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Malignant mixed tumour

A salivary gland tumour showing both carcinomatous and sarcomatous features

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Summary. Two malignant mixed tumours, in which both carcinomatous and sarcomatous features were present, are described. They arose in the palate in patients who had undergone surgery and irradiation for a pleomorphic adenoma at the same site 30 and 36 years previously.

The histological differential diagnoses of recurrent benign pleomorphic adenoma, pleomorphic adenoma resembling mesenchymal tumour, and carcinoma in (ex) pleomorphic adenoma are discussed. On the basis of their positive reaction for keratin with specific monoclonal antibodies it is suggested that the myoepithelial cells are of epithelial origin. Immunohistochemical studies together with the histological appearance of the neoplasms indicate that the carcinomatous as well as the sarcomatous elements were derived from modified myoepithelial tumour cells.

Irradiation may have been responsible for inducing a true malignant mixed tumour as distinct from the more common malignancy which may arise in pleomorphic adenoma, this being a simple carcinoma.

Key words: Malignant mixed tumour – Immunohistochemistry – Pleomorphic adenoma

Introduction

Ten to fifteen percent of all salivary gland tumours originate in the intraoral mucosa where the palate is the most common site (60%). Salivary gland neoplasms constitute nearly 50% of all palatal tumours and here, as in the major salivary glands, pleomorphic adenoma is by far the most common form (Eneroth 1964; Thackray and Lucas 1974).

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The frequency with which carcinoma will develop in pleomorphic adenoma is between 2% (Eneroth 1971) and 9% (Foote and Frazell 1953). This tumour entity (carcinoma in or ex pleomorphic adenoma) refers to an epithelial malignancy in which remnants of a pleomorphic adenoma can still be seen. Only one malignant epithelial component can be identified, usually a carcinoma of ductal type. The term malignant mixed tumour should not be used in these cases (Moberger and Eneroth 1968). However, true malignant mixed tumour is said to exist but is rare. Two forms are recognized: one a pathological oddity in which a histologically benign neoplasm metastasizes without any alterations in histological character (Youngs and Scheuer 1973; Chen 1978), and the other in which both epithelial and stromal elements are malignant and metastasize together (Batsakis 1979).

The purpose of this paper is to describe two cases of the rare entity of malignant mixed tumour in which both carcinomatous and sarcomatous elements are present in contrast to the much more common malignancy arising in pleomorphic adenoma, i.e. carcinoma in (ex) pleomorphic adenoma. The origin of the myoepithelial cells and their role in the histogenesis of the tumour cells is investigated by means of immunohistochemical methods, and also the possibility that this unusual development was induced by previous radiotherapy is discussed.

Materials and methods

Case 1. In 1954 a 25 year old woman presented with an eleven year history of a lump in the right side of the hard palate extending posteriorly into the soft palate. This tumour was removed and was a pleomorphic adenoma showing no features of malignancy. The patient was given a full course of radiotherapy. In 1976 she was seen again because of tooth problems, and at that time there was no sign of recurrence. In 1984, 30 years after radiotherapy and 41 years after the palatal swelling was first observed, a rapidly growing tumour presented at the same site in the palate. This lesion was biopsied and diagnosed histologically as chondrosarcoma. A palatal resection was performed.

Follow-up. One year after the operation in 1984 the patient is alive and without evidence of disease.

Case 2. In January 1985 a 64 year old male was admitted to hospital for surgery of a huge recurrent tumour causing breathing problems, facial nerve palsy and severe pain necessitating opiate analgesia. This patient had presented in 1948 with a swelling of his left palate which was excised under local anesthesia. The histological diagnosis was pleomorphic adenoma. In 1949 and 1950 further excisions were made and also a full course of radiotherapy was given. In 1970 and 1973 transpalatal excisions were performed, with the same histological appearances of pleomorphic adenoma. In 1973 spread to the parotid region and in 1978 to a lymph node was observed. These two biopsies are the only ones we have been able to trace and review; sections showed pleomorphic adenoma. Further excisions were made in 1977 and 1980, and debulking procedures in 1981, 1982 and 1984, all for recurrent pleomorphic adenoma. During 1984 the tumour grew considerably and in July/August he presented with severe pain, hoarseness and oropharyngeal swelling. When admitted to our hospital in January 1985 he had palsies involving cranial nerves VII, IX, X and probably also XI. The tumour mass extended from the left palate to the lateral nasal wall, oropharynx, nasopharynx, skull base and the retromandibular region.

Follow-up. When the patient was last seen, five months after the operation, there was no evidence of recurrent disease.

Light microscopy. The complete material of the two tumours was step-serially sectioned and the sections were stained with haematoxylin and eosin and periodic acid Schiff with and without diastase. Immunohistology was applied using the antibodies listed below. Sections of normal elastic cartilage (pinna), a pleomorphic adenoma of the parotid, and a laryngeal chondrosarcoma were used as controls.

Before applying the antibodies to sections of formalin fixed, paraffin embedded tissue, the sections were dewaxed and taken through graded alcohols to water and then endogenous peroxidase was inhibited by hydrogen peroxidase in methanol. The sections were further digested by 0.1% trypsin for 10–20 min at 37° C to unmask antigenic sites (Makin et al. 1984; Mepham et al. 1979). The indirect immunoperoxidase method was used for the monoclonal antibodies and the sandwich technique for S-100. Antibodies used were:

S-100 (Dakopatts, Mercia Brocades Ltd, Brocades House, Pyrford Road, West Byfleet, Weybridge, Surrey KT14 6RA, Great Britain) is a polyclonal antiserum to S-100 protein derived from ox brain (Moore 1965). It stains brain glial cell and ependymal cell tumours, tumours of Schwann cell origin (Ludwin et al. 1976; Yamaguchi 1980), melanocytic tumours (Gaynor et al. 1980), and pleomorphic adenoma of the salivary glands (Nakazato et al. 1982; Regezi et al. 1985).

CAM 5.2 (Kindly provided as a gift from Dr CA Makin, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, Great Britain) is an IgG2a murine monoclonal immunoglobulin and it identifies the lower (50,43 & 39 kD) molecular weight keratins (Makin et al. 1984).

PKK1 (Labsystems (UK) Ltd, 12 Redford Way, Uxbridge, Middlesex UB8 1SZ, Great Britain) is a monoclonal antibody raised against cytoskeletal proteins from a pig kidney epithelial cell line and reacts with 52.5, 45 and 40 kD polypeptides (Holthofer et al. 1983 and 1984).

Vimentin (Labsystems (UK) Ltd, 12 Redford Way, Uxbridge, Middlesex UB8 1SZ, Great Britain) is a monoclonal antibody raised against cytoskeletal proteins from a pig kidney epithelial cell line and is said to react only with 58 kD vimentin, the main subunit protein of intermediate filaments in fibroblastoid cells (Franke et al. 1979; Virtanen et al. 1981).

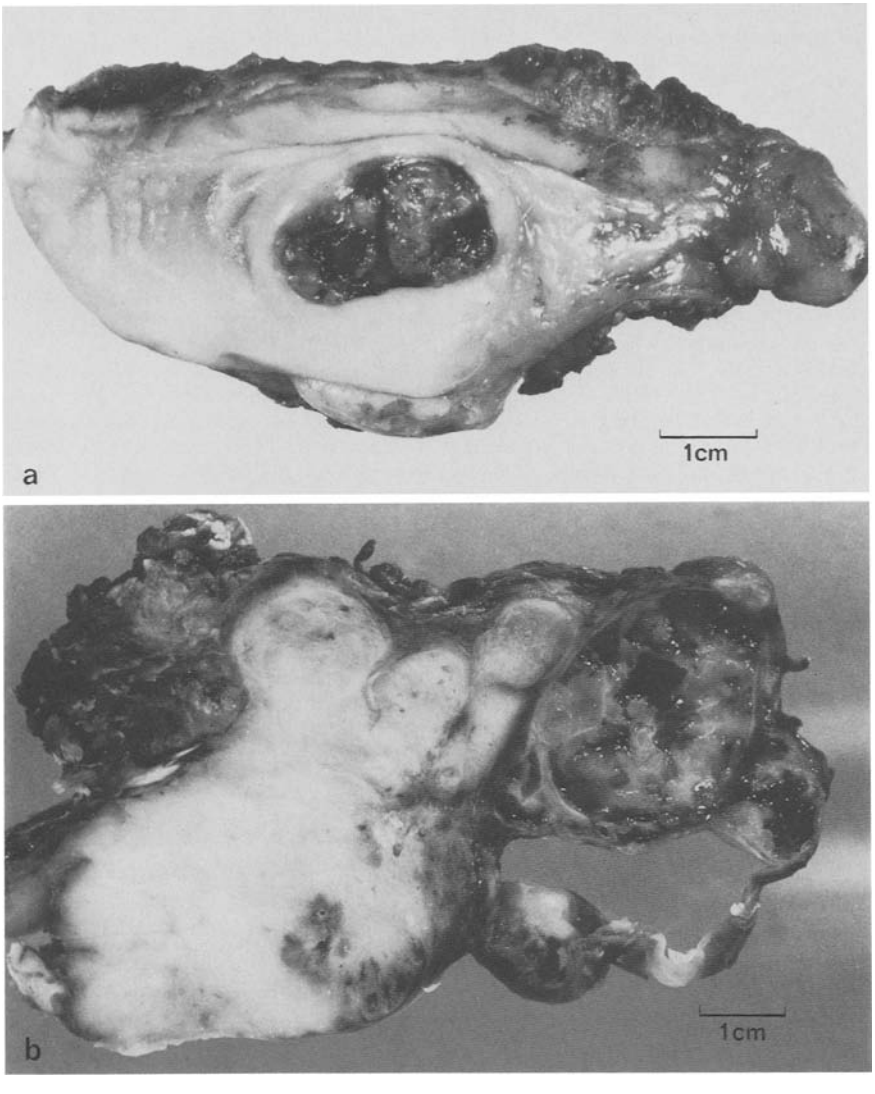
Results

Gross

The palatal tumour in Case 1 was a firm lesion situated in the right side of the hard palate. It measured $20 \times 15 \times 15$ mm. It was protruding and partly ulcerated (Fig. 1 a). It was not encapsulated and the cut surface was greyish with small areas of haemorrhage. The other palatal tumour (Case 2) measured $13 \times 7 \times 5$ cm, was partly encapsulated and presented a cut surface that varied from area to area. The major part of the tumour had a greyish, firm cut surface while other areas were dark and cyst-like (Fig. 1 b).

Light microscopy

Case 1. The complex histological nature of the tumour was discovered when the whole operation specimen was examined. The tumour consisted of two malignant elements. One was a carcinoma of ductal type (Fig. 2) and the other a chondrosarcoma of similar appearance to that of the preooperative biopsy specimen in 1984 (Fig. 3). Between these areas there were myxoid



Figs. 1 a, b. Palatal resection specimen in Case 1 with the protruding and partially ulcerated tumour **a** and cut surface of the large tumour in Case 2 **b**. To the left there is a solid area (carcinoma) and to the right a more cystic, mucinous area (chondrosarcoma)

as well as cellular foci with spindle shaped, loose lying cells. These cells resembled myoepithelial cells and appeared in some places to merge with malignant cartilage (Fig. 4). A few remnants of benign pleomorphic adenoma could also be identified.

Case 2. The morphological picture of this tumour was very similar to that of Case 1. In large areas the tumour was a carcinoma, partly poorly differen-

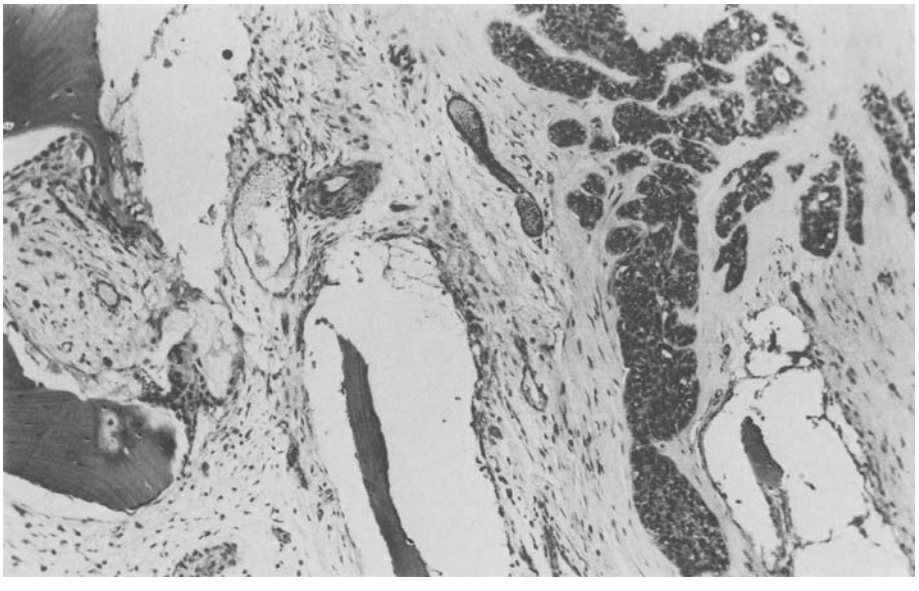


Fig. 2. The resected palatal tumour in Case 1 shows a carcinomatous element, partly of ductal type and invading bone (H&E, ×100)

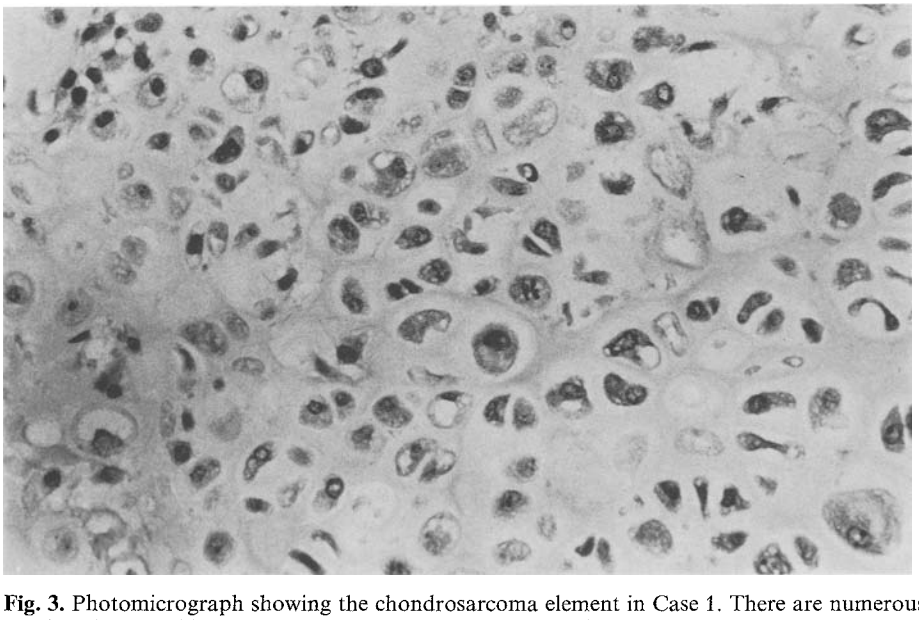


Fig. 3. Photomicrograph showing the chondrosarcoma element in Case 1. There are numerous atypical, large and irregular chondrocytes and also mitotic figures (H&E, ×320)

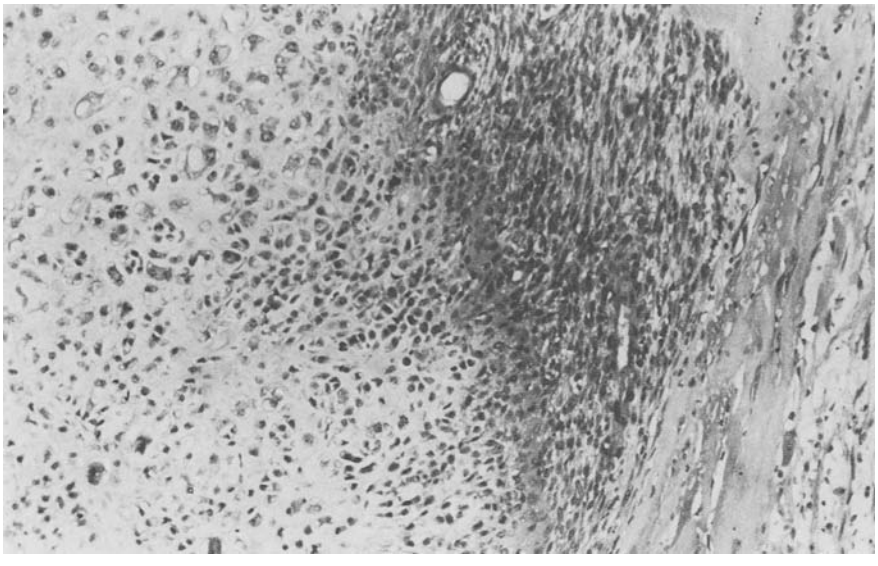


Fig. 4. Photomicrograph of resected palatal tumour (Case 1) showing myoepithelial cells merging with malignant cartilage (H&E, ×135)

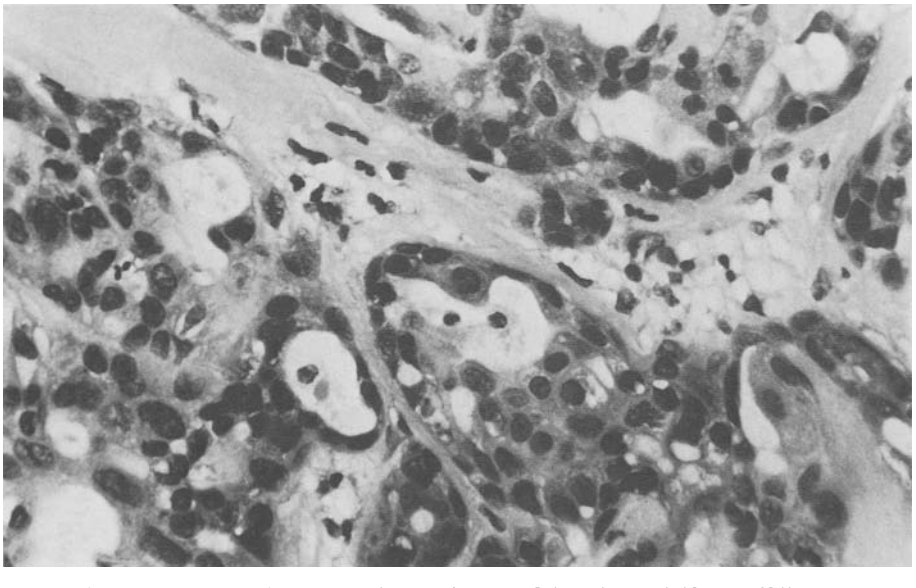


Fig. 5. The palatal tumour in Case 2 with carcinoma of ductal type (H&E, ×400)

tiated, partly of ductal type (Fig. 5). The mesenchymal component was a chondrosarcoma and between these two elements a large number of malignant, proliferating myoepithelial cells were seen. These cells merged into the malignant cartilage in a similar fashion to Case 1.

Table 1. Immunohistochemical staining of the tumours and three controls

	Malignant mixed tumours						Controls		
	Carcinoma		Myoep.cells		Cartilage		Cartilage		
Case	1	2	1	2	1	2	Pinna	Pleom. adenoma	Chondro-sarcoma
CAM 5.2	pos	pos	pos	pos	weakly pos	weakly pos	neg	weakly pos	neg
PKK1	pos	pos	pos	pos	weakly pos	weakly pos	neg	pos	neg
S-100	neg	pos	weakly pos	pos	neg	pos	pos	pos	pos
Vimentin	neg	neg	neg	neg	neg	neg	weakly pos	neg	pos

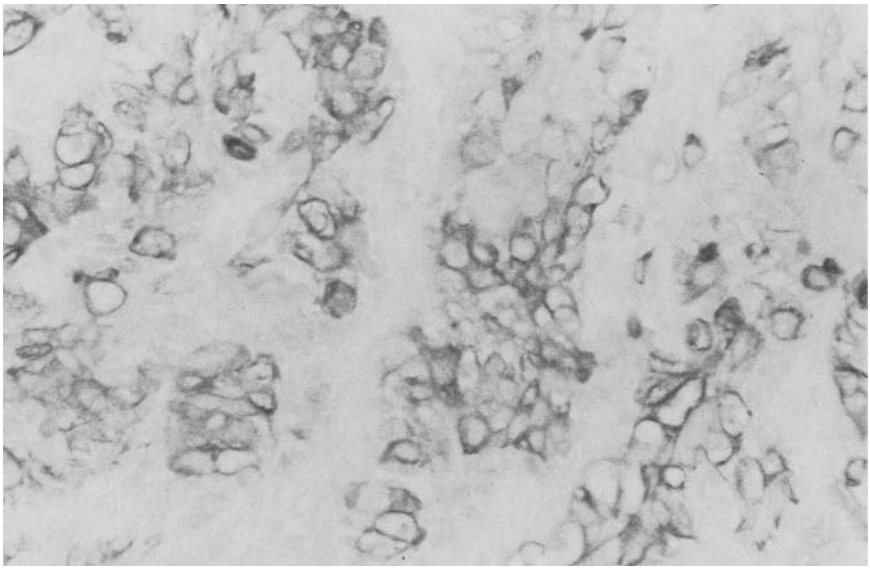


Fig. 6. Immunohistochemical staining for keratin in the carcinomatous area in Case 1. The majority of the cells are positive (PKK1, × 320)

Immunohistology

The results of immunohistochemical studies are shown in Table 1. The negative controls were negative in all cases; with S-100 there was a very weak, if any, background staining. Inbuilt positive controls were present in all cases. The monoclonal epithelial markers gave positive reactions in the carcinoma and myoepithelial cells in both tumours, weakly positive reactions

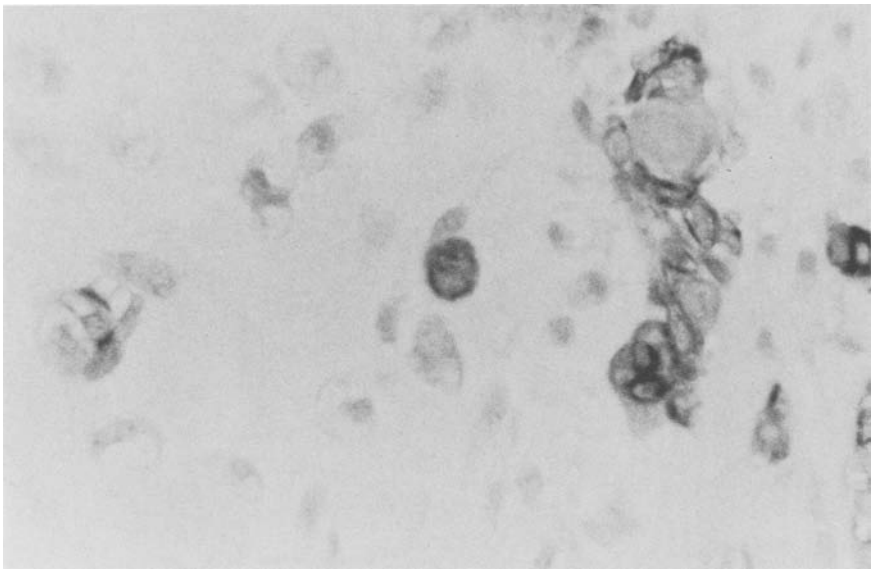


Fig. 7. A cluster of cells shows positivity for keratin in the malignant cartilaginous element of the tumour in Case 2 (PKK1, ×400)

in the cartilage cells of both the malignant mixed tumours and the pleomorphic adenoma but negative reaction with normal cartilage and the laryngeal chondrosarcoma (Figs. 6 and 7).

Discussion

In large series with satisfactory follow-up (Foote and Frazell 1953; Chaudhry et al. 1961; Eneroth 1964; Moberger and Eneroth 1968; LiVolsi and Perzin 1977) lesions described as malignant mixed tumours in fact were not tumours with both carcinomatous and malignant mesenchymal elements but most likely carcinomas in pleomorphic adenoma (Batsakis 1979). However, Spiro et al. (1977) do mention that six out of their eight palatal malignant mixed tumours had probably arisen de novo and consisted of both epithelial and mesenchymal malignant components. Three out of the 40 malignant mixed tumours reported by Tortoledo et al. (1984) were also in fact true malignant mixed tumours with two malignant elements.

Remnants of pleomorphic adenoma were found in our palatal malignancies, although not until a meticulous histopathological examination of the complete neoplasms had been made. The morphological picture of these palatal tumours with invasive ductal carcinoma, malignant cartilage, prominent proliferating myoepithelial cells with malignant appearance, and of adenomatous structure, is not, however, compatible with ordinary carcinoma in pleomorphic adenoma, but suggests true malignant mixed tumour. In some areas of the tumours there were spindle cells but they were not prominent and no palisade arrangement was recognized as described in

spindle cell pleomorphic adenoma or so called pleomorphic adenoma resembling mesenchymal tumours (Merino and LiVolsi 1977). As the present tumours were very cellular, which often may be the case in pleomorphic adenomas, especially in the minor salivary glands, and as the atypical cartilage theoretically could have been induced by previous radiotherapy, a diagnosis of recurrent benign pleomorphic adenoma had to be considered. However, the pronounced cellular atypia of the epithelial component and its growth pattern with invasion in surrounding tissue is not compatible with a recurrent benign pleomorphic adenoma. Furthermore, the bizarre bi- and multinucleated cartilaginous cells with mitoses, and invasive growth, make it extremely unlikely that the cartilage was reactive and not neoplastic.

There is evidence that the myoepithelial cells of the salivary glands are of epithelial origin (Radnor 1972; Cutler and Chaudhry 1974; Caselitz et al. 1981; Dardick et al. 1982; Krepler et al. 1982; Erlandson et al. 1984). The myoepithelial cells of the present tumours stained positively with two antikeratin antibodies as well as with S-100, and in all cases the myoepithelial cells stained negatively with vimentin (Table 1). The negative staining with vimentin does imply an absence of that filament. The report by Caselitz et al. (1984) suggests the coexpression of keratin and vimentin in the tumour cells of adenoid cystic carcinoma and that may theoretically also be the case in the malignant counterpart of a pleomorphic adenoma. However, the negative staining with vimentin in our cases is probably real and not due to the nature of the paraffin embedded tissue, as inbuilt controls for vimentin were positive. So the results do support the concept that the myoepithelial cells in these two tumours are of epithelial nature. The malignant cartilage of the palatal tumours stained, although weakly, with both PKK1 and CAM 5.2. The laryngeal chondrosarcoma used as a control did not stain with epithelial markers thus suggesting a difference in origin between these malignant cartilaginous growths. Vimentin reacted positively with the laryngeal chondrosarcoma and the cartilage of the pinna but negatively with the cartilage of the pleomorphic adenoma and the malignant mixed tumours (Table 1). However, sometimes the myxoid areas in an ordinary pleomorphic adenoma may be positive for vimentin (Krepler et al. 1982). The results suggest that the malignant cartilage of the present tumours may not only be derived by malignant transformation of benign cartilage in the original pleomorphic adenoma, but that it may be produced by the myoepithelial cells. This is supported not only by positive staining with epithelial markers but by the histological appearance of myoepithelial cells merging into the malignant cartilage, thus constituting a transitional zone with the sarcoma element. We thus suggest that the malignant elements in these malignant mixed tumours are derived from cells of epithelial origin, i.e. myoepithelial cells. This is in agreement with the concept that the principal tumour cell in an ordinary benign pleomorphic adenoma is a structurally modified myoepithelial cell (Takauchi et al. 1975; Dardick et al. 1982; Dardick et al. 1983; Erlandson et al. 1984).

The three cases of true malignant mixed tumour mentioned by Batsakis (1979) did not have any prior irradiation (Batsakis personal communica-

tion). The treatment of benign pleomorphic adenoma by radiotherapy followed by malignant mixed tumours after 30 and 36 years respectively, does, however, raise the possibility that the present tumours are radiation-associated. It is most likely that small remnants of the pleomorphic adenoma were left after the initial operations and the subsequent radiotherapy may have induced the myoepithelial cells to produce carcinoma and malignant cartilage, but the case remains unproven. In order to evaluate this a large series of cases of malignancy in pleomorphic adenoma should be investigated with special regard to whether or not prior radiotherapy was given to the original pleomorphic adenoma.

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