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# Specific Demonstration of Actin and Keratin Filaments in Pleomorphic Adenomas by Means of Immunoelectron Microscopy* **

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**Summary.** Human pleomorphic adenomas of the parotid gland were investigated by means of immunocytochemistry. Antibodies against the filaments of the actin and the keratin type were used on the light and electron microscopical level. For the immunoelectron microscopic procedure, the pre-embedding technique was applied. Actin and keratin were demonstrated in tumour cells. These observations provide further informations to the ambiguous nature of these neoplastic cells.

**Key words:** Pleomorphic adenoma – Actin – Keratin – Immunoelectron-microscopy

Filamentous systems e.g. actin and keratin filaments are major protein components present in cell populations of the normal parotid gland and in pleomorphic adenomas (Drenckhahn et al. 1977; Franke et al. 1980; Schlegel et al. 1980; Caselitz et al. 1981). These observations had been made on the light-microscopical level.

Hitherto ultrastructural analysis of filament systems in the parotid gland was restrained to the measurement of filament diameters in conventional electron microscopy (Hübner et al. 1971; Seifert and Donath 1976).

However, because of their wide variations in size and their capacity to aggregate these filaments cannot easily be defined by routine electron microscopy (Breathnach 1975; Hübner et al. 1971; Ghadially 1980).

The study of the distribution of these filaments needs further methods to identify the true nature of these systems on the level of electron microscopy.

For this reason immunoelectron microscopy would be a helpful tool to study the distribution of actin and keratin filaments in parotid gland tumours. Until now, immunoelectron microscopic investigations of filaments were concentrated on

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cultured cell systems (Osung et al. 1980; Henderson and Weber 1980; Willingham et al. 1981).

To our knowledge the present study represents the first immuno-electron microscopic study of actin and keratin filaments in pleomorphic adenomas.

## Material and Methods

Fresh tissue specimens of 3 pleomorphic adenomas were obtained from operations of the Ear, Nose and Throat Clinics of the University of Hamburg[1]. The material was chopped and immediately fixed in 3% paraformaldehyd for 4 h. After fixation the material was preserved in phosphate buffered saline over night (Schmitt et al. 1977; Schmitt 1979; Osung et al. 1980). Additionally 5 pleomorphic adenomas were investigated by immunohistochemistry in light microscopy.

*Specific Immune Sea.* Human antiactin serum was kindly provided by Dr. Astrid Fagraeus (Fagraeus and Norberg 1979).

Guinea-pig keratin antiserum was a kind gift of Prof. Thivolet (Viac et al. 1980a, 1980b).

Both antisera were used at the dilution 1 : 200.

*Peroxidase Conjugate.* Rabbit antihuman IgG conjugated with peroxidase was obtained commercially from Dakopatts (Copenhagen).

Goat anti-guinea-pig IgG conjugated with peroxidase was obtained commercially from Nordic Immunological Supplies.

Both antisera were used at a dilution 1 : 20.

*Immuno-enzymatic Procedure.* The light microscopic methods were described in previous papers (Löning et al. 1980; Caselitz et al. 1981). The minced slices of the tumours were labelled by the following technique (MacDonald et al. 1978; Gray et al. 1977).

1. Primary specific antiserum (3 h).
2. Link antiserum (2 h).
3. Fixation with 2% glutaraldehyde in cacodylate buffer (30 min).
4. DAB reaction (3,3 diaminobenzidine, Sigma) (20 min).
5. Postfixation with Osmiumtetroxid (20 min).

After each step the slices were centrifuged 10 min at 800 g to improve the penetration of immune sera and fixatives. At stage 1, 2 and 4 the samples were incubated at 37° C.

The specimens were thoroughly washed in phosphate buffered saline after step 1, 2 and 4 and in Tris HCl-buffer (pH 7.6) after step 3.

The material was dehydrated and embedded in Epoxy medium. Thin and ultrathin sections were cut with either glass or diamond knives on a Reichert Om-U-2 Ultramicrotome.

The grids were examined with a Zeiss Em 9 without contrast.

*Control Studies.* Control reactions were done by omitting the primary antisera and using normal sera instead (Osung et al. 1980). Absorption techniques and use of preimmune sera had been previously done (Viac et al. 1980a, 1980b).

## Results

### *1. Microfilaments of the Actin-Type*

*Light Microscopy.* Actin filaments could be demonstrated in pleomorphic adenomas. The tumour cells were stained positively by the immunoperoxidase technique (Fig. 1a).

[1] We gratefully appreciate the intense collaboration with Prof. Dr. Herberhold, Director of the Ear, Nose and Throat Clinics of the University of Hamburg

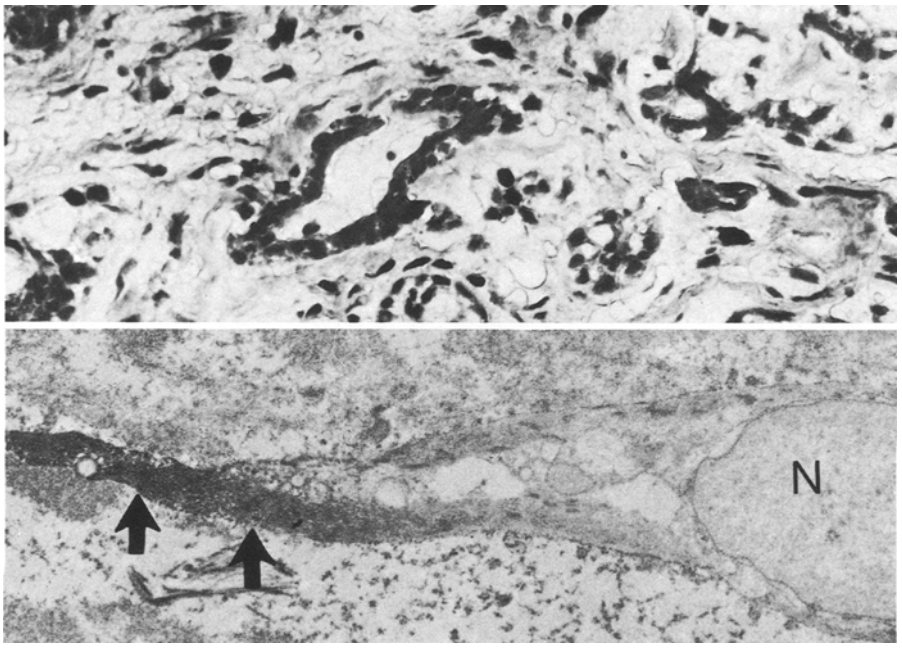


**Fig. 1. a** Pleomorphic adenoma in light microscopy. Positively stained tumour cells. Immunoperoxidase staining for actin. ×90. **b** Spindle shaped cell in pleomorpic adenoma. Densely arranged, intensely stained actin filaments at the periphery of the tumour cell (*arrows*). Non-stained nucleus (*N*). ×6,500

*Immunoelectron Microscopy*. The tumor cells showed a good immunoreactivity with actin antisera. Staining of bundles arranged according to the long axis of the cells was seen. The bundles were predominately located at the periphery of the cells and showed a concentration in the cytoplasmic elongations. Other cytoplasmic structures were not labelled at all (Fig. 1b). In the controls, no staining of the cytoplasmic filaments was seen.

### 2. *Intermediate Filaments of the Keratin-Type*

*Light Microscopy*. Keratin filaments, too, were observed in the tumour cells of pleomorphic adenomas (Fig. 2, inset) on the light microscopical level. There was a similar distribution as seen in the case of actin.

*Immunoelectron Microscopy*. Filament bundles of the keratin type were densely arranged in the periphery and sometimes in the centre of the tumour cells. In contrast to epidermal cells, the amount of keratin filaments was obviously lower. They were concentrated in distinct parts of the cell. The nuclei and other cell organelles did not react with anti-keratin sera (Fig. 2). In the controls, no staining of the cytoplasmic filaments was seen.

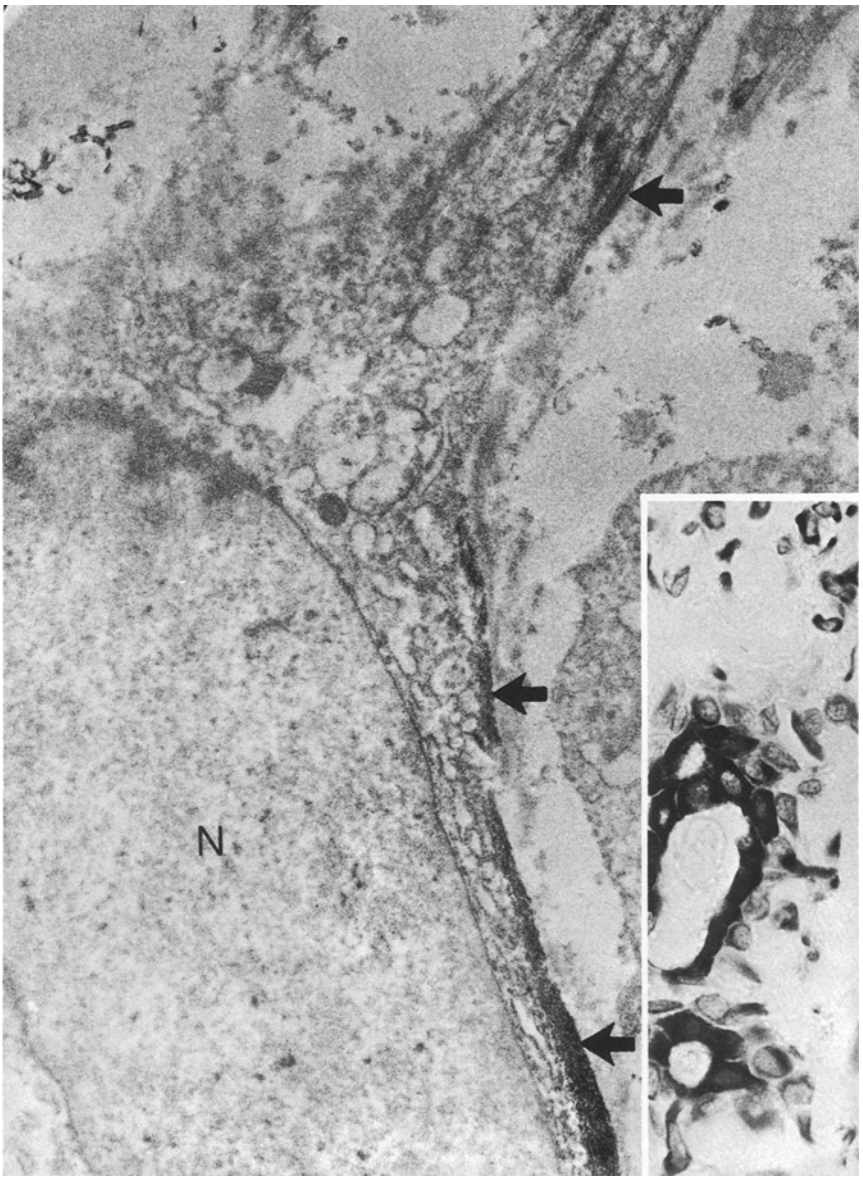


**Fig. 2.** Spindle shaped cell in pleomorphic adenoma. Densely arranged, intensely stained keratin filaments at the periphery of the cells (*arrows*). Non-stained nucleus (*N*). Immunoperoxidase staining for keratin. ×12,200. *Inset:* Pleomorphic adenoma in light microscopy. Positively stained tumour cells. Immunoperoxidase staining for keratin. ×340

## Discussion

Some technical aspects of the relevation of filamentous systems shall be discussed beforehand, since this study was concerned with entire tissue specimens and not with isolated cells.

The major problems arise from the accessibility of many antigens to the reagents and from the chemical interaction of the antigen with the fixative and embedding medium. In order to minimize these problems, we used the preembedding staining (Williams 1977; Sternberger 1979) using chopped tissue specimens for the incubation with the antisera. Since the tissue slides were minced by the procedure of chopping, the accessibility of the antisera seemed to be sufficient, although some of the membranes and the cellular cohesion may still represent a certain barrier (Gray et al. 1977).

In comparison with the postembedding staining, the preservation of the tissue and the minimal degree of pollution with reagents was an important argument for the preembedding procedure (Sternberger 1979). As fixative, paraformaldehyde seems to be the most suitable one (Sabatini et al. 1963; Heath and Dunn 1978), since Osung and coworkers (1980) have shown a good preservation of actin filaments in fibroblasts with this fixative.

Our studies have shown that immunoperoxidase findings with anti-actin and anti-keratin sera are readily reproduced with immunoelectron microscopy.

As for actin, we showed a concentration at the periphery of the tumour cells. This observation may correlate with some functional properties of the tumour cells (Gabbiani et al. 1975).

The peripheral distribution of actin filaments may be attributed to their function in the cell mobility, which is thought to be important in cancer cells (Gabbiani et al. 1975).

The presence of keratin-filaments in some tumor cells is a strong argument for their epithelial nature (Eversole 1971; Batsakis 1980; Caselitz et al. 1981).

Some labelled neoplastic cells resembled myoepithelial cells with regard to their elongated spindle-like shape and their filament content.

These cells are thought to be an important component of pleomorphic adenomas. The specific ultrastructural analysis of the cytoskeleton may help to elucidate the ambivalent properties of these cells which may be regarded not only as a reserve cell, but also as a differentiated epithelial cell sui generis.

Insofar, immunoelectron microscopy of the cytoskeleton provides new insights in cellular pathology.

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