Virchows Arch [Pathol Anat] (1981) 394:49–60

Virchows Archiv A

Immunohistochemical Detection of Carcinoembryonic Antigen (CEA) in Parotid Gland Carcinomas*

Analysis of 52 Cases

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Summary. The presence of CEA in parotid gland tumours was studied by immunohistochemical methods. 52 cases were analysed. 7 of 8 adenocarcinomas, 3 of 5 cystadenocarcinomas, 3 of 4 adenoid cystic carcinomas and all 3 salivary duct carcinomas were positive for CEA. 5 of 8 squamous cell carcinomas and 9 of 21 carcinomas in a pleomorphic adenoma were also positive for CEA.

The anaplastic carcinomas were negative. The distribution pattern of the presence of CEA in the carcinomatous and the adjacent normal or inflamed tissue was analysed. The results are discussed with regard to their histogenetic and diagnostic implications.

Key words: Carcinoembryonic antigen – Parotid gland carcinomas – Immunohistochemical detection

Introduction

The nomenclature and classification of salivary gland tumours have been based upon histological patterns and biological characteristics (Thackray and Sobin 1972; Thackray and Lucas 1974; Eneroth 1976; Seifert and Donath 1976a; Seifert and Donath 1976b; Woods et al. 1977). New methods of immunocytochemistry have opend the field of analysis of different tissue antigens (Avrameas 1969; Taylor et al. 1978; De Lellis et al. 1979; Sternberger 1979) and thus the presence of immunoglobulins and other substances have been shown in human salivary glands (Brandtzaeg 1957; Fleischer et al. 1980).

In special tumour pathology, new insights may be expected from the study of tumour markers. Among the great variety of these substances it is carcinoem-

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* Supported by Hamburger Stiftung zur Förderung der Krebsbekämpfung and by the Deutsche Forschungsgemeinschaft

** Prof. Dr. med. Dr. med. h.c. Karl Lennert, Kiel, dedicated on the occasion of his 60th birthday

0340-1227/81/0394/0049/$02.40

bryonic antigen which has been most extensively studied since its discovery (Gold and Freedman 1965; von Kleist and Burtin 1969; Lo Gerfo et al. 1971; Hansen et al. 1974; Goldenberg et al. 1976; and others). In spite of the extensive investigation of CEA in malignant and non-malignant lesions and in normal tissues there is an apparant lack of CEA studies in salivary gland tumours. Only Goldenberg and coworkers (1978) mention a squamous cell carcinoma of the parotid gland.

The present study on a collection of 52 parotid gland carcinomas was undertaken in order to attempt to answer the following questions:

1. Is the presence of CEA in parotid gland carcinoma related to the specific nature of the tumour epithelium, its presence being of value for histological diagnosis?

2. Is there a correlation between the differention of the tumour and the presence of CEA in the tissue?

3. Is there a correlation between the degree of malignancy of a given neoplasm and its tissue content of CEA?

Materials and Methods

52 carcinomas of the human parotid gland were collected during the years 1976 to 1978 in the Salivary Gland Register. The different types are shown in Table 1.

The tumour tissue was prepared for light microscopical and immunohistological investigation by conventional techniques. Slides were stained by haematoxylin-eosin, PAS-reaction and astra blue.

The demonstration of CEA was done by the triple layer method (Sternberger 1979). The method included the blocking of the endogenous peroxidase, incubation with rabbit anti-CEA at a dilution 1 : 1,000 (serum purchased from Dakopatts, Kopenhagen), incubation with goat antirabbit serum, incubation with rabbit antiperoxidase-peroxidase complex, DAB reaction. The details are given in a previous paper (Caselitz et al. 1980). The controls were carried out by omitting the primary antiserum, by using anti-CEA antiserum absorbed with human spleen tissue and by using primary antisera against other antigens (immunoglobulins) for comparative purposes.

Results

1. Normal and Inflamed Parotid Tissue

CEA was demonstrated in the normal and the inflamed parts of the parotid tissue which was found in the vicinity of the tumors.

Table 1. Classification of 52 parotid gland carcinomas

Type of tumour	Total number *n*
Adenocarcinomas	8
Cystadenocarcinomas	5
Adenoid cystic carcinomas	4
Salivary duct carcinomas	3
Squamous cell carcinomas	8
Carcinomas in pleomorphic adenomas	21
Anaplastic carcinomas	3
Sum	52

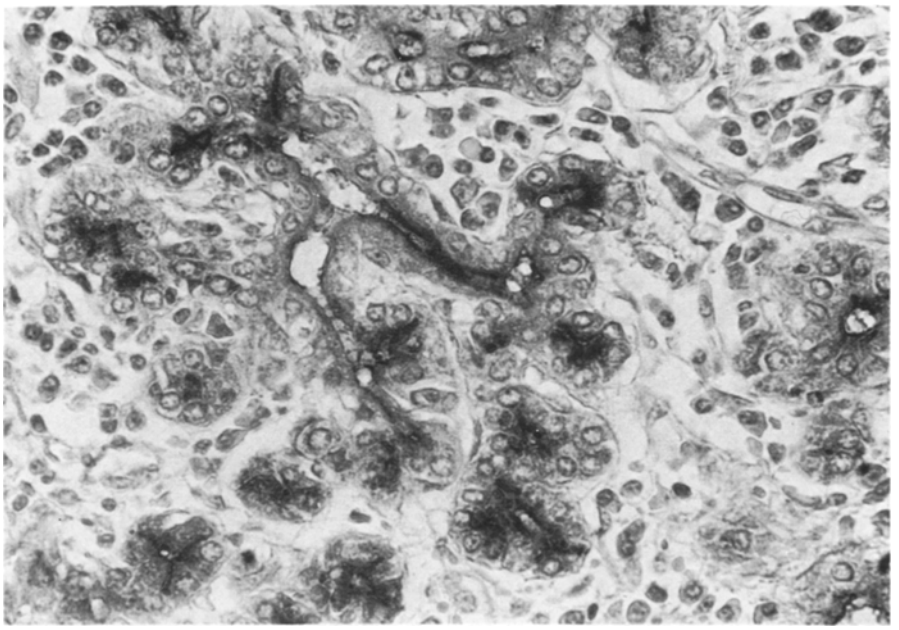

Fig. 1. Chronic parotitis: Cuboidal duct cells with dark staining of CEA at the apical border and in the cytoplasm. Immunoperoxidase staining of CEA. ×480

In normal glandular tissue which was neither affected by tumour infiltration nor by obstruction CEA was observed at the apical border of the acinar cells and the intercalated duct cells, in most cases. The chronically inflamed parotid gland showed a more intense staining of the indifferent duct cells. These cells also showed intracytoplasmatic staining (Fig. 1).

2 Neoplastic Parotid Gland Tissue

The result of all cases studied are shown in Table 3. The different types of tumours are dealt with below:

2.1 Adenocarcinomas. Among the 8 adenocarcinomas, there were six poorly differentiated and two highly differentiated ones. Seven of the cases were positive for CEA (Figs. 2, 3).

CEA was seen at the apical border of the infiltrating malignant cells which imitated ductular structures. The secretion product in the lumina was positive for CEA. The staining of the cytoplasm was not uniform, there were only single positive tumour cells among the group. In one case, lymph node metastasis was observed and was positive for CEA.

The presence of CEA was often related to better differentiated cells which produced a secretion product.

2.2 Cystadenocarcinomas. Three of the five cystadenocarcinomas were positive for CEA. The carcinoma cells, which were arranged in a tubular manner, showed an

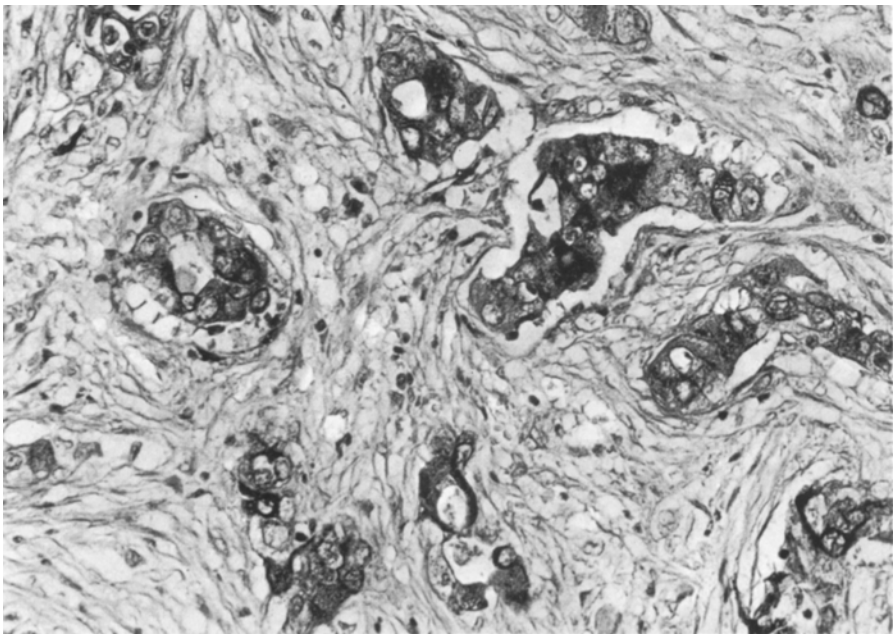

Fig. 2. Adenocarcinoma of the parotid gland: Tumour cells with different intensity of CEA-staining, most cells being clearly positive. Immunoperoxidase staining of CEA. ×300

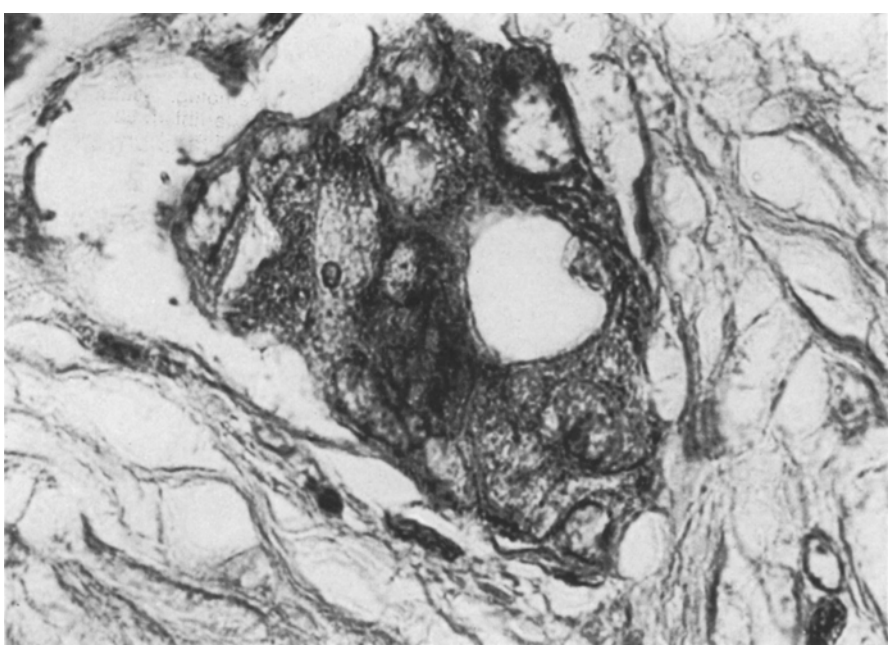

Fig. 3. Adenocarcinoma of the parotid gland: CEA-positive cells around a primitive lumen. Intense staining of the cytoplasm. Immunoperoxidase staining of CEA. ×1,200

Table 2. Immunohistological evidence for CEA in 52 parotid gland carcinomas

Tumour type	Demonstration of CEA[a] *n*
Salivary duct carcinomas	3/3
Adenocarcinomas	7/8
Adenoid cystic carcinomas	3/4
Squamous cell carcinomas	5/8
Cystadenocarcinomas	3/5
Carcinomas in pleomorphic adenomas	9/21
Anaplastic carcinomas	0/3
Sum	30/52

[a]First number indicates the CEA-positive cases, second number the entire group of the particular tumour

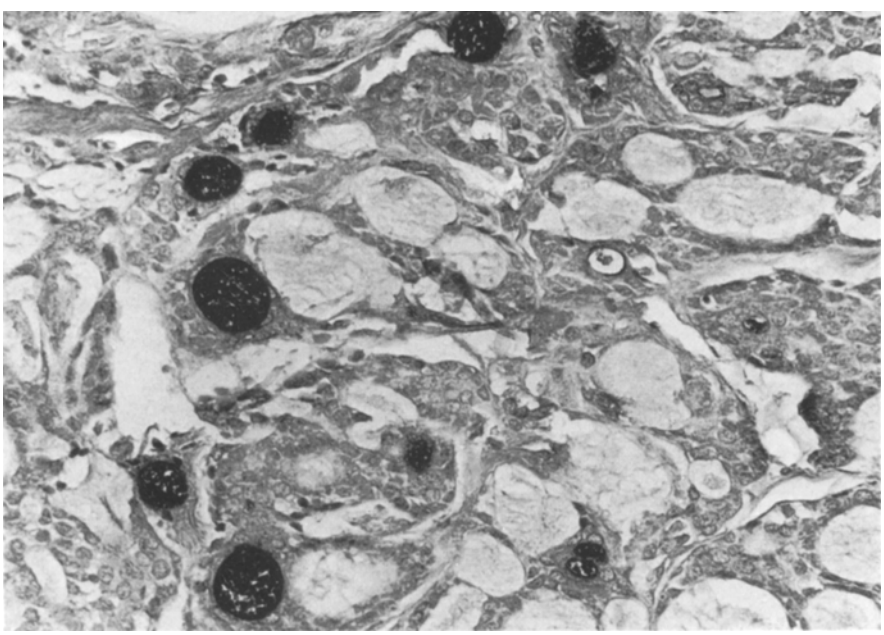

Fig. 4. Adenoid cystic carcinoma of the parotid gland: Typical cribriform pattern. Intense staining of the secretory material. Immunoperoxidase staining of CEA. ×300

intracytoplasmic staining. This staining was strong at the apical border of the cells. One case represented the coincidence of a high differentiated cystadenocarcinoma with cystadenolymphoma. Only the carcinoma cells were positive for CEA, in contrast to the negative cells of the cystadenolymphoma.

2.3 Adenoid Cystic Carcinomas. Four adenoid cystic carcinomas were investigated. There were three highly differentiated ones and one poorly differentiated one.

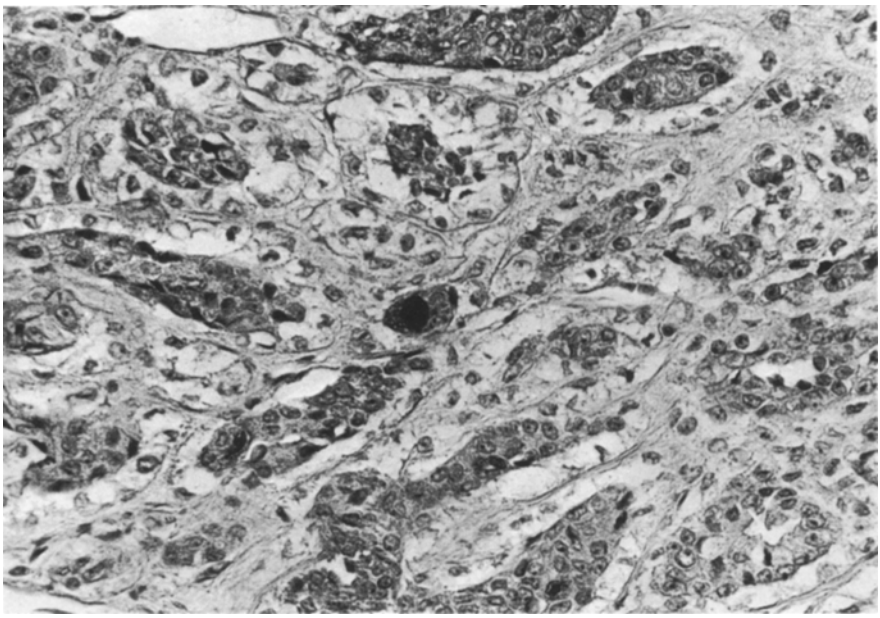

Fig. 5. Salivary duct carcinoma of the parotid gland: Intense staining of the cuboidal cells near a lumen. Immunoperoxidase staining of CEA. ×300

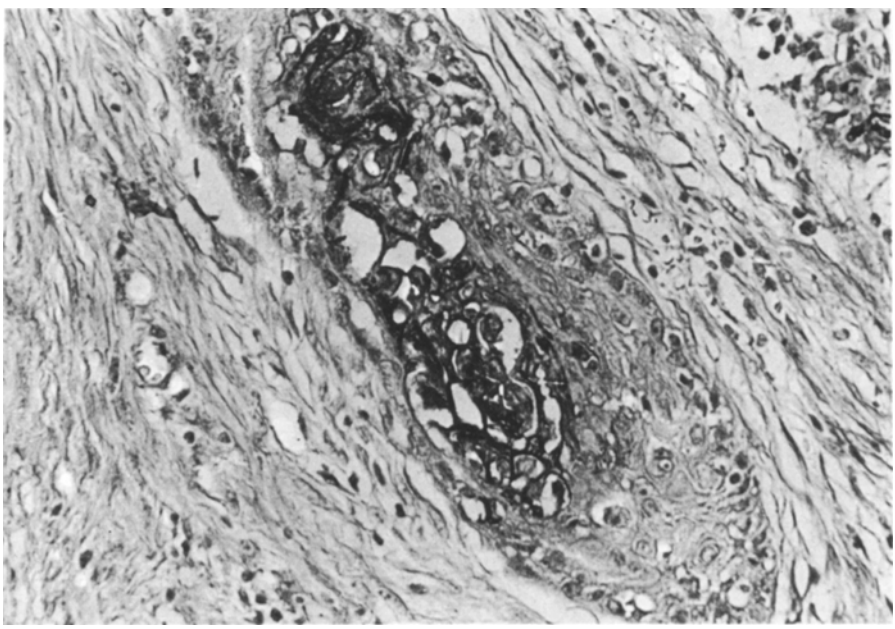

Fig. 6. Squamous cell carcinoma of the parotid gland: Intensely stained tumour cells in the center. Immunoperoxidase staining of CEA. ×300

Table 3. Review of immunocytochemical detection of CEA in different tissues

Organ	Type of tissue	Authors
Colon	normal tissue	Tappeiner et al. 1973; Burtin et al. 1973; Wagener et al. 1978
	inflammation	Goldenberg et al. 1976; Goldenberg et al. 1978
	carcinoma	Denk et al. 1972; Burtin et al. 1973; Tappeiner et al. 1973; Goldenberg et al. 1976; Goldenberg et al. 1978; Wagener et al. 1978
Appendix	carcinoid	Goldenberg et al. 1978
Jejunum	normal tissue	Issacson and Judd 1972
Stomach	dysplasia	Burtin et al. 1973; Wurster and Rapp 1979
	carcinoma	Burtin et al. 1973; Goldenberg et al. 1976; Goldenberg et al. 1978
Pancreas	carcinoma	Goldenberg et al. 1976; Goldenberg et al. 1978
Mamma	carcinoma	Goldenberg et al. 1978; Heymer et al. 1979; Shousha et al. 1979; von Kleist et al. 1980
Uterus	carcinoma	Goldenberg et al. 1976; Goldenberg et al. 1978; Lindgren et al. 1979; Wahlström et al. 1979
Ovary	carcinoma	Van Nagell et al. 1978; Goldenberg et al. 1976; Goldenberg et al. 1978; Heald et al. 1979
Thyroid gland	normal tissue	Kodama et al. 1980
	carcinoma	Burtin et al. 1979
Lung	carcinoma	Goldenberg et al. 1978
Bronchus	carcinoma	Goldenberg et al. 1976; Goldenberg et al. 1978;
Urinary bladder	carcinoma	Goldenberg et al. 1978

The highly differentiated tumours showed a predominantly glandular cribriform pattern (Fig. 4). Among these there was one case with distinct staining for CEA in almost all tumour cells. CEA was present in the cytoplasm at the apical cell border and in the lumen of the microcysts. In the other cases the positive cells were scattered among the tissue of the tumour. The CEA negative tumour was of the glandular cribriform type. In contrast to the CEA positive tumours, the single cells of this tumour were smaller and had a nucleus with a denser chromatin structure.

2.4 Salivary Duct Carcinomas. All three salivary duct carcinomas were positive for CEA. The antigen, however, could be demonstrated in single groups of cells only (Fig. 5). The CEA positive cells were arranged in small tubules and were situated

Table 4. Carcinoma in pleomorphic adenoma

Type of carcinoma	Total number *n*	Number of CEA-positive cases *n*
Mucoepidermoidcarcinoma	2	1
Adenocarcinoma	4	1
Adenoid cystic carcinoma	3	2
Salivary duct carcinoma	4	2
Anaplastic carcinoma	8	3[a]

[a] The positive cells in one anaplastic carcinoma belonged to the pleomorphic adenoma

adjacent to the lumina. The clear cells at the periphery of the tubules were always negative.

In comparison with the adenocarcinomas the staining of the salivary duct carcinomas was discrete. CEA was seen in the cytoplasm and at the apical cell border.

2.5 Squamous Cell Carcinomas. The eight squamous cell carcinomas had different degrees of differentiation; two were poorly differentiated.

In five cases, CEA was found in the tissue. The highest intensity was observed in the highly differentiated squamous carcinomas. The epidermoid formations were strongly stained (Fig. 6). The number and the distribution of the CEA positive cells varied with regard to the tumour and within the different regions in one tumour. CEA was demonstrated at the apical rim and in the cytoplasm of the malignant cells and also in the interstitial tissue around some malignant cells. The stromal infiltrate was negative for CEA.

2.6 Carcinomas in Pleomorphic Adenomas. 21 cases were investigated. The types of the neoplasms were divided into different groups, as shown in Table 3.

The parts of the carcinomas positive for CEA were generally arranged in an epidermoid or tubular manner. Sometimes the distinction between the different aspects of the pleomorphic adenoma and the carcinoma was difficult. This was due to the multifold morphology of the pleomorphic adenoma and to the non-homogenous appearance of the carcinomatous part. The malignant part of the tumours was designated after the quantitatively most impressive part.

2.7 Anaplastic Carcinomas. All three anaplastic carcinomas were negative for CEA.

Discussion

Carcinoembryonic antigen has been extensively studied in serological and morphological work (Lo Gerfo et al. 1971; Goldenberg et al. 1976; Rutanen et al. 1978; Burtin et al. 1979; Wahlström et al. 1979). It has not been studied in parotid gland tumours in detail, although there are two indices which are in favour for a presence of this antigen in the parotid gland. Firstly, CEA is present in human saliva

(Martin and Devant 1973) and it is elevated in saliva of patients with mucoviscidosis (Macsween and Gillespie 1980). Secondly, CEA can be found in tumours of the mammary gland which is a similar organ in terms of histological structure (von Kleist et al. 1980).

For the demonstration of CEA in tissue specimens, the triple layer method (Sternberger 1979) has been proven to be most useful (Wagener et al. 1978) since it may be used even on paraffin embedded material. The collection of salivary gland tumours that we analysed showed a distinct pattern of distribution of CEA. To begin with the normal parts of the glands, CEA was demonstrated in the acinar and intercalated duct cells of the normal gland and in the cuboidal cells of the inflamed gland. This observation is described in detail elsewhere (Caselitz et al. 1980) and may explain the fact that CEA is present in human saliva (Martin and Devant 1973).

The observations in the different tumour groups should be regarded in relationship to their histological pattern.

The types of tumours which have closest relationship to the original salivary gland are adeno- and cystadenocarcinomas. They were positive in 7 of 8 and 3 of 5 of the cases, respectively. Cytological analysis revealed that CEA was not restrained to the apical border of the glandular cells as in other observations of the parotid gland (Caselitz et al. 1980) and of the jejunum (Isaacson and Judd 1977) but was found in the cytoplasm and in the secretion products in the tubular lamina. Strong staining of CEA was often associated with well marked differentiation of the tumour.

Goldenberg and coworkers (1978) and Wahlström and coworkers (1979) showed the presence of CEA in adenocarcinomas of the gastrointestinal tract, bronchus, ovary, cervix and urothelium. CEA was also present in invasive carcinomas of the mammary gland (Shousha et al. 1979; von Kleist et al. 1980). The histogenetic relation of the adenocarcinomas to the original parotid gland tumours is seemingly underlined by the presence of CEA in these tissues.

The adenoid-cystic carcinomas were positive in 3 out of 4 of the cases. These carcinomas are derived from an indifferent duct cell which develops into an epithelial and myoepithelial carcinoma cell.

The salivary duct carcinomas were positive in all cases, although the distribution of CEA followed a distinctive pattern. A large part of the tumour was negative for CEA. The CEA positive parts were scattered among the tissue. In general, it was the duct cells adjacent to the lumen which were positive for CEA. The clear cells at the periphery, which are generally derived from the myoepithelial cells, were negative for CEA. CEA could also be found in the lumen of the tubules. Obviously, the capacity for producing CEA is limited to part of the tumour cells only, supposing our method is sensitive enough.

Squamous cell carcinoma is a tumour which is more common in other parts of the body. This tumour type was positive when originating from the bronchus, urinary bladder, cervix (Goldenberg et al. 1978), but was negative in other organs, like skin, oesophagus, anus and others (Goldenberg et al. 1978). Our findings demonstrate the presence of CEA in a part of the squamous cell carcinomas of the parotid gland. As a general rule, CEA was often combined with a squamous cell carcinoma which was highly keratinized and well differentiated.

In epidermoid lesions of the cervix an increasing occurrence of CEA was observed from normal to premalignant and malignant lesions (Lindgren et al. 1979). In this context, CEA seems to reflect a malignant potential but the application of these observation to another region of the human body has to be done with some caution.

Concerning the carcinoma in a pleomorphic adenoma, 9 of 21 cases were positive for CEA. In most cases strong intensity of CEA staining was found in the malignant part of the tumour. Thus, CEA may be helpful in analysing pleomorphic adenomas with regard to the presence of a carcinoma. This observation, however, is limited by the fact that a considerable part of the carcinoma in pleomorphic adenoma is clearly negative for CEA.

Putting the different facts together, one may conclude that the presence of CEA in parotid neoplasms is related to the glandular origin of the tumour. Since the acini and intercalated ducts are positive for CEA, the presence of CEA may indicate a histogenetic relationship between this part of the normal gland and adenocarcinomas, cystadenocarcinomas, adenoid-cystic carcinomas and salivary duct tumours.

With respect to the histogenetic schemes (Eversole 1971) a pool of reserve duct cells is regarded as the origin of most parotid gland tumours. Our findings support this hypothesis by indicating that perhaps the terminal duct cells are the progenitor cells for some tumours. This conclusion is underlined by the fact that the cuboidal duct cells which appear in chronic parotitis are positive for CEA, their staining being stronger than in the normal cells (Caselitz et al. 1980).

It is difficult to undertake a clear grading of the tumours with respect to their malignancy and to their clinical behaviour, for the biological behaviour of a tumour is dependent on the mode of treatment. As a general rule, a high amount of CEA in the tissue is a sign for a tumour with glandular character. A low amount of CEA may be seen in some tumours which have lost many characteristics of the original tissue. A study of CEA in mammary tumours showed that patients with CEA-negative tumours had significantly higher five and ten years survival rates (Shousha et al. 1979). Our morphological studies on the parotid gland carcinoma show that a further clinical evaluation of the parotid gland carcinoma would be useful with regard to the different amounts of CEA.

Acknowledgement. The authors gratefully appreciate the skilful technical assistance of Mrs. M. Dieckmann and B. Krüger.

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Accepted May 22, 1981