A Histopathological and Immunohistochemical Study of High-Molecular-Weight Cytokeratin, Mitochondrial Protein and Gross Cystic Disease Fluid Protein-15 in Pleomorphic Adenomas –With Special Reference to Epithelial Metaplastic Changes–

Toru Yoshihara, and Tadahiko Utsunomiya

Department of Oral Pathology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan

Correspondence to :

Tadahiko Utsunomiya

E-mail : utsunomiya.tadahiko@

nihon-u.ac.jp

Abstract

Pleomorphic adenoma, which is the major histopathological type of salivary gland tumor, shows polymorphous features including proliferating epithelial components with tubulo-ductal and/or solid nests and mesenchyme-like components with myxomatous, myxo-chondromatous, and/or fibrohyalinized tissues. The process of metaplasia, which involves progressive changes in tissues, results in the transformation of fully differentiated cells of one type into differentiated cells of another type in various organs and diseases. Therefore, metaplastic change is an important morphological factor that determines the individual characteristics and typical histogenesis of various lesions. The purpose of the present study was to investigate metaplastic changes histopathologically and immunohistochemically using antibodies against high-molecular-weight keratin (34 β E12), mitochondrial protein (MIT) and gross cystic disease fluid protein-15 (BRST-2) in order to clarify the morphological nature and mechanism of the metaplastic change in pleomorphic adenomas. These antibodies are known to be useful markers of squamous, oncocytic and apocrine-like metaplasia, respectively. Histopathologically, the parenchyma of pleomorphic adenoma consisted of double-layered epithelial elements showing luminal and abluminal arrangements, continuously adjacent to mesenchyme-like elements with myxomatous, myxochondromatous and/or hyalinized tissues. The epithelial parenchyma contained squamous eddies with polygonal cells and/or horny keratin pearls (10/10 cases), eosinophilic cuboidal and/or columnar oncocytic cells (8/10 cases), and also apocrine-like features (3/10 cases). Immunohistochemically, 34 β E12-positive cells corresponded to the squamous eddies, whereas MIT- and BRST-2-positive cells corresponded to the eosinophilic, oncocytic cells and apocrine-like cells, respectively. In addition, positive immunoreactivity for 34 β E12, MIT and BRST-2 was evident at luminal sites, and 34 β E12 was expressed at abluminal sites in this tumor. These results suggest that metaplasia of squamous epithelium is associated with both ductal or luminal differentiation and abluminal differentiation of basal and myoepithelial cells, whereas, metaplastic changes of oncocytes and apocrine cells are related to ductal, luminal proliferation, comprising the typical polymorphous features of pleomorphic adenoma.

Keywords :

metaplasia, pleomorphic adenoma, squamous metaplasia, oncocytic metaplasia, apocrine metaplasia

Introduction

Pleomorphic adenoma, the major histopathological type of salivary gland tumor, is characterized histopathologically by polymorphous features (1, 2). The tumor

consists of epithelial and mesenchyme-like components, especially myxomatous, myxo-chondromatous, and/or fibrohyalinized tissues.

The process of metaplasia, which involves progressive

Table 1. Primary antibodies used in the present case and immunohistochemical results

Primary antibody	Source	Clonality	Dilution	Result			
				Luminal cell		Abluminal cell	
				SC	OC	AC	SC
Cytokeratin, HMW (34 β E12)	DakoCytomation	M	1 : 100	+	—	—	+
Mitochondrial protein (MIT)	Chemicon International	M	1 : 20	—	+	—/+	—
GCDFP-15 (BRST-2)	Signet Pathology Inc.	M	1 : 10	—	—/+	+	—

Abbreviations: HMW; high molecular weight, GCDFP; gross cystic disease fluid protein, M; monoclonal, SC; squamous cell, OC; oncocytic cell, AC; apocrine-like cell, +; positive immunoreactivity, —/+; positive immunoreactivity in less number of cells, —; negative

changes in tissues, results in the transformation of fully differentiated cells of one type into differentiated cells of another type in response to abnormal stimuli (3). Epithelial and mesenchymal tissues are particularly involved. In epithelial tissues, squamous metaplasia is common in the presence of chronic bronchitis and bronchiectasis, and also in smokers. The metaplasia in respiratory organs involves replacement of the ciliated columnar cell lining of the respiratory tract by stratified epidermal epithelium (3). Such metaplasia also occurs in the duct epithelium in response to chronic inflammation or hyperplasia, and in neoplastic lesions, especially pleomorphic adenoma, that affect the salivary gland (1, 2, 5). The epithelial metaplastic change also involves oncocytic features, which are commonly identified in breast tumors (6). Therefore, these metaplastic changes are an important biological determinant of the morphological characteristics of neoplastic lesions.

Although metaplastic change is one of the factors responsible for tumor polymorphism, there have been no detailed morphological analyses of the polymorphous and metaplastic features of pleomorphic adenoma. The purpose of this study was to investigate the metaplastic changes in pleomorphic adenoma by histopathology and immunohistochemistry with antibodies against high-molecular-weight cytokeratin (34 β E12), mitochondrial protein (MIT) and gross cystic disease fluid protein-15 (BRST-2) in order to clarify the nature and mechanism of metaplasia in pleomorphic adenoma.

Materials and Methods

Ten cases of pleomorphic adenoma, diagnosed at the Department of Oral Pathology, Nihon University School

of Dentistry at Matsudo, were used for the present study. 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 formalin, and then made into paraffin-embedded blocks, which were sectioned at a thickness of 4 μ m for histopathological and immunohistochemical observation. The sections were stained with hematoxylin-eosin (HE) after deparaffinization.

The envision polymer + kit (DakoCytomation, Glostrup, Denmark) was used for immunohistochemistry. The sections were deparaffinized, and then after a thorough washing, they were incubated with target retrieving solution (DakoCytomation, Glostrup, Denmark) at 95 °C, 40 minutes for antigen retrieving. After cool down at room temperature, 20 minutes, the sections were incubated with the primary antibodies, which are listed in Table 1. Positive control tests for all antibodies were performed on normal salivary gland tissue and breast cancers. For negative controls, the primary antibodies were replaced with universal negative control-mouse (DakoCytomation, Glostrup, Denmark) and no staining reactions were obtained. Finally, the immunohistochemical sections were counterstained with Mayer's hematoxylin.

Results

Histopathological findings

The tumor consisted of epithelial elements arranged in nests, with trabecula or tubule formation, and mesenchyme-like elements with myxomatous, myxochondromatous and/or hyalinized tissues. The epithelial elements showed double-layered ductal structures of various

Fig. 1. Squamous metaplastic change in pleomorphic adenoma. Squamous eddies with keratinous horny pearl appears in fibro-hyalinized tissue (a). Luminal structure shows epidermal cyst-like appearance (b). Polygonal cell clusters are found in abluminal area (arrows) (c). Positive immunoreactivity for 34βE12 is identified in squamous eddies (d), ductal cells of epidermal cyst-like luminal area (e) and polygonal cells in abluminal cells (arrows) (f). (original magnification (a)–(e): x400, (f): x600)

Fig. 2. Oncocytic change in pleomorphic adenoma. Ductal cells have eosinophilic cytoplasm resembling oncocytes (arrows) (a). Positive immunoreactivity for MIT is observed in ductal cells (arrows) corresponding to oncocytic cells by HE (b). (original magnification (a): x400, (b): x600)

Fig. 3. Apocrine-like change in pleomorphic adenoma. Eosinophilic cells of ductal, luminal structure have apocrine-like projections in intraluminal side (arrows) (a). Positive immunoreactivity for BRST-2 appears in the projection, intraluminal side and/or cytoplasm of ductal cells (arrows) corresponding to apocrine-like cells and a few number of oncocytic cells by HE (b). (original magnification (a), (b): x600)

sizes (Figs. 1a-c, 2a, 3a). The inner side had a luminal structure lined by cuboidal or columnar-shaped cells admixed with an amorphous, secreted substance, whereas the outer side had abluminal features with polygonal and/or round cells (Figs. 2a, 3a). Squamous epithelium-like polygonal cell clusters, sometimes with central horny pearl formation, were also observed in all 10 cases (Fig. 1a-c). The squamous structures were evident in the luminal zone and abluminal areas. Some luminal zone showed epidermal cyst-like appearance (Fig. 1b). Ab-

luminal, hypercellular zone involved polygonal cells (Fig. 1c). Some parts of the luminal cells had eosinophilic cytoplasm resembling oncocytes (8/10 cases) (Fig. 2a) and with apocrine-like projections (3/10 cases) (Fig. 3a). The outermost zone of the epithelial elements showed a gradual transition to myxomatous, chondromyxomatous or hyalinized stroma.

Immunohistochemical findings

Positive immunoreactivity for 34 β E12, MIT and BRST

-2 was partly observed and/or scattered in the parenchyma of pleomorphic adenoma, and the incidence of positive cases corresponded to that of histopathological findings in HE-stained sections.

Positive immunoreactivity for 34 β E12 was observed in horny pearls and in the cytoplasm of clustered polygonal cells. Some 34 β E12 positivity was distributed in some squamous cells of the luminal zone and polygonal cells in abluminal areas (Fig. 1d-f). MIT positivity was distributed in the eosinophilic, oncocytic cells (Fig. 2b) and less number of apocrine-like cells in ductal luminal structures, stained by HE. Positive immunoreactivity for BRST-2 was identified in the apocrine-like cells (Fig. 3b) and less number of oncocytic cells in the luminal zone, stained by HE. In addition, BRST-2 immunoreactivity was distributed in the cytoplasm and/or luminal side of ductal cells and in secreted substances (Fig. 3b). No reactivity for MIT and BRST-2 was evident in the abluminal area.

The immunohistochemical profile is summarized in Table 1.

Discussion

Epithelial metaplasia in salivary gland tumors involves squamous, oncocytic and apocrine-type changes (4, 6-8). Squamous metaplasia is a well known feature of mucoepidermoid carcinoma, basal cell adenoma/adenocarcinoma and pleomorphic adenoma (1, 2). Similar to epidermal squamous epithelium, the cells involved are polygonal in shape admixed with keratinous substances forming squamous eddies. The characteristic immunohistochemical features of the squamous metaplasia are positivity for high-molecular-weight keratin (34 β E12) (corresponding molecular weight: 68, 58, 56.5 and 50 kD), as was the case in this study (9). In addition, the present study showed that immunoreactivity for 34 β E12 was also evident in the ductal cells of the luminal zone and the polygonal and/or round cells of the abluminal components. These findings suggested that the squamous metaplasia was associated with both proliferating sites of ductal differentiation and the basal or myoepithelial compartments in pleomorphic adenoma.

Oncocytic cells are a component of the parenchyma of Warthin tumor and oncocytoma/oncocytic carcinoma,

and are also present in the parenchyma of salivary duct carcinoma (10, 11). This type of cell is characterized by a plump, columnar or cuboidal shape and eosinophilic microgranules. Ultrastructurally, the microgranules correspond to overgrowth of degenerated mitochondria (4, 6). Because oncocytes are a remarkable feature in salivary ducts, especially those of aged individuals, it has been suggested that they may be related to senile changes in the salivary glands (4). Immunohistochemically, the antibody against mitochondrial protein (MIT) used in this study is known to be a useful marker of oncocytic parenchyma in such tumors (12). In the present study, immunoreactivity for MIT was observed in the columnar or cuboidal ductal cells with eosinophilic cytoplasm in luminal areas, whereas negative immunoreactivity was found in abluminal zone. These findings suggested that this type of metaplastic change is associated with ductal proliferation sites, indicating the presence of polymorphous features in pleomorphic adenoma.

GCDFP (gross cystic disease fluid protein)-15 (also known as "BRST-2"), a 15-kD monomer protein, is a product of fluid secretion from breast cysts (13). Although the antibody against this protein is a useful marker of apocrine sweat glands and breast tumors with apocrine features, as BRST-2-producing cells are primarily those with apocrine function, the protein is also expressed in minor salivary glands and serous cells of the submandibular glands (13, 14). Swanson et al. (8) observed the expression of BRST-2 in salivary gland tumors and indicated that pleomorphic adenoma has a biological relationship to lesions involving cutaneous apocrine glands and breast, since the tumor cells showed immunoreactivity for BRST-2. In the present study also, positive immunoreactivity for BRST-2 was identified in ductal cells and secreted substances, suggesting that pleomorphic adenoma has apocrine gland-like activity. In addition, the present study showed that positive immunoreactivity for MIT was observed in less number of apocrine-like cells, whereas positivity for BRST-2 was identified in less number of oncocytic cells. The results suggest that a ductal tumor cell of pleomorphic adenoma has an ability of differentiation into both cell types, although the detail mechanism has not been clarified, as yet.

Previously, metaplasia was thought to involve direct and/or indirect transformation from one type of differentiated cell to another (3). Recently, however, it has been recognized that metaplasia occurs after differentiated cells become undifferentiated or pluripotent (15). Therefore, it has also been referred to as “transdifferentiation”. Ihrler et al. (15) examined the process of regenerative and metaplastic changes in the salivary duct using non-neoplastic surgical materials and found that metaplastic changes originated from proliferating and/or pluripotent basal cells.

The present immunohistochemical findings demonstrated 34 β E12-positive squamous metaplasia in both luminal and abluminal areas, whereas MIT-positive oncocyctic metaplasia and BRST-2-positive apocrine metaplasia were found only in the luminal zone. These results suggest that squamous, oncocyctic and apocrine-like metaplastic changes are associated with luminal proliferation of ductal cells, and that squamous change is also related to abluminal proliferation of myoepithelial or basal cells, thus accounting for the typical polymorphism of pleomorphic adenoma. Furthermore, the authors hypothesize that in pleomorphic adenoma both pathways of metaplasia may be mediated by stem cell-like, pluripotent cells, although further study of molecular biological behavior will be needed to clarify in detail the mechanism of metaplastic changes in this tumor type.

Conclusion

The present study investigated metaplastic changes histopathologically and immunohistochemically using antibodies against high-molecular-weight keratin (34 β E12), mitochondrial protein (MIT) and gross cystic disease fluid protein-15 (BRST-2) in order to clarify the morphological nature and mechanism of the metaplastic change in pleomorphic adenomas, and the following results were obtained.

1. Histopathologically, epithelial parenchyma of pleomorphic adenomas contained squamous eddies with polygonal cells and/or horny keratin pearls (10/10 cases), eosinophilic cuboidal and/or columnar oncocyctic cells (8/10 cases), and also apocrine-like features (3/10 cases).
2. Immunohistochemically, 34 β E12-positive cells corre-

sponded to the squamous eddies, whereas MIT- and BRST-2-positive cells corresponded to the eosinophilic, oncocyctic cells and apocrine-like cells, respectively.

3. Positive immunoreactivity for 34 β E12, MIT and BRST-2 was evident at luminal sites, and 34 β E12 was expressed at abluminal sites in this tumor.

These results suggest that metaplasia of squamous epithelium is associated with both ductal or luminal differentiation and abluminal differentiation of basal and myoepithelial cells, whereas metaplastic changes of oncocytes and apocrine cells have relation to ductal, luminal proliferation, comprising the typical polymorphous features of pleomorphic adenoma.

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