A Histopathological and Immunohistochemical Study of Necrotizing Sialometaplasia

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Abstract
Necrotizing sialometaplasia (NSM), a benign tumor-like lesion of the salivary glands, often resembles a malignant tumor in terms of clinical and histopathological features. In general pathology, metaplasia results from a kind of progressive change after chronic abnormal stimuli, and NSM is thought to arise after ischemic necrosis. However, the nature and pathogenesis of NSM remain unclear. The purpose of the present study was to investigate the morphological characteristics of metaplastic change in NSM by histopathological and immunohistochemical analyses using primary antibodies for cytokeratins (CKs) and MIB-1. Positive immunohistochemical reactivities for CK13 and MIB-1 staining in the squamous metaplasia of NSM were observed. Positive reactivity for CK13 was observed in prickle cells of the stratified squamous epithelium. The mean MIB-1 index was 6.0%. These results suggest that duct epithelial cells are responsible for metaplasia in NSM, because ducts in the palatal glands showed stratified squamous-like epithelium near areas of NSM and immunohistochemical findings resembled either salivary glands or oral mucosa covered with the lesion.

Introduction
Metaplasia represents the process of transformation of fully differentiated cells of one type into differentiated cells of another type in response to abnormal stimuli (1). Metaplastic lesions such as squamous metaplasia, sebaceous cell metaplasia, mucous cell metaplasia, and oncocytic metaplasia can arise in the salivary glands.

Necrotizing sialometaplasia (NSM) was defined by Abrams et al. (2) in 1973 as a rare reactive necrotizing inflammatory process involving minor salivary glands of the hard palate. NSM presents as a swelling, ulcerating tumor, and can be quite difficult to discriminate from squamous cell carcinoma or mucoepidermoid carcinoma of the minor salivary glands. Although metaplasia after ischemic necrosis involving glandular tissue has been hypothesized as a cause of NSM, the nature and pathogenesis of this entity have yet to be fully understood.

The purpose of the present study was to investigate squamous metaplastic change of NSM using histopathological and immunohistochemical investigations with antibodies against cytokeratins (CKs) and MIB-1 and comparing the results with the immunoreactivities of cells from salivary glands near the lesion and oral mucosa covered by the lesion.

Materials and Methods
Specimens from three cases of NSM diagnosed in the Department of Oral Pathology at Nihon University School of Dentistry, Matsudo, Japan, were used for the present study. Palatal glands around the lesion and oral mucosa were used as control tissues. Consideration was given to patient privacy, diagnosis, and the management and prognosis of the lesions (Ethics committee recognition number: EC 05–006).

The resected specimens were immediately fixed in 10% neutral-buffered formalin solutions, then embedded in paraffin and sectioned at a thickness of 4 µm for histopathological and immunohistochemical observation. Sections were stained with hematoxylin and eosin (HE) after deparaffinization using the usual methods.

A ChemMate ENVISION kit (DakoCytomation, Glostrup, Denmark) was used for immunohistochemistry. Sections
were deparaffinized, and then after thorough washing and blocking for endogenous peroxidase reaction with 3% H2O2, were incubated with the primary antibodies for pan-CK, CK10, CK13, CK17, CK18, CK19 and MIB-1 (Table 1). Positive control tests for all antibodies were performed on normal salivary gland tissue. For negative controls, primary antibodies were replaced with citrate buffer solution (pH 6.0) and no staining reactions were obtained. Finally, immunohistochemical sections were counterstained using Mayer’s hematoxylin.

Results

Clinical findings

NSM in the present study was located in the buccal mucosa (Case 1), palate, located in not other specified (Case 2), and soft palate (Case 3). The patients were a 60-year-old man (Case 1), a 28-year-old woman (Case 2), and a 39-year-old man (Case 3).

A summary of the present cases is shown in Table 1.

Histopathological findings

The lesions consisted of scattered squamous cell nests without atypical changes or invasive growth, separated by fibrous tissues with inflammatory cell infiltration and dilatation of the vessels, partially involving ductal lumina consisting of ductal cells and mucous cells in squamous islands and nests.

Immunohistochemical findings

The immunohistochemical profile is summarized in Table 2.

NSM

Squamous metaplastic cells showed strong immunoreactivity for pan-CK and CK13, with moderate-to-slight immunoreactivity for CK17 and CK19 in parts. Ductal cells displayed strong-to-moderate immunoreactivity for pan-CK and moderate immunoreactivity for CK18 in parts. Mucous cells exhibited strong immunoreactivity for pan-CK, slight immunoreactivity for CK10, CK13, CK17 and CK18, and moderate immunoreactivity for CK19 in parts. MIB-1 index was 6.0%.

Salivary glands around NSM

Ductal cells showed moderate immunoreactivities for pan-CK, CK18 and CK19, whereas mucous cells showed no immunoreactivity for these primary antibodies. The MIB-1 index was 1.0%.

Oral mucosa

Prickle cells showed strong or moderate immunoreactivities for pan-CK, CK10 and CK13, and slight immunoreactiv-
Fig. 1. Squamous metaplastic change in NSM. The lesion separated by fibrous tissues. (original magnification: ×200)

Fig. 2. Squamous metaplastic change with ductal space. (original magnification: ×400)

Fig. 3. The squamous metaplastic cells showed strong immunoreactivity for CK13. (original magnification: ×400)

Fig. 4. The squamous metaplastic cells showed negative immunoreactivity for CK19. (original magnification: ×400)

Fig. 5. The squamous metaplastic cells with a ductal space showed strong immunoreactivity for CK13. (original magnification: ×400)

Fig. 6. The ductal luminal cells showed moderate immunoreactivity for CK19 in part. (original magnification: ×400)

Fig. 7. Positive immunoreactivity for MIB-1 was observed in squamous metaplastic cells (arrows). (original magnification: ×400)

Fig. 8. The ductal cells showed negative immunoreactivity for CK13. (original magnification: ×400)

Fig. 9. The ductal cells showed moderate immunoreactivity for CK19. (original magnification: ×400)

Fig. 10. The prickle cells showed strong immunoreactivity for CK13. (original magnification: ×400)

Fig. 11. The prickle cells showed negative immunoreactivity for CK19. (original magnification: ×400)
ity for CK17 in parts, whereas basal cells were identified with moderate immunoreactivity only for pan-CK among CKs used in the present study. The MIB-1 index was 9.7%.

Discussion

NSM is a benign disease, but clinical and histopathological features are similar to those in malignant tumors such as squamous cell carcinoma or mucoepidermoid carcinoma. The disease manifests as a deep-seated ulcer. NSM mainly affects white men, predominantly in the 50th decade of life. The average age at diagnosis is 46 years (3). In the present cases, the ages of patients ranged from 28 to 60 years, with an average of 42.3 years. The male-to-female ratio was 2:1, similar to reported cases.

The detailed pathogenesis of NSM has yet to be fully elucidated, but metaplasia after avascular necrosis of the vasculature supplying the salivary gland lobules has been the most widely accepted theory. Etiological factors resulting in ischemia have been reported, including ill-fitting dentures, drinking, smoking, pharmacotherapy, radiation exposure, upper respiratory infection/allergy, intubation and surgical procedures for other associated lesions (3). The histopathological changes in NSM were described by Abrams et al. (2), as follows: a) lobular infarction or necrosis; b) bland-appearing nuclear morphology of the squamous cells; c) simultaneous metaplasia of ducts and mucous acini; d) prominent granulation tissue and inflammatory components; and e) maintenance of the general lobular morphology despite fairly extensive inflammatory and metaplastic changes, often involving more than one lobule. In addition, pseudoepitheliomatous hyperplasia of the overlying or adjacent epithelium is a common feature (3–5).

The present cases corresponded to the above-mentioned theories b–e as bland-appearing nuclear morphology of squamous cells, simultaneous metaplasia of ducts and mucous acini, prominent granulation tissue and inflammatory components, maintenance of the general lobular morphology despite fairly extensive inflammatory and metaplastic changes, although necrosis was not particularly noticeable. Lobular necrosis is likely to occur in the earliest stages and therefore may not always be seen by the time the patient undergoes biopsy. Also, since the present cases did not show any atypism, invasive growth or markedly high MIB-1 index, histopathological findings were used to distinguish the lesion from malignant tumors such as mucoepidermoid carcinoma and squamous cell carcinoma.

In the present study, CK13-positive reactivity was found not only in the squamous metaplastic cells, but also in the ductal cells of NSM, and negative findings were seen in the ductal cells in normal salivary glands. CK13 is known to exist in basal cells of the stratified squamous epithelium, but no observation of salivary duct cells has been reported previously (6). The present results thus suggest that the histogenesis of NSM is associated with squamous metaplastic changes in salivary ductal cells. In addition, the present study examined MIB-1 index, which is well known as a useful marker for assessing malignancy and/or neoplastic characteristics (7). This was because the MIB-1 index is generally >10% higher in malignant tumors such as squamous cell carcinoma and high-grade mucoepidermoid carcinoma than in benign tumors or non-neoplastic lesions (8–10). The present results for MIB-1 index showed positive reactivity in NSM (6.0%), suggesting that NSM represents a benign lesion and the histogenesis is related to progressive changes in salivary ductal cells.

Mucous cells were also observed in NSM, and showed different positive immunoreactivities for keratins from normal salivary glands, such as strong reactions for pan-CK, slight reactions for CK10, CK13, CK17 and CK18, and moderate reactions for CK19 in parts, since mucous cells were affected by squamous metaplasia. We concluded that the histogenesis of NSM might be associated with squamous and progressive changes in salivary duct cells based on the results of immunohistochemical analysis.

References

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