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Pleomorphic adenomas, adenoid cystic carcinomas and adenolymphomas of salivary glands analysed by a monoclonal antibody against myoepithelial/basal cells* **

An immunohistochemical study

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Summary. Myoepithelial and basal cells were identified by a monoclonal antibody raised against keratin. This antibody (CK B1) which detects myoepithelial cells in normal salivary glands, labels spindle shaped and polygonal cells in pleomorphic adenomas. Most cells in adenoid cystic carcinomas and some basal cells in adenolymphomas were also positive for this antibody. The oncocytic epithelium of adenolymphoma was negative.

An inverse reaction was seen with an antibody against cytokeratin 18.

The antibody CK B1 seems to be of interest for the detection of myoepithelial/basal cells in salivary glands and salivary gland tumours.

Key words: Myoepithelial cells – Monoclonal antibodies – Keratin – Salivary gland – Pleomorphic adenoma – Adenoid cystic carcinoma

Introduction

Several studies have dealt with the role of myoepithelial cells in salivary gland tumours. Most of these papers have been based on electron microscopy (Mylius 1960; Hamperl 1970; Hübner et al. 1971; Batsakis 1974; Dardick et al. 1983a, 1983b; Kahn et al. 1985).

The identification of myoepithelial cells is generally difficult (for review: Pinkstaff 1980), although some markers like actin have been applied to

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identify them (Bussolati et al. 1980). Since the labeling of myoepithelial cells is possible using cytokeratin staining (Franke et al. 1980; Caselitz et al. 1981; Erlandson et al. 1984) there has been a search for monoclonal antibodies with sufficient specificity for myoepithelial cells (Palmer et al. 1985; Caselitz et al. 1986). A monoclonal antibody CK B1, which detects myoepithelial and basal cells (Caselitz et al. 1986) has been used to delineate these cells in some salivary gland tumours.

Among the various types of salivary gland tumours pleomorphic adenomas are a major component (for review see Seifert et al. 1984). Adenoid cystic carcinomas of the salivary glands display a similar morphology to pleomorphic adenomas (Batsakis 1974; Seifert et al. 1984) and, in addition, adenolymphoma (Warthin's tumor) (Seifert et al. 1984) is relatively common.

We have chosen the pleomorphic adenoma and adenoid cystic carcinoma as the type of a "mixed" salivary gland tumour where the histogenesis may be from myoepithelial/basal cells (Caselitz et al. 1984). The adenolymphoma, which has a quite different morphology and histogenesis, provides a good basis of comparison for the other tumours.

Using different antibodies against "total" keratin, against keratin 18 and against myoepithelial/basal cells we analysed the different cellular patterns in these salivary gland tumours.

Materials and methods

The tumour tissue was obtained during surgery and was quick frozen in liquid nitrogen. 15 pleomorphic adenomas, 5 adenoid cystic carcinomas and 10 cystadenolymphomas were included in this study. 5 µm sections were cut on a Reichert cryostat OM U. Most of the sections were stained directly, some sections were stained after lyophilization at −20° C. The specimens were fixed in acetone for 10 min at −10° C.

The following mouse monoclonal antibodies were used:

1. *Antibody KL1* is a commercially available "broad" range antikeratin antibody (purchased from Dianova, Hamburg). It stains most epithelia and most epithelial tumours.

2. *CK 5* is similar to the CK 1–CK 4 monoclonals (Debus et al. 1982). It recognizes the keratin 18 polypeptide characteristic of simple epithelia. It has been characterized in immunoblots (Tölle et al. 1985).

3. *CK B1* is a monoclonal antibody raised against human callous keratins. It has been characterized elsewhere (Caselitz et al. 1986).

Tissue sections were stained by the indirect immunofluorescence technique with FITC labeled goat anti-mouse Ig Gs (Capell Laboratories, Cochranville, PA, USA).

Additionally, the avidin-biotin system was used (Vectastain kit, Vector Laboratories, USA), and the peroxidase was visualized by the diaminobenzidine reaction.

Finally, the alkaline phosphatase anti-alkaline phosphatase reaction was used (APAAP technique). The antibodies were purchased from Dianova (Hamburg). Tests for specificity of staining were performed by control sections incubated without the primary antibody. All controls were negative.

Results

Normal salivary glands (parotid gland and submandibular gland) adjacent to the tumours were investigated as the normal matrix for the antibody reaction. With KL 1 (total keratin) antibody the epithelial part of the sali-

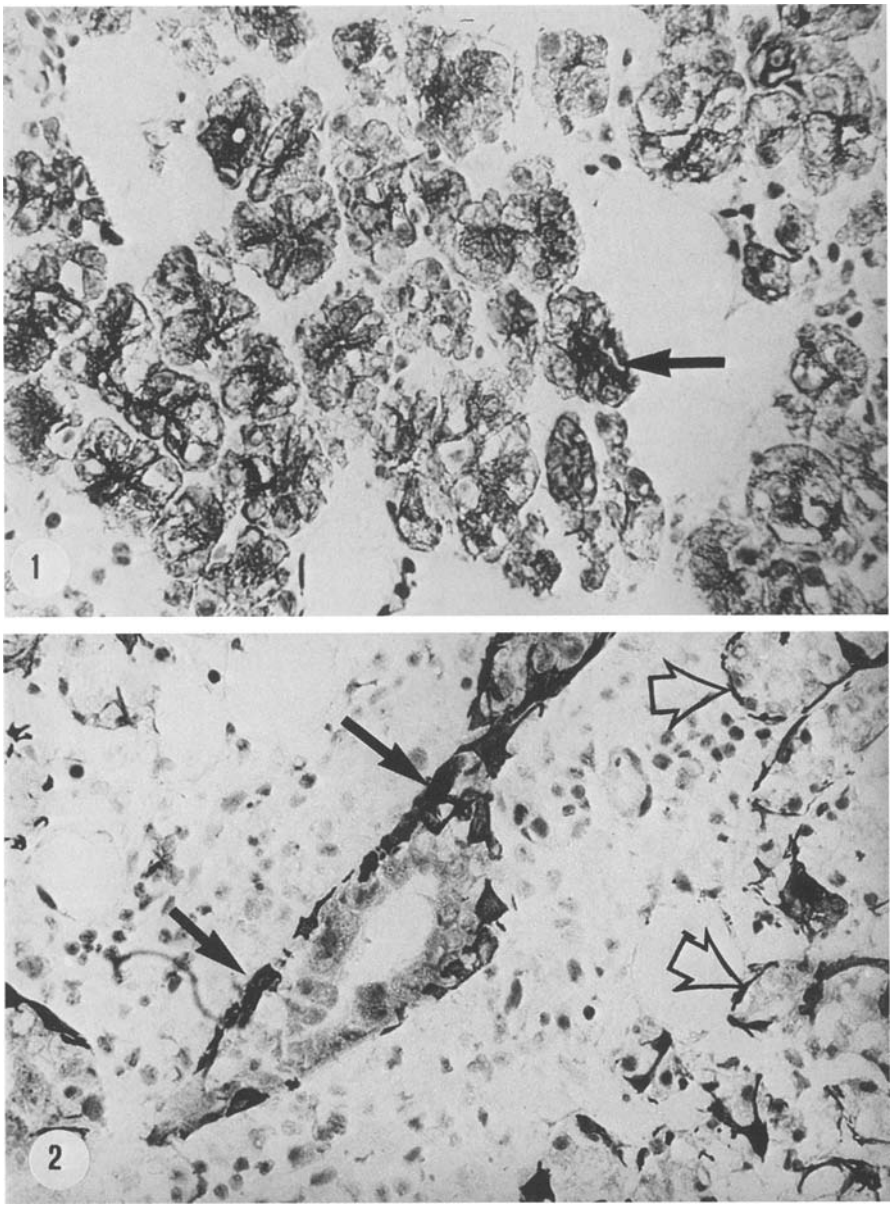

Fig. 1. Parotid gland. Acinic cells (*arrow*) and duct cells positive for cytokeratin 18. Monoclonal antibody CK 5. APAAP technique. × 300

Fig. 2. Parotid gland. Positively stained myoepithelial cells (*open arrows*) and positive basal cells around the ducts (*arrows*). Monoclonal antibody CK B1. APAAP technique. × 300

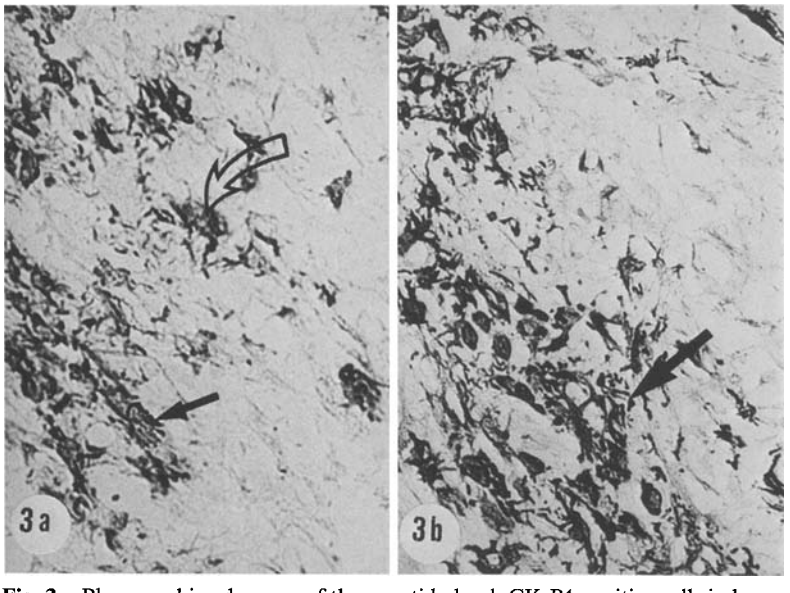

Fig. 3a. Pleomorphic adenoma of the parotid gland. CK B1 positive cells in loose arrangements (*arrow*). Some single polygonal positive cells (*open arrow*). **b.** Pleomorphic adenoma of the parotid gland. CK B1 positive tumour cells (*arrow*) in solid and loose arrangments. **a** and **b**: Monoclonal antibody CK B1. APAAP technique. × 300

vary glands was positively stained. The intensity was strongest in the ductal compartment. Keratin 18 was demonstrated (Fig. 1) by the use of the monoclonal antibody CK 5. The acinic cells and the duct cells were labelled by this antibody. In contrast to the staining obtained with the antibody CK 5, the monoclonal antibody CK B1 stained a subpopulation of cells in the salivary gland parenchyma (Fig. 2). The acinic cells and the duct cells lining the luminal border were generally negative. The basket cells around the acini were clearly decorated by the antibody CKB 1. At the border of the intercalated ducts, striated ducts and excretory ducts there were triangular shaped cells positive for this antibody. These cells were somewhat similar to the true myoepithelial cells, but could be separated by their different localization (ducts versus acini) and by their shape which was more triangular, the cells having no branches.

The 15 pleomorphic adenomas showed the typical heterogenous morphology, being composed of myxoid and epithial areas.

A large proportion of tumour cells was positive with KL 1 (total keratin). The staining was intense in the tubular and solid parts, but positive cells were also identified in the myxoid parts. The staining of the polygonal cells in the myxoid parts was weaker.

Many cells were positive for cytokeratin 18 with CK 5. The staining, however, was restrained to the tubular parts of the tumours. The cells at the border of the tubular parts were generally not labeled by this antibody. However, some cells were positive in the solid and myxoid areas.

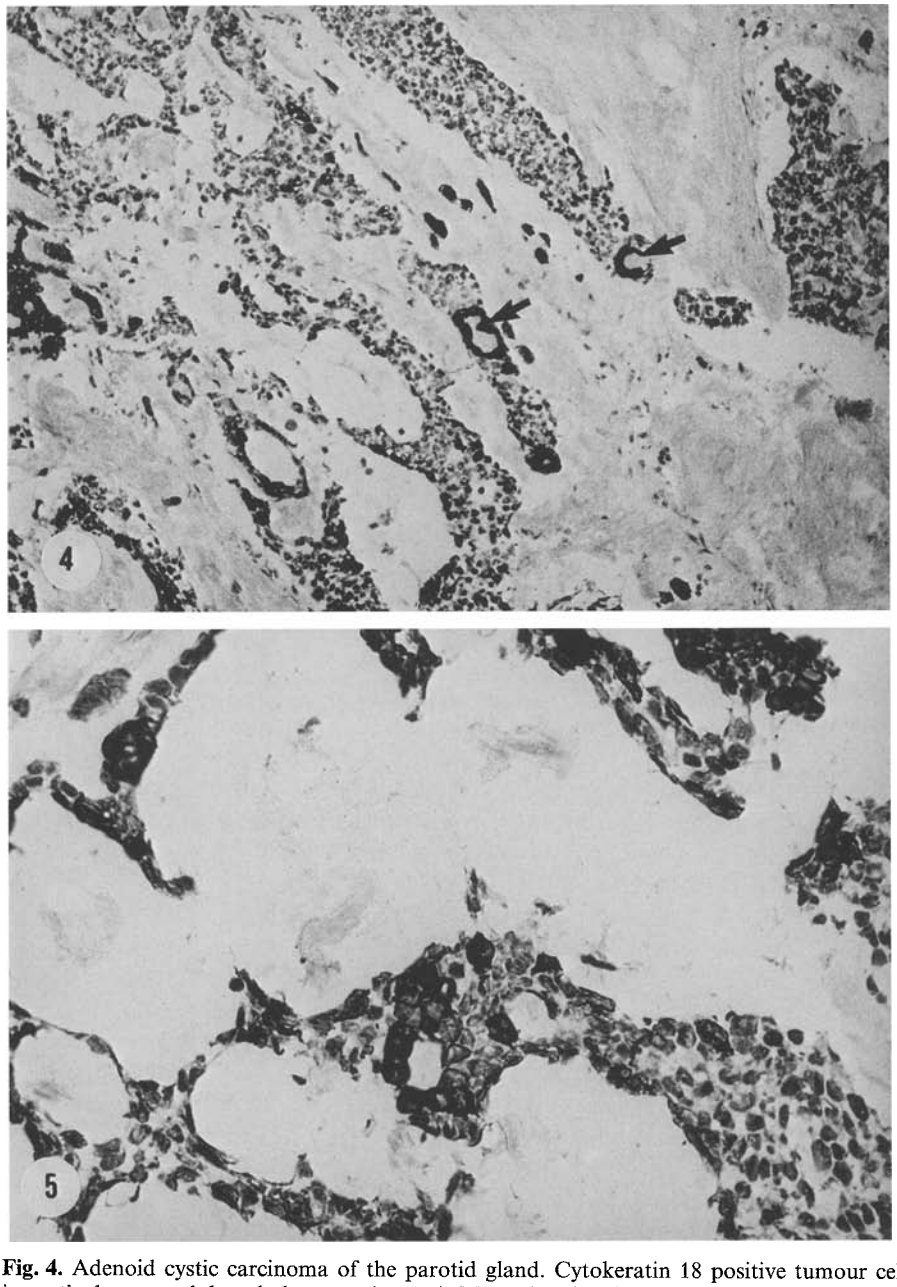

Fig. 4. Adenoid cystic carcinoma of the parotid gland. Cytokeratin 18 positive tumour cells, in particular around the tubular parts (*arrows*). Monoclonal antibody CK 5. APAAP technique. ×120

Fig. 5. Adenoid cystic carcinoma of the parotid gland. CK B1 positive tumour cells. Stromal part negative. Monoclonal antibody CK B1. APAAP technique. ×300

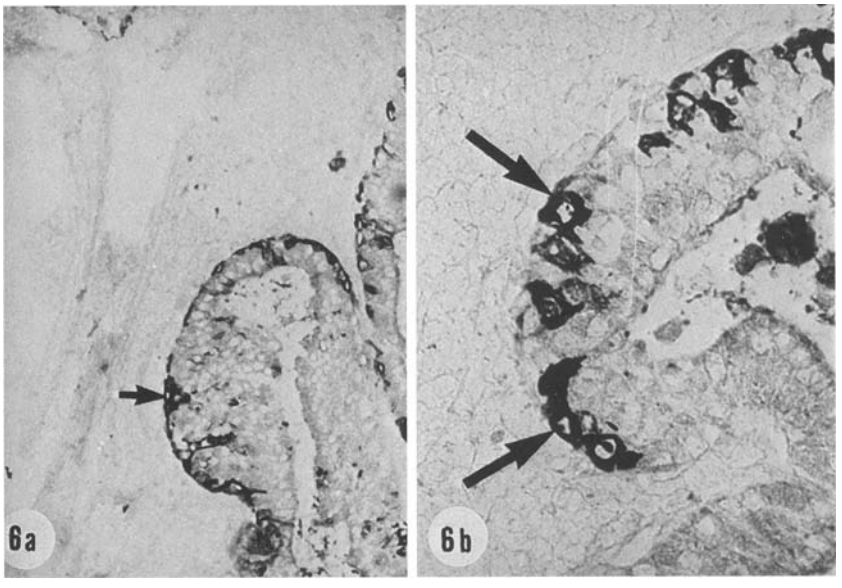

Fig. 6a. Cystadenolymphoma of the parotid gland. CK B1 positive tumour cells (*arrows*) at the base of the oncocytic epithelium which is negative. Negative stroma. Monoclonal antibody CK B1. APAAP technique. × 120. **b.** Cystadenolymphoma of the parotid gland. CK B1 positive tumour cells (*arrows*) at the base of the oncocytic epithelium which is negative. Negative stroma. Monoclonal antibody CK B1. APAAP technique. × 300

Some groups of cells were stained by CK B1 (Fig. 3). Some of the positive cells surrounded the tubular parts. Other positive cells were seen in the solid parts, and some of them were detected in the myxoid areas.

Almost all tumour cells of adenoid cystic carcinomas displayed a strong staining for KL1. Thus, the presence of keratin and the epithelial nature of the tumour cells was established. The tumours were also positive for cytokeratin 18. In particular, the tubular parts in this tumour were positive (Fig. 4).

The antibody CK B1 labelled many tumour cells in adenoid cystic carcinoma (Fig. 5). In particular, those cells resembling "myoepithelial cells" as reported in the literature, were positive for this antibody. In some areas, more than 90% of the cells were positive.

The histological pattern of adenolymphoma is quite different from pleomorphic adenoma and the distribution pattern of the antibodies against keratins were studied for comparative reasons. The epithelial part of the tumour was clearly positive for KL1, whereas the lymphoid stroma was constantly negative. The staining intensity in the oncocytic epithelium was sometimes weak, due to the large amount of mitochondria found in the cells. The epithelial part of these tumours, consisting mainly of oncocytic cells was positive for CK 18. These cells were clearly different from the negative stroma. The basal cells were only weakly stained or even negative. The true oncocytic cells were negative for the antibody CK B1. In contrast,

the basal cells were focally positive (Fig. 6). At the basal part, there were some triangular shaped cells, situated mostly in a single layer, sometimes in small groups. These cells were distinctly stained by the antibody CK B1, whereas the stroma, again, was negative.

Discussion

Antibodies against keratins have proved to be a useful marker in differential diagnosis of tumours (e.g.: Osborn et al. 1982; 1984; Lane 1982; Holzer et al. 1982; Osborn and Weber 1983; Osborn et al. 1984; Von Overbeck et al. 1985). The introduction of monoclonal antibodies into the study of the subgroups of keratins offered new insights in the analysis of cellular differentiation. It has become possible to subdivide epithelia cells according to their keratin content (Tseng et al. 1982; Moll et al. 1982; Wu et al. 1983). One of these antibodies has been characterized on two dimensional gel electrophoresis and is specific for cytokeratin 18 (Debus et al. 1982; 1984). This antibody and other antibodies against cytokeratin 18 bind to luminal cells in salivary gland tissues and in mammary glands (Debus et al. 1982, 1984; Bartek et al. 1985; Nagle et al. 1985).

In our material, we made similar observations in normal salivary gland tissue. This tissue reacted with "broad" antikeratin antibodies, polyclonal antibodies (Caselitz et al. 1981) and monoclonal ones like KL1 and LU 5. The tumours of the salivary glands which we investigated were all of glandular character. Hence, in all tumours the presence of cytokeratin 18 was demonstrated by the antibody CK 5 (Debus et al. 1984). The distribution pattern of the cytokeratin positive cells, however, was a special one. The most intense staining was generally found in the tubular parts of pleomorphic adenomas and adenoid cystic carcinomas.

The oncocytic cells of adenolymphoma were also positive for cytokeratin 18. This is in accordance with the truely glandular character of the epithelium in this kind of tumour. Previous reports on adenolymphomas have shown that the epithelial part was positive with broad specificity keratin antibodies (Caselitz et al. 1982). Further analysis of subclasses of keratins in these tumours have not been done.

Myoepithelial cells and basal cells of normal glands were negative for cytokeratin 18 (Caselitz et al. 1986). Cells which correspond to the "myoepithelial" cells in pleomorphic adenomas (Hübner et al. 1971) are generally negative for cytokeratin 18. In this way, the absence of cytokeratin 18 may be interpreted as a sign for myoepithelial differentiation. However, since the absence of a marker is not a good feature by which to identify special cell types, we have looked for a monoclonal antibody which selectively picks up myoepithelial and basal cells in salivary glands and salivary gland tumours. The antibody CK B1 seems to meet these criteria (Caselitz et al. 1986) and has been used for the analysis of salivary gland tumours.

In normal salivary glands, typical myoepithelial cells around the acini are labeled by this antibody. Basal cells, which display a more triangular shape and which are localized at the peripheral border of the ducts (interca-

lated ducts, striated ducts, excretory ducts), were equally positive for this antibody.

The term "myoepithelial" cell has been used variably in the literature. Whereas Garrett and Emmelin (1979) and Palmer et al. (1985) favor a rather strict use of this term (designating only "basket" cells around the acini), the term "myoepithelial cell" has been used in a broader sense (including basal and reserve cells) by other authors, especially in those papers dealing with the role of myoepithelial cells in certain tumours (Nathrath et al. 1982; for further discussion see: Pinkstaff 1980). The difficulty of identifying myoepithelial cells is mainly due to technical reasons. On the electron microscopical level (Mylius 1960), some criteria for the identification of myoepithelial cells have been established (Hübner et al. 1971; Seifert and Donath 1976). The electron microscopic criteria are not present in every section, however, and interpretation may be difficult especially in neoplastic lesions, in which the normal localization of myoepithelial cells is disturbed (Kahn et al. 1985). Histochemical approaches have been useful in a limited field, but neither alkaline phosphatase nor ATPase are markers which are present in all myoepithelial cells (Garrett and Harrison 1971; Pinkstaff 1980). In immunohistochemistry, actin and myosin have been shown to stain myoepithelial cells (Bussolati et al. 1980; Palmer et al. 1985). Actin, again, is not a unique marker for these cells. Since myoepithelial cells have been identified as epithelial cells by application of antibodies against prekeratin or "total" keratin (Franke et al. 1980; Caselitz et al. 1981; Krepler et al. 1982; Kahn et al. 1985) we have tried to look for antibodies which detected subsets of keratins only present in myoepithelial cells. One monoclonal antibody of this kind has been presented by Knight et al. (1985) and by Palmer et al. (1985). Their antibody does not label "true" myoepithelial cells surrounding the acini, but the basally located triangular shaped "reserve" cells. The antibody which we used detects not only "true" myoepithelial cells, but also the triangular shaped reserve cells. Interestingly, "basal" cells of other epithelia are stained by this antibody, as well (Caselitz et al. 1986). We would like to designate this marker as a common marker for the myoepithelial/basal cell system. The results may be interpreted as showing that there is a common expression of epitopes in this type of cells.

In pleomorphic adenomas, different cells were stained by CK B1. Some positive cells surrounded the tubular parts of pleomorphic adenomas and are similar to the antibody 16 a positive cells reported by Palmer et al. (1985). Since only a special group of basal cells is labeled by the antibody 16 a, only a small number of cells is stained in pleomorphic adenomas. Interestingly, the peripheral cell layer of the tubular parts of pleomorphic adenomas is positive for antibody 16 a, but is myosin negative. Since Palmer et al. (1985) take the presence of myosin as an important feature of myoepithelial cells, they conclude that these cells are rather more "reserve" cells than true myoepithelial cells.

The antibody CK B1 stains the whole range of "true" myoepithelial cells and "basal" cells. The staining pattern is much broader than that of the antibody 16a (Palmer et al. 1985). Thus, more cells are stained in

pleomorphic adenomas: those cells at the outer layer of ducts, some cells in the solid parts and some cells in the myxoid parts. The data point to the fact that there might be a similar differentiation in the myoepithelial and basal cells in normal salivary glands and pleomorphic adenomas. Our antibody cannot distinguish between these two components, but can show, on the other hand, the number of cells that display a myoepithelial/basal cell differentiation in this tumour. This distinction may be helpful for the identification of "myoepitheliomas".

The role of myoepithelial cells in pleomorphic adenomas has been studied in several papers (Erlandson et al. 1984; Kahn et al. 1985; Caselitz et al. 1986). The epithelial origin of these cells is based on their keratin content (Franke et al. 1982; Caselitz et al. 1981). The special differentiation pattern is underlined by the double expression of keratin and vimentin in some of these cells (Caselitz et al. 1981; Krepler et al. 1982; Erlandson et al. 1984; Caselitz et al. 1984; Kahn et al. 1985) and by the fact that myoepithelial cells seem to be associated with the occurrence of basal membrane associated material (Palmer et al. 1985; Caselitz et al. 1986).

In some reviews myoepithelial cells are said to be involved in the histogenesis of pleomorphic adenomas and adenoid cystic carcinomas (Batsakis 1980). Other authors favor the hypothesis of a disturbance of the entire ductular-acinar unit (Dardick et al. 1983a, b). Apart from the problem of origin and histogenesis, most authors agree that the differentiation pattern corresponds to that of "myoepithelial/basal" cells, which may be transformed. A critical discussion of the term "myoepithelial cells" is given by Palmer et al. (1985) and by Caselitz (1986) and it is this type of myoepithelial/basal cell which is identified by the antibody CK B1. The differentiation pattern of "mixed" tumours is clarified by this antibody. Interestingly, the antibody does identify other basal cells in those tumours where one does not expect "true" myoepithelial cells. Whether the amount of myoepithelial/basal cells reflects some kind of proliferation may be answered by further studies.

Adenoid cystic carcinomas are tumours with a great similarity to pleomorphic adenomas, although the clinical behaviour is quite different (Perzin et al. 1978; Chilla et al. 1981; Szanto et al. 1984; Seifert et al. 1984; Caselitz 1986).

The antibodies we used showed a similar distribution pattern in this group. CK 18 was found in the tubular parts of the tumours. Tubular differentiation is generally interpreted as indicating a favorable prognosis (Perzin et al. 1978; Chilla et al. 1981) and the application of this antibody may be useful in establishing the amount of tubular differentiation in adenoid cystic carcinomas. However, many cells of adenoid cystic carcinoma reacted with the antibody CK B1; in some cases more than 80% of the tumour cells were positive. These tumours exhibited mostly a cribriform or solid pattern. These observations point to the fact that myoepithelial/basal cell differentiation is a leading constituent of adenoid cystic carcinomas. The antibody CK B1 may be useful in the identification and quantitative evaluation of this cell type in different subtypes of adenoid cystic carcinoma.

Adenolymphomas have been analysed for comparative reasons, since the morphology and– seemingly – their histogenesis is quite different from that of pleomorphic adenomas (Eversole 1971; Batsakis 1980; Seifert et al. 1984). The epithelial part of this tumour is positive for keratin as has been shown by several observations (Caselitz et al. 1982). Subtyping of keratin in adenolymphomas has not been previously performed. The antibody CK5 shows that all adenolymphomas are positive for cytokeratin 18. This keratin is present in the oncocytic cells of the epithelial parts of the tumour. The staining seems to be somewhat more prominent at the apical part. In contrast to these findings, basal cells are negative. CK B1, nevertheless does not label the oncocytic epithelium, but picks out single basal cells and sometimes basal cells in groups. This type of cell in adenolymphoma has been detected both light- and electronmicroscopically, but there has been no antibody which specifically decorates this kind of cell. Since they cannot be designated as "myoepithelial" cells, they should be named "basal cells".

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