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Multiple expression of tissue markers in mucoepidermoid carcinomas and acinic cell carcinomas of the salivary glands*

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Summary. The distribution of various tissue antigens was studied in mucoepidermoid carcinomas ($n=74$) and acinic cell carcinomas ($n=38$) by means of immunocytochemistry. Mucoepidermoid carcinomas were generally positive for cytokeratin and showed double expression for cytokeratin and vimentin in 31.1% and triple expression for cytokeratin, vimentin and GFAP in 24.1%. CEA was studied using new monoclonal antibodies which distinguish between epitopes that are present on CEA alone and those which are present on nonspecific cross reacting antigens as well. The monospecific CEA antibody was completely negative in mucoepidermoid carcinomas, while nonspecific cross reacting antigens (NCAs) were positive in mucoepidermoid carcinomas to a varying degree. Alpha1-antichymotrypsin, a marker formerly thought to be specific for tissues for histiocytic origin, was positive in 85.1% of mucoepidermoid carcinomas. Twenty three percent of mucoepidermoid carcinomas showed focal infiltration by S-100 positive dendritic stromal cells, tumour cell being negative. Leu-M1 antigen was positive in 58.1% of mucoepidermoid carcinomas. Acinic cell carcinomas were generally positive for cytokeratin and in single cases showed double expression for cytokeratin and vimentin and triple expression for cytokeratin, vimentin and GFAP. Monospecific CEA antibody positivity could be demonstrated in 24.2% of acinic cell carcinoma, while nonspecific cross reacting antigens (NCAs) were positive in acinic cell carcinomas to a varying degree. Alpha1-antichymotrypsin was positive in 97.4% of acinic cell carcinomas. 2.5% of acinic cell carcinomas showed focal infiltration by S-100 positive dendritic stromal cells, 2.5% of acinic cell carcinomas were positive for S-100 protein with no dendritic stromal cells present. Leu-M1 antigen was positive in 86.8% of acinic cell carcinomas. For S-100 protein and Leu-M1, no correlation with the clinical course, as reported previously for other tumours, could be observed.

Key words: Salivary gland tumours – Mucoepidermoid carcinoma – Acinic cell carcinoma – Tissue markers

Introduction

The distribution of tissue antigens determined by immunocytochemistry has gained increasing interest for the elucidation of histogenesis, differential diagnosis and prognosis of human tumours. Intermediate sized filaments, for example, can give insight into the histogenesis and can be helpful in differential diagnosis and identification of tumours (Altmannsberger et al. 1982; Osborn and Weber 1983). For salivary gland and other tumours, there have been reports on double expression of keratin and vimentin, and even triple expression of keratin, vimentin and GFAP, in pleomorphic adenomays and adenoid cystic carcinomas for example (Achtstätter et al. 1986; Caselitz et al. 1982; Caselitz et al. 1984; Ramaekers et al. 1983). Alpha1-antichymotrypsin has been regarded as a histiocytic marker, but was subsequently reported to be present in a number of different non-histiocytic tissues including salivary gland tumours (Leader et al. 1987; Permanetter and Meister 1984). Carcinoembryonic antigen (CEA) as an oncofetal antigen has been studied thoroughly in various tumours including salivary gland lesions (Caselitz et al.

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1981a, b) but the recognition of specific CEA epitopes and epitopes that cross react with non CEA molecules (nonspecific cross reacting antigens – NCAs) makes it necessary to reconsider these results (Bätge et al. 1986; Schröder and Klöppel 1987). By means of three distinct commercially available monoclonal antibodies, the precise distribution of antigens that are present on NCAs 55 and 95 and CEA, on the one hand, and NCA 95 and CEA on the other, can be assessed. A further antibody used is specific for an epitope present on CEA alone (Bosslet et al. 1985). Finally, S-100 protein and Leu-M1 antigen, two rather different antigens, have proven their usefulness as a prognostic tool for various neoplastic diseases. A large number of S-100 positive dendritic Langerhans cells within the stromal infiltrate of papillary thyroid carcinomas, lung carcinomas, nasopharyngeal carcinomas and gastric carcinomas is correlated with good prognosis (Furukawa et al. 1985; Nomori et al. 1986; Schröder et al. 1988a; Tsujitani et al. 1987), whereas the extent of positivity in tumour cells for the leukocytic antigen Leu-M1 in papillary and medullary thyroid carcinomas is correlated with a poor prognosis (Schröder et al. 1987, 1988b).

The aim of the present study was to define the extent of intermediate sized filament expression, to demonstrate alpha1-antichymotrypsin in salivary gland tumours and underline its nonspecificity as a histiocytic or general tumour marker, to update data on CEA with the newly developed monoclonal antibodies against CEA and NCA, and to evaluate the prognostic significance of S-100 and Leu-M1 in salivary gland tumours.

Materials and methods

The tissue materials were taken from the Salivary Gland Registry, Institute of Pathology, University of Hamburg, FRG. This registry contains today more than 12000 cases of salivary gland diseases, amongst which more than 4000 tumours. Mucoepidermoid carcinomas and acinic cell carcinomas were evaluated. The cases had been collected during the years 1965–1980. Precise clinical follow up data for assessing the prognostic significance of S-100 protein and Leu-M1 were available. Paraffin embedded material from 112 tumours (74 mucoepidermoid carcinomas and 38 acinic cell carcinomas) was evaluated by immunocytochemistry.

Immunocytochemistry was performed using a commercially available alkaline phosphatase detection system (Vectastain, Vector Laboratories, Burlingame, Calif., USA). 4–5 µm thick paraffin sections were cut and dried at 60° C overnight. Sections were deparaffinated in xylene and rehydrated in descending alcohol concentrations. Neutral serum was applied for 20 min to inhibit nonspecific binding and was followed by overnight incubation of primary antibody. Secondary antibody linked to alkaline phosphatase, was incubated for 30 min. APAAP complex was then applied for 45 min. Immunocytochemical reactions were visualized by incubation with hexazotized new fuchsin solution for 30 min, followed by hemalaun counterstain. Controls were performed by replacing the specific primary antibody by phosphate buffered saline.

Sources and dilutions of the applied antibodies are shown in Table 1. The immunocytochemical reactions were assessed by semiquantitative evaluation (e.g. 10 microscopical fields were assessed quantitatively using a distribution score of (1) = 1–25%, (2) = 25–50%, (3) = 50–75%, (4) = 75–100% of tumour tissue).

Table 1. Applied antibodies, sources and dilutions

Antigen	Source	Species	Dilution
Cytokeratin (KL1)	Dianova	mouse	1:200
Vimentin	Dako	mouse	1:10
GFAP	Dako	rabbit	1:100
CEA/NCA	Behring	mouse	1:20
– MAB 374/14 (CEA/NCA 55–95)			
– MAB 250/183 (CEA/NCA 95)			
– MAB 431/31 (CEA)			
Alpha1-antichymotrypsin	Dako	rabbit	1:100
S-100	Camon (Biogenex)	rabbit	1:200
Leu-M1	Becton Dickinson	mouse	1:30

(Dianova, Hamburg, FRG; Dako, Copenhagen, Danmark; Behring, Marburg, FRG; Camon, Wiesbaden, FRG; Becton Dickinson, Mountain View, California, USA)

Table 2. Distribution of antigens in mucoepidermoid carcinomas ($n = 74$)

Antigen	Cases positive (%)	Score
Cytokeratin (KL1)	72/74 (97.3)	2–4
Vimentin	23/74 (31.1)	1–3
GFAP	36/58 (62.1)	1–3
CEA/NCA		
– 374/14 (CEA/NCA 55–95)	51/74 (68.9)	1
– 250/183 (CEA/NCA 95)	15/74 (20.3)	1–4
– 431/31 (CEA)	negative	
Alpha1-antichymotrypsin	63/74 (85.1)	2–4
S-100	negative	
	17/74 (23.0)	focal expression of S-100 positive stromal cells
Leu-M1	43/74 (58.1)	1–4

Results

The distribution of different antigens in summary is shown in Table 2 and 3. For technical reasons, the number of cases examined show variations.

Of mucoepidermoid carcinomas all but two tumours (97.3%) were positive for cytokeratin, 25 up to 100% of tumour cells being positive. 23 tu-

Table 3. Distribution of antigens in acinic cell carcinomas ($n=40$)

Antigen	Cases positive (%)	Score
Cytokeratin (KL1)	36/40 (90.0)	2–4
Vimentin	3/38 (7.9)	1–2
GFAP	12/40 (30.0)	1–4
CEA/NCA		
– 374/14 (CEA/NCA 55–95)	16/31 (51.6)	1–4
– 250/183 (CEA/NCA 95)	6/32 (18.8)	1–4
– 431/31 (CEA)	8/33 (24.2)	1–4
Alpha1-antichymotrypsin	38/39 (97.4)	1–4
S-100	1/40 (2.5)	2
	1/40 (2.5)	focal expression of S-100 positive stromal cells
Leu-M1	33/38 (86.8)	1–4

mours (31.1%) were positive for vimentin, 18 cases showing focal antigen expression, 4 cases up to 50% of cells and one case up to 75% of cells, all of which showed double expression with cytokeratin. 36 cases were positive for GFAP (62.1%; Fig. 1). In 27 cases, antigen expression was only focal or seen maximally up to 25% of cells, in 6 cases up to 50%, 1 case up to 75% and 2 cases up to 100% of cells. Triple expresison of cytokeratin, vimentin and GFAP was seen in 24.1% of cells.

For CEA, MAb 374/14 was positive in 51 tumours (68.9%), quantity of staining ranging from focal to 25% of cells. MAb 250/13 was positive in 15 cases (20.3%) the quantity of staining ranging from focal to 25% in all but one case, the latter one being 100% positive. MAb 431/31 was completely negative in all cases.

63 tumours (85.1%) stained positive for alpha1-antichymotrypsin, 25 cases up to 25% of cells, 24 cases up to 50%, 9 cases up to 75% and 5 cases up to 100% of cells. Occasionally, inflammatory cells (histiocytes) were also positive.

In 17 cases (23.0%), focal infiltration of S-100 positive dendritic stromal cells could be identified in mucoepidermoid carcinomas. Tumours cells were negative. It was not possible to demonstrate a correlation with the clinical course.

43 cases (58.1%) were positive for Leu-M1, 28 cases up to 25%, 9 cases up to 50%, 5 cases up to 75% and one case up to 100%. It was not possible to demonstrate a correlation with the clinical course (Fig. 2).

In acinic cell carcinomas, 36 cases (90%) were positive for cytokeratin, 18 cases up to 25% of cells, 13 cases up to 50%, 4 cases up to 75% and one case up to 100%. 3 cases (7.9%) were positive for Vimentin (Fig. 3), one case up to 25%, two cases up to 50%, all others being negative. 7.9% showed double expression with cytokeratin. GFAP was positive in 12 cases (30%), 7 cases up to 25%, 3 cases up to 50%, one case up to 75% and 100% each. 5.3% showed triple expression for cytokeratin, vimentin and GFAP.

For CEA, MAb 374/14 was positive in 16 cases (51.6%), 6 cases up to 25% and 50% each, one case up to 75% and three cases up to 100% of cells. MAb 250/183 was positive in 6 cases (18.8%), 2 cases up to 25%, 2 cases up to 50%, one case up to 75% and one case up to 100%. MAb 431/31 was positive in 8 cases (24.2%), one case up to 25%, 6 cases up to 50% and one case up to 100% of cells (Fig. 4).

38 cases (97.4%) were positive for alpha1-antichymotrypsin, 22 cases up to 25%, 12 cases up to 50%, 3 cases up to 75% and one case up to 100%. Occasionally, inflammatory cells (histiocytes) were positive, too.

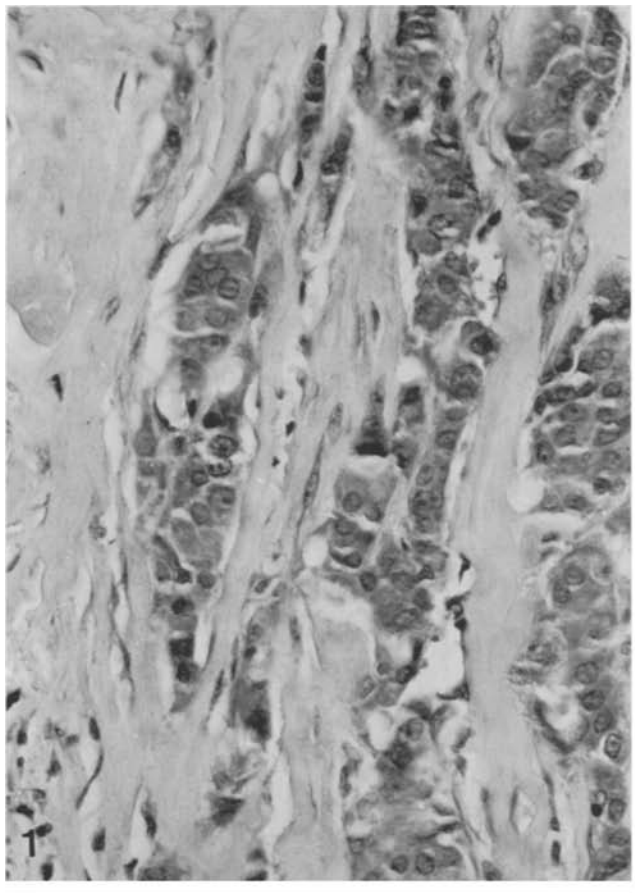
One case (2.5%) was positive S-100, up to 50%. Another case (2.5%) showed focal infiltration of S-100 positive dendritic stromal cells. No correlation to the clinical course was observed.

33 cases (86.8%) were positive for Leu-M1, 12 cases up to 25%, 16 cases up to 50%, 3 cases up to 75% and 2 cases up to 100%. No correlation to the clinical course was observed.

Discussion

Salivary gland tumours are rare neoplasms that have widely been studied by immunocytochemistry. In the present study, several tissue markers were assessed. The presence of cytokeratin in epithelial tumours is no surprise, as salivary gland tumours are usually derived from the terminal duct segment (Caselitz 1987). Vimentin was found to be positive in 31.1% of mucoepidermoid carcinomas, and in 7.9% of acinic cell carcinomas, at least focally. This fact has been described for many others tumours, including those of the salivary glands but not for mucoepidermoid carcinomas (Caselitz et al. 1982; Seifert and Caselitz 1985).

GFAP was positive in 62.1% of mucoepidermoid carcinomas and 30% of acinic cell carcinomas, all of which showed double expression with cytokeratin. 24.1% of mucoepidermoid carcinomas and 5.3% of acinic cell carcinomas were positive for all three filaments. In an earlier study, GFAP was demonstrated in pleomorphic adenomas, but not in mucoepidermoid carcinomas (Regezi et al. 1985). The epithelial cells of the normal parotid gland of myoepithelial aspect showed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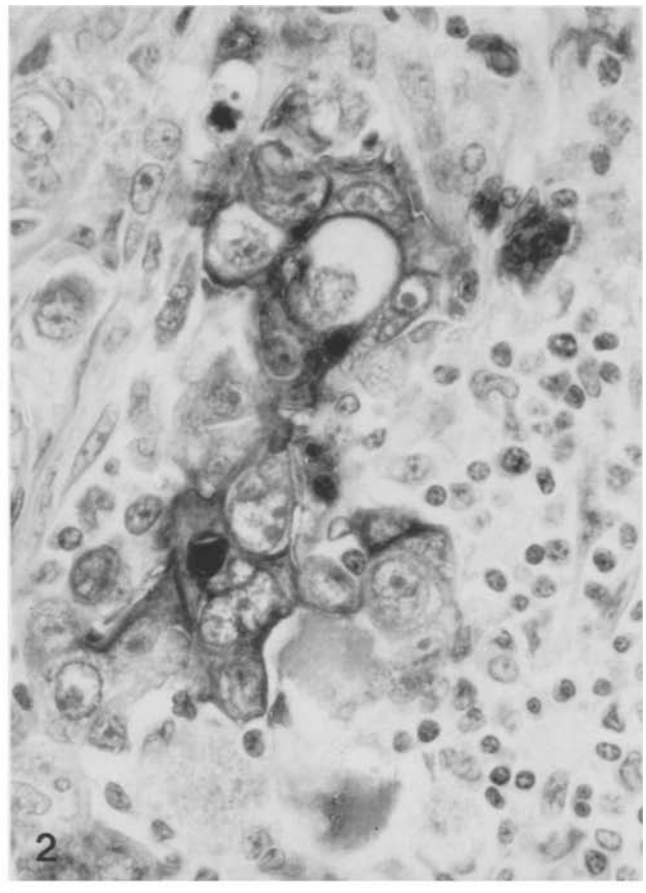
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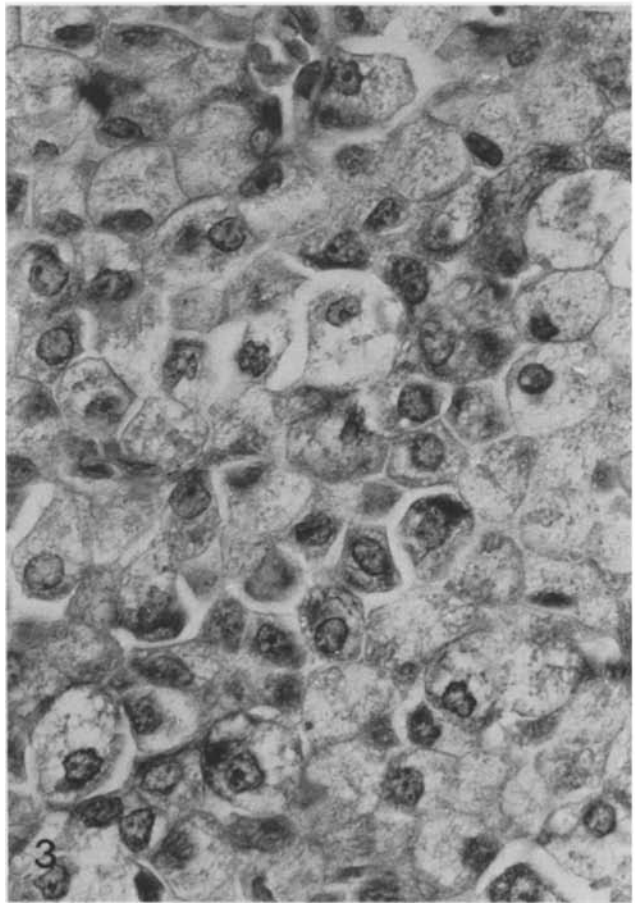
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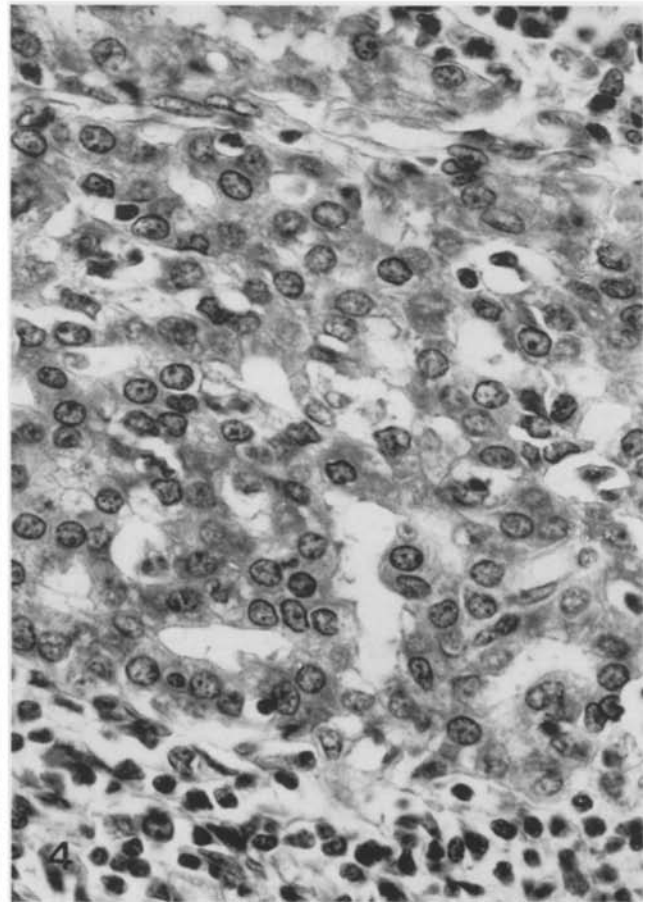
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double expression of cytokeratin and GFAP, those of pleomorphic adenoma showed a triple expression of cytokeratin, vimentin and GFAP (Achtstätter et al. 1986). According to these authors, double expression of vimentin and GFAP can be found in tissues of different origins. A possible explanation for double or triple expression of intermediate sized filaments could be a stepback into embryological behaviour and thus more primitive differentiation of tumour cells. Cross reaction between intermediate sized filaments due to common antigenic determinants has been described, however (Pruss et al. 1981) and differences in results in comparison with other tumours may be due to the fact that in previous studies only very small collections of mucoepidermoid carcinomas and acinic cell carcinomas have been evaluated.

Carcinoembryonic antigen (CEA) has been studied in many different human tissues and tumours (Denk et al. 1972; Terry et al. 1974). In salivary glands, normal tissue, inflammatory lesions and tumours have all been analyzed (Caselitz et al. 1981a, b; Toto and Hsu 1985; Tsukitani et al. 1985). All these studies were done using polyclonal antiserum which does not distinguish between CEA and NCA. Recently, CEA specific antisera have been used for immunocytochemistry (Bätge et al. 1986; Schröder and Klöppel 1987; Sumimoto et al. 1987) and these studies indicate that the results obtained by polyclonal antibodies have to be reconsidered. Polyclonal CEA has been demonstrated in mucoepidermoid carcinomas (Seifert and Caselitz 1985). In the present study, the antibody specific for CEA showed completely negative results, whereas cross reacting antibodies were positive to varying degrees in mucoepidermoid carcinomas. In a previous study, one case of acinic cell carcinoma was shown to be slightly positive for monospecific CEA, the polyclonal CEA antibody showing much more positivity (Sumitomo et al. 1987) which is consistent with our results. So, mucoepidermoid carcinomas apparently contain no monospecific CEA, acinic cell carcinomas do so to a certain degree, and nonspecific cross reacting antigens are present in both groups. Monospecific CEA reactivity, which might be related to a certain component of glandular function, could thus be interpreted as reflecting greater specialization in acinus-type cells of acinic cell carcinoma in contrast to duct-type cells of mucoepidermoid carcinoma.

Alpha 1-antichymotrypsin has been regarded as a marker of histiocytic tissues and was proposed as aid in differential diagnosis (Heerde et al. 1984; Motoi et al. 1980). Alpha 1-antichymotrypsin has been demonstrated in many tumours of nonhistiocytic origin, amongst which salivary gland tumours (pleomorphic adenomas, cystadenolymphomas and, in one case, adenocarcinoma in pleomorphic adenoma, Aroni et al. 1984; Kittas et al. 1982a, b; Murase et al. 1985; Permanetter and Meister 1984; Sehested et al. 1985; Takahashi et al. 1988). The doubt about its usefulness as histiocytic marker was stressed by a large study of various sarcomas, carcinomas and melanomas (Leader et al. 1987). In the present study, alpha 1-antichymotrypsin was demonstrated in 85.1% of mucoepidermoid carcinomas and 97.4% of acinic cell carcinomas and thus underlines its worthlessness in differential diagnosis of tumours of histiocytic or different origin.

Leu-M1 and S-100 protein, two rather different antigens, have been widely studied and considered to be useful in prognosis. Expression of Leu-M1 antigen, which has been demonstrated in various tumours (Hsu and Jaffe 1984; Kornstein et al. 1986; Sheibani et al. 1986), showed a positive correlation with unfavorable prognosis in various thyroid carcinomas (Schröder et al. 1987, 1988b). For salivary gland tumours, no data are available. In the present study, Leu-M1 was demonstrated in 58.1% of mucoepidermoid carcinomas and in 86.8% of acinic cell carcinoma. No correlation with clinical outcome was observed. The presence of Leu-M1 in salivary gland tumours thus underlines the "nonspecific" character of this antigen.

The extent of S-100 positive dendritic cells within the stromal infiltrate of various carcinomas was correlated with a good prognosis (Furukawa et al. 1985; Nomori et al. 1986; Schröder et al. 1988a; Tsujitani et al. 1987). In a previous study (Zarbo et al. 1986), S-100 positive dendritic cells were shown in nine out of 18 mucoepidermoid car-

Fig. 1. GFAP in poorly differentiated mucoepidermoid carcinoma. Strong cytoplasmic positivity of tumour cells. APAAP method, ×300

Fig. 2. Leu-M1 in poorly differentiated mucoepidermoid carcinoma. Focus of strong cytoplasmic positivity of tumour cells, some inflammatory stromal cells being positive, as well. APAAP method, ×480

Fig. 3. Vimentin in typical acinic cell carcinoma. Dispersed granular and filamentous cytoplasmic positivity of tumour cells. APAAP method, ×480

Fig. 4. CEA MAb 431/31 in ductular type of acinic cell carcinoma. Mostly homogeneous cytoplasmic positivity of tumour cells. APAAP method, ×300

cinomas, but no information was available as to the clinical course. Dendritic stromal cells were also observed in cystadenolymphoma, but not in mucoepidermoid carcinomas or acinic cell carcinomas by Hara et al. (1983). In the present study, S-100 positive dendritic stromal cells were observed in 17 mucoepidermoid carcinomas (23%) and in one acinic cell carcinoma (2.5%). A correlation to the clinical course was not observed. No positivity of tumour tissue could be demonstrated for 18 mucoepidermoid carcinomas and 2 acinic cell carcinomas in the study of Zarbo (1986), whereas Hara (1983) demonstrated rare occurrence of S-100 in mucoepidermoid carcinomas and acinic cell carcinoma.

Nakazato et al. (1985) demonstrated S-100 positivity in acinic cell carcinoma. In our collection, no case of mucoepidermoid carcinoma and one case (2.5%) of acinic cell carcinoma showed cytoplasmic positivity for S-100. The interpretation of this finding remains speculative. The presence of S-100 protein is interpreted as being related to myoepithelial differentiation, for example in pleomorphic adenoma (Mori et al. 1987; Nakajima et al. 1982). Thus cells with myoepithelial differentiation may play a role in acinic cell carcinomas, a fact which might be explained by the pluripotentiality of the terminal duct segment, from which these tumours arise.

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