**VIMENTIN CHARACTERISTICALLY EXISTS IN GRANULAR
CONVOLUTED TUBULES OF HAMSTER
SUBMANDIBULAR GLANDS
—IMMUNOHISTOCHEMICAL STUDIES ON COMPARATIVE
LOCALIZATION OF FILAMENT PROTEINS
IN SALIVARY GLAND DUCTS—**

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Immunocytochemical detection of vimentin class of filament proteins except for keratin is reported in ductal segments of rodent submandibular glands (SMGs) and compared to stainabilities of different ductal systems; intercalated duct (ICD), granular convoluted tubule (GCT), striated duct (SD) and excretory duct (ED), and in the major salivary glands; parotid (PG), submandibular (SMG) and sublingual glands (SLG). Filament proteins; vimentin, desmin, filamin, actin and myosin were examined by immunocytochemical technique. Vimentin characteristically was confined to the GCT cells of hamster SMGs, and was distributed in luminal surface of the SD cells of guinea pigs, where it was negative in the SMGs of rats and mice, but positive in SLG duct cells. Vimentin was also positive in the ICD cells of hamster SLG.

Desmin was slightly positive in the duct cells of hamsters, and intensely positive in luminal side of the SD cells of SMG and PG in guinea pigs, and of SLG in rats. Trace amounts of desmin were also noted in the GCT cells of mice and rats.

Filamin was strongly positive in smooth muscles of blood vessels and trace or slightly positive in ductal cells from all the specimens. Actin was also strongly evident in smooth muscles, and slightly reactive to hamsters GCT cells, guinea pigs, and rats ductal cells and was negative in mice. Myosin was slightly positive in the GCT cells of hamsters and in the SD cells of guinea pig.

The constitution of cytoskeleton or cytoplasmic matrix is composed of three fiber systems, microfilaments, microtubules and intermediate filaments, hence immunocytochemical studies of those fibrillar structures are well represented in many cells and tissues. Intermediate sized filament proteins are generally classified into keratin (40,000–68,000) which specifically distributed in epithelial cells (4, 10), vimentin (58,000) in mesenchymal cells (3, 5, 10) and desmin (53,000–56,000) in muscular cells (10, 15). Keratin is associated with heteropolymers, vimentin and desmin homopolymers. Microfilaments (actin, filamin, myosin) are formed in an average diameter of 60Å and have function stress fibers that distribute network at

the cellular periphery which may be related to cell motility and movement (9, 10, 13, 14).

Keratin polypeptides were demonstrated by use of antikeratin antiserum in salivary glands, ductal epithelia of intercalated duct (ICD), striated duct (SD) and excretory duct (ED), however, granular convoluted tubule (GCT) cells of submandibular glands (SMGs) do not contain keratin proteins (20). In GCT cells in murine SMGs (mouse, rat, and hamster) particular structures exist for biosynthesis and storage of salivary gland growth factors (EGF and NGF), renin, kallikrein and esteroprotease (1, 7, 8, 12, 18). The preceding papers have reported that ductal segments of hamster SMGs were crossreacted with mouse EGF (11) and slightly positive to keratin proteins from immunohistochemical method (20). However, filament protein localizations excluded in keratins in hamster salivary glands as well as rodent glands have not been pointed out previously. The present study was undertaken to observe comparatively the immunohistochemical detectable filament proteins such as vimentin, desmin, actin, filamin and myosin in ductal segments including GCT cells from different kinds of rodent specimens; hamsters, guinea pigs, rats and mice. The main purpose of this investigation was done to identify the immunohistochemical distributions of those different types of filament proteins, including either microfilament or intermediate filament proteins, except for keratin polypeptides in ductal segments. It has been pointed out that vimentin and desmin are distributed in smooth muscle and fibrous cells (3, 5, 10, 15, 17) and actin, filamin and myosin in muscle fibers and sometimes it has been noted to co-exist among them (9, 10, 13–15), however, no report has been made concerning the histochemical localization in the salivary gland.

MATERIALS AND METHODS

Animals: Adult male hamster (Syrian), guinea pig (Hartley), rat (Wistar) and mouse (ddY) were examined.

Salivary Glands: The three major salivary glands were extirpated immediately after sacrifice (12 hr after fasting).

Tissue Sections: Salivary glands were cut into small blocks and fixed in 10% formalin for 12 hr at 20°C, or Bouin's solution for 12 hr at 4°C. Serial sections of 4 μ m were made to detect vimentin, desmin, actin, filamin and myosin.

Immunohistochemical Methods: Deparaffinized sections were; 1) immersed in a 0.3% H₂O₂/methanol solution for 20 min to inactivate endogenous peroxidase activity, and rinsed in phosphate-buffered saline (PBS) for a short time. 2) immersed in normal swine serum (1 : 20, Dakopatts, Denmark) for 30 min. 3) reacted with rabbit-antivimentin (1 : 100), antidesmin (1 : 250), antiactin (1 : 400), antifilamin (1 : 200) and antimyosin (1 : 400) for 60 min in a moist chamber at 20°C. All antigens purchased from Transformation Research, Inc., USA. 4) rinsed in PBS for 15 min and reacted with swine anti-rabbit IgG (1 : 50, Dakopatts, Denmark) for 30 min. 5) rinsed in PBS for 15 min and then reacted with peroxidase anti-peroxidase complex (PAP, 1 : 200, Dakopatts, Denmark) for 30 min in moist chamber. 6) rinsed in PBS and immersed in diaminobenzidine (DAB) solution for 10 min, and then dehydrated and mounted.

According to documentation of Transformation Research, Inc., vimentin was obtained from cultured human fibroblasts and purified vimentin did not stain microfilaments and microtubules. Antigen source of desmin was chicken gizzard and antidesmin serum showed no reaction to fibroblasts and epithelial cells. Microfilaments, actin, filamin, and myosin were purified from chicken gizzard and chicken breast muscle (myosin), and antisera were crossreacted with skeletal and cardiac muscle of mammals and aves.

RESULTS

Vimentin: GCT cells in hamster SMGs were characterized by the existence of strong vimentin staining (Fig. 1). SD cells of the PG, SMG and SLG were moderately reactive to vimentin (Fig. 2), and ICD cells of the SLG were strongly reactive. In the guinea pig salivary glands, staining intensities to vimentin were usually slight to moderate in SD, whereas they were strong in the luminal surface of the SD (Fig. 7). In the rats and mice, vimentin staining in GCT and SD cells of SMGs were trace (Figs. 11, 13).

TABLE 1. *Immunohistochemical detection of vimentin, desmin, actin, filamin, myosin in salivary glands*

	Vimentin	Desmin	Actin	Filamin	Myosin
Hamster					
PG SD	1	1	0	0	0
SMG GCT	4	1	1-2	1-2 (muscle 3)	1
SD	1	1	0	0	1
SLG SD	1 (ICD 4)	1	0	0	0
Guinea pig					
PG SD, ED	1-2 (luminal 3-4)	1-2 (luminal 3)	± (muscle 3)	0 (luminal 2) (muscle 3)	1 (luminal 2)
SMG SD, ED	1-2 (luminal 3-4)	1 (luminal 2-3)	± (muscle 3)	0	0
SLG SD	1-2	1	1	±	±-1
Rat					
PG SD, ED	±	±	±	±	0
SMG GCT	±	±	1	1	0
SD, ED	±	±	±	±	0
SLG SD	1 (luminal 3)	1 (luminal 4)	1	± (muscle 4)	0
Mouse					
PG SD	±	±	±	0	0
SMG GCT	±	±	±	0	0
SD	±	±	± (muscle 2)	0	0
SLG SD	1	2	±-1	0	0

0: negative, ±: trace, 1: slight, 2: moderate, 3: strong, 4: strongest

FIGS. 7-10. Male Guinea pig SMG. $\times 200$

FIG. 7. Strong vimentin reaction exists in luminal side of the SD cells.

FIG. 8. Positive desmin reaction is located only luminal surface of the SD cells.

FIG. 9. Strong actin reaction is found in the smooth muscle of blood vessels, but negative in ductal and acinar components.

FIG. 10. Slight-to-moderate myosin reaction is limited to luminal border in the SD cells.

FIGS. 1-6. Male hamster. $\times 200$

FIG. 1. Significantly high vimentin staining is confined to the GCT cells.

FIG. 2. Moderate vimentin staining is found in the SD and ICD cells of the SLG.

FIG. 3. Desmin staining.

FIG. 4. Filamin staining.

FIG. 5. Actin staining.

FIG. 6. Myosin staining. There are slight reaction in the GCT cells.

Figs. 11, 12. Male rat SMG. $\times 200$

FIG. 11. Vimentin staining.

FIG. 12. Filamin staining. There is trace-to-slight reaction in the GCT cell.

Figs. 13, 14. Male Mouse. $\times 200$

FIG. 13. Mouse SMG lacks vimentin staining.

FIG. 14. Moderate desmin reaction in the SD cells of SLG.

Desmin: Epithelial cells from the hamster ductal system including GCT cells were slightly positive to desmin staining (Fig. 3). Ductal epithelia of the guinea pig showed slight desmin staining, and the luminal border of the SD cells displayed a comparatively intense staining (Fig. 8). Desmin reaction to the GCT and ductal cells in rats and mice was only trace, however, the luminal side of the SD cells in rats SLGs showed a high staining reaction (Fig. 14).

Actin: Hamster GCT cells were only slightly to moderately positive to actin reaction and other ductal segments were negative (Fig. 5). Ductal epithelia of the guinea pig specimens were observed to be negative to trace in actin staining and the smooth

muscle of blood vessels magnified strong reaction (Fig. 9). GCT cells of rat SMGs displayed a very weak staining, whereas those in mice were trace or slight in their amounts. Muscle layer and the myoepithelium indicated a strong positive reaction to actin.

Filamin: Hamster GCT cells revealed a slight-to-moderate filamin staining which was similar to that of actin staining (Fig. 4). The smooth muscle showed a marked staining to filamin. SD cells of the guinea pigs showed an almost negative response while the luminal side of ductal epithelia was moderate. Ductal segment in rat salivary glands was negative or trace, and GCT cells showed only a slight reaction to filamin (Fig. 12). Mouse glandular ducts showed negative results.

Myosin: Myosin staining was localized in hamster GCT cells (Fig. 6), and in guinea pig SD cells with a slight degree of myosin staining (Fig. 10). Epithelial elements in ducts from rats and mice were not reactive to myosin staining.

In conclusion, vimentin, desmin, actin, filamin and myosin distributions in rodent salivary glands, particularly the GCT cells from hamster SMG, characteristically indicated the highest staining to vimentin, and they showed a comparatively slight staining to desmin, actin, filamin and myosin. Guinea pig salivary glands have no GCT cells in SMGs, however, vimentin and desmin were distributed in luminal border of SD cells. GCT cells from rats SMG only showed actin and filamin staining with slight or moderate levels. The ductal system, including GCT cells of mice SMG, showed negative-to-slight evidence of 5 kinds of filament proteins.

DISCUSSION

GCT cells in the salivary glands are unique cells which markedly exist in the SMG in mature male mice, and secretory granules are the main source of salivary gland growth factors such as EGF and NGF (1, 8, 12). GCT cells in rodent SMGs were found in mice, rats and hamsters, and EGF also exists in the rat GCT cells, however, the biological roles of hamster GCT cells are not yet understood. As compared with other rodent SMGs, GCT cells in matured mice were the most well developed, as well as rich in biological active peptides, whereas keratin proteins did not exist in these granules (20) and the hamster GCT did not contain many polypeptides (11). However, the vimentin type of filament proteins had a significantly strong reaction, in addition to filament proteins of desmin, actin, filamin and myosin which were slightly positive in hamster GCT cells. In general, under biochemical and immunocytochemical investigation of intermediate sized filament proteins, keratin filaments characteristically occurred in epithelial cells (4, 16, 19), vimentin in mesenchymal cells and desmin in smooth, skeletal and cardiac muscle cells (10, 15). Contractile proteins; actin, filamin and myosin participated strongly in cellular movements and contractions (9, 10, 13, 14, 15). In reviewing the excellent reports of such biochemical studies, no report was observed concerning GCT cells from hamster SMGs having abundant positive reaction to vimentin.

Immunocytochemical detectable keratin in salivary glands has described in the ductal epithelia except for GCT cells that displayed marked staining (2, 4, 16, 19), whereas GCT cells were devoid of keratin reaction (20). It is very interesting to know why GCT cells in the SMGs of mice, rats and hamster were negative or very weak to keratin proteins. On the contrary, vimentin was confined very strongly to

GCT cells of hamster SMG, and desmin, actin, filamin and myosin also showed a little slight staining in the GCT cells. It has been previously reported that two or more filament proteins were found in co-existence with the same cells (10). It has been concluded therefore that vimentin and desmin may co-exist in the muscular cells (6, 21). The simultaneous existence of vimentin, with other filament proteins which have a slight reaction in hamster GCT cells were probably related to the co-existence phenomenon.

It has been described that vimentin filaments are found particularly in the perinuclear area or nuclear membrane of the cells, and keratin proteins are significantly abundant near desmosomes in epithelial cells. Lazarides (10) speculated that vimentin distribution in nucleation area may be regulated by proteolytic mechanism rather than by polymerization-depolymerization equilibrium though vimentin is insoluble in buffers and highly susceptible to proteolysis. Vimentin in ductal cells of guinea pig SMG has been shown to be strongly deposited in luminal side. Kallikrein like enzyme and proteolytic activity with strong levels have been reported in salivary gland duct cells (7, 18), however, no detailed report has been made in GCT cells of hamster SMG.

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