

Comparison of immunohistochemical markers between adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma

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Abstract: Adenoid cystic carcinoma (AdCC) and polymorphous low-grade adenocarcinoma (PLGA) have several common histological and clinicopathological features that may create diagnostic difficulties. In this study, 10 AdCCs, 8 PLGAs, and 5 normal minor salivary glands as a control group were selected. Sections prepared from each tumor were stained using the streptavidin-biotin system for seven marker antigens: carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA), muscle-specific actin (MSA), vimentin, S100, p53, and Ki-67. Data analysis showed high expression of CEA, MSA and Ki-67 in AdCCs compared with PLGAs, although CEA expression was limited to luminal cells. Ki-67 was expressed in both luminal and non-luminal cells and MSA only in non-luminal cells. Vimentin and S100 showed stronger expression in PLGAs, the expression of vimentin was more noticeable, being focal and widespread. The immunoreactivities of EMA and P53 were not helpful for distinguishing between the two tumors, although the EMA expression pattern in AdCCs was limited to luminal cells, whereas it was present in both luminal and non-luminal cells in PLGAs. Thus, immunohistochemistry can be helpful for

differential diagnosis of AdCC and PLGA, particularly that for CEA, vimentin, and Ki-67. (J Oral Sci 51, 509-514, 2009)

Keywords: adenoid cystic carcinoma; malignant salivary gland tumor; polymorphous low-grade adenocarcinoma; immunohistochemistry.

Introduction

Tumors of the salivary glands are an important entity in the field of oral and maxillofacial pathology. Different classifications of salivary gland tumors have been devised, but these are being continuously updated as new lesions, such as polymorphous low-grade adenocarcinoma (PLGA), become recognized (1,2).

Although adenoid cystic carcinoma (AdCC) is a high-grade malignant tumor, in most cases, it does not show cell atypia and mitotic features, except in the solid type (3). Histologically, two main types of cells have been observed in this tumor: ductal epithelial cells and myoepithelial cells, which typically have an angular and hyperchromatic nucleus and clear cytoplasm (4). The tumor shows extensive local recurrence and widespread metastasis, so may require rigorous treatments including combined surgery and radiotherapy. Despite these treatments, however, the tumor tends to have a poor prognosis due to widespread metastasis, mostly to the lungs and bones (1,3).

Malignant epithelial cells in polymorphous low-grade adenocarcinoma (PLGA) have cytologic uniformity, diverse

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morphology, and an infiltrative growth pattern with low metastatic capability (4). A variety of histological forms of this lesion has been described, including lobular, papillary cystic, cribriform, and trabecular patterns. It has the propensity to attack nerves and blood vessels, and can sometimes metastasize to distant sites (5,6).

From clinical and epidemiologic viewpoints, both of these tumors have a high propensity to recur in minor salivary glands. The most commonly affected site for both tumors is the palate. As both have a predisposition to attack nerves, pain is a common symptom (4-7).

Differentiation between these tumors is based mainly on histological findings. The cells in PLGA are cubic and cylindrical and have vesicular nuclei with scanty eosinophilic cytoplasm, thus lacking the basaloid appearance of the cells in AdCC. Unlike PLGA, papillary and fascicular growth is rare in AdCC. In contrast to AdCC (especially the solid type), in the cellular area of PLGA, nuclear polymorphism, necrosis, and mitotic activity are inconspicuous (4). However, in some cases, differentiation between these tumors is impossible. Considering the difference in treatment planning and prognosis between these two tumor types, techniques such as immunohistochemistry (IHC) may be beneficial for distinguishing them (4,8). The aim of this study was to examine the usefulness of IHC for differentiation between AdCC and PLGA.

Materials and Methods

This study included ten cases of AdCC and eight cases of PLGA from patients referred to the Department of Oral Pathology, Mashhad Faculty of Dentistry, between 1982 and 2007. All of the resected specimens were fixed in 10% formalin and embedded in paraffin blocks. Serial sections 4 μ m thick were prepared from each case for hematoxylin and eosin (HE) staining and IHC evaluation. After conducting a histopathologic study and confirmation of the previous diagnosis by two independent pathologists, the tumors were divided into two categories: AdCC with a mixed microscopic appearance and PLGA. For IHC, the labeled streptavidin-biotin technique (L-SAB) was used.

Antibodies against Ki-67 (H7241 clone NIB1), p53 (H7213 clone Do-7), EMA (H7097 clone E29), CEA (H7096 clone 11-7), MSA (H7114 clone IA4), S100 (Hoo66), and vimentin (H7095 clone V9) were utilized. All were obtained from Dako Cytomation (Eskan Teb Asia, Tehran, Iran).

After deparaffinization in xylene, the tissue sections were mounted on slides. Then the slides were immersed in 90% ethanol for 10 min, followed by Tris buffer solution. They were then microwaved at 700 W for 7 min, followed

by 350 W for 15 min, and left to cool for 15 min. Finally, the slides were rinsed in running tap water. For inhibition of internal peroxidase, hydrogen peroxide was added for 10 min, followed by rinsing in Tris buffer solution (pH 7.6, 20 mM) and NaCl solution (145 mM). Each slide was then incubated with the primary antibody, and then with biotinylated linking antibody, followed by streptavidin/peroxidase as a chromogen, and Mayer's hematoxylin (for background staining). Finally, the slides were dehydrated in absolute alcohol and mounted with Entellan glue.

The membranous, cytoplasmic and nuclear expression of each antigen was estimated in one hundred cells at magnifications of $\times 10$ and $\times 40$, and the stained cells were counted.

After preparation of IHC slides, the samples were independently examined by two pathologists. Then the expression of each antigen in one hundred cells was studied and evaluated in comparison with the internal control (natural mucosa of minor salivary glands).

Statistical analysis of the data was done using *t*-test and Mann-Whitney test at a significance level of $P < 0.05$. Then, in order to differentiate the tumors, we tried to derive a suitable cut-off point and sensitivity for each marker by using the receiver operating characteristic (ROC) curve (Fig. 1 and Table 1).

Results

In the control group (minor salivary glands), expression of CEA and EMA was observed around intercalated and striated ducts; however, the expression of EMA was more marked. Focal expression of EMA was evident around mucus acini, whereas this was not the case for CEA.

Myoepithelial cells around intercalated and striated ducts were immunostained for S100, and myoepithelial cells around intercalated ducts and acini were immunostained for MSA. Blood vessels were also strongly positive for MSA.

Ki-67 and p53 also were expressed around ducts, but expression of Ki-67 was more intense. Vimentin was expressed in a few stroma-associated glands.

Examination of Ki-67 and p53 indicated that only Ki-67 expression was useful for differentiation of PLGA from AdCC ($P < 0.001$) (Fig. 2), whereas p53 expression was not significantly useful ($P = 0.45$).

In AdCC, a mean of 22.5% of tumor cell nuclei were positive for Ki-67, compared with 3.88% for PLGA. Therefore, a cut-off point of 9.5% for Ki-67 gave a sensitivity of 100% and 87.5% for diagnosis of AdCC and PLGA, respectively.

Although expression of the epithelial markers CEA and

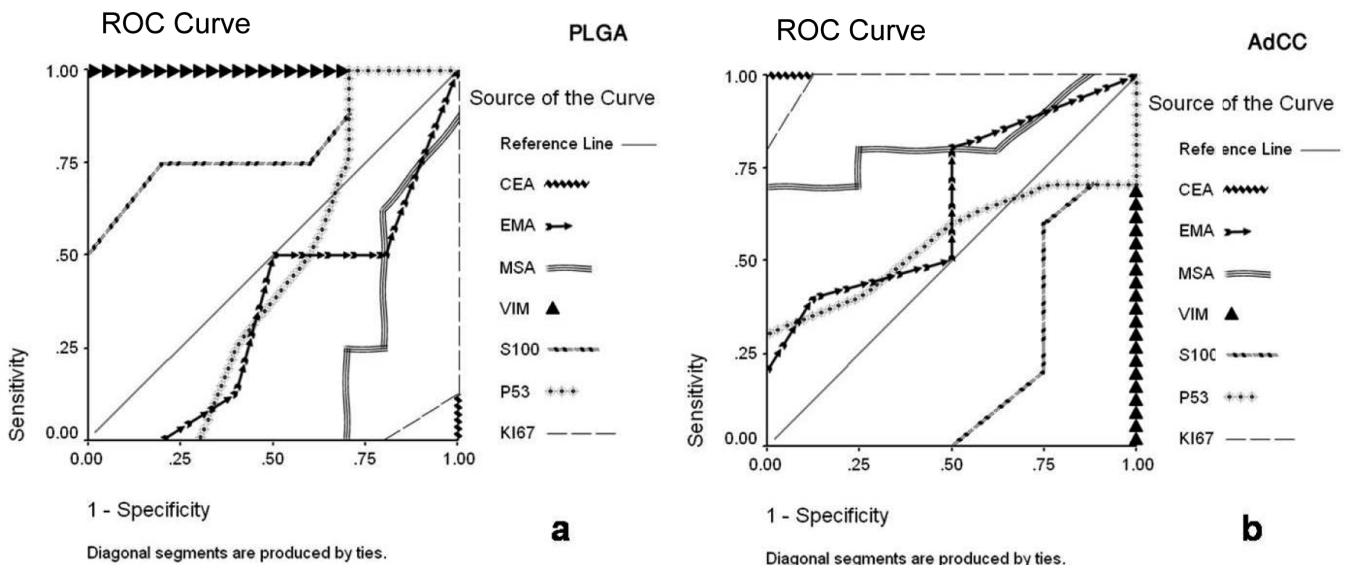


Fig. 1 Diagnostic value of the present markers based on cut-off points determined by the receiver operating characteristic (ROC) curves: (a) for PLGA and (b) for AdCC.

Table 1 Comparison of the expression of different markers (Mann-Whitney test)

Marker	PLGA		AdCC		P-value
	Mean	Standard deviation	Mean	Standard deviation	
Ki-67	3.88	3.3	22.5	9.2	<0.001
p53	12	11.44	17.1	15.85	0.764
CEA	1.88	1.13	10.5	7.62	<0.001
EMA	16.88	10.33	26.5	19.44	0.274
MSA	27.5	10.35	52.5	22.64	0.034
S100	48.75	15.53	30.3	18.94	0.027
Vimentin	65.63	13.48	16.4	13.61	<0.001

EMA was more obvious in AdCC, the inter-tumor difference was greater for CEA (Fig. 3). A cut-off point of 4.5% for CEA gave 100% sensitivity for diagnosis of both tumors. Regarding EMA, no significant relationship was evident ($P = 0.22$), and even when the cut-off point was increased to 20, an equal degree of positivity between the tumors remained, with no sensitivity for differentiation between them. Although the expression patterns of both markers were similar, both being expressed around luminal areas in AdCC, in PLGA expression of CEA was often focal around lumina, whereas EMA was expressed around both luminal and non-luminal cells and ducts.

Myoepithelial and mesenchymal markers showed differences in expression. Expression of vimentin in PLGA was 65.63%, whereas it was 16.4% in AdCC ($P < 0.001$) (Fig. 4). On the other hand, by considering the cut-off point of 45, this marker showed 100% sensitivity for diagnosis

of both tumors. However, considering the widespread roles of myoepithelial cells, MSA was more widely expressed in AdCC; the average MSA expression level in AdCC was 52.5%, compared with 27.5% in PLGA ($P = 0.011$). On the other hand, AdCC expressed MSA in tumor cells and cells surrounding the luminal space, whereas in PLGA more focal expression around cystic spaces was observed.

S100 also showed distinct expression in luminal cells, but its expression in AdCC was not as apparent as in PLGA. Furthermore, in PLGA, strong focal expression of this marker was evident. The average expression level of S100 in PLGA was 48.75%, compared with only 30.3% in AdCC ($P = 0.041$).

Table 2 indicates the diagnostic values of the various markers. For example, using a cut-off point of 9.5%, Ki-67 had a sensitivity of 100% and 87.5% for diagnosis of

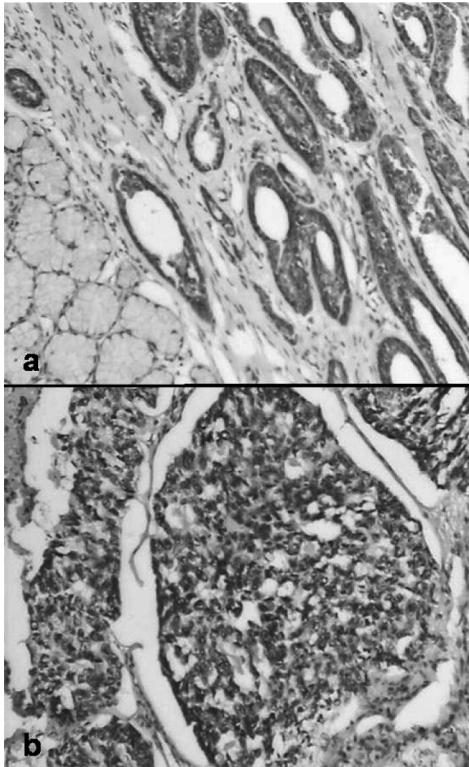


Fig. 2 Ki67 expression in AdCC (a) and in PLGA (b).

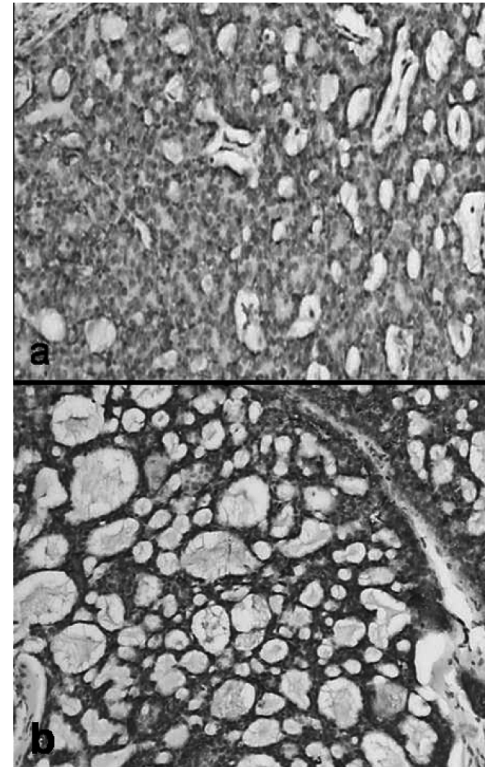


Fig. 3 CEA expression in AdCC (a) and in PLGA (b).

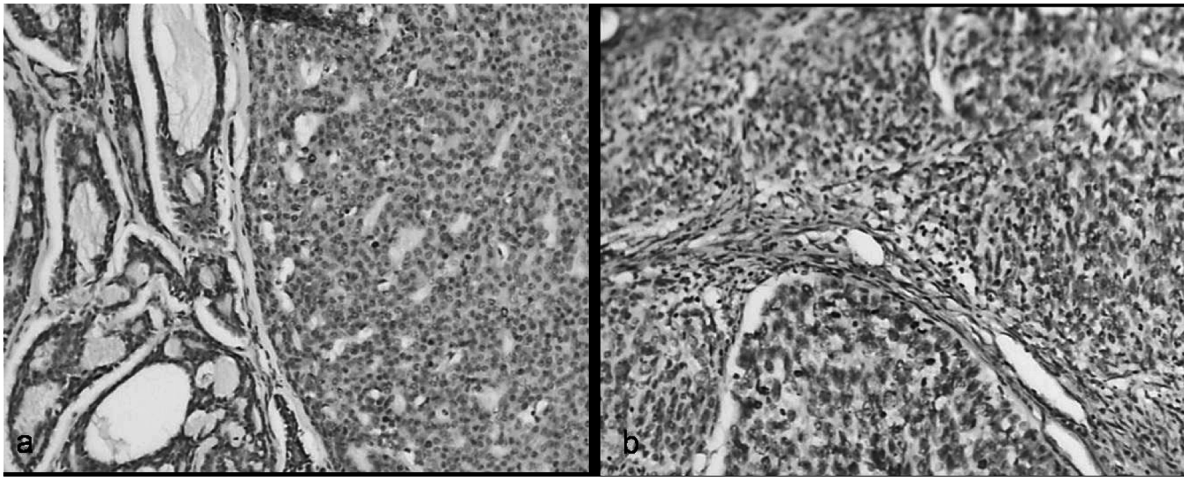


Fig. 4 Vimentin expression in AdCC (a) and in PLGA (b).

Table 2 Diagnostic value of various markers

Marker	AdCC		PLGA	
	Diagnosis of PLGA	Diagnosis of AdCC	Diagnosis of PLGA	Diagnosis of AdCC
Ki-67 (cut off point = 9.5)	0	10	7	1
p53 (cut off point = 5)	4	6	4	4
CEA (cut off point = 4.5)	0	10	8	0
EMA (cut off point = 20)	5	5	4	4
MSA (cut off point = 25)	2	8	5	3
S100 (cut off point = 50)	10	0	4	4
Vimentin (cut off point = 45)	0	10	8	0

AdCC and PLGA, respectively.

Discussion

In this study, we tried to differentiate between PLGA and AdCC by using IHC. AdCC is a very malignant tumor with a marked capacity for invasion, and requires intensive treatment, whereas surgical excision and follow-up are usually sufficient for PLGA (1,6). This study indicated a significant role of Ki-67 for separation of these two tumors, as its average expression in AdCC was 22.5%, whereas that in PLGA was 3.88%. This finding is in accord with Skálová et al. (9) and Gnepp (2). Indistinct and lower expression of Ki-67 in PLGA has also been confirmed in other studies (10,11). However, regarding p53, no specific and adequate relationship has been found (4). This ill-defined relationship may be due to the lack of role of p53 in tumors such as AdCC shows no relationship between p53 gene alterations and immunostaining (12).

Regarding epithelial indices, although the expression of both EMA and CEA was greater in AdCC than in PLGA, it appears that the role of CEA is more distinct than that of EMA for differentiating between these tumors on the basis of IHC. Also, both markers showed the same degree of expression around luminal cells in AdCC, similar to the study of Gnepp et al. (13). However, compared to AdCC, focal expression of CEA often appears around luminal cells in PLGA, whereas expression of EMA is more widespread.

Vimentin tended to have more power for distinguishing between the two tumors; its average expression in PLGA was 65.63%, whereas that in AdCC was 16.6%. This finding is in accordance with Darling et al. (10) and a study the WHO (4).

S100 showed higher expression in PLGA than in AdCC. Ferreiro (14) stated that the expression of S100 in PLGA was moderate to intense, whereas that in AdCC was mild to moderate. However, Simpson et al. (15) reported that expression of S100 was negative in AdCC, but intense in PLGA.

Greater expression of MSA was seen in AdCC than in PLGA, the latter showing a level almost half of that in the former, being approximately 27.5%. This finding was in accord with some other studies (10,11,15). On the other hand, the expression pattern of MSA in AdCC was similar to that reported by Chen et al. (16), being observed not only around the pseudocystic spaces, but also in tumoral and non-luminal cells, especially those with a tubular growth pattern.

Currently, some other indices are used for differentiation between PLGA and AdCC on the basis of IHC (17). Perhaps for this reason, Darling et al. (10) believes that distinction between these tumors using IHC is still uncertain

and requires further investigation. Ultimately, it appears that more studies of this issue are needed, and hopefully initial data like those obtained here will be useful for this purpose.

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