Virchows Arch [Pathol Anat] (1984) 403:337–344

*Virchows Archiv A*


# Coexpression of keratin and vimentin filaments in adenoid cystic carcinomas of salivary glands

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**Summary.** Six cases of adenoid cystic carcinoma of salivary glands have been examined with antibodies specific for either keratin or vimentin. Tumor cells in all six cases showed coexpression of keratin and vimentin.

**Key words:** Intermediate filaments – Keratin – Vimentin – Adenoid cystic carcinoma – Double immunofluorescence – Salivary glands

Adenoid cystic carcinoma is a special entity within salivary gland tumours because of both its morphological features and its biological behaviour (Berdal et al. 1970; Eneroth 1976; Seifert and Donath 1976; Nochomowitz and Kahn 1977; Gläser 1979; Chilla et al. 1980; Seifert et al. 1980; Lawrence and Mazur 1982). Although this type of tumour may be found in other organs, such as the lacrimal glands, breast, tracheobronchial glands, larynx, and Bartholini's glands (Gamel and Font 1982; Lawrence and Mazur 1982) it plays one of its most important roles in salivary glands. Adenoid cystic carcinoma accounts for 3–6% of all tumours observed in the major salivary glands and 16–22% in the minor salivary glands (Eneroth 1976; Seifert et al. 1980). They constitute 14–25% of all tumours of the oral and nasal cavity and of the pharynx (Kleinsasser 1969). The clinical behaviour of the tumour is malignant, because it infiltrates nerves, bones and other structures. Special problems may occur in the diagnosis of adenoid cystic carcinoma when its most typical feature, namely the cribriform pattern, is seen only in distinct parts of the tumour. In order to get further information on the histogenesis and biology of this tumour, histochemical studies of the intercellular substance (Azzopardi and Smith 1959; Bloom et al. 1977; Chomette et al. 1982; Nochomowitz and Kahn 1977) and electronmicros-

Supported by the Deutsche Forschungsgemeinschaft (Grant Ca 93 1/2) and by the Hamburger Stiftung zur Förderung der Krebsbekämpfung

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copical investigations have been undertaken (Kleinsasser 1969; Hübner 1971; Seifert and Donath 1976; Lawrence and Mazur 1982).

In the last few years, because of progress in molecular and cellular biology a new tool has been developed for the analysis of tumour tissue: antibodies against the intermediate filaments (keratin, vimentin, desmin, glial fibrillary acidic protein, and neurofilaments) can be applied in immunohistochemistry to distinguish major tumour groups (Schlegel et al. 1980; Löning et al. 1980; Altmannsberger et al. 1981; Caselitz et al. 1981; for review see: Osborn and Weber 1983). Thus far intermediate filament typing of tumours of the parotid gland has shown that while mucoepidermoid tumours and squamous cell carcinomas are keratin-positive and vimentin-negative (Caselitz et al. 1981), pleomorphic adenomas contain cells which coexpress both keratin and vimentin (Caselitz et al. 1981 and 1982; Krepler et al. 1982). Here we extend the use of intermediate filament typing to adenoid cystic carcinomas, and show that cells of this tumor type also appear to stain positively with both the keratin and vimentin antibodies.

## Materials and methods

The adenoid cystic carcinomas were removed by surgery. Three of the patients were male and 3 were female, the mean age was 44 years. The youngest patient was 33, the oldest was 55. All tumours were located in the palate. The specimens were frozen in liquid nitrogen immediately after removal and were stored at −70° C. Cryostat sections were cut at −20° C and were either used after 1–24 h at room temperature or were stored at −70° C before use.

*Antibodies and immunofluorescence microscopy*

The following antibodies specific for different intermediate filament types were used:

1. The keratin antibody was produced in a guinea pig using keratin purified from cow hoof (Franke et al. 1978; Osborn et al. 1982). It was affinity purified on the same antigen coupled to Sepharose 4B. It has a broad specificity recognizing both keratinizing and non-keratinizing epithelium.

2. Two vimentin antibodies were available. The first vimentin antibody was produced in a guinea pig using vimentin purified from mouse 3T3 cells (Franke et al. 1978) and was affinity purified on Sepharose 4B coupled to vimentin from rabbit chondrocytes (Osborn et al. 1982).The second antibody which was used for the double labelling was raised in sheep against vimentin purified from rabbit chondrocytes and affinity purified on the same antigen coupled to Sepharose 4B.

3. Desmin antibodies were made in a rabbit against desmin purified from chicken gizzard and were affinity purified on the same antigen coupled to Sepharose 4B (Osborn et al. 1981).

These antibodies have been extensively used on normal and neoplastic human material (for references see: Altmannsberger et al. 1981; Caselitz et al. 1981).

Second antibodies were FITC goat anti-rabbit IgG (Miles, Israel), FITC goat anti-guinea pig IgG and rhodamine goat anti-sheep IgG (both Cappell Laboratories). The second antibodies were sometimes absorbed on an acetone powder either of human parotid gland or of human large bowel to reduce the background.

Sections were fixed in acetone at −10° C for 10 min and air dried. After application of the first antibody, sections were incubated for 45 min at 37° C and washed in phosphate buffered saline. The second antibody was applied thereafter and the specimens were incubated for a further 30–45 min at 37° C. After a further wash, specimens were mounted in Mowiol 4–88. For double immunofluorescence microscopy both primary antibodies were given together. The specimens were then washed and then both second antibodies were applied.

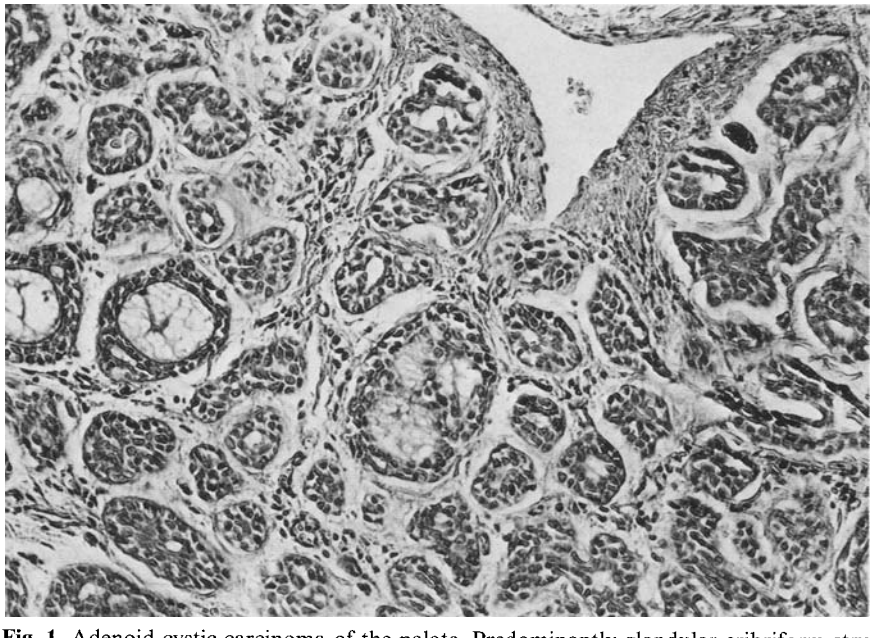

**Fig. 1.** Adenoid cystic carcinoma of the palate. Predominantly glandular-cribriform structure, party tubularly arranged cells. HE, ×180

## Results

All tumours showed the typical features of adenoid cystic carcinoma with cribriform, tubular and solid regions (see Fig. 1). The most typical diagnostic feature, the cribriform pattern, was found in every tumour, but in varying amounts, and the cords of small cells with small amounts of cytoplasm are visible in Fig. 1.

The results of using antibodies specific for either keratin or vimentin are illustrated in Fig. 2 and 3.

The tumour cells showed strong staining by keratin antibodies (Fig. 2a). The tumour cells, arranged in a cribriform, a tubular or a solid pattern, were distinctly stained (Fig. 2a). In some cases, there seemed to be some differences in the intensity: cells lining the inner part of the tumour groups were stained more strongly for keratin than the outer cell layer (Fig. 2a). The keratin antibodies did not stain cells in the stroma. The application of vimentin antibodies revealed a distribution of filaments which was unexpected. The tumour cells of the adenoid cystic carcinoma appeared positive for vimentin filaments (Figs. 2b and 3b). Vimentin positive filaments were observed in the cribriform, solid and tubular areas of the tumour (Fig. 3). The outer cell layer seemed to be slightly more intensively stained than the inner cell layer of the tumour sheets (Figs. 2b and 3b). The stromal cells were, as expected, strongly positive with vimentin (Fig. 2b).

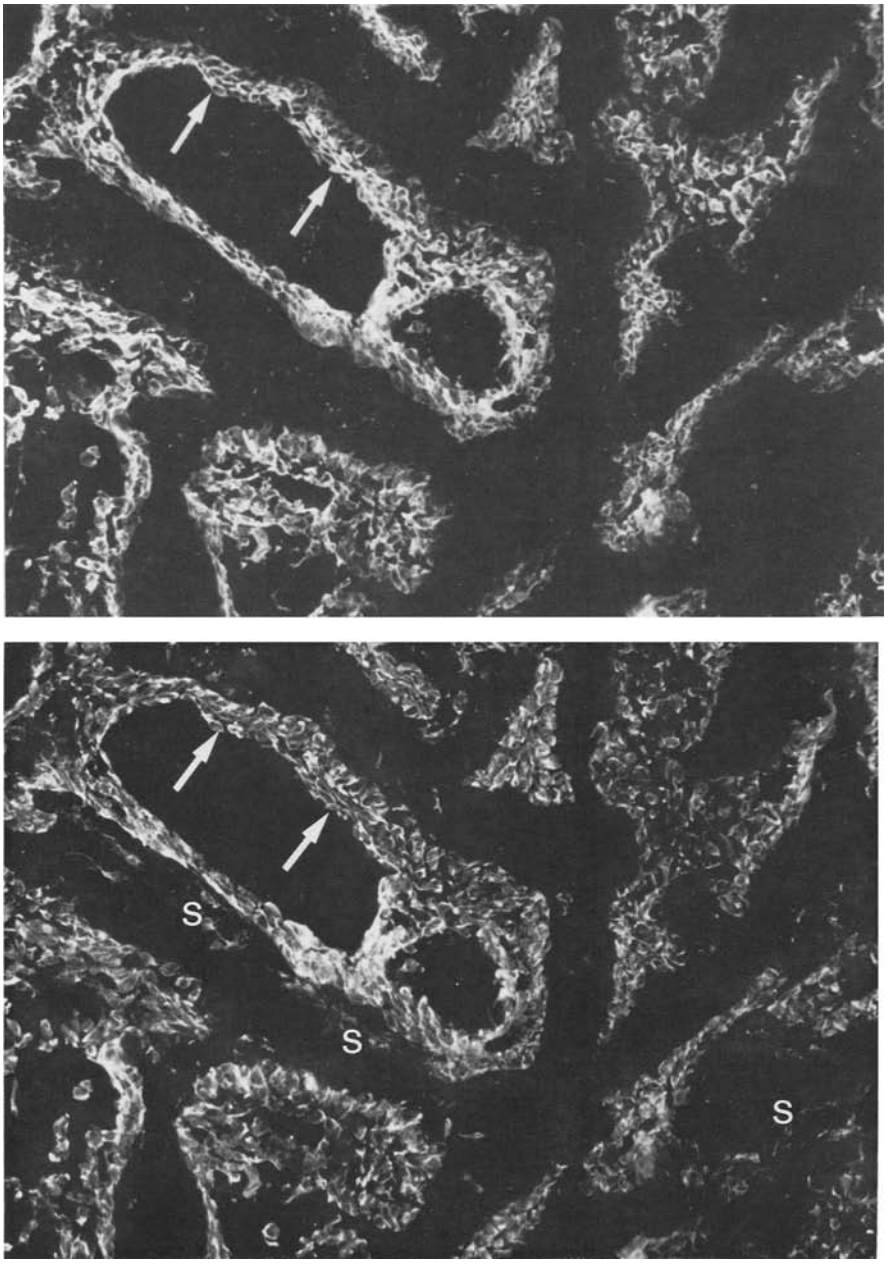


**Fig. 2.** Adenoid cystic carcinoma of the palate. **a** Strong staining for keratin in the tumour cells, intense staining particularly of the luminal cells (*arrows*). **b** Identical slide to **a**. Staining for vimentin in the tumour cells and in the stromal cells (*S*). Luminal cells show a weaker staining for keratin than for vimentin (*arrows*). **a** Immunofluorescence for keratin **b** Immunofluorescence for vimentin. Double immunofluorescence on the same slide, × 210

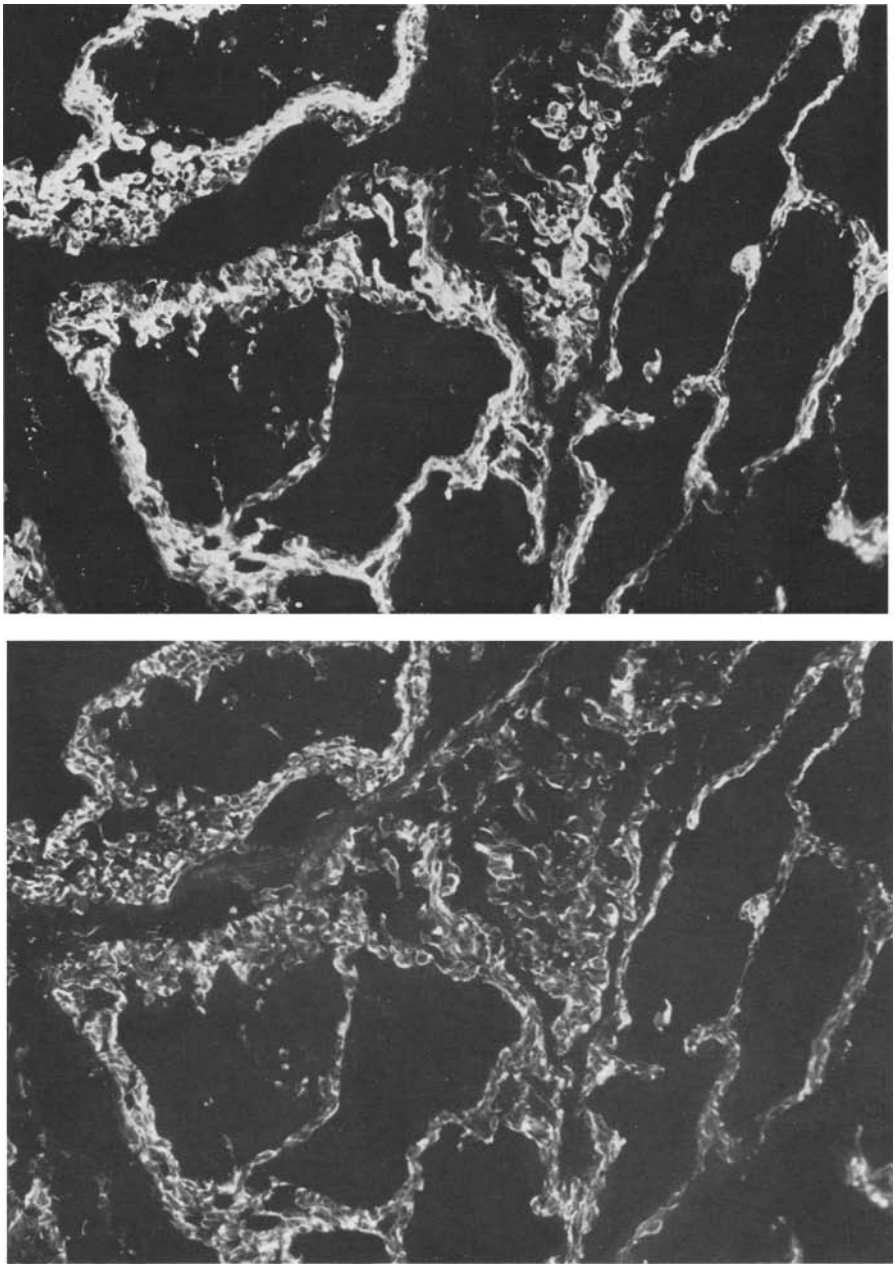

**Fig. 3.** Adenoid cystic carcinoma of the palate. **a** Strong staining for keratin in a more cystic area of an adenoid cystic carcinoma. **b** Same slide as 3a, strong staining of vimentin in the tumour cells, but also in the stromal cells. **a** Immunofluorescence for keratin. **b** Immunofluorescence for vimentin. Double immunofluorescence on the same slide, ×210

Double immunofluorescence microscopy with the keratin and vimentin antibodies confirmed the presence of keratin and vimentin filaments in most of the tumour cells (compare Figs. 2a with 2b and 3a with 3b).

The tumour cells were negative for desmin filaments, although some blood vessels were clearly positive.

## Discussion

Different subtypes of adenoid cystic carcinoma have been described (Seifert 1966; Nochomowitz and Kahn 1977; Gläser 1979; Chilla et al. 1980; Seifert et al. 1980). These include solid (or basaloid), cribriform, and tubular forms (Evans and Cruickshank 1970; Nochomowitz and Kahn 1977; Chomette et al. 1982; Gamel and Font 1982). The cell of origin of adenoid cystic carcinoma has been considered to be an undifferentiated duct cell able to differentiate along the two pathways of myoepithelial and epithelial development. With respect to histogenesis, two main cell types have been suggested: myoepithelial and undifferentiated duct cells (Eneroth et al. 1968; Hamperl 1970; Hoshino and Yamamoto 1970; Hübner 1971). Thackray and Lucas (1974) point to the fact that there is a relationship between adenoid cystic carcinoma and pleomorphic adenoma, since for both tumours the role of an undifferentiated duct cell as a potential stem cell and the special role of the myoepithelial cell is discussed in histogenesis.

Our results identify keratin filaments in adenoid cystic carcinomas, a fact which parallels earlier findings in certain other salivary gland tumours (Caselitz et al. 1981; Krepler et al. 1982) and in a single case of adenoid cystic carcinoma in the uterine cervix (Mazur and Battifora 1981). The observation that more keratin filaments are observed in the "inner layer" of tumour cell groups may be explained by a certain differentiation of these inner cells into duct cells. From their cytological characteristics and from the presence of keratin filaments the duct-like cells resemble those described in pleomorphic adenomas (Caselitz et al. 1981) and the duct-lining type of cells as well as the duct-isolated epithelial cells of Krepler et al. (1982). Additionally, as shown here, the myoepithelial-like cells in adenoid cystic carcinomas are positive for keratin as found previously for myoepithelial-like cells in the pleomorphic adenoma (Caselitz et al. 1981; Krepler et al. 1982) and for normal myoepithelial cells (Franke et al. 1980).

We have also observed vimentin filaments in the tumour cells of adenoid cystic carcinoma, suggesting that in these cells vimentin and keratin are coexpressed. The vimentin antibodies have been used in a variety of other studies and in general do not crossreact with the keratins present in a variety of normal tissues or in carcinomas (Altmannsberger et al. 1981; Caselitz et al. 1981). The presence of both keratin and vimentin filaments in the same cells in pleomorphic adenomas (Caselitz et al. 1981) has been confirmed by Krepler et al. (1982). The latter group performed experiments with antibodies against vimentin absorbed on a mixture of keratins and still found a decoration in the indifferent pleomorphic cells, whereas other cell types show a lesser decoration by the crossabsorbed antibodies. Al-

though, because keratins are a large heterogenous family, we cannot exclude the presence of an unusual keratin present in pleomorphic adenoma and in adenoid cystic carcinoma which shares an epitope with vimentin, we feel this is unlikely.

The histogenetic implication of coexpression of keratin and vimentin is currently hard to assess. Coexpression is very rare in the adult mammal, although interestingly it has been reported during embryogenesis where cells of the parietal endoderm coexpress keratin and vimentin (Lane et al. 1983; Lehtonen et al. 1983) and is very common in epithelial cells growing in culture. In tumours coexpression of keratin and vimentin has so far only been reported in pleomorphic adenomas (Caselitz et al. 1981 and 1982; Krepler et al. 1982) and in certain carcinomas growing as ascites tumours (Ramaekers et al. 1983). Carcinomas growing as solid tumours in situ seem only to express keratin (for review and original references see Osborn and Weber 1983). Thus the finding of coexpression of keratin and vimentin in tumour cells in adenoid cystic carcinoma as well as in pleomorphic adenoma provides further evidence that these two tumour types are closely related. Whether both tumour types are derived from the triangular duct cells described in the normal salivary glands in which coexpression of both keratin and vimentin has also been observed (Caselitz et al. 1981 and 1982; Krepler et al. 1982) remains to be determined.

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Accepted February 15, 1984