The Value of Immunohistochemical Markers in Pleomorphic Adenoma and Adenoid Cystic Carcinoma of the Salivary Gland

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Abstract

**Background:** In recent years, immunohistochemical markers have provided considerable contribution to salivary gland tumor diagnosis and classification. The aim of this study was to evaluate diagnostic value of various cytokeratin markers and smooth muscle actin (SMA) in differential diagnosis of pleomorphic adenoma (PA) and adenoid cystic carcinoma (ACC).

**Methods:** In this retrospective study, 35 cases of salivary gland tumors composed of 19 PA and 16 ACC were selected and immunohistochemical staining with six cytokeratin markers (CK7, CK8, CK13, CK14, CK17 and CK18) and SMA were performed. The positivity of these markers in both typical and atypical areas of each tumor was compared. Positivity was defined if 10% or more of the cells were immunostained.

**Results:** In the PA group, there was no difference between luminal and stromal expression of CK7, CK8, CK13, CK14, CK17, and CK18 in both typical and atypical areas except SMA that was not expressed in the atypical area. No statistically significant differences were found between the expression of CK7, CK8, CK14 and CK18 in both typical and atypical areas of ACC group, with no expression of SMA in atypical area.

**Conclusion:** It seems that the immunoprofile of the atypical area of both PA and ACC are similar to each other and IHC does not help to differentiate them.

**Keywords:** Salivary gland; Tumors; Immunohistochemistry

Introduction

Salivary gland tumors have a special status in human neoplasia and probably have the most complex histopathologic feature of the body organs and are very heterogeneous. Their remarkable morphologic variability combined with rarity renders these tumors difficult to diagnose.¹ Accurate diagnosis is necessary for perfect treatment and prediction of prognosis. In this way, immunohistochemical markers have provided useful contributions in the salivary gland tumor diagnosis.¹⁻⁷ In the totally excised specimen, the diagnosis of salivary gland tumors can be made without difficulty.⁸ In the small biopsy specimens which have the solid cellular patterns, the exact diagnosis is difficult and four main differential diagnoses including pleomorphic adenoma (PA), adenoid cystic carcinoma (ACC), basal cell adenoma and poorly differentiated mucoepidermoid carcinoma are present. Mucoepidermoid carcinoma can be determined by the presence of glandular structures composed of mucinous, transitional and squamous cells. Basal cell adenoma has numerous endothelial lined channels, which distinguishes it from ACC.⁸ However, with histologic features alone, the differentiation between PA and ACC cannot be easily made.¹ According to previous studies,⁸⁻¹³ the pattern of cytokeratin markers has been established in typical morphologic types of PA and ACC tumors; however, the above-mentioned pattern has not been
defined in atypical morphologic varieties of these tumors which is the most difficult aspect of histopathologic diagnosis. In this study, we evaluated the cytokeratin markers and smooth muscle actin (SMA) in PA and ACC and compared the results both in typical and atypical morphologic areas. We hope this study helps the physicians to diagnose the definite type of tumor in small biopsies with atypical morphologic features that are otherwise difficult or sometimes impossible on histological criteria alone.

Material and Methods

In this retrospective study, all the epithelial salivary gland tumors (benign and malignant) involving minor and major glands were retrieved from the pathology files of Khalili Hospital affiliated to Shiraz University of Medical Sciences, during 2002-2006. Our criteria for selection were the presence of typical and atypical areas in each tumor. We defined the atypical area as an area with patternless or solid sheet of tumoral cells without any known architecture with occasional duct-like structure, so the diagnosis of tumor type based only on this area is impossible. One hundred twenty tumors (PA and ACC) were reviewed and 40% of our cases had at least one focus of atypical area. 19 PA and 16 ACC were fitted in our criteria, which were defined above. The proper hematoxylin and eosin stained slides of each case including both areas were selected. Serial sections with 3 µm thickness were obtained from each specimen and mounted on poly-L lysine coated slides for immunohistochemical evaluation. Immunohistochemistry was performed on selected representative paraffin blocks of each case by using streptavidin-biotin peroxidase complex detection system (LSAB kit, Dako, Denmark) with the presence of adequate and appropriate positive and negative controls. For negative controls, all steps of the procedure were performed without primary antibodies. Statistical analyses were performed with SPSS software (version 15, Chicago, IL, USA). The data were analyzed, using Chi-Square and Kruskal-Wallis test. The source, clone, concentration, and incubation periods of the primary monoclonal antibodies are summarized in Table 1. Cells labeled by these antibodies display a cytoplasmic staining pattern. Two independent pathologists evaluated the slides. Tumors were considered positive when the percentage of tumor cells with brown stained cytoplasm was higher than 10%. Overall, the normal salivary glands are tubuloacinar exocrine glands composed of two cell types: Luminal and non-luminal cells. The luminal cells of the acini are the secretary unit of the gland that produces serous and/or mucous secretions. Non-luminal acinar cells are mainly composed of myoepithelial cells. In this study, the luminal cells in both tumors (PA and ACC) are those cells that line the duct-like or glandular structure. The other cells that compose the rest of tumor cells beyond the luminal cells and those in the stroma are considered as non-luminal cells. Immunopositivity distributions in different cellular compartments of the various tumors were recorded.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone/Source</th>
<th>Dilution</th>
<th>Pretreatment</th>
<th>Positive control</th>
<th>Source</th>
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</thead>
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<tr>
<td>CK7</td>
<td>Monoclonal OV-TL 12/30</td>
<td>Predilute</td>
<td>Boiling in citrate buffer</td>
<td>Glandular epithelium endometrium</td>
<td>Dako</td>
</tr>
<tr>
<td>CK8</td>
<td>Monoclonal 35 BH 11</td>
<td>Predilute</td>
<td>Boiling in citrate buffer</td>
<td>Gastric mucosa</td>
<td>Dako</td>
</tr>
<tr>
<td>CK13</td>
<td>Monoclonal DE-K13</td>
<td>1: 300</td>
<td>Boiling in citrate buffer</td>
<td>Tongue mucosa</td>
<td>Dako</td>
</tr>
<tr>
<td>CK14</td>
<td>Monoclonal CK B1</td>
<td>1: 300</td>
<td>Boiling in citrate buffer</td>
<td>Myoepithelial cells of salivary glands</td>
<td>Sigma</td>
</tr>
<tr>
<td>CK17</td>
<td>Monoclonal E3</td>
<td>1: 30</td>
<td>Boiling in citrate buffer</td>
<td>Suprabasal cell of esophageal epithelium</td>
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</tr>
<tr>
<td>CK18</td>
<td>Monoclonal De −10</td>
<td>1: 80</td>
<td>Boiling in citrate buffer</td>
<td>Kidney tubules</td>
<td>Dako</td>
</tr>
<tr>
<td>SMA</td>
<td>Monoclonal 1A4</td>
<td>Predilute</td>
<td>Boiling in citrate buffer</td>
<td>Myoepithelial cells of salivary glands and blood vessels</td>
<td>Dako</td>
</tr>
</tbody>
</table>
Results

Thirty five patients were included with an age range of 27 to 50 years and mean age of 32.67±11.21 years. Among them, 20 cases (57.14%) were female and 15 (42.86%) were male. The average tumor size was 3.1±1.2 cm in PA group (n=19) and 2.3±1.3 cm in ACC group (n=16). The parotid, submandibular and the soft palate glands were major tumor sites. Immunostaining findings of atypical area in PA and ACC with the panel of antibodies used were summarized in Table 2, and examples are shown in Figure 1 and 2 (A&B). Briefly,

Table 2: Immunostaining of cytokeratins and smooth muscle actin in both typical and atypical parts of pleomorphic adenoma & adenoid cystic carcinoma

<table>
<thead>
<tr>
<th></th>
<th>SMA</th>
<th>CK7</th>
<th>CK8</th>
<th>CK13</th>
<th>CK14</th>
<th>CK17</th>
<th>CK18</th>
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<tbody>
<tr>
<td>PA</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Luminal</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+^a</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nonluminal</td>
<td>+^a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+^a</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Stromal</td>
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<td>+^a</td>
<td>+^a</td>
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<tr>
<td>Atypical</td>
<td>+^a</td>
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<td>+</td>
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<td>+^a</td>
<td>+^a</td>
<td>+^a</td>
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<td>ACC</td>
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<tr>
<td>Luminal</td>
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<tr>
<td>Nonluminal</td>
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<td>Atypical</td>
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Figs. 1A and 1B: Immunoreactivity of CK7 (1A) and CK14 (1B) in ductal structures of pleomorphic adenoma (Streptavidin-Biotin complexÔ 400)

Figs. 2A and 2B: Immunoreactivity of CK7 (2A) and Smooth muscle actin (2B) in ductal structures of adenoid cystic carcinoma. (Streptavidin-Biotin complexÔ 400)
IHC in salivary gland tumors

structures similar to the intercalated duct were found in PA and ACC. In these structures, luminal cells expressed CKs 7, 8, 17, 18 and 14 exhibiting the same immunoprofile as the luminal cells of the intercalated duct of the normal salivary gland. The outer cell of the ductal structures exhibited SMA, but CK14 was rare. Non-luminal cells of PA occasionally expressed SMA and CK14. It is noteworthy that positivity of CK13 was rarely detected in the atypical area of PA and its expression was solely in the areas of squamous metaplasia. The immunoprofile of non-luminal cells of ACC was expression of SMA, CK14, CK17, and CK18. CKs 7, 8 and 14 were observed in the cells of the atypical area in pleomorphic adenoma with variable expression of CKs 17, 18, and SMA. The same results were also observed in the atypical area of ACC except CK17 that was not expressed at all.

Discussion

It is well known that histopathologic diagnosis of salivary gland tumors especially those with atypical morphologic features may be a challenge for the pathologists. The distinction between both tumor types can be difficult because of an enormous variation in histologic appearance, with either type showing partially overlapping morphologic features. In this study, in typical areas of both PA and ACC, the luminal cells of ductal structure are positive for CK7, CK8, CK19 consistently and CK14 variably. Non-luminal cells express SMA consistently and CK14 variably. Our results confirm those of previous studies in which the immunoprofile of typical PA and ACC was discussed.1,2,7,9,10

According to Gurrbuz et al.’s findings,14 in PA, CK14 and SMA were observed in all cases in both epithelial and myoepithelial cells. In our study, the luminal cells were negative for SMA. Basal cells of mature ducts and myoepithelial cells are derived from common primordial cells.14 The myoepithelial and ductal basal cells are the proximal and distal compartments of the same continuum that extend from the acinus to the distal ducts. This can explain the CK14 expression of both of these cells.14 This CK14 expression in our case is an indicator of both basal and myoepithelial differentiation. CK14 was the most intensely expressed cytokeratin in PA. All the tumors were uniformly negative for CK13. In the PA group, there was no difference between the expression of CK7, CK 8, CK 13, CK 14, CK 17, and CK 18 in both typical and atypical areas except SMA that was not expressed in the atypical area.

In the ACC group, there was no difference between the expression of CK7, CK 8, CK 14, and CK 18 in both typical and atypical areas except SMA that was not expressed in the atypical area. The CK17 was not expressed in this group. Ihrler et al. emphasized complete or preponderant negativity of CK7 with positivity of CK18 in ACC.15 In our study, we found expression of CK7 in both luminal and atypical area of ACC.

CK7 has been shown in this study to be expressed in both typical and atypical portions of PA and ACC and, more specifically and intensely, in the luminal cells of the salivary ducts, whereas non-luminal and stromal cells stain with less intensity or not at all. In this regard, it seems that the immunoprofile of the atypical area of both tumor types are similar to each other and IHC could not differentiate between these two histological types in salivary gland tumors. Therefore, meticulous tumor sampling from different parts with careful histopathological evaluation is yet the best way for diagnosis.

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Conflict of interest: None declared.

References


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