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MYOEPITHELIAL CELL AND ITS PROSPECTIVE ROLE IN SOME SALIVARY GLAND TUMORS (AN IMMUNOHISTOCHEMICAL STUDY)

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ABSTRACT

Salivary gland tumors are characterized by a wide variety of histological types which makes their classification and diagnosis difficult. It should be noted that the diversity in the occurrence and dilemma regarding the pathogenesis of salivary gland tumors is due to lack in distinguishing the cells participating in its oncogenesis, especially the Myoepithelial cells (MECs). MECs are normal constituent of the major and minor salivary glands, they are found between the basal lamina and the acinar or ductal cells. Proper and extensive studies regarding MECs are varied and thus have posed difficulty for a pathologist to understand this cell. Numerous functions of MECs have been described, the most important of them being important for contraction of the glands, also, it has been found to prevent tumor progression. In this study we try to make a thorough description of this cell and its role in some salivary gland tumors. 24 formalin-fixed, paraffin-embedded specimens of salivary gland tumors (7 cases of pleomorphic adenoma, 6 cases of mucoepidermoid carcinoma, 6 cases of Adenoid cystic carcinoma, and 5 cases of clear cell carcinoma) were used in this study. The specimens were cut at 5µm thick for H&E staining as a routine stain and calponin immunostaining. Calponin is a 34 kDa protein, it is a family of actin filament-associated proteins and it is a sensitive immunohistochemical marker for MECs. The present study revealed positive staining reaction for calponin in pleomorphic adenoma and adenoid cystic carcinoma and negative calponin reaction in mucoepidermoid carcinoma and clear cell carcinoma. These findings demonstrate that myoepithelial cells play a role in the pathogenesis of some salivary gland neoplasms and no or minimal role in others.

KEY WORDS: Myoepithelial cell, Calponin, salivary gland tumors.

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INTRODUCTION

In many exocrine glands such as salivary, mammary, sweat, lacrimal and bronchial glands, the secretory end pieces and the ducts are partly covered by cells with long processes that form an interlacing network. These cells are considered as epithelial cells as they are found to contain keratin intermediate filaments but at the same time having smooth muscle like property as they contain a large number of myofilaments, so these cells present a double phenotype of epithelial and smooth muscle cells thus they are referred as myoepithelial cells (MECs).[1] MECs are important components of salivary gland structure, they are closely related to the secretory units and proximal ducts being located between the basal lamina and the acinar or ductal cells. The shape of the MECs suggested that their role includes contraction when the secretory function of the gland is stimulated, compressing and strengthening the cells of the glandular parenchyma and assisting the expulsion of saliva.[2,3] Association of tumors with MECs was determined when scientists found that a variety of tumors occurred in salivary glands and breast as compared to pancreas and concluded that this was because of presence of MECs in the former two glands.[4]

Salivary gland tumors (SGTs) which comprise about 5% of head and neck cancers are a morphologically and clinically diverse group of lesions and may present considerable diagnostic challenge to the pathologist. The most common SGT is pleomorphic adenoma with an incidence of 70% and the most common malignant tumors are mucoepidermoid carcinoma (35%) and adenoid cystic carcinoma (20%). [5,6] SGTs show wide spectrum of clinical and histopathological characteristics therefore diagnosis and treatment of SGTs is a challenge to the pathologists and surgeons.[7] Final diagnosis is essentially based on the histopathological findings by hematoxylin-eosin (H&E) stained sections. However, some tumors demonstrate common histopathological features and subsequently definite diagnosis is very difficult. Therefore using other pathologic techniques such as Immunohistochemistry seems to be useful to discern similar tumors and support histological assessment.[8]

The role of MEC in salivary gland tumors is well established. It can be said that MEC has the capacity to differentiate into epithelial as well as the mesenchymal components in a tumor and these tumor cells have been termed the neoplastic MEC. The ability of neoplastic MEC to provide a mesenchymal component to salivary gland tumors can be due to fact that MEC exhibits features of smooth muscle.[9] It has to be understood that the neoplastic MEC has the ability to provide a wide spectrum of cytological and extracellular matrix differentiation, it can also produce different architectural patterns in a tumor.[10]

Positive identification of salivary gland MECs on routine microscopic examination is very difficult. Many immunohistochemical investigations have pursued differentiation markers, especially of myoepithelium to assist in classification such as S-100 protein and vimentin but non-specific in their reactivity. However, there is promise for some of the newer myoepithelial smooth muscle markers like α-smooth muscle actin (SMA), smooth muscle myosin heavy chain, calponin, and p63 in select diagnostic situations.[11-14] Calponin is a 34 kDa (Kilo Dalton) protein, it is a family of actin filament-associated proteins expressed in smooth muscle and non-muscle cells.[15] Calponin antibody is a sensitive immunohistochemical marker for MECs.[16] In this study we used calponin marker to make a thorough description of this cell and its role in salivary gland tumors.

MATERIALS AND METHODS

This study comprised 24 formalin-fixed, paraffin-embedded specimens of salivary gland tumors: 7 cases of pleomorphic adenoma, 6 cases of Adenoid cystic carcinoma, 6 cases of mucoepidermoid carcinoma and 5 cases of clear

cell carcinoma. They were selected from the stored blocks of Pathology Department, National Cancer Institute, Cairo University and Oral Pathology Department, Faculty of Dental Medicine, Al-Azhar University (Assiut branch). The specimens were cut at 5μm thickness for Hematoxylin and eosin (H&E) staining for histopathological examination and immunohistochemical staining.

Immunohistochemistry

The sections were mounted on silicon-coated glass slides, deparaffinized in xylene and rehydrated with a descending series of ethanol. After blocking endogenous peroxidase activity with methanol containing 0.3% H_2O_2 for 30 min, antigenicity was retrieved by microwave heating for a period of 5 min (2-3 times) in a citrate buffer (pH 6), then non-specific staining blocking reagent for 10 min. The sections were incubated with anti-calponin antibodies (Dako, Carprinteria, CA, USA) at 4ºC overnight at dilution 1:100. After rinsing with phosphate buffer saline (PBS), secondary antibody was then applied followed by incubation with streptavidin peroxidase for 30 minutes. To develop brown reaction, the sections were stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and washed with PBS. The slides were counterstained with Mayor's hematoxylin and washed in running tap water. Finally the slides were dehydrated in ascending grades of alcohol, mounted and covered with cover slips. Omission of primary antibody was employed as negative control and bowel tissue was used as positive control.

RESULTS

In pleomorphic adenoma

Histologically, the epithelial cells arranged in sheets, islands and duct-like structures, in addition to a mantle of MECs (the spindle-shaped and plasmacytoid cells). These cells appear to be gradually blended with a variable chondromyxoid matrix (Figure 1a). Immunohistochemically, all examined cases showed positive calponin immunoreactivity. Immunopositivity was strong in MECs surrounding the ductal epithelial cells and some of the neoplastic cells. This immunopositivity was either cytoplasmic or both cytoplasmic and nuclear in localization (Figure 1b).

In adenoid cystic carcinoma

Histologically, anastomosing cords of small deeply staining cells producing cyst-like spaces surrounded by hyalinized connective tissue stroma giving the tubular and cribriform patterns and masses of cells giving the solid pattern were seen (Figure 2a). Immunohistochemically, All examined cases of adenoid cystic carcinoma showed positive immunoreactivity for calponin. Immunoreactivity was nuclear and cytoplasmic in localization. Immunopositivity was strong in MECs along the periphery of the tubular, cribriform and solid growth patterns and cells lining the cyst-like spaces. (Figure 2 b&c).

In mucoepidermoid carcinoma

Histologically, sheets and nests of epidermoid and mucous cells with microcyst formation were seen (Figure 3a). Immunohistochemically, all examined cases were negative to calponin (Figure 3b).

In clear cell carcinoma

Histologically, there were a uniform solid nests and islands of tumor cells with a clear cytoplasm surrounded by dense bands of fibrous connective tissue (Figure 4a). Immunohistochemically, all examined cases showed negative calponin immunoreactivity except some of the stromal cells were immunopositive (Figure 4b).

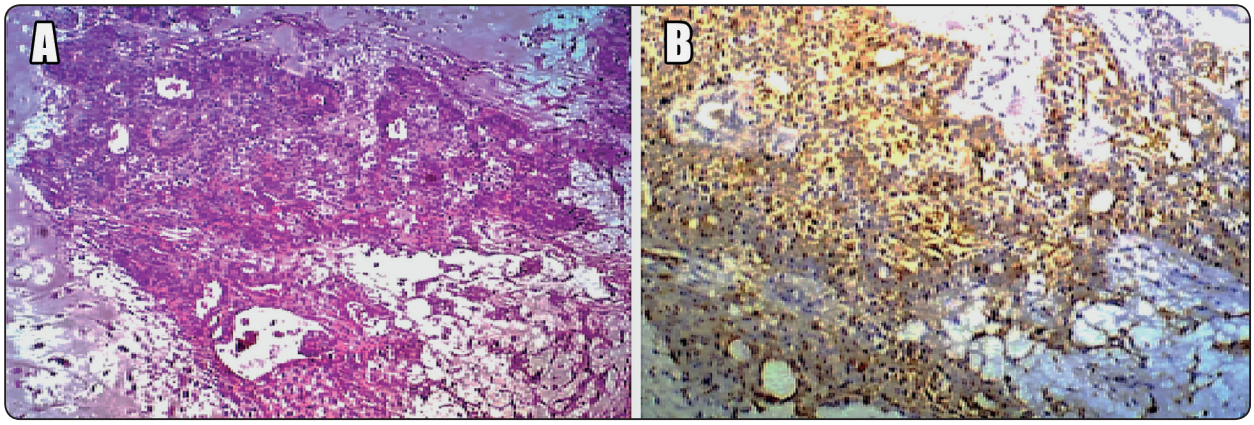

Fig. (1) Photomicrograph showing stained section of pleomorphic adenoma with (a) H&E staining (X 100), (b) positive calponin immunostaining (x200).

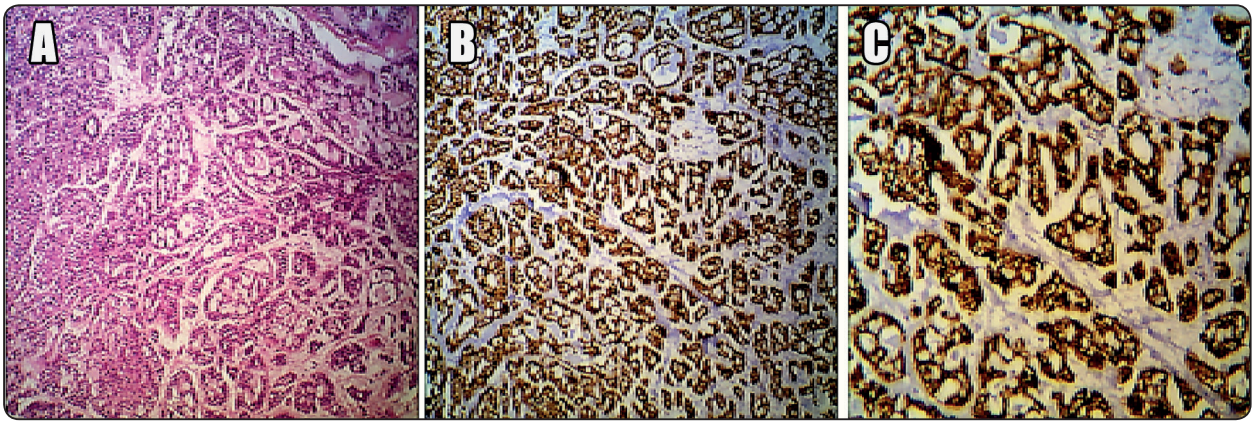

Fig. (2) photomicrograph showing stained section of adenoid cystic carcinoma with (a) H&E staining (X 100), (b) positive calponin immunostaining in the tubular, cribriform and solid patterns (x100), (c) positive calponin immunostaining in the lining of the cyst-like spaces (x200).

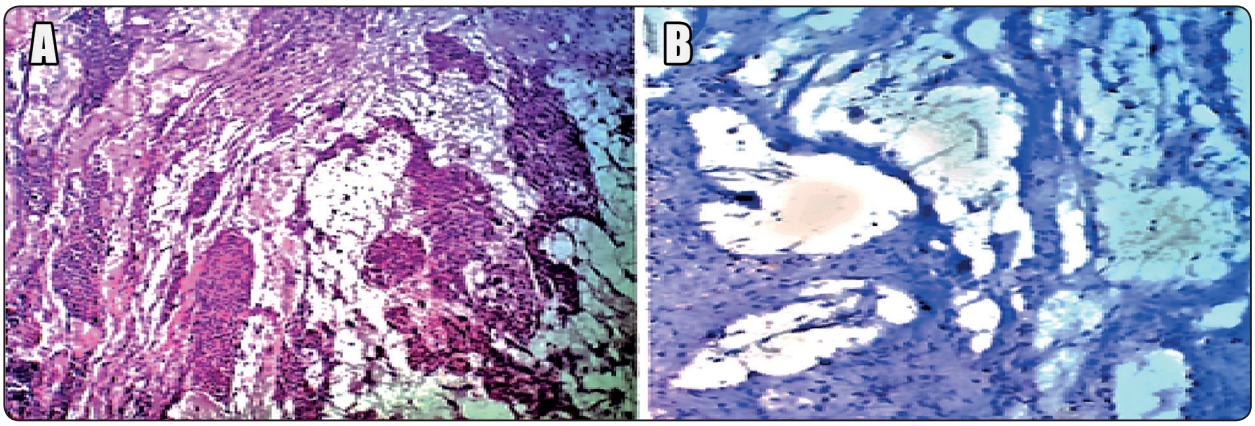

Fig. (3) Photomicrograph showing stained section of mucoepidermoid carcinoma with (a) H&E staining (X 100), (b) negative calponin immunostaining (x200).

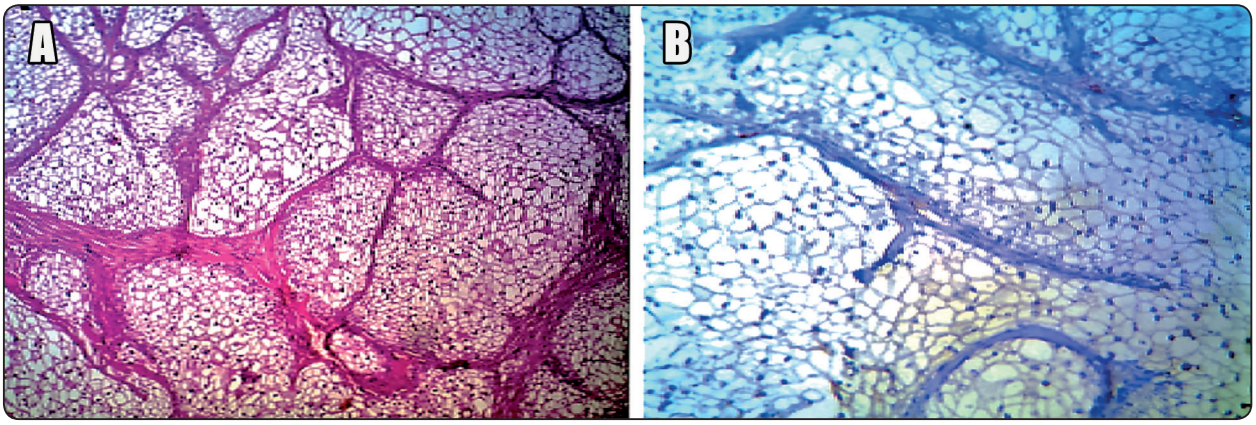

Fig. (4) Photomicrograph showing stained section of clear cell carcinoma with (a) H&E staining (X 100), (b) negative calponin immunostaining (x200).

DISCUSSION

Neoplastic MECs are considered to be the key cellular participant in morphogenetic processes responsible for the variable histological appearances of many salivary gland tumors.[17] Early attempts to identify myoepithelial cells in normal salivary glands included immunohistochemical procedures with anti α-smooth muscle myosin. Later, muscle-specific actin was demonstrated in normal myoepithelial cells and became the most important marker of the tumor myoepithelial cell. Recently, calponin which is a protein isolated from smooth muscle and non-muscle cells has been used to identify myoepithelial cells in salivary gland tumors.[18,19] Of note, calponin was recently reported to play an active role in tumor suppression as well.[20]

In pleomorphic adenoma, the most common benign salivary gland tumor in which MECs form the principal cell type, these cells can assume a variety of cytological forms such as hyaline, myxoid or epithelial cells, but they frequently occur as angular, slightly separated cells surrounding ducts or forming variably sized clusters or sheet-like patterns and do not present the classical features of normal MECs. Initially, these cells are related to duct luminal cells. However, with proliferation, they are gradually separated by increasing amounts of matrix material, resulting in the development of the myxoid and chondroid areas.[21] Strong expression of calponin was observed in most specimens examined in the present study. Calponin immunopositivity was observed in MECs as well as the neoplastic cells forming strands, masses and duct-like structures along with some of the stromal cells. These findings are in agreement with other studies showed calponin positivity in most cases of pleomorphic adenoma. The reactivity was concentrated in almost all non-luminal cells of tubules and ducts as well as in polygonal, plasmacytoid cells and spindle-shaped cells irrespective of tumor arrangement into sheets, nests or cords. [22-24] Melissa et al., [25] stated that high levels of calponin may affect neoplastic MECs contrary to acting as tumor suppressors, it may cause these cells to induce growth, migration and invasion.

Adenoid cystic carcinoma is a basaloid tumor consisting of a mixture of MECs and ductal cells that can have a varied arrangement. Three major patterns are recognized (cribriform, tubular and solid patterns), usually a combination of these is seen and the tumor is classified according to the predominant pattern.[26] positive reaction for calponin was principally observed in adenoid cystic carcinoma at the periphery of the cribriform and

tubular patterns and scattered in the solid pattern. It was also seen within some cells lining the cystic-like spaces of cribriform patterns. These findings are consistent with the distribution of MECs in adenoid cystic carcinoma and with findings described by Prasad et al.[27] and others.[16,23] Furuse et al.[23] showed calponin positivity in cases of solid adenoid cystic carcinoma considering that the cells of solid adenoid cystic carcinoma are poorly differentiated and possess the ability to differentiate into ductal luminal and myoepithelial cells.

Salivary gland tumor classifications separate mucoepidermoid carcinoma from other neoplasms on the basis of a number of histological features, in particular, the lack of participation of neoplastic MECs. The relationship of intermediate cells to the luminal cells in mucoepidermoid carcinoma is of prime importance, which is remarkably similar to that seen between modified MECs and luminal cells in pleomorphic adenoma. These means that intermediate cells of mucoepidermoid carcinoma are the counterpart of the modified MECs of pleomorphic adenoma.[28] The present study confirmed a negative staining reaction for calponin in tumor cells of mucoepidermoid carcinoma and this is indicative of no or low level of MECs differentiation in the histogenesis of mucoepidermoid carcinoma. According to this result, it can be hypothesized that MECs have no or minimal role in the pathogenesis of mucoepidermoid carcinoma. The loss of calponin reaction can be helpful for differentiation between mucoepidermoid carcinoma and pleomorphic adenoma as well as other adenocarcinmas which their diagnosis with usual methods of H&E may sometimes be problematic, these findings are agree with the study of Foschini et al.[29]

Primary clear cell tumors of salivary glands comprise a subgroup of salivary tumors which are distinct in terms of histogenesis, tumor biology and clinical behaviour.[30] The differential diagnosis of clear cell salivary neoplasms encompasses a broad range of possibilities.[31] The diagnostic term "clear cell carcinoma" is a diagnosis of exclusion, applied only after other specific tumors with clear cell morphology are excluded.[32] In the differential diagnosis among different clear cell tumors, histology is often of little use mainly when no definitive evidence of myoepithelial differentiation is found due to the high morphological similarities observed in the different "clear cell" entities. So, immunohistochemistry may be helpful in revealing the cell of origin of the tumor. [33]

Clear cell carcinoma is a low grade carcinoma, recently included in WHO classification.[34] It is rare salivary gland tumor found primarily in the minor salivary gland, especially the palate and less commonly in other intraoral sites.[35] This tumor was speculated to be previously called or confused with monomorphic variants of epithelial-myoepithelial carcinoma, mucoepidermoid carcinoma, and myoepithelial carcinoma. It was separated from these entities because of its lack of apparent squamous, mucinous, and myoepithelial differentiation.[36] The present study showed negative reaction for calponin in clear cell carcinoma indicating lack of myoepithelial differentiation. This means that myoepithelial cells play no or minimal role in the pathogenesis of this tumor. This findings is in agreement with the studies of Suzuki et al.,[32] Lai et al.,[37] and Yamashita et al.[38]

CONCLUSION

The present study showed positive immunoreactivity to calponin in pleomorphic adenoma and adenoid cystic carcinoma. On the basis of this finding we can concluded that MECs were involved in the development of pleomorphic adenoma and adenoid cystic carcinoma which mean that MECs play a role in their pathogenesis. On the contrary, this protein was negative in mucoepidermoid carcinoma and clear cell carcinoma which is indicative that MECs have no or minimal role in their pathogenesis.

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