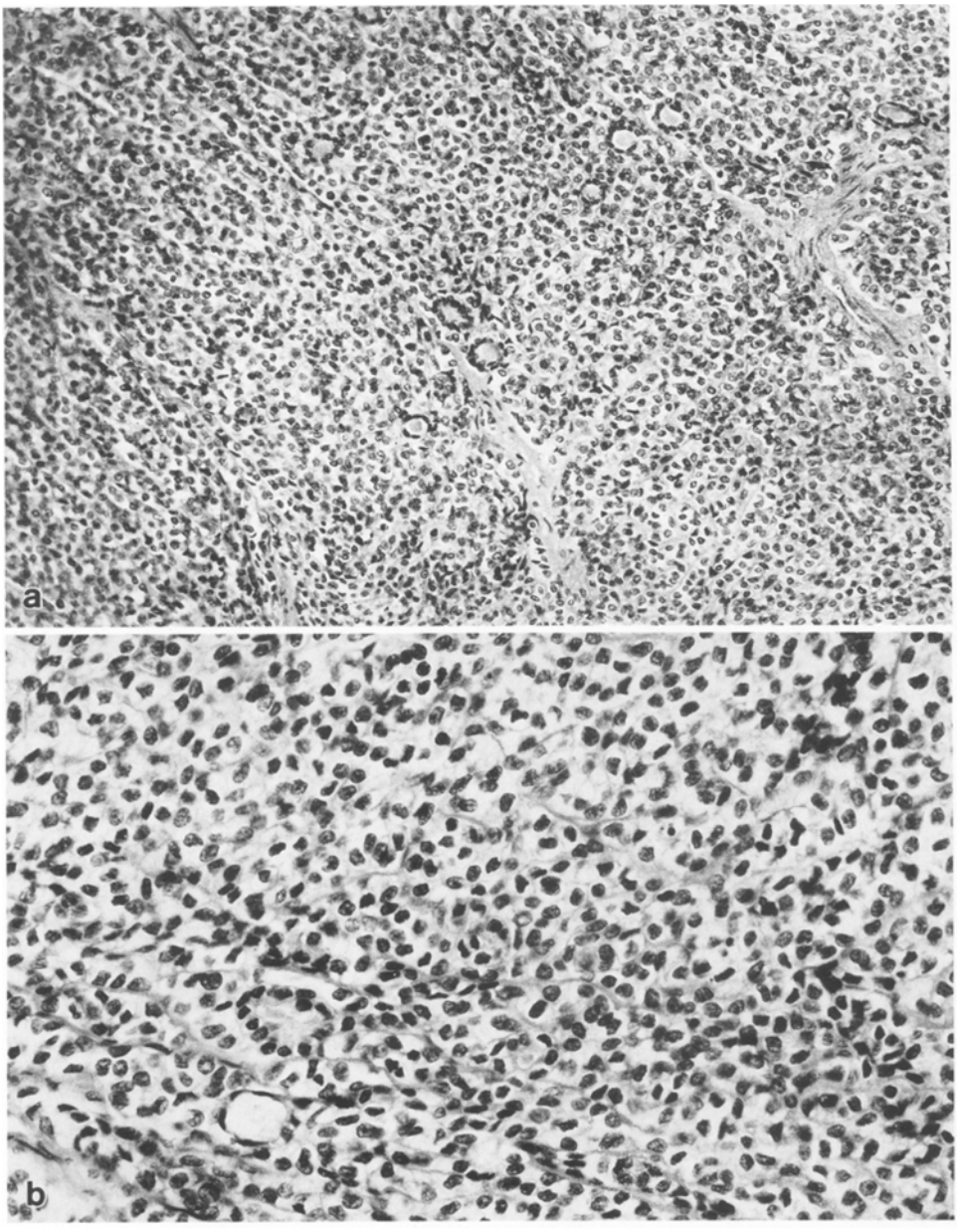

Fig. 1 a Solid arrangements of the tumour cells with thin fibrotic septa delineating an ill-defined lobular organization. A few ductular structures lined by epithelial-type cells are discernible (H & E, original magnification × 163). **b** Higher magnification of the tumour of **a**. There is inconspicuous tubule formation (H & E, original magnification × 327)

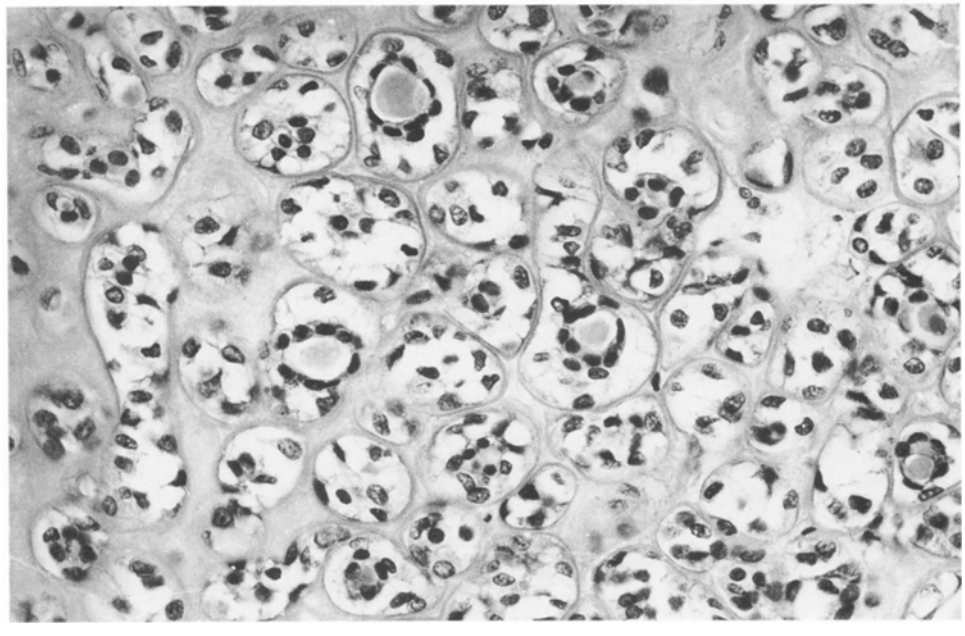

Fig. 2. Well formed tubular pattern with hyaline-like material surrounding the tubules. These are formed by an inner layer of dark duct-like cells and an outer layer of clear and large myoepithelial-like cells (H & E, original magnification × 327)

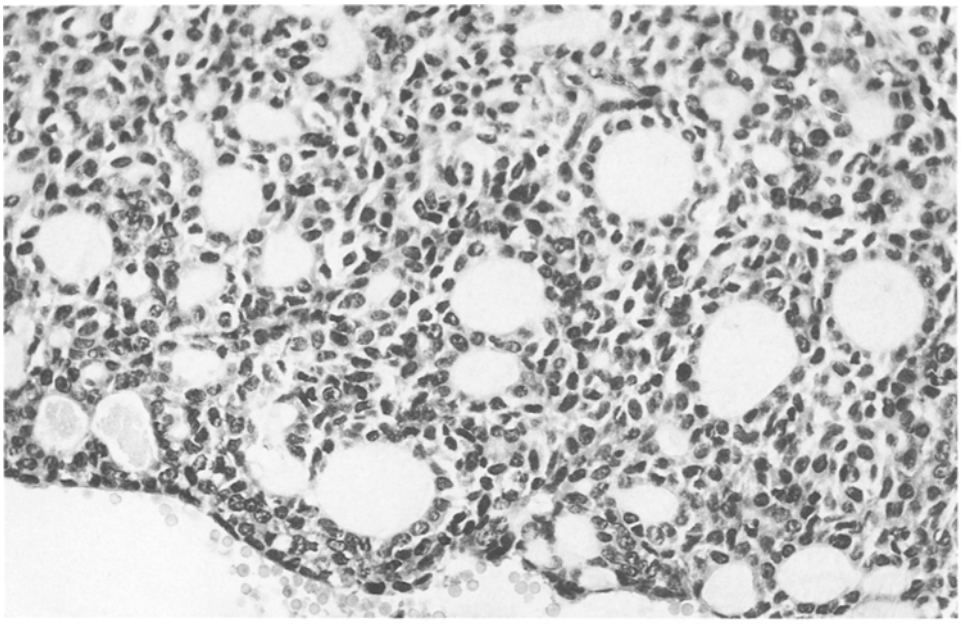

Fig. 3. A tumour cell nest with cribriform arrangement simulating an adenoid cystic carcinoma. However, there is no hyaline material within the round and oval spaces. Although not conspicuous, two cell populations are apparent (H & E, original magnification × 327)

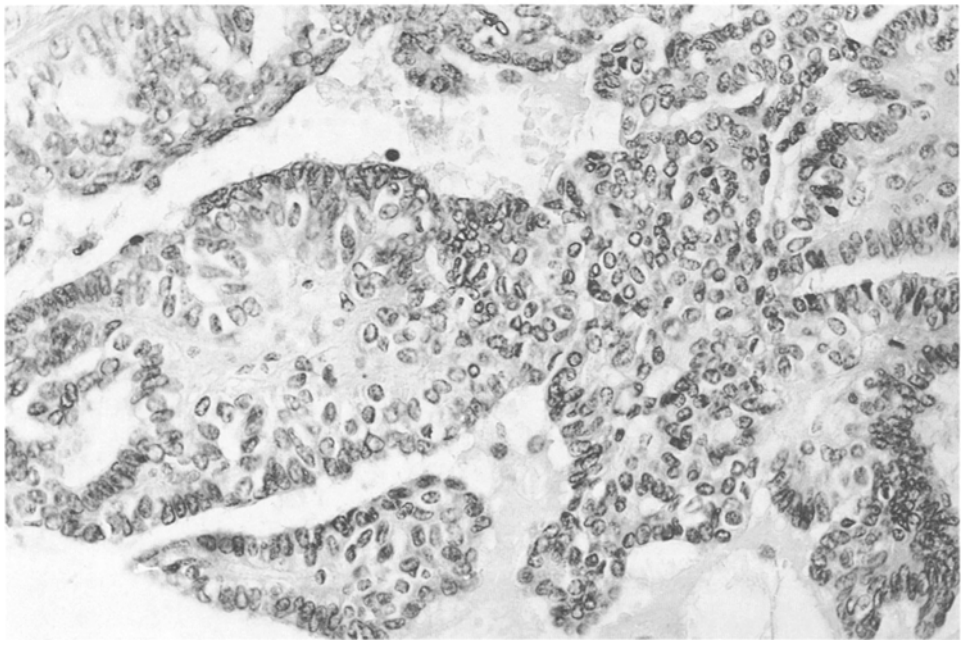

Fig. 4. A branching organization was typical of the papillary-type EMC. The dark cells lined the papillae over the clear myoepithelial cells that, however, were not disclosed in some areas (H & E, original magnification × 327)

and muscle specific actin antisera was demonstrated in most cells. Some also expressed faint CAM 5.2 staining.

Four distinct architectural types were identified according to the predominant cellular organization: solid, tubular, cribriform and papillary. The solid type, found in 13 cases, was formed by multiple nodules mostly composed by clear cells. In the centre of the nodules the duct-like structures were recognized by their smaller size as well as by a darker appearance of the cytoplasm of the cells forming them (Fig. 1). In the tubular type, found in 6 cases, there were tubules regularly formed by an inner layer of duct-like cells and an outer layer of clear, myoepithelial cells, looking like the usual organization of normal intercalated ducts (Fig. 2). Two cases displayed a cribriform appearance that mimicked adenoid-cystic carcinoma (Fig. 3). The papillary type, represented by a single tumour, was formed by thin papillae lined by the typical double-layered structure (Fig. 4). None of the 22 tumours was, however, pure, and the final classification in each of the four microscopic subtypes was always made according to the prevalent architectural pattern.

Two cases were lost to follow-up. Follow-up information was obtained in the remaining 20 cases, for periods that ranged from 2 to 174 months (mean of 81.2 months). The group with favourable prognosis included 4 patients. In the group with unfavourable prognosis, recurrence occurred in 12 cases in 6 of them more than once. Disease-free periods ranged between 1 and 9 years. Metastases occurred in 7 cases: 5 cases to the

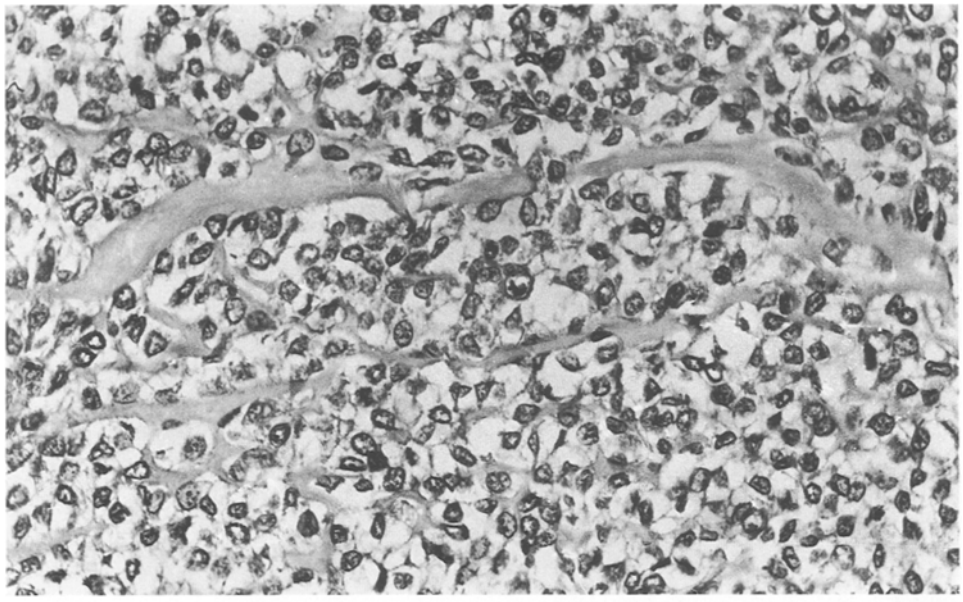

Fig. 5. Solid EMC with cell atypia and mitosis (H & E, original magnification × 327)

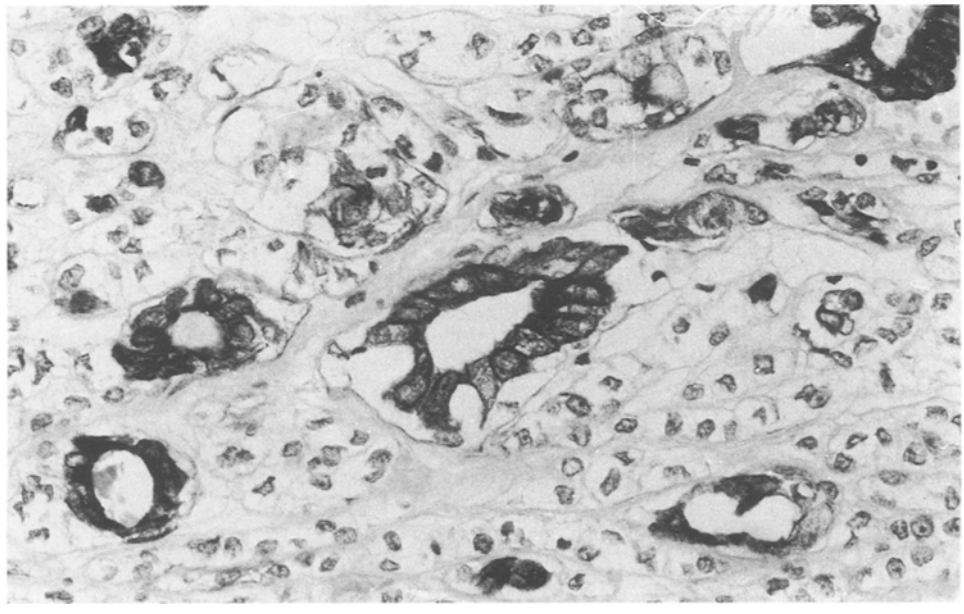

Fig. 6. Tubular type EMC stained with CAM 5.2. The inner layer of epithelial duct cells is strongly positive and the clear, myoepithelial cells are mostly negative (CAM 5.2, original magnification × 327)

cervical lymph nodes only, 1 to the cervical lymph nodes and the skin of the skull and 1 to the lung. In 3 patients there were both recurrence and metastases. Eight patients died of the disease, due to local extension of the tumour (5 cases), metastases (1 case) and both (2 cases). Cumulative survival at 5 years was of 87.1% and at 10 years of 67.5%.

Nuclear atypia, as defined in the materials and methods section was present in 16 cases (Table 1). Fourteen cases had no mitosis in 10 HPF and 8 cases had one or more mitoses per 10 HPF. The mitotic figures were only observed in the clear cell population of myoepithelial elements.

Necrosis was documented in 8 cases, always in the centre of the tumour nodules, sometimes exhibiting a comedo-like appearance. This change tended to be confluent.

Neural invasion was present in 14 cases and absent in 2 cases. In the remaining 6 cases the available tissue samples enabled us to undertake proper evaluation of this feature. Lymphatic permeation was documented in 6 cases. Fibrosis was slight or absent and was observed only at the periphery of the neoplastic nodules in the solid type of EMC.

The assessment of DNA ploidy of the tumour cells was performed in 18 cases. In the remaining 4 cases there was no available tissue for analysis. Diploidy was observed in 15 cases and aneuploidy in 3 cases (Table 1).

The morphological variables evaluated (nuclear atypia, necrosis, neural invasion and lymphatic permeation) and the DNA pattern were correlated with the two prognostic groups: favourable and unfavourable (Table 2). There were no differences between the two groups regarding neural invasion ($p=0.36$) and the presence of

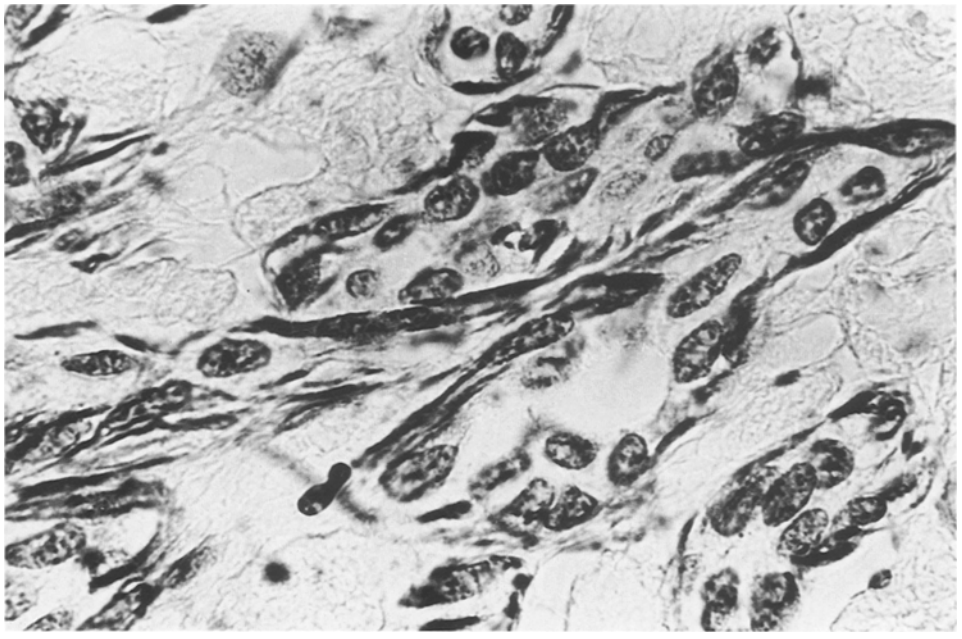

Fig. 7. Smooth-muscle actin decorates the outer layer of the neoplastic tubules that are formed by myoepithelial cells (anti-actin, original magnification × 818)

necrosis ($p = 0.53$). Lymphatic invasion was not observed in any of the 4 cases without disease complications but it occurred in 6 out of the 14 cases with morbidity. However, the difference between the two groups has no statistical significance ($p = 0.18$). Nuclear atypia was the only variable that showed to be significantly different in the two prognostic groups ($p = 0.002$).

Atypical DNA ploidy histograms were observed only in patients that died of the neoplastic disease.

Discussion

EMC was recognized in the updated revision of WHO salivary gland tumour classification as an intercalated duct originated tumour of low-grade malignancy (Seifert and Sobin 1991, 1992).

It is a rare but distinct clinicopathological entity and its individualization is mostly related to its peculiar morphology. Areas with histologically typical double-layered appearance usually allow differential diagnosis from the other clear cell salivary carcinomas, namely dedifferentiated acinic cell, mucoepidermoid carcinomas of clear-cell type and malignant myoepitheliomas (Batsakis and Luna 1989; Batsakis et al. 1992; Eveson 1992).

In the presence of an adenocarcinoma of the salivary glands with predominant clear-cell component it is necessary to search for a pattern of dual cell differentiation using immunocytochemical markers. Duct-like cells are positive for low-molecular weight cytokeratins and the neoplastic myoepithelial cells react with S100 protein and muscle specific actin antiserum (Luna et al. 1987; Palmer 1985). These antibodies distinguish solid EMC from other clear-cell tumours of salivary glands allowing the correct categorization of the neoplasm. The demonstration, at the EM level, of reduplicated basal lamina also contributes to the differential diagnosis of EMC from other clear cell tumours (Daley et al. 1984).

Most EMC of the present series originated in the parotid gland, affected patients in their 5th to 8th decades of life, and were more frequent in women. This epidemiological profile is similar to that previously reported in other series (Luna et al. 1987). As it is shown in Table 3, the mean age of the patients recorded in the accumulated series is similar. The same occurs with sex, there is a slight predominance of women in all but the Simpson et al. series (Simpson et al. 1991). The prevalent affection of the parotid gland is, however, less constant and varies from series to series. Two rare locations of

Table 3. Summary of accumulated data on EMC

	n	Age (ys)	Sex (F:M)	Local (Mj:mi)	Fav	Unfav	DOD
Corio et al. 1982	16	61.5	1.6:1	4:1	3	6	0
Luna et al. 1985	9	64.2	2:1	8:1	4	5	2
Daley et al. 1987	4	68.0	3:1	4:0	2	–	–
Hamper et al. 1989	21	59.3	1.1:1	2:1	16	5	0
Simpson et al. 1991	4	76.3	1:1	4:0	3	1	0
Present series	22	61.4	2.1:1	1.4:1	4	16	8

Mj, major glands; mi, minor glands; Fav, favourable outcome; Unfav, unfavourable outcome; DOD, dead of disease

EMC have been collected in this study: the sublingual gland and the minor glands of the maxillary sinus.

In our series, 41% of the cases recurred. This rate is similar to that (39%) found by Luna et al. (1985, 1987) and 5 out of 8 cases by Corio et al. (1982) but we observed a higher metastatic rate than previously reported (Corio et al. 1982; Daley et al. 1984; Hamper et al. 1989b; Luna et al. 1985; Simpson et al. 1991; Stiernberg et al. 1986). Metastases occurred mostly through lymphatics with tumour deposits at the regional lymph nodes. In the 2 cases with distant deposits we could not demonstrate overt venous invasion. This may be due to the difficulty in distinguishing venous from lymphatic capillaries in standard H & E sections. Neural invasion was demonstrated in 63.6% of the cases and it is our experience that, similar to what occurs in prostate neoplasms this neurotropic growth or direct invasion of neural structures is not always associated to lymphatic permeation around nerves.

Death related to the neoplastic disease was found in 40% of the cases, but was reported only in 2 cases of the Luna et al. series (1985) (Table 3). This probably is due to the fact that our series is an oncological hospital-based one and which therefore includes a higher number of aggressive cases, admitted frequently for the treatment of recurrences. Consequently the general assumption that EMC is a low-grade neoplasm should be questioned; a significant number of cases develop disease complications and behave as intermediate-degree or aggressive tumours. This contrasts with the bland histological appearance of the tumours. This is misleading and may induce an inexperienced pathologist to make a benign diagnosis. The duct-like cells component exhibits an almost benign appearance, and we could not demonstrate any atypicallity in this cell population in any of the 22 cases. However, if one carefully scrutinizes the nuclear characteristics of the myoepithelial-like cells, a certain degree of mild to moderate nuclear atypia is present in many cases and this feature was, in our series, significantly related to the prognosis ($p=0.002$) (Table 2).

The relative rarity of EMC makes it difficult to establish reliable prognostic markers, either clinical or morphological and justifies the report of further series with long follow-up periods in an attempt to accumulate adequate experience of their behaviour. Like adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma of salivary gland origin, despite many attempts to establish prognostic criteria it is still not possible to predict the outcome of the individual case (Corio et al. 1982; Daley et al. 1984; Hamper et al. 1989b; Luna et al. 1987; Simpson et al. 1991). We also verified that the microscopical features, with the exception of nuclear atypia of the myoepithelial-like cells, did not show a significant association to disease evolution.

There is only one study attempting to evaluate the value of DNA ploidy information on the evolution of EMC (Hamper et al. 1989b). This concludes that the usual rule that associates, aneuploidy with a worse prognosis in most tumour types does not fit well with either overall survival or disease recurrence in most low-grade adenocarcinomas of the salivary glands, in contrast to mucoepidermoid carcinoma (Fonseca et al. 1993; Hamper et al. 1989c) and the adenocarcinomas of unspecified type of the salivary glands (Hamper et al. 1989a).

In their study of a series of 21 EMC, Hamper et al. (1989b) found that all tumours were diploid and excluded DNA ploidy assessment value in prognosis for this tumour type. Using similar methodology, we also confirmed that DNA ploidy did not correlate with disease survival. However, unlike Hamper et al. (1989b) we observed three aneuploid cases that recurred and killed the patients. They showed a solid type with prevalence of clear myoepithelial-like cells. This suggests that aneuploidy should be looked on as an ominous sign of disease evolution and its association with the clear solid EMC deserves further investigation.

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