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The pleomorphic adenoma of salivary glands transplanted on athmymic mice

A lightmicroscopical and immunohistochemical investigation*

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Summary. 10 pleomorphic adenomas of the human parotid gland were transplanted on several groups of nude mice. For comparative reasons, 10 other pleomorphic adenomas, a neurinoma and a chordoma and transplants of squamous cell carcinomas and of normal salivary gland tissue were also analysed.

In the primary tumours and in the transplants, the presence of keratin, carcinoembryonic antigen, tissue polypeptide antigen, lactoferrin, lysozyme, immunoglobulins, secretory component, amylase, fibronectin and of several lectin-receptors (PNA, WGA, HPA, Ulex europaeus) was sought.

The immunohistological observations show that many of the features of a pleomorphic adenoma are constant under the conditions of transplantation. In the transplanted tumour, the same heterogeneity as in the primary tumours can be observed. Autoradiographic studies show little labelling with 3-H thymidine, which is in good accordance with the biological behaviour of the tumour.

The distribution of fibronectin shows an interesting association with myoepithelial-like cells.

Our results support the hypothesis that the histogenetic origin of the pleomorphic adenoma is a cell pool of the terminal ductal segment. A differentiation towards ductal cells (with production of secretory substances) and towards myoepithelial cells (associated with large amounts of basal membrane like substances) is observed.

Key words: Athymic mouse – Pleomorphic adenoma – Immunohistochemistry – Myoepithelial cells

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Introduction

Pleomorphic adenomas are the commonest epithelial tumours of salivary glands (Seifert et al. 1976; Gläser 1979; Seifert et al. 1984). The main histological feature of these tumours is the simultaneous appearance of epithelial and mesenchymal structures (Eversole 1971) and the quantity and distribution of both components show considerable variation (Seifert et al. 1976).

In several papers, the histiogenesis of these tumours has been discussed (e.g. Fasske 1960; Mylius 1960; Hamperl 1970; Chisholm et al. 1974; Harrison and Auger 1982; Dardick et al. 1983; Erlandson et al. 1983).

A new approach towards the histogenesis of pleomorphic adenoma is given by cell and tissue culture methods which may show the proliferative behaviour and the cytodifferentiation of these tumours (Kondo et al. 1971; Shirasuna et al. 1980). The first successful transplantation of a human adenocarcinoma into athymic mice (Rygaard and Povlsen 1969) illustrated a model for experimental oncology (for review see: Fogh and Giovanella 1978; Bastert et al. 1981; Lindenberger 1981) which we have exploited. The aim of this study is to answer the following questions:

1. What is the behaviour of pleomorphic adenomas after heterotransplantation in comparison with the primary tumour?
2. What are the changes in the distribution pattern of tumour markers?
3. What is the proliferative behaviour?

Material and methods

This investigation is based upon 20 pleomorphic adenomas which were surgically removed in the Ear- Nose- and Throat Clinic and in the Clinics of maxillofacial surgery at the University of Hamburg (Table 1).

For comparative reasons the following other tumours were investigated: neurinoma (1 case), chordoma (1 case), squamous cell carcinoma (1 case) and normal salivary gland tissue (5 cases).

The female athymic mice were purchased from the Zentrale Versuchstieranstalt in Hannover (Western Germany).

After operation, the material of 10 pleomorphic adenomas and of the neurinoma and chordoma was transplanted subcutaneously on nude mice (dorsolateral part of the thorax). The transplanted pieces had a size of 1 to 5 mm and the size of the transplants was measured every week.

The material of the 10 tumours was transplanted into 51 mice. The animals were sacrificed under ether anaesthesia after different time periods (4 mice after less than 7 days, 9 mice after 7 to 30 days and 28 mice with transplants after more than 30 days). The tumours and the mouse tissue (heart, lungs, liver, lymph nodes, intestine and skin) were frozen in liquid nitrogen, fixed in Bouin's solution and glutaraldehyde. Material from the original tumours was prepared in the same way. For light microscopical and immunohistological investigations, the material was embedded in paraffin and staining for the following substances was performed by immunohistological methods: Keratin, tumour markers (carcinoembryonic antigen, tissue polypeptide antigen), other markers (lactoferrin, lysozyme, immunoglobulins, secretory component, amylase, fibronectin).

Lectin receptors were sought by affinity histochemistry (Peanut agglutinin, wheat germ agglutinin, Helix pomatia agglutinin, Ulex europaeus agglutinin).

The immunohistological methods were performed in the following manner:

After preincubation with normal serum (goat) and after blocking the endogenous peroxidase, the sections were incubated with the primary antibody (e.g. against CEA from the rabbit),

Table 1. Primary tumours

Tumour	Patient		
	male	female	age (years)
Pleomorphic adenoma			
type 1		×	41
	×		44
type 2	×		17
	×		31
		×	58
		×	51
	×		71
	×		22
	×		19
		×	44
		×	44
	×		58
	×		42
	×		27
		×	16
	×		26
type 3	×		37
	×		53
type 4		×	61
		×	37
Neurinoma	×		23
Chordoma	×		44

Table 2. Transplanted tumours

Tumour type	Primary tumour number	Transplant number	Necrosis (scar) number
Pleomorphic adenoma			
type 1	1	9	3
type 2	7	28	15
type 3	1	8	2
type 4	1	6	2
Chordoma	1	1	1
Neurinoma	1	3	
Squamous cell carcinoma		2	
Normal salivary glands		5	

with the second antibody, which was directed against the species of the primary antibody (e.g. anti-rabbit-antibody from the goat, so-called link antibody), with the PAP complex (rabbit peroxidase-antiperoxidase). The presence of the peroxidase complex, and consequently the presence of the primary antibody was visualized by the reaction with diaminobenzidine. Each step of the aforementioned incubations was followed by washing the sections in PBS buffer.

Table 3. Origin of the different antibodies

Antigen	Origin
Keratin	Dakopatts, Kopenhagen
Amylase	Behringwerke, Marburg
Fibronectin	Behringwerke, Marburg
Immunoglobulins A, G, M	Behringwerke, Marburg
Secretory component	Dakopatts, Kopenhagen
Lactoferrin	Dakopatts, Kopenhagen
CEA	Dakopatts, Kopenhagen
Lectins coupled to peroxidase (PNA, WGA, HPA, Ulex)	Medac, Hamburg
TPA	Prof. Björklund, Stockholm

A counterstaining was generally performed by haemalaun. The origin of the different antibodies is indicated in Table 3.

The demonstration of the lectin receptors (the specific sugar residues) was done by peroxidase coupled lectins and by the DAB-reaction.

Each specimen was controlled by omitting the specific primary antibody and substituting by normal serum instead.

Part of the tumour transplants were analysed by autoradiography. Methyl-3-H-thymidine was injected intraperitoneally (with a specific activity of 25 mCi/mmol) into athymic mice. The injections were performed every 2 h (4×2 µg/g body weight). 2 h after the last injection (after the total period of 8 h) the mice were prepared under anaesthesia. The tumours and the organs were taken and fixed in Bouin's solution. Part of the material was stained by HE, another part was stained by immunhistochemical methods (for TPA).

The sections were analysed by the stripping film technique (Kodak Fine Grain autoradiographic stripping Plate AR 10). The period of exposure was 21 days. The labelling index was indicated in percentage of a given cell population. Nuclei with more than 5 silver grains were regarded as positive. For every section, at least 500 cells were counted. Since the transplants were sometimes small, this was the maximum number of cells which could be identified. In order to verify the correlation of TPA and autoradiographic labelling, we evaluated the labelling index of the TPA positive cells.

Some transplants were analysed by the semithin section technique. For this investigation the specimens were incubated in glutaraldehyde and in cacodylate buffer and were postfixed in osmium tetroxyde. The sections were stained by toluidine blue.

Results

In principle, the size of the transplanted pleomorphic adenomas was constant after 1 month. In contrast, the squamous cell carcinoma showed an exponential growth after an adaption period of 3 weeks. After 6 weeks, the malignant tumours were three times larger than the original size. The transplanted tumours generally preserved the subtype of the primary tumour (Table 4).

Conventional light microscopy

Pleomorphic adenoma, type 1 and type 2. The pleomorphic adenomas of the type 1 consisted of a stroma rich and a stroma poor part, whereas in type 2,

Table 4. Correlation of primary tumour and tumour transplants

Primary tumour	Transplanted tumours			
	type 2	type 1	type 3	type 4
type 2	+ + + + + + + +	+ + + +	+	
type 1	+ + +	+ + +		
type 3	+ + +	+		
type 4		+		+ + +

Each cross means one case

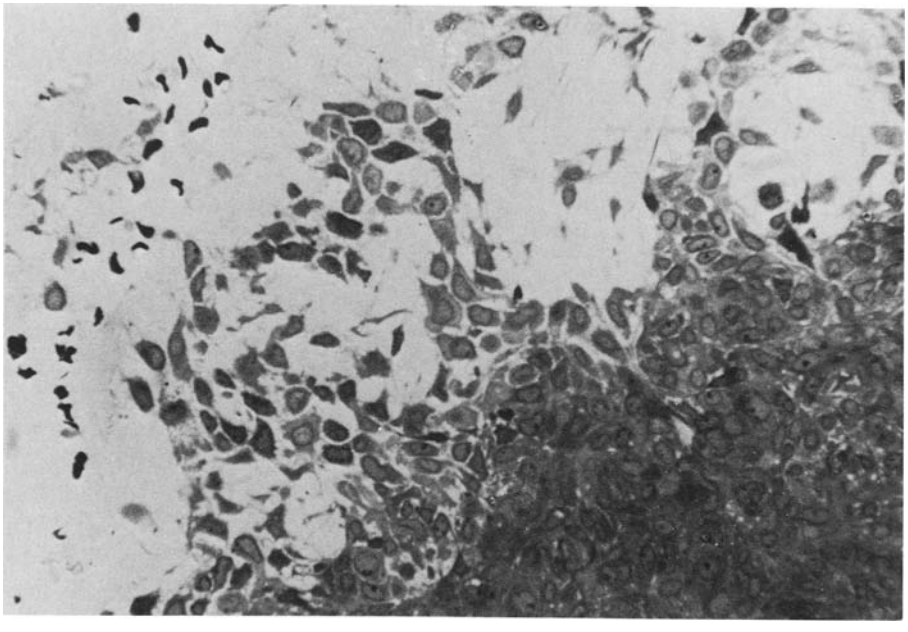

Fig. 1. Pleomorphic adenoma, subtype 1. Epidermoid parts. At the margin there is transition to a dispersed pattern (spindle shaped cells). Semithin section, toluidine blue. × 300

the "stromal" part was more prominent. Changes in the different periods after transplantation were similar so that both types are described together.

The stroma of the tumours consisted of mucoid substances and chondroid substances. In these parts, generally spindle shaped cells and some hyaline cells were found. The predominant differentiation of the "epithelial" parts was into tubular and solid arrangements (Fig. 1). In some parts, there was squamous metaplasia. The tubules were built up of a cuboidal epithelium and sometimes by myoepithelial cells (Fig. 2). The cells of the solid

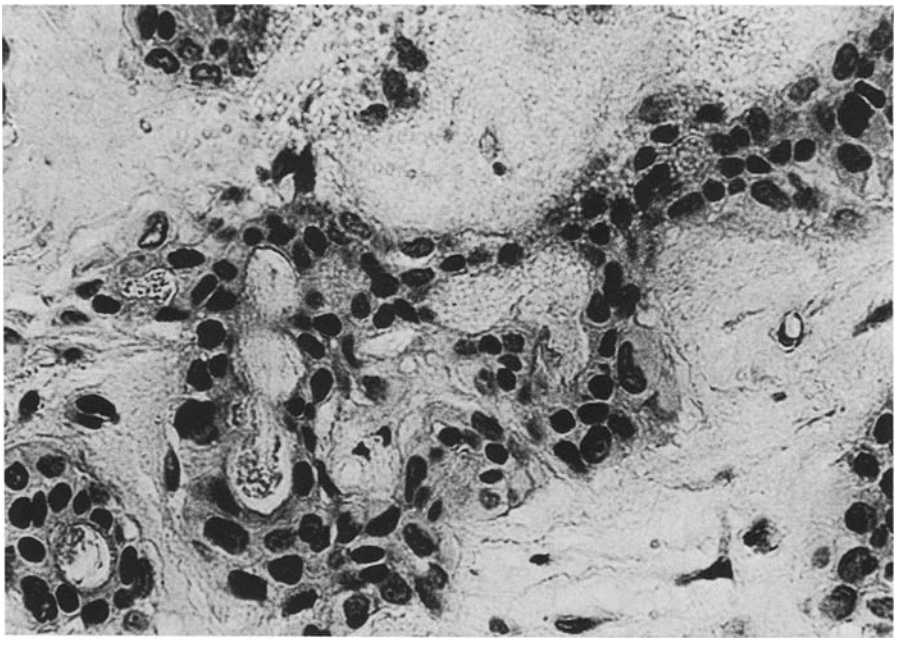

Fig. 2. Pleomorphic adenoma, subtype 2. Tubules of duct-like tumour cells. At the outer border some spindle shaped myoepithelial cells which seem to "flow" into the stroma. Semithin section, toluidine blue. × 300

areas were similar to the nondifferentiated duct cells with an uniform, oval nucleus, which was sometimes indented, and a distinct nucleolus. The shape of the cells was polygonal, and the cytoplasm was clear. At the border of the solid parts, a more myoepithelial like cellular type predominated. These cells were often stellate and were often seen in the mucoid parts.

The transplants which were only some days old displayed phenomena of regression. The less vascularized areas of the stroma rich parts showed large areas of necroses and sometimes infiltrates of inflammatory cells. In the chondroid parts, the cells were generally necrotic. The peripheral zone of 10 cells was generally viable.

After more than one month, necrotic changes were rarely seen. The tumours displayed the same morphology as the primary lesions (Fig. 3). There were several septa of connective tissue between the nodules, and fibroblasts, fibrocytes and capillaries were seen. The cellular areas were generally seen at the border of the transplant with mucoid and chondroid parts in the center. After more than three months, there was no change in the transplanted tumours, which displayed the typical appearance of pleomorphic adenomas with mucoid and cellular parts. There was a slight capsule of connective tissue around them.

Pleomorphic adenoma, type 3 and 4. Both types have a predominant epithelial component, type 4 having a more monomorphic aspect. Some areas dis-

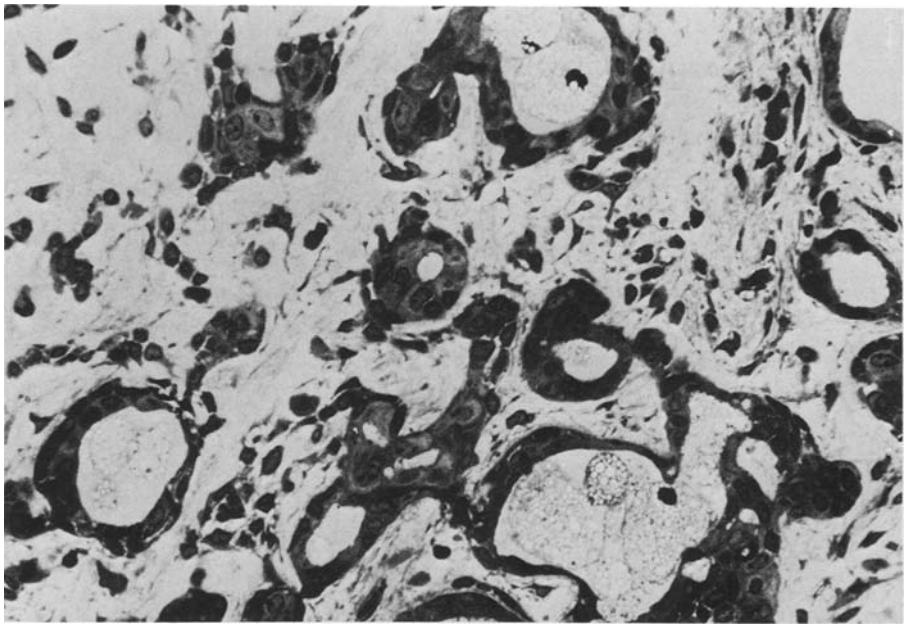

Fig. 3. Transplanted pleomorphic adenoma, subtype 1. Tubularly arranged cells. At the margin there is transition to a dispersed growth pattern. Mucoid stroma. Semithin section, toluidine blue. × 300

played the typical features of a pleomorphic adenoma, others showed more monomorphic features. They were generally formed by duct like cells, arranged in tubules. The basal membranes were thickened. In the transitional zone between the monomorphic part and the stroma rich parts the basal membranes lost their original appearance and were more dispersed. "Clear cells" were sometimes observed. The basal membrane between the trabecular areas was thickened (Fig. 4).

The transplants showed the same appearance as the primary tumours. Most cells were arranged in solid areas, single cells were oval with a clear and basophilic cytoplasm. The nuclei were generally oval (Fig. 5). In the stroma rich parts of the tumour, there were some dispersed spindle like cells. The stroma was only poorly developed, displaying a mucoid or a hyaline aspect.

Immunohistochemistry (Table 5)

1. Keratin. Keratin was regularly identified in the epithelial parts of the tumours. Tubular areas, including duct cells and basal myoepithelial like cells, and squamous areas were positive for keratin, although the intensity of the staining varied. In the "stromal" areas, some of the polygonal and spindle cells were positive for keratin, thus their epithelial nature was demonstrated.

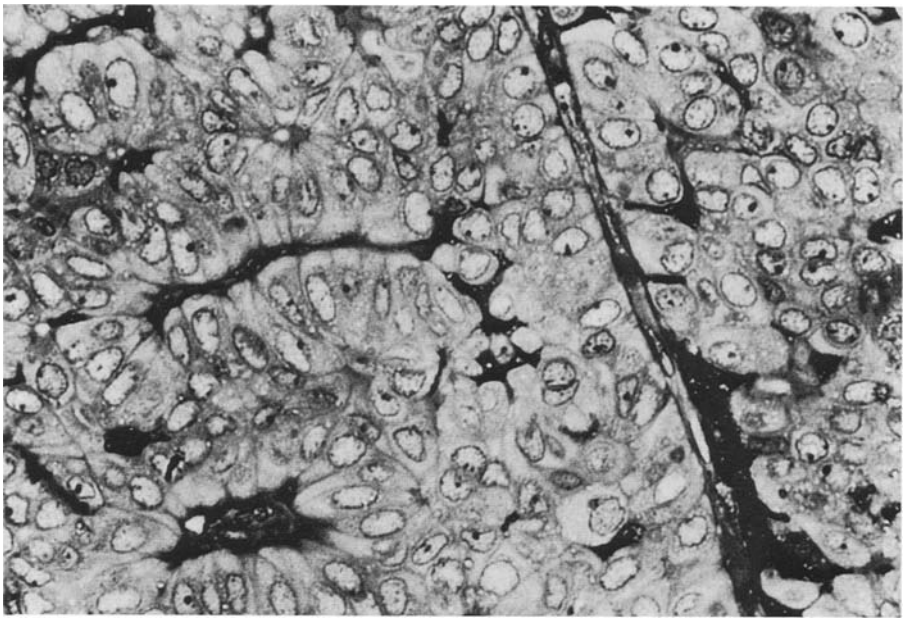

Fig. 4. Pleomorphic adenoma, subtype 4. Monomorphic aspect with large cells in tubular and solid arrangements. Semithin section, toluidine blue. × 600

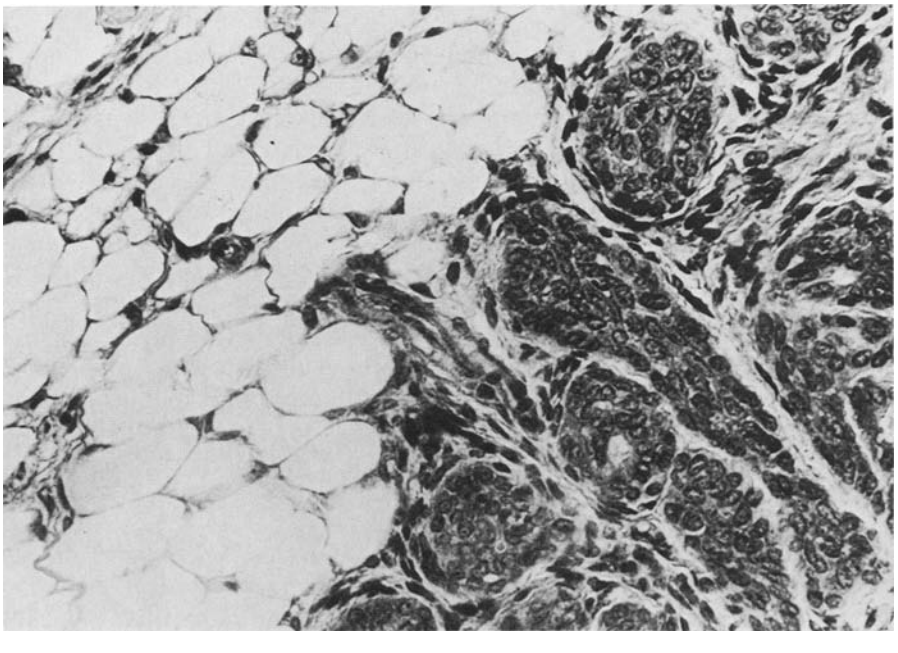

Fig. 5. Transplanted pleomorphic adenoma, subtype 4. Tumour cells in solid and tubular arrangements. At the border there is mouse connective (fat) tissue without inflammatory reaction. Haematoxylin-eosin. × 300

Table 5. Results in immunohistochemistry

Type of tissue	Keratin	CEA	TPA	LF	LZ	SC	IGA	IGG	IGM	FN
Pleomorphic Adenoma Primary tumour	+	+	+	+	+	+	+	−	−	+
Pleomorphic Adenoma Transplant	+	+	+	+	+	+	−	−	−	+

Abbreviations: CEA (Carcinoembryonic antigen), TPA (Tissue polypeptide antigen), LF (Lactoferrin), LZ (Lysozyme), SC (Secretory component), IG (Immunglobulin), FN (Fibronectin)

In the transplanted tumours, the staining of keratin was most intense in the "epithelial" areas. Cells in the dispersed arrangements were generally less intensely stained for this marker.

2. Tumour markers. CEA. CEA was generally detected in the tubular and in the solid areas of the primary tumours. The staining in the tubular areas was of varying intensity and generally at the apical border. In the transplanted tumours, CEA was found in a similar distribution pattern.

TPA. In the primary tumours, TPA was found only in the epithelial area of the tumours. In the double layered tubules, the cuboidal cells near the lumen were generally more intensely stained than the outer cell layer (Fig. 6). Some polygonal cells of the solid areas were also positive for TPA. Mesenchymal cells were never positive.

The same pattern for the TPA distribution was found in the transplanted tumours. In comparison with the primary tumours, TPA staining was augmented in quantity and quality.

Lactoferrin. In the primary tumours, lactoferrin was only seen in some areas, especially in those which showed a tubular differentiation. There was a strong intracytoplasmatical staining of the cuboidal cells lining the lumen. In the transplanted tumours, lactoferrin was detected in the same tubular areas, although the staining seemed sometimes to be stronger than in the primary tumours (Fig. 7). The stromal areas were generally negative for TPA.

Lysozyme. In the primary tumours, lysozyme was generally not found, or at least in very small amounts. In the normal glandular tissue, lysozyme was detected in the acinic cells and in some cells of the intercalated ducts. In the transplanted tumours, lysozyme was only found in very small amounts.

IG A. IG A was detected in some of the primary tumours. It was generally localized at the apical border of the cuboidal cells at the lumen. Sometimes

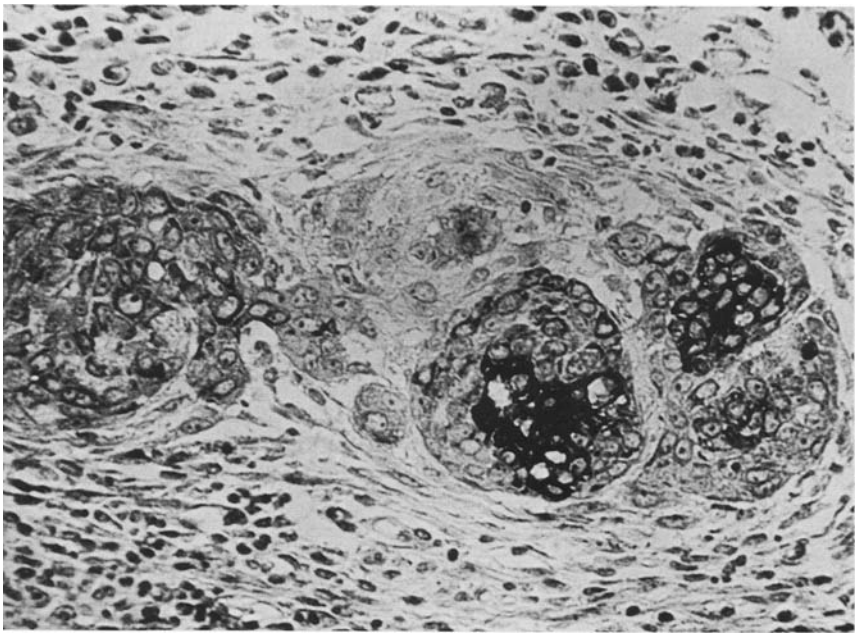

Fig. 6. Transplanted pleomorphic adenoma, subtype 2. TPA positive cells in the solid parts of the tumour. Immunoperoxidase reaction for TPA. × 300

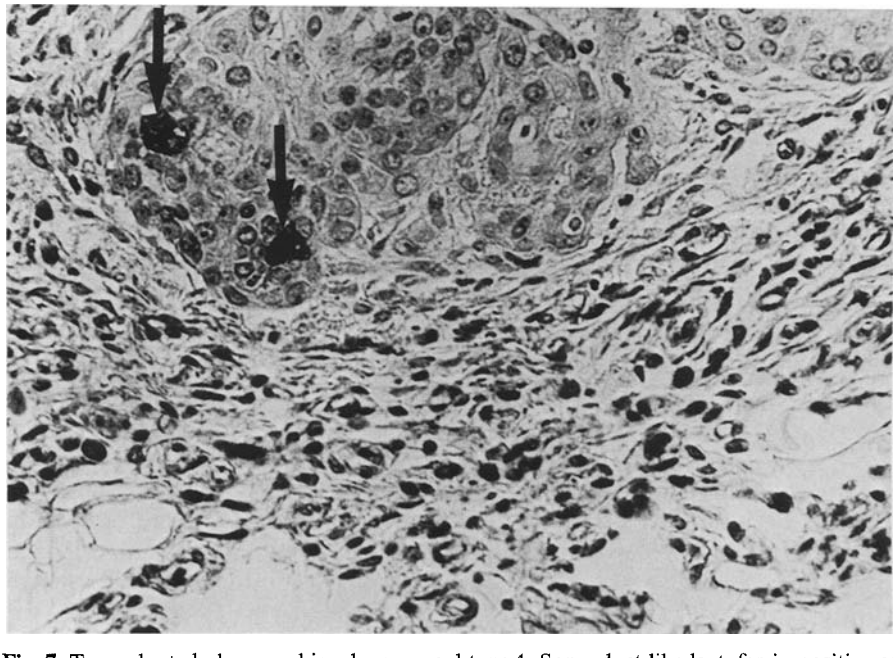

Fig. 7. Transplanted pleomorphic adenoma, subtype 1. Some duct like lactoferrin positive cells (*arrows*). Cells in the solid parts negative for lactoferrin. Immunoperoxidase reaction for lactoferrin. × 300

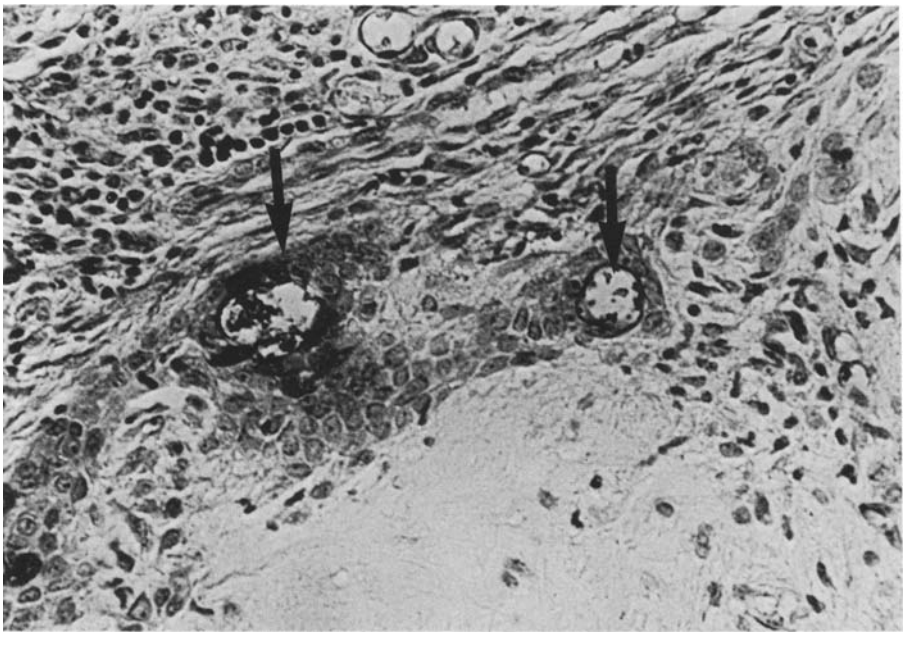

Fig. 8. Transplanted pleomorphic adenoma, subtype 2. Labelling for secretory component at the apical border of duct-like cells (*arrows*). No secretory component in the mucoid stroma. Immunoperoxidase reaction for secretory component. × 300

the secretory material was negative. Some plasma cells seemed to be positive for IG A. The transplanted tumours were never positive.

IG G and IG M. IG G and IG M were not detected in the primary tumours nor in transplants.

Secretory component. In the primary tumours, secretory component showed a typical distribution pattern, as detected by immunohistochemistry. It was seen at the apical border of the ductal cells and in the lumen of the tubules. Sometimes, small amount of secretory component were found in the cytoplasm.

The transplanted tumours were positive for secretory component. In some transplants, secretory component was detectable in up to 70% of the cells, generally arranged in a tubular pattern (Fig. 8). The staining seemed to be stronger than in some of the primary tumours. Due to the heterogeneity of pleomorphic adenomas, there were some transplants which revealed only traces of secretory component and these usually had a large amount of stroma.

Amylase. No primary tumour was positive for amlyase. The transplants were also negative.

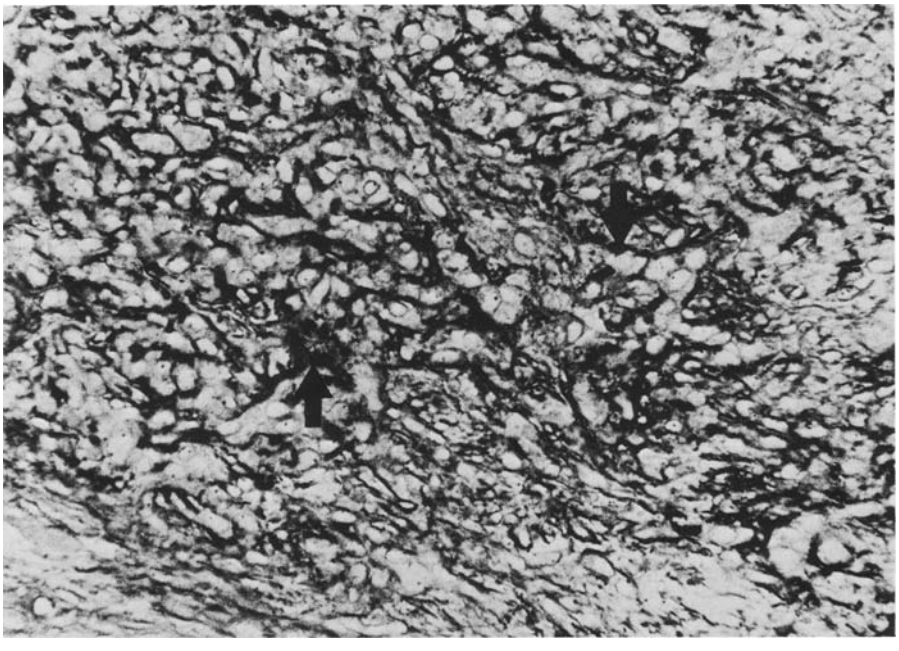

Fig. 9. Transplanted pleomorphic adenoma, subtype 2. Transitional zone of a solid to a dispersed part. Fibrillar arranged deposits of fibronectin (*arrows*). Immunoperoxidase reaction for fibronectin. × 300

Fibronectin. In the primary tumours fibronectin was regularly detected with a similar distribution pattern in all primary tumours.

At the outer layer of tubular structures where generally myoepithelial cells were concentrated, and at the margins of solid areas with more dispersed pattern where myoepithelial like cells seemed to "flow" into the stromal area (Fig. 9), strong staining was seen on the aspect of myoepithelial cells which was directed towards the stromal area. The distribution of fibronectin showed a fibrillar, sometimes granular pattern. It could be found in the dispersed polygonal cells in the stroma, but to a lesser extent.

The basal membrane around the tubularly arranged cells was sometimes thickened, and had an intense staining for fibronectin. Some basal membranes around the tubules were negative. Fibronectin was generally found in the stroma, but only the myoepithelial cells were positively stained. The transplanted tumours showed a similar distribution pattern. The epithelial cells (duct-like cells) were negative or only stained very weakly for fibronectin. Myoepithelial like cells – at the border of the ducts or of the solid areas – showed a relatively intense staining for fibronectin. These cells were mostly found at the transition zone between epithelial and "stromal" differentiation.

Affinity histochemistry with lectins

Peanut Agglutinin (PNA). PNA "receptors" were detected in most pleomorphic adenomas which we analysed. They were generally seen in epithelial

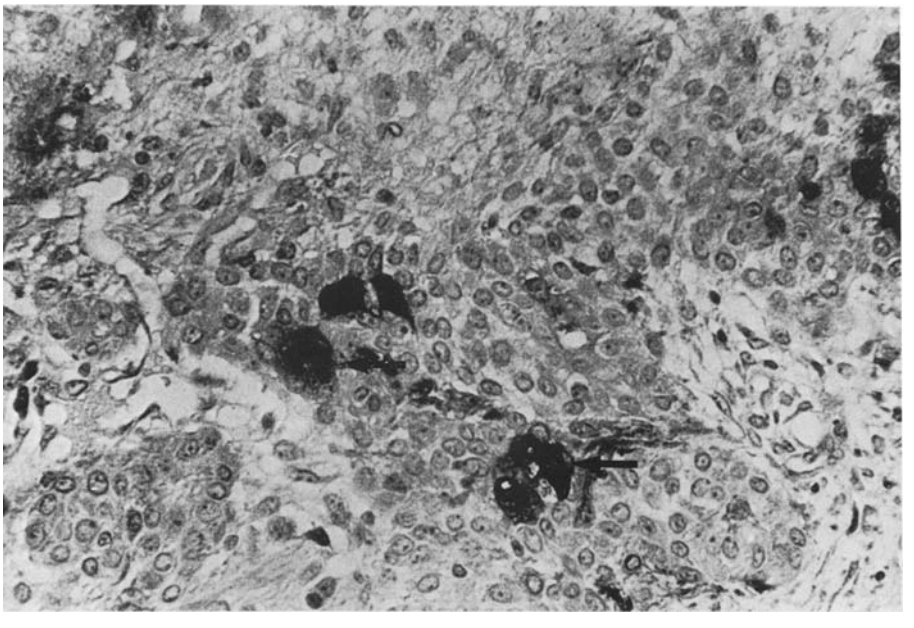

Fig. 10. Transplanted pleomorphic adenoma, subtype 1. Some PNA positive cells (*arrows*) in the solid part of the tumour. Affinity histochemistry with peroxidase labelled PNA. × 300

differentiated structures, but could also be observed in some areas of the stroma and in some dispersed cells. The duct cells showed generally a strong staining of the apical cellular membrane, other cells showed large positive vacuoles in the cytoplasm. The lumina of some tubules were positive.

Of the dispersed cells in the stroma, only a few were weakly PNA positive in the cytoplasm. A PNA positive halo was sometimes detected around the dispersed cells, and was attributed to a nonspecific binding mechanism. Treatment of the sections by neuraminidase detects those binding sites of PNA which are normally covered by neuraminic acid and in primary tumours, a similar pattern was observed to that obtained before the reaction, but the staining intensity and the number of positive cells was higher. Prominent vacuoles were observed in some cells. In the transplants, strong staining of the tubular areas was seen with cytoplasmic staining of some cells. A vacuolar pattern was observed more often than in the primary tumours. After treatment with neuraminidase, the same distribution pattern was observed, but there was generally a more intense staining in the tubular and in the solid areas (Fig. 10). The dispersed cells were generally negative or only very weakly stained.

Wheat germ agglutinin (WGA). In the primary tumours, a positive staining with WGA was only seen at the apical cellular border of the tubularly arranged cells. The solid and the dispersed areas were generally negative for WGA. Sometimes a weak fibrillar staining of the stroma was observed. The same pattern was observed after digestion with neuraminidase.

Table 6. Results in autoradiography

Type of tissue	Period of transplantation	3-H-Thymidine-positive cells
Normal salivary glands	more than 30 days	1.1%
Pleomorphic adenomas	less than 30 days	4%
Pleomorphic adenomas	more than 30 days	3%
Squamous cell carcinomas	more than 30 days	26%

In the transplanted tumours, the same structures were positive for WGA, namely the tubular areas of the pleomorphic adenoma. The dispersed and polygonal cells were negative or only very weakly stained. The same pattern was observed after digestion with neuraminidase.

Helix pomatia agglutinin (HPA). This lectin showed only a weak staining in the primary as well as in the transplanted tumours. Although its staining was strong in the ductal areas in both kind of tumours, some other areas were also positive. After digestion with neuraminidase, there was only little change in the staining intensity, but none in the distribution pattern.

Autoradiography

The labelling indexes of the different tissues are indicated in Table 6. In the normal tissue which was transplanted to nude mice we found a labelling index of about 1 percent.

Pleomorphic adenomas showed a slightly elevated labelling index of about 3%. This index was a little higher in the "younger" transplants (4%). The transplants showed a relative heterogeneity of the labelled areas. The most intensely labelled were the solid and the tubular epithelia, whereas in the more dispersed areas there was only about 1% labelled cells.

The labelling index of 2 squamous cell carcinomas contrasted with that of the pleomorphic adenomas with a value of more than 20%.

In some sections we analysed the labelling index of slides which were immunohistologically stained for TPA. The labelling index of the TPA positive cells was slightly higher than that of the other cells, but no statistically significant difference was found in this material. Generally, there were more positive cells for TPA than for 3-H-Thymidine.

Discussion

The histiogenesis of pleomorphic adenomas of the salivary glands has been the topic of many articles (for review see: Lang 1929; Mylius 1960; Hamperl 1970; Eversole 1971; Hübner et al. 1971; Batsakis 1980; Dardick et al. 1982; Dardick et al. 1983; Erlandson et al. 1983; Seifert et al. 1984).

There are few experimental investigations on pleomorphic adenomas since there are no appropriate animal models, but several other groups have applied either cell culture or tissue culture methods (Zymbal 1933;

Favata 1948; Kondo et al. 1971; Shirasuna et al. 1980) which had the disadvantage that the milieu in which the cells are investigated is artificial so that factors such as the interaction of epithelial and "stromal" compartments are lost. The nude mouse offers a new approach for the analysis of tumours under conditions which are much more similar to the natural milieu (Bastert et al. 1981).

Pleomorphic adenomas have rarely been transplanted into nude mice (Sharkey et al. 1978; Lindenberger 1981). Sharkey et al. (1978) reported that the transplants of pleomorphic adenomas showed a proliferative behaviour. In our material, we observed a "steady state" of long term transplants in good accordance with the clinical behaviour.

The growth curve of a pleomorphic adenoma resembles that of normal salivary gland tissue transplanted to nude mice rather than that of a squamous cell carcinoma (Sendler et al. 1984). The nude mouse model allows the possibility of labelling human material with 3-H-thymidine. In our experiment the labelling index of the long term transplants of the pleomorphic adenomas was only 3% with normal tissue showing a labelling index of about 1%. The squamous cell carcinoma, which we analysed for comparative reasons, had a labelling index of more than 20%.

The histological structure of the pleomorphic adenoma was generally preserved in the nude mouse transplants. In some cases there was a slight change of the tumour subtype (according to Seifert et al. 1976). The rare observation of a shift in the tumour subtype is interpreted as being the consequence of the given heterogeneity of the primary pleomorphic adenoma.

Immunohistologically, we analysed several *marker substances* in the tumours (Seifert and Caselitz 1983). *Keratins* are regarded as the markers of the epithelial nature of a given tissue. In diagnostic application, antibodies against keratin can be used to identify the epithelial nature of a given tissue (Schlegel et al. 1980; Gabbiani et al. 1981; Krepler et al. 1982; Osborn and Weber 1983). We found keratin in the primary and in the transplanted tumours with staining strongest in the ductal, in the solid and in the epidermoid parts of the tumours. Keratin could only be found in some dispersed cells of the stromal parts of this tumour. This is in accordance with our previous observations on salivary gland tumours (Caselitz et al. 1981c; Erlandson et al. 1983).

Amylase is detected in acinic cells (Kraus and Mestecky 1971; Korsrud and Brandtzaeg 1982) and is found in acinic cell tumours (Caselitz et al. 1983b). We could not detect amylase in the primary and in the transplanted tumours. Lactoferrin and lysozyme are generally found in the terminal duct system of the salivary glands. They are detected in the cells of the intercalated ducts as well as in the acinic cells (Reitamo et al. 1980; Caselitz et al. 1981b; Seifert and Caselitz 1983). In the primary and transplanted tumours, we detected these substances only focally, and lactoferrin was more often seen than lysozyme. The presence of this substance was generally noted in areas and was less evident in the transplanted tumours.

Immunoglobulins and secretory component have a special role in salivary glands (Brandtzaeg 1975; Roitt and Lehner 1980). The production of the IgA secretory component may be regarded to be a glandular function of the tissue. In the primary tumours, we found secretory component bound to the tubular differentiated areas where it was detected in the cytoplasm and the lumen of the ducts. Ig A was detected in similar areas of the pleomorphic adenoma, but generally to a lesser degree. Since the transfer of the pleomorphic adenoma to the nude mice does not affect the tumour itself, we detected secretory component in the same areas of the pleomorphic adenomas. The reaction for Ig A was generally negative as no human Ig A was produced by the tumour itself. We interpreted the presence of secretory component as the expression of a preserved glandular function in the pleomorphic adenoma.

Carcinoembryonic antigen (for review see: Uhlenbruck and Wintzer 1981) was only analysed by commercial antibodies. In the primary and in the transplanted pleomorphic adenomas, it was generally found in the tubular areas. The myoepithelial cells and the polygonal cells of the stromal area were generally negative for CEA. The general distribution pattern was similar to that of secretory component. Our observations are similar to those made previously (Caselitz et al. 1981 a, d).

Tissue polypeptide antigen was detected as a "common tumour antigen" by Björklund (Björklund 1980; Björklund and Björklund 1983; Lüthgens et al. 1980; Skryten et al. 1981). Caselitz et al. (1983 a) showed the presence of TPA in pleomorphic adenomas, especially in the tubular and in the solid areas, whereas the mesenchymal areas were negative. We saw a similar distribution of TPA in the primary and the transplanted tumours which we investigated. Thus, the distribution pattern of TPA was similar to that of keratin.

Simultaneous immunohistochemistry and autoradiography showed that many more cells were positive for TPA in the cytoplasm than were labelled by 3H-thymidine. The 3-H-thymidine positive cells (about 3%) were generally TPA positive. We interprete our observation to suggest that most proliferative cells are found in the solid and the tubular areas of the pleomorphic adenomas, and that TPA should be interpreted as a marker of differentiation.

There was a focal increase of *fibronectin* (review for fibronectin: Pearlstein et al. 1979; Ruoslahti et al. 1980; Klingemann 1982) in the pleomorphic adenomas, where it appeared to be arranged in coarse, sometimes granular bundles. In regions rich in fibronectin, spindle shaped or polygonal "myoepithelial" cells were seen with an apparent dispersion of these cells from the more solid areas of the tumour. This distribution pattern was found in the primary and in the transplanted pleomorphic adenomas.

In pleomorphic adenomas, there seems to be a specific disorder of the distribution pattern of fibronectin, which is focally augmented. Fibronectin which is often found in the vicinity of myoepithelial like cells may form

the substrate for the special biological behaviour of this tumour, with its tendency for recurrences. New studies in cytogenetics show that the gene locus for fibronectin is located in chromosome 8, where cytogenetic disturbances of many pleomorphic adenomas are found (Stenman 1985).

The distribution pattern of the staining with *lectins* (Nicolson 1974) varied greatly. Wheat germ agglutinin labelled the apical cellular membrane of tubular areas in the pleomorphic adenomas. In the solid areas, there was only a weak or no staining.

With pea nut agglutin (Klein et al. 1979; Newman et al. 1979; Böcker et al. 1984), we found a staining in the tubular areas, where the apical cellular membrane and the luminal contents were positive. In the solid areas of the pleomorphic adenomas, PNA labelled the cytoplasm primarily. In some cells, a vacuolar pattern was detected.

The distribution pattern of the marker substances which we analysed is similar in the primary and the transplanted pleomorphic adenomas. Part of these substances are bound to the tubular areas of the pleomorphic adenomas (lactoferrin, secretory component) and indicate a relationship to the terminal duct system of the normal salivary gland tissue. However, there was a specific disorder of the basal membrane as visualized by the presence of fibronectin which was correlated with myoepithelial like cells.

In accordance with other authors (Eversole 1971; Regezi and Batsakis 1977; Batsakis 1980) we think that the proliferative cell pool in pleomorphic adenomas is the duct like and indifferent cells of the solid and the tubular areas of tumours.

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