Study of Myoepithelial Cell Markers in Pleomorphic Adenoma and Mucoepidermoid Carcinoma of Salivary Glands

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ABSTRACT

Introduction: The application of immunohistochemical method has resulted in marked improvement of the microscopic diagnosis of neoplasms combined with H&E staining. Although unique cellular antigens have not been found in salivary gland neoplasms, multiple less specific immunomarkers have been used and may be helpful in elucidating the role of myoepithelial differentiation in those neoplasms. The aim of this study was to evaluate immunohistochemical myoepithelial markers (GFAP, actin, vimentin, and S100) in mucoepidermoid carcinoma and pleomorphic adenoma of salivary glands for differential diagnosis of these tumors and specification of their histogenesis.

Methods and Materials: Formalin-fixed and parafin embedded tissue sections of 25 pleomorphic adenoma and 25 mucoepidermoid carcinoma were immunohistochemically analyzed for the presence of actin, vimentin, GFAP, and S100 protein. A standard biotin-streptavidin procedure was used after antigen retrieval. Immunoreactivity of myoepithlial cells and chondromyxoid areas in pleomorphic adenoma and mucus cell, epidermoid cells, and intermediate cells in mucoepidermoid carcinoma were evaluated and immunoreactivity was scored on a scale of 0 to +4 (Regezi method) with o as negative, 1+ as scattered staining, 2+ as 25% to 50% of positive tumoral cells, and 4+ as more than 50% positive cells. The data were analyzed with chi-square test, and significance level was considered as 0.05 ($P<0.05$).

Results: In 25 pleomorphic adenomas, all nonluminal cells and chondromyxoid areas were positive (+4) for GFAP and vimentin and (0→+3) for muscle-specific actin (12:0, 12:+1, 1:+3) and (+1→+4) for S100 protein (3:+1, 3:+2, 18:+3, 1:+4). But all mentioned markers were negative for all mucoepidermoid carcinomas, regardless of their grades ($P<0.001$) and there were no immunohistochemical difference in major and minor salivary glands neoplasms.

Discussion: Expression of myoepithelial cell-associated markers in pleomorphic adenoma have confirmed role of myoepithelial the cells in histogenesis of this tumor and lack or limited expression of these antigens in mucoepidermoid carcinoma, indicate the minimal myoepithelial differentiation in this tumor. Therefore, evaluation of myoepithelial cell markers can be helpful in differential diagnosis of salivary gland neoplasms with myoepithelial cell differentiation, and also specification of histogenesis of these tumors.

Key words: Pleomorphic Adenoma, Mucoepidermoid Carcinoma, S100, GFAP, Muscle specific actin, Vimentn, Immunohistochemistry.

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Introduction

The application of immunohistochemical method in pathology has been resulted in marked improvement in microscopic diagnosis of neoplasms and more exact realization of histopathologic features, histogenesis, and pathogenesis of those lesions.Also,

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this method is important in determination of prognosis of neoplasms.

Immunohistochemical method is used for differential diagnosis of salivary gland tumors, but unique cellular antigens have not been defined in any salivary gland neoplasms, so multiple less specific immunomarkers may be used in surgical pathology [1].

Because neoplastic cells express those antigens that are found on their normal cell counterparts, knowledge of immunotypes of salivary gland cells and in particular, myoepithelial cells may be helpful in elucidating of myoepithelial cell roll and its level of participation and differentiation in these neoplasms [2].

Pleomorphic adenoma (PA) is the most common benign and mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland neoplasms. Many investigations have showed that myoepithelial cells play a major role in histogenesis of pleomorphic adenomas and may also be important in many mucoepidermoid carcinomas [3-5].

Many researchers have studied on the presence of S100, actin, vimentin, and GFAP immunomarkers in pleomorphic adenoma and mucoepidermoid carcinoma.

Kahn and Baumal (1985) have suggested that myoepithelial cells in normal salivary glands have immunostaining with CK, S100, actin, and vimentin antibodies. This immunoreactivity was also present in modified myoepithelial cells in pleomorphic adenoma [6].

In numerous studies by Ishida and Nakazato (1985), Zarbo and Regezi (1986), Crocker and Campbell (1988), Huang (2000), and Curran and white (2001), they have evaluated the presence and distribution of S100 and GFAP proteins in normal and neoplastic salivary glands and their results show the presence of S100 and GFAP in cytoplasm and nucleus of chondromyxoid and cellular areas of pleomorphic adenoma[7-11].

VC Araujo and NS Araujo (1990) have suggested that vimentin is one of the early indicators of neoplastic myoepithelial differentiation [12]. Also VC Araujo and Carvalho

(1994), and Dardic and Takai (1995) have stated that nonluminal cells in pleomorphic adenoma have a positive immunoreactivity by vimentin but they aren't stained with muscle-specific actin and are found only in the capillary walls, so actin was suggested to be partially or totally replaced by vimentin in neoplastic myoepithelial cells [13, 14].

The results of studies of Furukawa and Nishimura (1991), and Murakami and Makino (1993) showed that in pleomorphic adenomas, there was positive immunoreactivity to GFAP, S100, and vimentin which was not observed in normal salivary glands. Therefore, they concluded that expression of these antibodies is related to oncogenesis [3, 15].

About mucoepidermoid carcinoma, Hassanin and Ghosh (1989) have suggested that intermediate cells have a positive immunoreactivity for actin, vimentin, and S100 and these cells have similar features with myoepithelial cells of normal salivary glands[16].

But in studies of Zarbo, Batsakis and Regezi (1991), and Sousa and Loyola (1998), mucoepidermoid carcinomas were nonreactive for actin, vimentin, GFAP ,and S100 [1, 17, 18], and in Regezi study ,various grades or subtypes of mucoepidermoid carcinoma didn't exhibit any significant differences in immunohistochemical staining [1].

Foschini and Marucci (2002), showed the keratin positive epithelial cells in mucoepidermoid carcinoma but smooth-muscle actin which is an indicator of myoepithelial cell differentiation was negative in this tumor. This researchers also suggested also that anti-mitochondrial antibody was diffusely positive in MEC, therefore they concluded that immunohistochemical cell profile of MEC is similar to normal striated duct cells [19].

The purpose of the present study was to determine the presence and distribution of myoepithelial cells related immunomarkers

(actin, vimentin, GFAP, and S100) in pleomorphic adenoma and mucoepidermoid carcinoma and to evaluate the role of myoepithelial cells in histogenesis of these tumors.

Methods and Materials

This study was an analytical-descriptive study, without direction. Cases of this study were paraffin blocks of 25 pleomorphic adenomas and 25 mucoepidermoid carcinoma, which were retrieved from the files of oral pathology department of Isfahan School of Dentistry and also department of pathology of Alzahra hospital in Isfahan and Amir Alam hospital in Tehran, Iran.

The selected blocks were cut from of pleomorphic adenoma (14 major and 11 minor salivary glands) and MEC (17 major and 8 minor salivary glands) with normal salivary glands near those tumors and were stained with hematoxylin-eosin to confirm the accuracy of the diagnoses and also sections of MEC were cut and stained with periodic-acid-schiff (PAS) to determine grades of that tumor.

Immunohistochemical staining was carried out with biotin-streptavidin method with the use of antibodies such as anti-vimentin (clone V9, zymed CN:08-0052 RTU), anti-muscle–specific-actin (clone: HHF3, zymed CN:08-1262 RTU), anti-GFAP (clone:2-2B10,Zymed CN:13-0300,dilution 1/100), and anti-S100 (clone: zy44 ,zymed CN: 18-0046, dilution 1/100). Sections were studied by two pathologist separately.

In pleomorphic adenoma, myoepithlial cells, ductal cells, and chondromyxoid areas and in MEC, mucus cells, squamous cells, clear cells, and intermediate cells were immunohistochemically evaluated and immunoreactivity of tumoral cells was scored on the basis of Regezi method with O as negative or nonreactive, 1+ representing scattered spotty staining, 2+ indicating up to 25% of tumor cells positive, 3+ indicating 25% to 50% tumor cells positive, and 4+ indicaing more than 50% of tumor cells positive.

The datas were analyzed with chi-square test and significance level was considered as 0.05.

Results

Results of this study are demonstrated in two parts:

Histopathology

The histopathologic structure of pleomorphic adenoma generally consisted of both the mesenchymal (chondromyxoid area) and the epithelial components in arrangement of cellular sheets and two layered ductal structures in which myoepithelial cells layed out in exterior layer.

Mucoepidermoid carcinomas typically exhibited an infiltrative growth pattern and consisted of mucus cells, epidermoid cells, intermediate cells and clear cells. Microcystic spaces were frequently seen in the well differentiated lesions. 8 cases of these tumors were classified as low grade tumor which contained numerous PAS+ mucus cells and cystic spaces.

14 lesions were intermediate grade tumors which characteristically contained fewer mucus cells and exhibited a more solid growth pattern with mild dysplasia and in 3 tumors that were diagnosed as high grade MEC, few mucus cells and nuclear pleomorphism and dysplasia were seen.

Immunohistochemistry

In all normal salivary glands, the myoepithelial cells on the bases of mucus and serous acini were positive (4+) for GFAP, Actin, and Vimentin, but reaction for S100 was weakly positive (1+).

All pleomorphic adenomas were positive (4+) for GFAP and Vimentin in the cytoplasm of myoepithelial cells and chondromyxoid areas, although it was only somewhat positive (0→3+) for actin (12 cases negative, 12 cases 1+, and 1 case 3+) and for S100 it was variably positive (1+→4+)(3 cases 1+,3 cases 2+, 18 cases 3+, and 2 cases 4+).

All mucoepidermoid carcinomas were nonreactive to all applied antibodies and only the connective tissue stroma of the

tumor was stained with GFAP and vimentin ,but the stroma was nonreactive for S100 and stained by actin only in two cases.
Immunohistochemical staining was not different in minor and major salivary gland pleomorphic adenoma and mucoepidermaoid carcinoma.

Differences in immunohistochemical staining of pleomorphic adenoma and mucopepidermoid carcinoma are presented in tables [1-4].

In all immunohistochemical reactivities, there were significant statistical differences between pleomorphic adenoma and mucoepidermoid carcinoma (P<0.001).

Table 1: Comparative frequency of vimentin stainability in pleomorphic adenoma and mucoepidermoid carcinoma (P<0.001).

Tumor type	Frequency of Vimentin stainability					Total
	-	+	++	+++	++++	
Pleomorphic adenoma	0	0	0	0	25	25
Mucoepidrmoid Carcinoma	25	0	0	0	0	25
Total	25	0	0	0	25	50

0:unstained, 1+:Focal staining, 2+: Maximum %25 of cells positive, 3+:%25-50 of cells positive, 4+:Over than%50 of cells positive

Table 2: Comparative frequency of GFAP stainability in pleomorphic adenoma and mucoepidermoid corcinoma (P<0.001).

Tumor type	Frequency of GFAP stainability					Total
	-	+	++	+++	++++	
Pleomorphic adenoma	0	0	0	0	25	25
Mucoepidermoid carcinoma	25	0	0	0	0	25
Total	25	0	0	0	25	50

0:unstained, 1+:Focal staining, 2+: Maximum %25 of cells positive, 3+:%25-50 of cells positive, 4+:Over than %50 of cells positive

Table 3: Comparative frequency of S100 stainability in pleomorphic adenoma and mucopeidermoid carcinoma (P<0.001).

Tumor type	Frequency of S100 stainability					Total
	-	+	++	+++	++++	
Pleomorphic adenoma	0	2	3	18	2	25
Mucoepidermoid Carcinoma	25	0	0	0	0	25
Total	25	2	3	18	2	50

0:unstained, 1+:Focal staining, 2+: Maximum %25 of cells positive, 3+:%25-50 of cells positive, 4+:Over than %50 of cells positive

Table 4: Comparative frequency of actin stainability in pleomorphic adenoma and mucoepidermoid carcinoma (P<0.001).

Tumor type	Frequency of Actin stainability					Total
	-	+	++	+++	++++	
Pleomorphic adenoma	12	12	0	1	0	25
Mucoepidrmoid Carcinoma	25	0	0	0	0	25
Total	37	12	0	1	0	50

0:unstained, 1+:Focal staining, 2+: Maximum %25 of cells positive, 3+:%25-50 of cells positive, 4+:Over than %50 of cells positive

Discussion

The present results as positive reactivity (4+) for GFAP and vimentin and less intensivity for S100 and also nonhomogenous and incomplete reactivity for actin are in agreement with the studies of ishida and Nakazato (1985) [8], Regezi and Batsakis (1986) [1], Crocker and Campbell (1988) [7], Huang (2000) [10], and Curran and white (2001) [11], about immunoreactivity of GFAP and S100. Also obtained results for the presence of S100 and vimentin in pleomorphic adenoma and myoepithelial cells in normal salivary glands are consistent with previous study that have done by kahn and Baumal (1985) [6].

As elaborated by VC Araujo and NS Araujo (1990) [12], carvalho and VC Araujo (1994) [14], and Takai and Dardic (1995) [13], incomplete or absent expression of muscle specific actin is common in pleomorphic adenoma and positive immunoreactivity for muscle specific actin is seen only in the capillary wall.

According to the reports of Furukawa and Nishimura (1991) [3] and Makino and Murakami (1994) [15], GFAP, S100 and Vimentin which were not detected in normal salivary glands, were observed in pleomorphic adenoma. But in our study, positive immunoreactivities of GFAP, S100 and Vimentin were seen in both normal salivary glands and pleomorphic adenoma. Therefore probable expression of these antigens is not related to oncogenesis.

Also the study of VC Araujo and Carvalho [14], which have stated that in neoplastic myoepithelical cells, actin is partially or totally replaced by vimentin, is in contrast with our study in which positive immunoreactivity of vimentin was seen in both normal salivary glands and pleomorphic adenoma. But it must be noticed that immunoreactivities of GFAP, Vimentin, and S100 in normal salivary glands in our study, were observed near the pleomorphic adenoma.

Therefore although immunoreactivity of GFAP, Vimentin, and S100 in normal salivary glands were the same as pleomorphic adenoma, but in may be due to probability of oncogenic changes in ultrastructural and histochemical levels. Because of these evidences, it can not be exactly concluded that the same presence of these proteins in pleomorphic adenoma and normal salivary glands is indicative for non-connection of these antigens with oncogenesis.

Myoepithelial cell immunomarkers, especially GFAP, Vimentin, and lesser S100 are expressed in chondromyxoid areas and nonluminal cells in pleomorphic adenoma, so this reactivity is a reflex of presence of myoepithelial cells in their roll in histogenesis of pleomorphic adenoma and evaluation of these markers can be helpful in differentiation between salivary gland neoplasms and myoepithelial cell differentiation.

As mentioned before, negative or weakly positive staining of actin was seen in pleomorphic adenoma, whereas in normal salivary glands, actin was positive in the bases of acini. According to the ultrastructural study, it is obvious that neoplastic myoepithelial cells lose some features of myoepithelial cells such as

myofilaments, hemidesmosomes, micropinocy-totic vesicules, and etc [14, 17], so it is possible that incomplete expression of actin in pleomorphic adenoma is related to the stage and level of myoepithelial cell differentiation.

In our study there were no differences in expressions of actin, vimentin, GFAP, and S100 in major and minor salivary glands. So, despite of histological differences of pleomorphic adenoma in major and minor salivary glands, the immunological differences were not seen.

In mucoepidermoid carcinoma, the present results confirmed negative staining of all applied antibodies in tumoral cells and only the connective tissue stromal elements of the tumor were immunoreactive for vimentin and GFAP. Therefore, our findings are in agreement with results of studies of Batsakis, Zarbo and Regezi (1991) [1], Loyola and Sousa (1998) [18], and also Marucci and Foschini (2002) [19], but because we didn't apply antimitochondrial antibody, it was not possible to determine the exact origin of mucoepidermoid carcinoma and its relatin with striated ducts of salivary glands.

But our results were in contrast with Ghosh and Hassanin's study (1989) [16] that certainly may be related to technical differences.

Therefore, we can state that special immunomarkers of myoepithelial cells (GFAP, actin, vimentin, and S100) are minimally or not expressed in mucoepidermoid carcinoma, so it is indicative of no or low level of myoepithelial cell differentiation in the histogenesis of mucoepidermoid carcinoma.

In fact, loss of these markers can be helpful for differentiation between of MEC and PA and other adenocarcinmas with myoepthelial cell differentiation which their diagnosis with usual methods of H&E may sometimes be problematic.

In this research, it was elucidated that various subtypes of the mucoepidermoid carcinoma don't exhibit any significant immunohistochemical staining differences, indicating that applied immunomarkers in our study have no value in the subcalssifcation of these lesions.

Accorading tothe absence of myoepithlial cells in excretory and striated ducts of salivary glands and negative immunohistochemical staining of myoepithlial cell markers in MEC, it is suggested that MEC originates from excretory or striated duct components of salivary glands.

In contrast to MEC, the immunohistochemical positivity of myoepithelial cell antigens in pleomorphic adenoma is indicative for originating of this tumor from intercalated duct components or, with less probability, from acinus components of salivary glands.

So the origin of MEC and PA are different. In fact, PA originates from initial and MEC originates from intermediate or last parts of salivary gland ducts. Based on this findings probably, the role of environmental carcinogenic agents in incidence of MEC is probably more effective than PA.

Conclusion and Suggestions

Complete and homogenous expression of GFAP, Vimentin, and S100 are seen in all of PAs that are limited to nonluminal cells and chondromyxoid areas and incomplete expression of actin is seen in half of the PAs, but these proteins have no expression in MEC and its various subtypes don't exhibit any significant differences in immunohistochemical staining, so applied immunomarkers have no value in subclassification of these lesions and the absence of GFAP, actin, vimentin, and S100 in MEC indicate that the myoepithelial cells have no role in histogenesis of MEC.

It is concluded that pleomorphic adenoma originates from initial parts of salivary gland ducts or interclated ducts and mucoepidermoid carcinoma originates from intermediate or last parts of salivary gland ducts, namely striated or excretory ducts. Therefore, it is suggested to use antimitochondrial markers for determination of origin of mucoepidermoid carcinoma

from striated or excretory ducts of salivary glands.

In addition, it is suggested to perform epidemiologic study for specification of carcinogenic agents of MEC and also oncogenic antigens of these tumors or as a whole, salivary glands tumor antigens.

Also, it is suggested to use cellular cultivation for specification of histogenesis of these tumors and to perform more researches about the role of myoepithelial cells in development of salivary gland tumors with immunomarkers such as P-63 and also ultrastructural methods by electron microscopy.

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