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Proliferative Activity of Myoepithelial Cells in Mucoepidermoid Carcinoma

Faisal Mehsen Alali[1,2]*, Bassel Tarakji[1], Nasser Raqe Alqhtani[1], Abdullah Bin Nabhan[1], Ali Alrafedah[1], Adel Alenzi[1], Nabil Kochaji[2]

[1]Department of Oral and Maxillofacial Surgery, Diagnostic Sciences, Faculty College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia; [2]Department of Oral Histology and Pathology, Faculty of Dentistry, Damascus University, Syria

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***Correspondence:** Faisal Mehsen Alali, Department of Oral and Maxillofacial surgery, Diagnostic sciences, prince Sattam Bin Abdulaziz University, Faculty college of Dentistry, Saudi Arabia. E-mail: f.alali@ psau.edu.sa
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Abstract

AIM: The aim of the study is to investigate the role of myoepithelial cells in the pathogenesis of mucoepidermoid carcinoma (MEC) using the double immunohistochemical staining; α smooth muscle actin (α-SMA)as specific marker for the myoepithelial cell differentiation and proliferative cell nuclear antigen (PCNA) as a marker for proliferative activity of myoepithelial cells.

MATERIAL AND METHODS: Retrospective study of twenty salivary gland specimens (ten MEC and ten normal salivary glands) were studied using double immunohistochemical labeling for α smooth muscle actin α-SMA) and proliferative cell nuclear antigen (PCNA). The SPSS statistical package was used for data analysis (IBM SPSS Statistics for Windows, Version 20.0, Released 2011, IBM Corp, and Armonk, NY, USA).

RESULTS: In mucoepidermoid carcinomas, no positivity of α-SMA was seen in neoplastic cells (Frequent test), and it was just observed in the stroma of tumor, in the walls of blood vessels whereas, PCNA was positive, especially in high-grade tumors. In contrast, in normal salivary glands, the proliferating myoepithelial cells are stained by both α-SMA and PCNA.

CONCLUSIONS: We believe that the myoepithelial cells have no a role in the development of mucoepidermoid carcinoma.

Introduction

Mucoepidermoid carcinoma (MEC) is the most common malignant tumors of salivary glands [1]. Salivary glands show a complex tissue structure consisted of several types of cells in the secretory and ductal system including the ductal, acinar, or myoepithelial cells [2]. Myoepithelial cells have unique functions include; maintaining the structure and cell polarity of the acini, producing tumor suppressor proteins, anti-angiogenesis factors, and its role as a barrier against invasive adenocarcinomas [3], [4].

The role of myoepithelial cells in the pathogenesis of MEC is controversial [5], [6], [7].

Morphologically, neoplastic myoepithelial cells have several phenotypes include myxoid, basaloid, epithelioid, and clear cells in addition to many architectural patterns as microcystic or solid [7]. These cells have patterns resemble the histopathological features of mucoepidermoid carcinoma composed of mucous, epidermoid, intermediate, and clear cells with microcystic, solid, and sheet arrangement [8]. Hence, it was supposed that the abluminal intermediate cells of MEC are modified myoepithelial cells [7].

Furthermore, most studies have investigated either proliferation of all neoplastic cells without identifying their types [9], [10] or showed the existence of myoepithelial differentiation in the pathogenesis of salivary gland tumors without investigating their proliferative capacities [11].

The aim of this study is to investigate the role of myoepithelial cells in MEC using the double immunohistochemical staining; α smooth muscle actin (α-SMA) as specific marker for the cell differentiation [12] and proliferative cell nuclear antigen (PCNA) as a marker for its proliferative activity [9].

Materials and Methods

Tissue specimens

Twenty blocks of salivary gland specimens were retrieved from the archive of Department of oral

histology and pathology, faculty of dentistry, Damascus University. New five-micron sections of the specimens were cut and stained using H and E staining, then re-diagnosed as normal salivary glands (n=10) and mucoepidermoid carcinomas (n=10, six males, four females); low-grade tumors (n=3) show numerous large mucous cells surrounding a cystic spaces and high-grade tumors (n=7) show a sheet of squamous cells with occasional mucus cells. Microscopic slides stained with hematoxylin and eosin were reviewed by two pathologists to confirm the histopathological diagnosis and to reclassify the studied cases.

Deparaffinized slides were subjected to microwave pre-treatment with target retrieval solution (citrate buffer, pH 6, 15 minutes). Endogenous alkaline phosphatase and peroxidase activity were blocked with dual endogenous enzyme block containing hydrogen peroxide (0.05%). For staining for PCNA and α-SMA, the EnVision G|2 double stain system was applied. The first antibody (monoclonal anti-PCNA, ready-to-use, Dako) was applied at room temperature for 30 minutes and was visualized using HRP/DAB+. The second antibody (monoclonal anti-α-SMA, ready-to-use, Dako) was applied at room temperature for 30 minutes and was visualized using AP/Permanent Red. Mayer's hematoxylin was used as a counterstain. The vessels within the stroma were used as internal positive controls for α-SMA [13]. The positive controls for PCNA were the follicular tissues of the tonsil [14] (Figure 1).

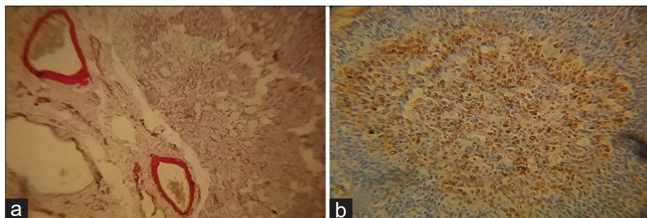

Figure 1: High power view of postive control of immunohistochemical staining. (a) Internal positive control of α-SMA around the blood vessels within a normal gland. (b) positive control of PCNA in the tonsil

The used criteria of the assessment is depending on the labeling index, the percentage of proliferative cells within a total of 400 cells was calculated [13], [15], identified by positivity for PCNA and α-SMA. The mean percentage of cellular proliferation and the standard deviation (SD) were calculated. The SPSS statistical package was used for data analysis (IBM SPSS Statistics for Windows, Version 20.0, Released 2011, IBM Corp, and Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

Results

Normal salivary glands

Red cytoplasmic positivity of α-SMA found in the myoepithelial cells around the acini and intercalated ducts. On the other hand, the brownish positivity of PCNA identified in the nuclei of cells where the proliferating myoepithelial cells are the ones that have shown double staining; α-SMA and PCNA, with proliferative activity (1.6%) (Figure 2).

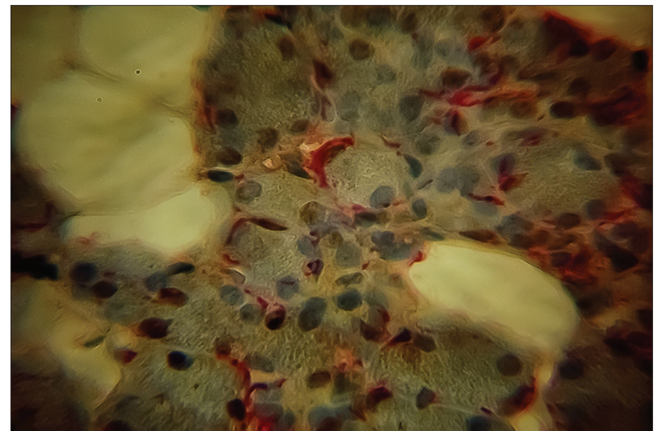

Figure 2: High power view of double immunohistochemical staining of proliferative myoepithelial cell in the parotid gland. PCNA appears brown in the nucleus, and the α-SMA in the cytoplasm is red

Mucoepidermoid carcinomas

No positivity of α-SMA was seen in the parenchyma of tumors, and it was just observed in the stroma of tumor, in the walls of blood vessels whereas, PCNA was positive, especially in high-grade tumors (Figure 3).

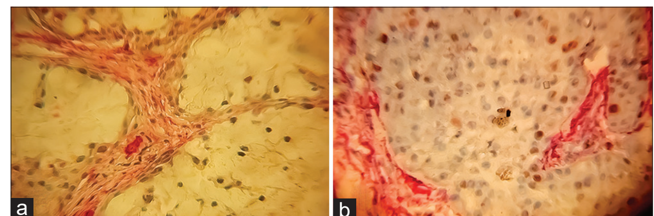

Figure 3: High power view of double immunohistochemical staining of mucoepidermoid carcinoma. (a) High-power view of low-grade mucoepidermoid carcinoma showing in which red positivity of α-SMA in the stroma of tumor only. (b) High-power view of high-grade mucoepidermoid carcinoma showing in which brown positivity of PCNA, whereas α-SMA is negative in the parenchyma of tumors

Discussion

We found in our study that the proliferative activity of myoepithelial cells in normal salivary glands is 1.6%. This result is supported by the multicellular theory of salivary gland tumors, which said that all of the cells of the salivary glands are able to proliferate [16].

It is thought that the intermediate cells in mucoepidermoid carcinoma are modified form of myoepithelial cells, so they are involved in the

pathogenesis of that tumor [7], [17]. In our study, however, α-SMA was negative. We believe that myoepithelial cells have no relationship with the pathogenesis of mucoepidermoid carcinoma or might be not express α-SMA due to malignant transformation. On the other hand, Ogawa reported that α-SMA is a specific marker of natural and neoplastic myoepithelial cells [18].

Three markers of myoepithelial cells were used; α-smooth muscle actin (α-SMA), smooth muscle myosin heavy chain (SMMH), and calponin which were all negative in mucoepidermoid carcinoma [11], while the S100 was positive in mucoepidermoid carcinoma [19]. and it has been confirmed that S100 is a specific marker of basal cells [13], [18] but not for myoepithelial cells [18], [20], [21]. Prasad *et al*. consider that the intermediate cells are closer to the basal luminal cells morphologically and immunophenotypically [11]. and it became clear that these cells differ from myoepithelial cells, and their location is confined to the striated and excretory ducts, while myoepithelial cells locate around the intercalated ducts and acini [20].

Ihrler *et al* have found that basal cells are the most proliferating (3.2±1.3%) in the normal salivary glands compared to myoepithelial cells (0.2%), acinar cells (2.0±0.9%), and intercalated duct cells (0.8±0.3%). Thus, the basal cells represent reserve cells that have a high ability to multiply and differentiate into multiple types. Therefore, they are responsible for the regeneration of the striated and excretory ducts, squamous cell metaplasia, mucous cell metaplasia, and basal cell hyperplasia [13].

Dardick explained the tumorigenesis of the salivary gland. He clarifies that mucoepidermoid carcinoma arises either from the excretory duct cells or from the basal reserve cells located in excretory ducts that have the ability to differentiate into mucous or squamous cells [21].

Moreover, mucoepidermoid carcinoma has no stromal secretions characterizing the tumors with myoepithelial differentiation, which are an important evidence of myoepithelial cells involvement and its differential diagnosis [22]. Although our sample size is limited and includes only ten cases of (MEC), this tumor is very rare and our sample size consistent with others [23], [24], [25].

Conclusion

We believe that the myoepithelial cells have no role in the development of mucoepidermoid carcinoma.

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