An immunohistochemical study was undertaken to determine the localization of involucrin in human salivary gland lesions and tumors. Normal glandular tissues and those with obstructive sialadenitis were negative for involucrin staining. In pleomorphic adenomas, the luminal surface of tubular, ductal, duct-like, and cystic structures was positive for involucrin, and some cells lining the cavities of these structures were also positive. In the outer layer of the duct-like structure, spindle-like cells having long anastomosing processes were negative. Squamously metaplastic cells and cells with ongoing keratinization strongly indicated the presence of involucrin. In Warthin's tumor specimens, cells strongly positive for involucrin were found, though rarely, scattered around the eosinophilic tumor epithelium. Immunohistochemically detected involucrin may be a specific marker for detecting squamous metaplasia or keratinizing change in the epithelia of salivary gland tumors.

Involucrin has been identified as a 92 kDa precursor of the cross-linked envelope protein found in human stratum corneum (1, 7). Studies on the immunohistochemical localization of involucrin have shown that cells of the basal and parabasal layers of squamous epithelia lack the protein, while lower spinous layer cells and those with ongoing keratinization show increasing stainability (1, 5, 15). Involucrin is considered a useful marker for both squamous differentiation and terminal keratinization. Various previously published histochemical data point to its presence in squamous-cell carcinomas or other epithelial tumors of the skin, oral cavity, cervicovaginal mucosa, lung, and urogenitalia (5, 8–10, 13, 14).

In several kinds of salivary gland lesions, including chronic sialadenitis due to sialolithiasis and obstruction, and in benign tumors such as pleomorphic adenomas, ductal epithelial cells may be transformed into squamous metaplastic cells displaying varying degrees of keratinization. In the present study, the immunohistochemical localization of involucrin in paraffin sections of specimens from chronic obstructive lesions and benign tumors of human salivary glands were examined. Furthermore they were compared with the keratin distribution patterns in salivary gland lesions and tumors already reported (2–4).
MATERIALS AND METHODS

Materials

A total of 60 cases of salivary gland lesions including sialadenitis (19 cases) and benign tumors (41 cases) were examined. The specimens of sialadenitis were obtained from the submandibular gland due to sialolithiasis. Histologically normal minor salivary glands and lip mucosa adjacent to mucous retention cyst (mucocele) were also tested. Salivary gland tumors consisted of pleomorphic adenomas (38 cases) and Warthin’s tumors (3 cases).

The materials were obtained immediately after surgery, fixed in 10% formalin solution for 12 hrs, and embedded in paraffin. Paraffin sections at 4 μm were prepared for immunohistochemical detection of involucrin.

Immunohistochemical Method

An INVOLUCRIN IMMUNO-KIT, which was purchased from Biomedical Technologies Inc., Cambridge, USA, was used to detect involucrin immunohistochemically. Deparaffinized sections at 4 μm thick were treated with methanol solution containing 0.3% H₂O₂ for 20 min for inactivation of endogenous peroxidase and then rinsed well. Next, the sections were treated with normal goat serum for 30 min and blotted with filter paper. They were reacted with rabbit anti-human involucrin for 1 hr and rinsed 3 times in PBS. The sections were then treated with HRP-labeled goat anti-rabbit immunoglobulin antiserum for 30 min and rinsed 3 times in PBS. Finally, they were immersed for 5 min in 0.05 M Tris buffer (pH 7.6) containing 0.03% 3,3’-diaminobenzidine tetrahydrochloride (DAB) and 0.005% H₂O₂. These reactions were done at room temperature. In this involucrin immuno-kit highly specific involucrin antibody is employed and the specificity is confirmed by immunostaining of normal skin and mucosa (11).

Control Method

Normal rabbit serum (1 : 20 dilution; Wheaton, USA) was used in place of rabbit anti-human involucrin as a control. The control were negative.

RESULTS

Normal Salivary Glands

Glandular components of both major and minor normal salivary glands were negative for involucrin staining, irrespective of their histologic nature (mucous or serous acinar or ductal segments).

Obstructive Sialadenitis

Histologically, this type of lesion was characterized by acinar atrophy and ductal enlargement in the early stage and with fibrous replacement in the late stage. Histologic changes were dependent on the progress of the obstruction. In general, the early stage of sialolithiasis showed rather marked cellular infiltration, while in the late stage, severe atrophy of acinar compartments and duct-like structures or cystic dilatation of ductal segments were evident, with or without inflammatory cells. Involucrin was not detected in any cellular structures during the course of obstructive sialadenitis.

Mucocele in Oral Mucosa

Mucous acinar cells and ductal segments showing moderate cystic dilatation
Involucrin in Salivary Gland Lesions and Tumors

Figs. 1A-D. Involucrin staining in tubular, duct-like and cystic structures of pleomorphic adenoma.

Fig. 1A. Epithelial cells of duct-like structures show strongly positive involucrin staining. ×100

Fig. 1B. Luminal border in small tubular space shows slight by positive staining. ×200

Fig. 1C. Luminal surface of ductal cavities shows positive involucrin staining. ×200

Fig. 1D. Some of the cystic epithelium cells are positively stained. ×200
with epithelial proliferation were devoid of detectable involucrin in all areas of the retention cyst.

**Pleomorphic Adenoma**

The salivary pleomorphic adenomas consisted histologically of epithelial strands and sheets with tubular, ductal, duct-like, cystic, and stromal tissue. Squamous metaplasia was occasionally observed. The epithelial cells in the

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Figs. 2A–D. Mucoepidermoid change and sebaceously metaplastic area. ×80

Fig. 2A. Mucoepidermoid transformation areas; sebaceous metaplasia and solid epithelial structures.

Fig. 2B. Serial section of Fig. 2A. Squamously differentiating regions show marked involucrin staining, and glandular or solid tumor structure is negative to involucrin staining.

Fig. 2C. Luminal side of glandular epithelia shows eosinophilic staining.

Fig. 2D. The cells located in luminal surface stain strongly to involucrin.
tubular, ductal, and duct-like structures in this tumor were arranged in two or more cell layers. Spindle shaped cells with long anastomosing processes were seen at the outer zone. Almost all the epithelial cells of these structures were negative for involucrin staining; however, involucrin immunoreactivity was often present along the luminal borders and in the luminal epithelial cells (Figs. 1A–D). The luminal surface appeared as very narrow lines in the tubular and duct-like structures and was positive for involucrin (Figs. 1A, B). The epithelial cells located on the

Figs. 3A, B. Involucrin staining in squamous differentiation and keratinized area of pleomorphic adenoma.

Fig. 3A. Epithelial proliferation into luminal cavity indicates positive involucrin staining. ×80
Fig. 3B. Higher keratinizing area indicates the strongest staining, and involucrin positive cells scattered in the tumor. ×80
Fig. 3C. Warthin’s Tumor
Intense involucrin staining cells scattered among eosinophilic tumor epithelia. ×80
luminal side of these structures also gave marked staining (Fig. 1A). In cystic structures or dilated ductal components, involucrin-positive cells were scattered throughout an otherwise negative cystic wall (Figs. 1C, D).

Pleomorphic adenomas sometimes showed granular or adenomatoid patterns with sebaceous-like metaplasia (Figs. 2A, C). Involucrin staining was strongly positive in these altered epithelial cells (Figs. 2B, D).

Squamous differentiation with varying degrees of keratinization was often noted in duct-like structures, solid masses, and centers of anastomosing cellular foci. In most cases of squamous differentiation, the cells were found at the luminal side of ductal or cystic structures, with proliferation into the luminal cavities. Involucrin staining was strongly positive in such cells undergoing squamous differentiation (Figs. 3A, B). The greatest staining intensity was found around the well-keratinized epithelial foci (Fig. 3B).

**Warthin’s Tumor**

Eosinophilic epithelial cells of Warthin’s tumors were usually devoid of involucrin although involucrin-positive cells of high intensity were scattered slightly in the epithelium. Stromal cells, including lymphoid cells, were negative for involucrin staining (Fig. 3C).

**DISCUSSION**

Involucrin is considered to be a marker of terminally differentiated epidermal cells, and thus immunoreagents specific for it are useful tools for detecting keratinization in epithelial tumors (1, 5, 8–10, 13–15). Immunohistochemically identified involucrin staining is generally limited to squamous epithelium in epidermis and mucous membranes where involucrin is usually localized in upper spinous and granular cells layers.

Waits et al. (13) have noted a positive involucrin staining in mucoepidermoid tumors but not in pleomorphic adenomas and Warthin’s tumors among 318 abnormal tissues examined. In the present study, ductal epithelia and acinar cells, either serous or mucous, of normal glands indicated negative staining as did duct-like structures in obstructive sialadenitis. Keratin staining is strongly evident in epithelial architecture, i.e., normal ductal segments of salivary glands and duct-like structures of sialadenitis (2, 3, 6, 12). This finding suggests that the duct-like structures in obstructive salivary lesions have less tendency to terminal keratinization. Pleomorphic adenomas of the salivary glands showed narrow-lined positive staining for involucrin along the luminal border of duct-like structures whereas the cells on the outer side of these structures lacked involucrin. This pattern may indicate the keratinization process or squamous differentiation occurring at the luminal surface of duct-like epithelial structures. In similar specimens, immunohistochemically detectable keratin proteins are found in all cells located on the luminal side of tubular and duct-like structures (2, 3).

Squamous differentiation and focal keratinization was found in some variants of pleomorphic adenomas and mucoepidermoid tumors. The involucrin reaction in those structures was markedly positive, and this current finding suggests that many types of epithelial tumors may have potential for keratinization, irrespective to histologic criteria.
Warthin's tumor is characterized by the presence of eosinophilic epithelia including numerous lymphoid cells, and such epithelia are not keratinized. However, involucrin-positive cells were sometimes, though rarely found intermingled within this eosinophilic epithelia, probably indicating the participation of individual cells in the keratinization process in the epithelia. The biological significance of such dotted keratinizing cells with strong involucrin staining is unknown.

REFERENCES