The Three-Dimensional Architecture of the Cribriform Pattern in Adenoid Cystic Carcinoma of Salivary Gland Origin

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The three-dimensional (3-D) architecture of the cribriform pattern in adenoid cystic carcinoma of salivary gland origin was studied by histological and immunohistochemical findings. Tumor cell nests had numerous spaces composed of both laminin negative true cystic spaces and laminin positive pseudocystic spaces with occasional interconnection to the outer stromal tissue by light microscope. By the 3-D architecture, in tumor cell nests with predominant pseudocyst, the true cystic spaces showed the isolated spherical shape, whereas the pseudocystic spaces showed the spherical and/or ovoid shape interconnected with other pseudocystic spaces. The mean number of argyrophilic nucleolar organizer regions (AgNORs) per nucleus of the tumor cells forming the pseudocyst was significantly higher than that forming the true cyst. This study supports that these 3-D features were useful for a better understanding of biological characteristics of epithelial tumor cell nests of adenoid cystic carcinoma.

Key words: Adenoid cystic carcinoma, Cribriform, Three-dimensional architecture, AgNORs

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Introduction

Adenoid cystic carcinoma (ACC) is the well-recognized malignant tumor of exocrine glands, first described by Billroth (1) in 1859 as the name "Zylindroma" (cylindroma) because the histological appearance of this tumor was similar to thyroid follicles composed of cylinders of hyalin and mucin surrounded by tubes of the epithelial elements. Many different names have been proposed such as "cribriform adenocarcinoma" (2), "basalioma" (3), and "adenocarcinoma of mixed tumor type" (4), although the term "cylindroma" is still in common use. More recently, however, the WHO subcommittee on classification of tumors recommended adoption of the term "adenoid cystic carcinoma", which was first used by Reid (5). One of the most characteristic and classical features of ACC is the cribriform pattern showing numerous cystic spaces surrounded by anastomosing cord of epithelial tumor cells, and that is considered to be an important factor responsible for the biological behavior of this tumor. Although this peculiar cystic feature has been described as "sieve", "cylinder", and "Swiss cheese" by light microscope, no stereographic architecture has been elucidated.

In order to clarify the morphological characteristics of the cribriform pattern in ACC, a recent, advanced stereographic computer-technology was applied for inves-

tigating the three-dimensional (3-D) structural attributes of ACC. Furthermore, the proliferative status of tumor cells constituting cystic spaces was evaluated to investigate the relation to cyst formation.

Materials and Methods

A total five cases of the cribriform pattern in ACC of salivary gland origin were retrieved from surgical pathology files of Tohoku University Graduate School of Dentistry (Sendai, Japan) for the present study. They were three women and two men of ages ranging from 56 to 76 years, with ACC originating in four minor salivary glands and one sublingual gland. Biopsy specimens had been fixed in 10% formalin solution, dehydrated in graded ethanol solutions and xylene, and embedded in paraffin. Over 200 serial sections of each specimen were made in a thickness of about 3 μ m with a microtome and mounted on glass slides. Hematoxylin and eosin (HE) staining, and argyrophilic nucleolar organizer region (AgNOR) staining (6) were performed. For AgNOR staining, paraffin sections were dewaxed in xylene and then hydrated through a graded series of ethanol to water. The AgNOR staining solution was a 2% solution of gelatin in 1% formic acid. One volume of this solution was combined with two volumes of 50% silver nitrate solution. The prepared solu-

tion was poured over the tissue sections and allowed to stand 30 minutes in a darkened room. The sections were then washed with deionized water, dehydrated to xylene, and mounted.

Immunohistochemical staining

Following deparaffinisation of sections, immunohistochemical staining was performed using antibodies directed against laminin (dilution 1:100, DAKO, Kyoto, Japan), muscle-specific actin (dilution 1:100, clone HHF 35, DAKO, Kyoto, Japan) and cytokeratin (dilution 1:200, clone AE 1/AE 3, Becton-Dickinson, San Jose, USA). Bound antibodies were visualized using the supersensitive streptavidin-biotin detection system (Biogenex, San Ramon, USA). The color was developed with diaminobenzidine and supplemented with hydrogen peroxide. The sections were finally counterstained with Mayer's hematoxylin.

Three-dimensional reconstruction

Representative sections were selected for the 3-D

reconstruction using a computer graphics analysis system ('TRI-P' Ratoc System Engineering Co., Ltd, Tokyo) after HE, AgNOR and immunohistochemical staining. A 35 mm black and white photographic serial film strip was prepared. The serial photographic images of the outlines of tumor cell nests and cystic spaces of ACC then traced manually on the computer screen were synthesized into computer-generated 3-D images suitable for examination from different perspectives.

Results

HE findings

The cribriform pattern in ACC was characterized by numerous cystic structures (Fig. 1-a). An abundance of mucinous substances were packed within the cystic spaces surrounded by tumor cells. The outer stroma surrounding the tumor cells nests was composed of dense fibrous connective tissue containing fibroblasts, collagen fibers and blood vessels. There were two types of cystic structures; one was surrounded by one or two layers of cuboidal and polygonal tumor cells (Fig. 1-b), and the

Fig. 1: (a) Cribriform pattern in ACC with numerous cystic spaces in the tumor nest. (HE, $\times 160$) (b, c) Two types of the cystic structure. (HE, $\times 540$) (b) Surrounding one or two layers of cuboidal and polygonal tumor cells. (c) Surrounding rather small polygonal and spindle-shape tumor cells.

other was surrounded by rather small polygonal and spindle-shape tumor cells (Fig.1-c). In some areas, the tumor cell nests were broken up to form thin strands, and the cystic spaces were connected more frequently to one another and the interstitial connective tissue surrounding tumor nests.

Immunohistochemical findings

Laminin staining was characterized by its presence in the basement membrane of the tumor nests and cystic spaces (Fig.2). The inner luminal surface surrounded by rather small polygonal and spindle-shape tumor cells strongly reacted for laminin, whereas that surrounded by cuboidal and polygonal tumor cells of one or two layers was negative (Fig.2). Furthermore, cuboidal and polygonal tumor cells of one or two layers were generally positive for cytokeratin and negative for muscle-specific actin, whereas rather small polygonal and spindle-shape tumor cells were usually negative for cytokeratin and positive for muscle-specific actin. These immunohistochemical results, as emphasized in prior literature (7-9), indicated that one or two layers of cuboidal and polygonal tumor cells were ductal epithelium-like cells forming the true cyst, whereas rather small polygonal and spindle-shape tumor cells were myoepithelium-like cells forming the

pseudocyst.

Three-dimensional findings

In the tumor cell nest with predominant pseudocyst (Fig.3, inset), the true cystic spaces showed the isolated spherical shape in the tumor nest, as visualized in Fig.3-a. The pseudocystic spaces showed the spherical and/or ovoid shape of varying sizes, as visualized in Fig.3-b. Many of the pseudocystic spaces were interconnected with each other.

AgNORs findings

AgNORs were observed within nuclei as black dots with a lighter background (Fig.4-a). Tumor cells containing 1 or 2 AgNOR dots were predominant in the true cyst (Fig.4-a, left), whereas tumor cells containing 2 or more AgNOR dots were widespread in the pseudocyst (Fig.4-a, right). The mean number of AgNORs per nucleus (AgNOR number) of tumor cells constituting true cysts like Fig.3-a and various sized pseudocysts like Fig.3-b was 1.6 ± 0.7 ($n=194$) and 3.1 ± 1.2 ($n=440$), respectively (Fig.4-b). It was significantly higher in the tumor cells constituting pseudocysts than in those that constituting true cysts ($p<0.001$).

Fig. 2: Negative staining at the border of the true cyst, whereas linear staining at the border of the pseudocyst in laminin staining. (Laminin, $\times 160$)

Fig. 3: (a) 3-D architecture of the true cystic spaces in the tumor cell nest of the cribriform pattern in ACC. bar=100 μ m (Inset; 2-D figure, HE, $\times 35$)
(b) 3-D architecture of the pseudocystic spaces in the same area. bar=100 μ m (Inset; 2-D figure, HE, $\times 35$)

Fig. 4: (a) Tumor cell nuclei containing silver-stained AgNOR dots forming a true cyst (left) and several small pseudocysts (right). (AgNOR staining, $\times 540$)
 (b) The mean AgNOR number of tumor cells according to site. T: isolated true cyst, P: interconnecting pseudocyst. (*; $p < 0.001$)

Discussion

Although there have been many publications about cystic spaces in ACC by light (10, 11) and/or electron (10, 12-14) microscope, we have dealt with the characteristic from a morphological viewpoint. Bloom *et al.* (10) and Toida *et al.* (11) pointed out that the two types of cystic spaces, the true cyst and the pseudocyst, include contents with different histochemical properties. Electron microscopically, Tandler (12) revealed that the true cyst was surrounded by cuboidal cells showing microvilli of apical poles, whereas the pseudocyst was lined by highly replicated basal lamina. The present 3-D architecture of the cystic space suggests that the true cystic spaces may come to be larger with duct-like epithelial lining, whereas the pseudocystic spaces may become larger without a definitive epithelial lining and may be interconnected with each other and/or connected to the outer stroma. The different 3-D features between the true cyst and the pseudocyst may reflect the basic biological behavior of each cyst.

Immunohistochemically, laminin has been shown to be confined to the basement membrane and can be used as a maker for the distinction between the true cyst and the pseudocyst forming the cribriform pattern in ACC (15-18). The true cyst is formed by ductal epithelium-like cells that retains salivary secretory material within the lumen, whereas the pseudocyst is formed by myoepithelium-like cells and contains an extracellular matrix in its lumen (19, 20). The present study reveals that the proliferative status significantly increased in myoepithelium-like cells than ductal epithelium-like cells. These facts suggest that myoepithelium-like cells play an important role in the growth and biological behavior of ACC. Furthermore, Fujita *et al.* (20, 21) showed that apoptosis is easily induced in ductal epithelium-like cells rather

than in myoepithelium-like cells. Therefore, myoepithelium-like cells forming the pseudocyst may be easier to reproduce than ductal epithelium-like cells forming the true cyst, and the tumor nest with predominant true cysts may transform into that with predominant pseudocysts. Chen *et al.* (7) proposed the following model of tumorigenesis of ACC: as neoplastic cells differentiate and proliferate, first the solid pattern is formed, next the cribriform or tubular pattern appears, and the latter two patterns may change into each other. Interestingly, they also indicated that the new tumor nest with a tubular pattern sprouts from the periphery of masses with a solid or cribriform pattern, and the outer cells of the tubular pattern, that is myoepithelium-like cells, may proliferate and form the pseudocysts. These findings may support the easy reproduction of myoepithelium-like cells forming the pseudocyst in comparison with ductal epithelium-like cells forming the true cyst.

The term "cribriform pattern" has been used in other diseases such as intraductal carcinoma of the breast (22), cribriform carcinoma of the prostate (23) and epididymal cribriform hyperplasia (24). Ohuchi *et al.* (22) pointed out the contrast between the cribriform pattern of carcinoma and the complex glandular pattern of the benign tumor of the breast by 3-D architecture. They reported that the cribriform pattern of the intraductal carcinoma seen in the 2-D figure corresponded to the porous structure observed in the 3-D architecture. The cribriform pattern of ACC in the present study was different from that of the intraductal carcinoma described by Ohuchi *et al.* (22) by 3-D architecture. It is therefore important to understand that there are great differences in the cribriform pattern of each neoplasm.

In conclusion, from the present 3-D feature of the

cystic spaces in the tumor cell nests of ACC of salivary gland origin, the terms "sieve" and/or "cylinder" were not suitable for 3-D structural attributes. This study supports that these 3-D features were useful for a better understanding of biological characteristics of ACC.

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