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Actin Containing Cells in Normal Human Salivary Glands

An Immunohistochemical Study

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Summary. A study of actin distribution in human salivary glands was performed, using smooth muscle antibodies from a patient with active chronic hepatitis.

Immunoperoxidase labelling methods were found to give a good staining intensity for actin containing cells. The peroxidase antiperoxidase (PAP) method gave a stronger reaction than the double layer method, but the latter was sensitive enough for our purpose and was less time consuming. Sections from formalin fixed and paraffin embedded specimens were negative for actin staining while frozen sections showed good staining results. Sections from specimens which were washed for 12 h at 4° C showed less background staining.

Strong staining was found in myoepithelial cells lying around the acini, intercalated ducts and parts of the striated ducts. The number of myoepithelial cells around acini increased in the following order: the parotid gland, the submandibular gland, the sublingual gland and the small glands of the lip. This distribution indicates the importance of myoepithelial cells in the process of physical expression of saliva. Cytoplasmic staining in the basal epithelial cells of the striated ducts illustrates that these cells may be involved in some sort of secretion. This staining might also suggest a histogenetic origin for myoepithelial cells.

Key words: Immunohistochemistry – Salivary glands – Actin – Myoepithelial cells

Introduction

Many questions on the occurrence, nature and the function of the myoepithelial cells in human salivary glands are still unresolved. Tamarin (1966), Tandler (1965) and Hamperl (1970) have proposed that myoepithelial cells in rats arise

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from either secretory epithelial cells or reserve cells of the epithelium. Donath et al. (1978), however, have suggested that they arise in the postnatal phase in human parotid glands, by mitotic division of other myoepithelial cells.

The amount and arrangement of the cytoplasmic fibrils have been the basis for the suggestion that myoepithelial cells resemble myofibroblast (Archer and Kao 1968).

Myoepithelial cells of the salivary glands present a problem of identification at the light microscopical level (Mason and Chrisholm 1975; Young and van Lennep 1978; Seifert and Donath 1978). Many special staining procedures such as phosphotungsic acid haematoxylin (PTAH) and iron haematoxylin have been used but their specificity for myoepithelial cells seems to be relatively low (Mylius 1960). Alkaline phosphatases and adenosine triphosphatases have also been considered to have a high activity in myoepithelial cells (Young and van Lennep 1978) but Garrett and Harrison (1970) have shown that neither of these techniques could be regarded as an universal marker for salivary myoepithelial cells.

By using fluorescein labelled antibodies towards actin positive staining has been shown in myoepithelial cells in man (Line and Archer 1972) and rat (Drenckhahn et al. 1977) salivary glands. Smooth muscle antibodies (SMA) occur in patients with active chronic hepatitis and are specific against actin. They mainly consist of the IgG class (Lidman et al., 1976) and have clear value in this type of study. Immunofluorescence has been used in most of the studies to demonstrate actin (Archer et al. 1971; Bottazzo et al. 1976; Gabbiani et al. 1976) but immunoperoxidase methods have distinct advantages over similar methods using fluorescein conjugated antibodies. The peroxidase preparations can be stored indefinitely and better correlation with the light microscopical morphology can be made. Additionally these techniques can be applied in the immuno-electron microscopy (Sternberger 1979). The present investigations were undertaken to study the myoepithelial cells in normal human salivary glands by means of SMA serum and peroxidase labelled antibody techniques and to find a method suitable for further studies of pathological lesions in salivary glands.

Materials and Methods

Both frozen and formalin fixed material from 6 patients which were operated on for neoplastic lesion in the mouth were studied. The parotid, submandibular and sublingual glands were studied together with small glands from the lower lip.[1] Specimens from human uterus and striated skeletal muscle were taken from autopsy material.

Small blocks from these tissues were made and prepared in the following ways:

1. Some were directly frozen at $-140°$ C precooled isopentane in liquid N_2.

2. Some were placed in gauze and washed in PBS at $+4°$ C for 12 h with slow rotation in the washing bath. The blocks were then frozen as for 1.

3. Some directly frozen blocks (as 1) were thawed and washed as 2.

4. Some blocks were fixed in buffered formalin (less than 4 h), dehydrated and infiltrated with paraffin keeping the temperature below 58° C, and stored at $-20°$ C.

5. Routine fixation in formalin and infiltration in paraffin were also performed on some blocks.

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1 We thank Dr. R. Schmitz, Nord-Deutsche Kieferklinik der Universität, Hamburg for the possibility to get fresh salivary glands

Frozen sections (7 μm) were cut from the first 3 groups and the sections were airdried in 30 min before incubation. Sections (7 μm) were cut from the paraffinblocks and deparaffinized in xylol and ethanol. The rehydrated sections were airdried and incubated in the same manner as the frozen sections. Parallel sections were always stained with haematoxylin and eosin.

Sera. The following sera were used:

1. Serum with smooth muscle antibodies (SMA) from a patient with chronic active hepatitis[2]. Titre against rat smooth muscle was 256. Tests for antimitochondrial and antinuclear antibodies were negative. The working dilution was found by chessboard titration to be 1:16. Both serum heated to 56° for 30 min and unheated serum were used.
2. SMA serum was absorbed with an homogenate of human uterus and used as in 1.
3. Peroxidaselabeled rabbit antihuman IgG (γ chain) (Dako-immunoglobulins P 1090) was used in the second layer. This serum was diluted 1:8 or 1:16 according to chessboard titration.
4. FITC labeled rabbit antihuman IgG (γ chain) (Dakoimmunoglobulins F 1090) was used to block the binding of the peroxidase labelled IgG and in the second layer in the PAP reaction. The working dilution was 1:8 or 1:16.
5. Goat antirabbit gammaglobulins (Behringswerke). Working dilution 1:13.
6. PAP complex (Behringwerke). Working dilution was 1:20.
7. Normal pooled human serum (NHS).

Incubation. 1. A double layer technique with SMA serum in the first layer and the peroxidase labelled rabbit antihuman IgG in the second layer was used. Incubation times were 45 min and the sections were washed 3 times for 10 min with PBS between each step. After the last washing the sections were incubated for 2 min in DAB (Graham and Karnovsky 1966) and the sections were postfixed in 1% OsO_4 in PBS for 15 min, dehydrated and mounted. Sections without postfixation were also used.

2. *Multilayer method (PAP)* (Sternberger et al. 1970) was performed with the following steps:

1. SMA (1:16 dilution)
2. Rabbit antihuman IgG FITC labeled antibodies (RAH) (1:16 dilution)
3. Goat antirabbit gammaglobulin (1:3 dilution)
4. PAP (1:20 dilution)

Washing procedures and treatment after the last incubation were as for the double layer method. Sections were examined without contrast staining or with a weak haematoxylin stain.

Controls for specificity were performed on the following tissues: 1. smooth muscle of human uterus, 2. small vessels in the sections of salivary gland, 3. Human skeletal muscle.

The binding of FITC conjugated RAH in the controls and the second layer in the PAP reaction were controlled in a transmitted fluorescence light microscope (Leitz: Filter 3 mm BG12 and K 520) before further incubation.

Results

The Methods

The staining pattern of actin could easily be correlated with the morphology of the gland both with or without counterstaining. The staining intensity was strongest with the PAP technique while the double layer method was sensitive enough for the detection of myoepithelial cells.

Washing of blocks reduced the background staining in the sections. This effect was most effective when the blocks were thawed first. After the prewashing some autolytic changes were seen, however. The strongest background was found in the fibrous septae of the glands and perivascularly. Paraffin embedded material showed negative staining for actin in all cases.

2 We wish to thank Dr. R. Matre, Broegelmann Research Laboratory for microbiology, Haukeland Hospital, Bergen, for the possibility to get the SMA serum

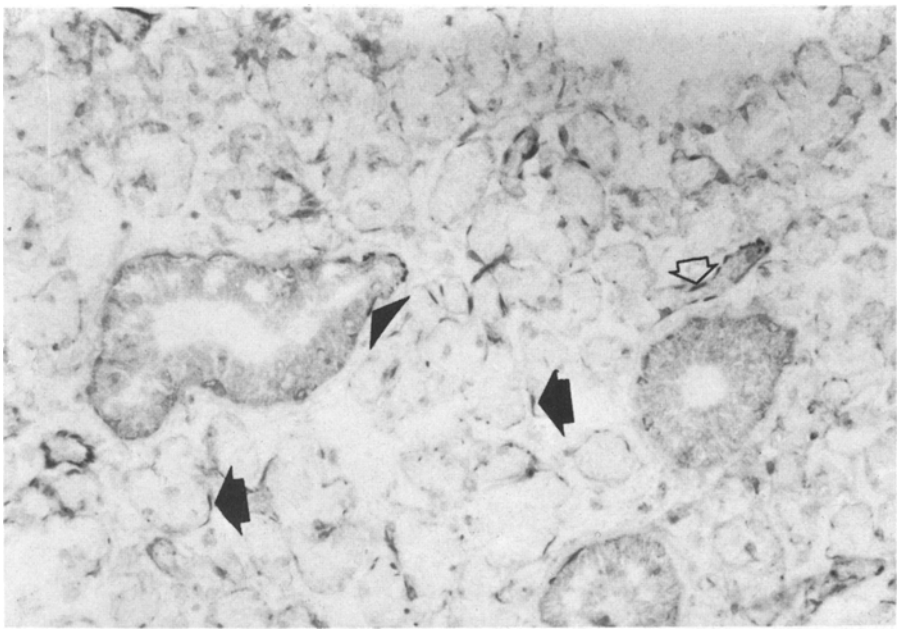

Fig. 1. Human parotid gland. SMA in a double layer method with and 20 s in haematoxylin. Actin containing cells around acini (*arrows*), intercalated duct (*open arrow*) and striated duct (*arrowhead*). Note positive cytoplasmic staining in the cells of striated ducts. (× 150)

Salivary Glands

The Parotid Gland (Fig. 1). Elongated strongly stained cells were found surrounding the acini. Sections through acini with areas lacking myoepithelial cells and transverse cuts of projections suggest a loose network of cells surrounding the acini. The acinic cells were not stained. Elongated peroxidase stained cells covered the intercalated ducts and seemed to be orientated along these ducts. The epithelium of the intercalated ducts stained moderately.

The elongated cells were also regularly observed in the peripheral part of the striated ducts, but striated ducts without myoepithelial cells in the periphery were also found.

The basal layer of the striated ducts and some bigger ducts regularly showed cuboidal strongly stained cells while the rest of the epithelial cells of these ducts stained more moderately. These strongly stained basal cells varied in number.

The Submandibular Gland (Fig. 2). The staining pattern resembled that of the parotid gland. There was, however, a more continuous anastomosing layer of elongated cells around the acini and the intercalated ducts. Such cells were found less regularly in the striated ducts of the submandibular gland than in the parotid gland. There was no staining in acinic cells while cells of the striated ducts had positive cytoplasmic staining. Strongly stained basal cells were also found in larger ducts.

Fig. 2. Human submandibular gland. SMA double layer method. Actin containing cells in the periphery of acini (*arrows*) and intercalated ducts (*open arrow*). (× 380)

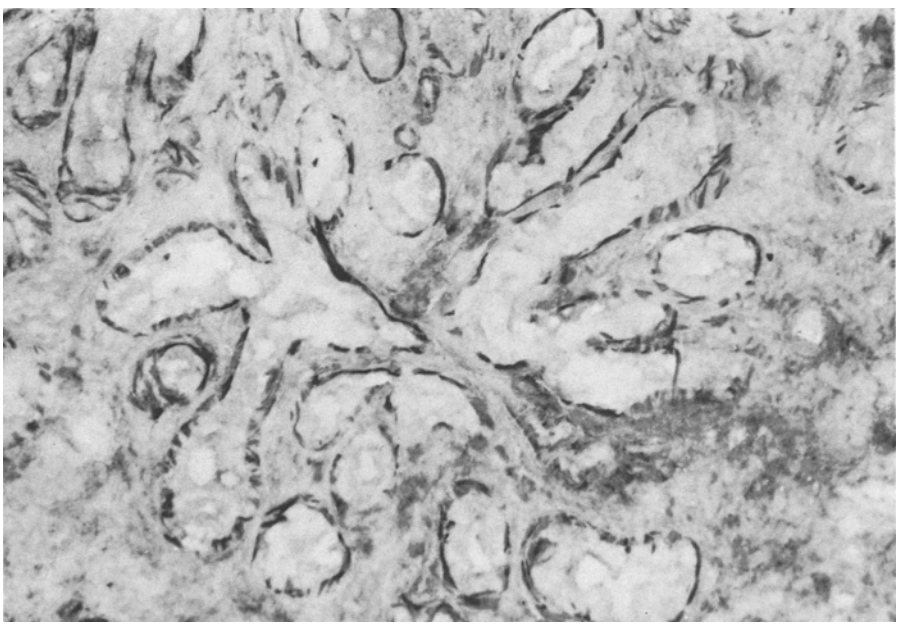

Fig. 3. Human sublingual gland. SMA in double layer method. Actin containing cells in the periphery of acini and ducts. (× 150)

Table 1

1. Incubation	2. Incubation	Peroxidase staining results
1) SMA	RAH peroxidase labelled	+
2) PBS	RAH peroxidase labelled	–
3) NHS	RAH peroxidase labelled	–
4) SMA_2	RAH peroxidase labelled	–
5) SMA	RAH FITC labelled before the RAH peroxidase labelled	–

The Sublingual Gland. There was an increased number of myoepithelial cells around acini and intercalated ducts when compared with the submandibular gland. The acinic cells showed no staining for actin, while the epithelial cells of the striated ducts had the same staining as the parotid gland.

The Small Glands of the Lip. These exhibited many elongated myoepithelial cells lying in the periphery of the acini and intercalated ducts.

In all glands smooth muscle cells of small vessels served as an intrasectional control. The results from the controls are shown in Table 1.

For the PAP method controls for specificity were performed in the same way as the double layered method, and gave the same results.

Discussion

The SMA serum seemed to have a high specificity for smooth muscle and myoepithelial cells. The specificity of SMA serum towards actin has previously been extensiviely studied by Lidman et al. (1976), who concluded that these sera were suitable for studies of actin containing cells.

Other methods to stain myoepithelial cells have been tried but the specificity and usefulness of these methods seem not to be as good as expected (Garrett and Harrison 1971). The use of peroxidase labelled antibodies gives several advantages in comparison with fluorescein labelled antibodies. Staining patterns are easily compared with the morphology and the sera are also stable during storage, which is the main disadvantages of fluorescein labelled antibodies. The present methods should therefore be suitable for studies of myoepithelial cells in inflammatory and neoplastic lesions in the salivary glands.

Demonstration of myoepithelial cells has previously been made on a morphological basis, or more recently by means of immunofluorescence techniques with antiactin and anti-actomyosin antibodies in rat salivary glands (Archer and Kao 1968; Line and Archer 1972; Drenckhahn et al. 1978). Bussolatti et al. (1980) have also stained material with peroxidase labelled antibodies in human glands. Our results in human salivary glands showed an easier correlation

between morphology and actin staining with peroxidase labelling than with immunofluorescence techniques.

The present results from salivary glands showed three types of stained cells: – 1. the elongated myoepithelial cells lying peripherally in the intercalated ducts, some of the striated ducts and the acini, 2. the more cuboidal stained cells in the basal part of the striated and larger ducts, and 3. the epithelium of the striated ducts. The staining for actin in the cells in the periphery of the acini and intercalated ducts showed a basket like pattern of cells. This distribution seems to be in good correlation with other light and electron-microscopical studies (Mylius 1960; Hamperl 1970; Donath 1976).

The increased number of myoepithelial cells in the parotid gland and progressive reduction in the submandibular, sublingual and the small glands of the lip, may illustrate the importance of myoepithelial cells in the propulsion of saliva, and may also be related to a decrease in additional muscle surrounding the glands and the increase in the viscosity of the saliva. Localization of these cells in acini and intercalated ducts seems to be in keeping with an immunofluorescence study with anti-actomyosin (Archer et al. 1971). The present study, however, also showed myoepithelial cells in the striated ducts of human parotid gland. This localization has previously been questioned (Garrett and Emmelin 1979) although it has been suggested from PTAH staining in the parotid gland (Mylius 1960). Actin containing cells in the ducts have been found in an early period of development in rat parotid gland (Line and Archer 1972) and also postnatally in the striated ducts (Caselitz et al. 1980). The myoepithelial cells may survive and subsequently proliferate during certain pathological processes in the salivary glands, and explain the finding of myoepithelial islands. Boquist (1970), however, was not able to find intermediate myoepithelial cells in ducts in a case of benign lymphoepithelial lesion. The "clear cells", however, which he has found in the epithelial islands resembled the cells which Tandler (1965) and Hamperl (1970) have described and thought might be able to transform to myoepithelial cells.

The positive staining which was observed in the cytoplasma of the epithelial cells of striated ducts is difficult to interpret. On the one hand it might reflect a possible relationship to myoepithelial cells (Caselitz et al. 1980), on the other hand it might be due to the synthesis and transport functions of these cells. The latter function has been proposed in relationship to actin in neural (Burridge and Philips 1975) and bone cells (Nilsen 1980).

The distribution of myoepithelial cells in salivary glands, demonstrated by immunoperoxidase techniques, seems to correlate well with electron-microscopic reports. These techniques should prove to be useful in further studies in inflammatory and neoplastic lesions of the salivary glands.

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