Review and Updates of Immunohistochemistry in Selected Salivary Gland and Head and Neck Tumors

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• **Context.**—Immunohistochemistry is a useful tool for diagnosing salivary gland and head and neck tumors.

Objective.—To review immunohistochemical markers, which can aid in the diagnosis of selected salivary gland and head and neck tumors.

Data Sources.—Literature review and authors’ personal practice experience.

Conclusions.—Salivary gland and head and neck tumors include a large diverse group of tumors with complex and overlapping histologic features. Immunohistochemistry plays an important role in resolving the differential diagnosis of some salivary gland and head and neck tumors and can provide information for the prognosis of certain tumors.

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### IMMUNOHISTOCHEMICAL MARKERS FOR CELLULAR DIFFERENTIATION OF SALIVARY GLAND TUMORS

There are 34 benign and malignant salivary gland epithelial tumors according to the 2005 third histologic classification of the World Health Organization.1 These tumors can show diverse morphology and overlapping histologic features. Although accurate diagnoses can be made on the basis of hematoxylin-eosin sections of most salivary gland tumors, immunohistochemistry can provide a powerful adjunct tool for pathologists to identify the cellular differentiation and assign correct classifications in difficult tumor cases.

The salivary glands include 3 major pairs of salivary glands (parotid, submandibular, and sublingual) and many minor salivary glands, which can be located throughout the upper respiratory tract. The glands are composed of acini (serous, mucinous, and mixed) and ducts (intercalated, striated, and excretory). The acini and intercalated duct are surrounded by myoepithelial cells. The striated duct and excretory duct are surrounded by basal cells. Most salivary gland tumors originate from acinar/ductal epithelial cells (luminal cells) and/or myoepithelial/basal cells (abluminal cells). Mono- and biphasic tumors have only 1 cellular component, either originating from acinar/ductal epithelial cells or from myoepithelial/basal cells. These include tumors such as myoepithelioma, acinic cell carcinoma, and salivary duct carcinoma. The tumors originating from both acinar/ductal epithelial cells and myoepithelial/basal cells are designated biphasic tumors, and this category includes tumors such as pleomorphic adenoma, epithelial myoepithelial carcinoma, and adenoid cystic carcinoma. Some tumors demonstrate other unique cellular differentiation, such as sebaceous adenoma/carcinoma, lymphadenoma, and mucoepidermoid carcinoma. Immunohistochemistry can be very helpful to determine the cellular differentiation of some of the more unusual tumors, or in variant morphologies of common tumors. Some tumors, such as salivary duct carcinoma and mammary analogue secretory carcinoma, demonstrate more specific immunohistologic profiles, which facilitate the diagnosis.

The acinar/ductal epithelial cells are positive for keratins (CK7 and CAM 5.2) and epithelial membrane antigen (EMA). They are focally positive or negative for high-molecular-weight keratins (HMWks; CK5/6 and 34bE12) and negative for p63, myoid markers (smooth muscle myosin heavy chain [SMMHC], smooth muscle actin [SMA], calponin), and CK20 (focal weak expression can be seen in rare salivary gland carcinomas). Myoepithelial cells are usually positive for p63, myoid markers (SMMHC, SMA, calponin), vimentin, S100, and HMWks (CK5/6, 34bE12), but only show weak expression for CK7 and CAM 5.2 and no expression for EMA. Basal cells are positive for p63 and HMWks (CK5/6, 34bE12), weakly positive or negative for CK7, CAM 5.2 and myoid markers (SMMHC, SMA, calponin), and negative for CK20, vimentin, S100, and EMA. S100 and vimentin are sensitive, but not specific, markers for myoepithelial cells. An important caveat in using p63 as a marker for myoepithelial or basal cells is that it also stains squamous epithelium (Tables 1 through 3; Figure 1, A through F; and Figure 2, A and B).2–11

The SRY-related HMG-box 10 (SOX10) protein is a transcription factor that is critical for formation and differentiation of neural crest cells,12 SOX10 is a more sensitive and specific marker for melanocytic and schwannian tumors than S100.13 Recently, Ohtomo et al14 found SOX10 was expressed in both luminal and abluminal cells of intercalated ducts of normal human major salivary glands.
The tumors with no SOX10 expression are salivary duct carcinoma, mucoepidermoid carcinoma, squamous cell carcinoma, oncocytic carcinoma, oncocytoma, and Warthin tumor, which are usually thought to resemble striated and excretory ducts. SOX10 expression was present in both neoplastic myoepithelial cells and neoplastic epithelial cells. SOX10 appears to be a potential marker for acinar and intercalated duct differentiation in the diagnosis of salivary gland tumors (Table 4 and Figure 3).

SELECTED SALIVARY GLAND AND HEAD AND NECK TUMORS

Acinic Cell Carcinoma

Acinic cell carcinoma demonstrates both serous acinar and intercalated ductal epithelial differentiation. The tumor has several growth patterns: solid/lobular, microcystic, papillary-cystic, and follicular. Most acinic cell carcinomas express CK7 and CAM 5.2. However, the tumors with acinar pattern are focally positive or negative for CK7. The normal acinar cells express amylase, but most acinic cell carcinomas are negative for this marker. Rare acinic cell carcinomas may acinar cells express amylase, but most acinic cell carcinomas are negative for this marker. Rare acinic cell carcinomas may express synaptophysin and mucin (MUC) 3. Acinic cell carcinomas from morphologic mimics, particularly mammary analogue secretory carcinomas (Figure 4).

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma is a malignant biphasic epithelial tumor composed of modified myoepithelial and ductal cells. Typically, patients with this tumor have a poor long-term prognosis. The tumor can have cribriform, tubular, and solid patterns; frequently, there are mixed growth patterns within the same lesion. Adenoid cystic carcinoma expresses both ductal and myoepithelial/basal cell markers, such as CK7, CAM 5.2, calponin, SMA, SMMHC, p63, SOX10, and S100.14,23–25

Most adenoid cystic carcinomas showed strong and diffuse expression of c-KIT, but studies have not identified c-KIT mutations in these tumors (Figure 5).26–32 c-KIT

### Table 2. Markers for Salivary Gland Tumors With Clear Cell Differentiation

<table>
<thead>
<tr>
<th></th>
<th>EMC</th>
<th>MEC</th>
<th>MC</th>
<th>CCC</th>
<th>ACC</th>
<th>OC/OCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>p63</td>
<td>+, outer layer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+, basal</td>
</tr>
<tr>
<td>Calponin/SMA/SMMHC</td>
<td>+, outer layer</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CK7/CAM 5.2</td>
<td>+, inner layer</td>
<td>-</td>
<td>-</td>
<td>or +</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>SOX10</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>-</td>
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</table>

Abbreviations: ACC, acinic cell carcinoma; CCC, clear cell carcinoma; CK, cytokeratin; EMC, epithelial-myoepithelial carcinoma; MC, myoepithelial carcinoma; MEC, mucoepidermoid carcinoma; ND, no data; OC/OCA, oncocytoma/oncocytic carcinoma; SMA, smooth muscle actin; SMMHC, smooth muscle myosin heavy chain; SOX10, SRY-related HMG-box 10.
expression is predominantly located in the inner ductal cells of the tubular and cribriform patterns and was homogeneously expressed in the solid form.\(^{33}\) This unique immunophenotype is useful in differentiating adenoid cystic carcinoma from some of its mimics. Some salivary gland tumors may show expression of c-KIT, such as basal cell adenocarcinoma, lymphoepithelial carcinoma, myoepithelial carcinoma, and basaloid squamous carcinoma.\(^{26,28,32}\) Tumors that only rarely expressed c-KIT include pleomorphic adenoma, sebaceous carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma, basal cell carcinoma, oncocytoma, and Warthin tumor.\(^{26}\) The expression of c-KIT in polymorphous low-grade adenocarcinoma is controversial. While some researchers reported that polymorphous low-grade adenocarcinoma rarely expressed c-KIT or had low expression of c-KIT, others have reported frequent expression of c-KIT in polymorphous low-grade adenocarcinoma.\(^{24,32,33}\) Further study is necessary to compare the expression of c-KIT in these 2 tumors.

Adenoid cystic carcinomas with high-grade transformation showed elevated Ki-67 and p53 labeling indices in the high-grade components. Overexpression of Ki-67 and p53 was associated with poor prognosis in the patients with adenoid cystic carcinoma.\(^{34,39}\) Also, these tumors lose the myoepithelial cell component and show an almost pure epithelial population.

Histone H3 lysine 9 trimethylation (H3K9me3) and acetylated histone H3 at lysine 9 (H3K9ac) are histone modifications that play an important role in the transcription of target genes. Xia et al.\(^{40}\) investigated H3K9me3 and H3K9ac expression in 66 cases of adenoid cystic carcinoma. They found that high levels of H3K9me3 expression was associated with solid pattern, higher incidence of distal metastasis, poorer disease-free survival rates and overall survival rates, while low H3K9Ac expression was associated with poor overall survival rates. High levels of H3K9me3 expression and solid pattern tumors were independent prognostic factors that significantly influenced overall survival. H3K9me3 expression was the only independent predictor of disease-free survival.

A recurrent t(6;9)(q22-23;p23-24) translocation was identified in adenoid cystic carcinoma.\(^{41}\) This translocation leads to the fusion of MYB and NFIB and the deregulation of the expression of Myb.\(^{42}\) A balanced translocation between 6p23 and 9q22-23 is not identified in other salivary gland tumors or nonsalivary gland neoplasms.\(^{43,44}\) Myb expression was found in 55% to 70% of adenoid cystic carcinomas, even in 46% to 61% of tumors without translocation.\(^{43,45}\) Myb expression was mainly located in the peripheral myoepithelial cells and was not identified in ductal cells of either the tubular or cribriform patterns.\(^{43,44}\) Myb expression was found predominantly in the cribriform (50%) and the tubular patterns (61%). Only 12% of the solid form expressed Myb. Myb expression was not detected in nontumoral salivary gland parenchyma.\(^{45}\) Strong Myb expression was seen in most of Myb-positive adenoid cystic carcinoma. Focal weak Myb expression was identified in other tested salivary gland tumors, including myoepithelial carcinoma, polymorphous low-grade adenocarcinoma, myoepithelioma, pleomorphic adenoma, and mucoepidermoid carcinoma. No Myb expression was seen in basal cell adenoma, oncocytoma, adenosquamous carcinoma, acinic cell carcinoma, and salivary duct carcinoma. A subset of breast carcinoma, seminoma, colorectal carcinoma, and thymoma showed strong Myb expression.\(^{46}\) Myb appears to be a valuable marker for differentiating a subset of adenoid cystic carcinomas from other salivary gland tumors.

### Mammary Analogue Secretory Carcinoma

Mammary analogue secretory carcinoma is a recently categorized salivary gland tumor that is histologically, immunohistochemically, and genetically similar to secretory carcinoma of the breast.\(^{46}\) The tumor has a t(12;15)(p13;q25) ETV6-NTRK3 translocation that is also present in breast secretory carcinoma. The tumor has a variety of growth patterns, including lobular, macrocystic, microcystic, papillary, cribriform, tubular, and solid microcystic. The tumor cells demonstrate low-grade nuclei and multivacuolated to eosinophilic granular cytoplasm. Historically, it was most often diagnosed as zymogen-poor acinic cell carcinoma and (cyst) adenocarcinoma and occasionally as mucin-producing signet ring adenocarcinoma or mucoepidermoid carcinoma.\(^{47,48}\) The tumor expresses S100, mammaglobin, CK7, CK8, CK18, CK19, 34βE12, EMA, gross cystic disease fluid protein 15 (GDFP-15), GATA3, signal transducer and activator of transcription 5a (STAT5a), MUC1, MUC4, and vimentin (Figure 6, A and B). Basal cell/myoepithelial cell markers, such as p63, calponin, SMA, and CK5/6, usually do not show expression in the tumor.\(^{46,49,52}\) However, the peripheral layer of residual basal cells surrounding the tumor nests could be highlighted by p63 staining.\(^{47,49}\) The tumor is negative for androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/neu). The MIB-1 indices range between 5% and 28%.\(^{46}\)

Compared to mammary analogue secretory carcinoma, acinic cell carcinoma rarely expresses mammaglobin, CK8, and CK19. Only a small percentage of acinic cell carcinomas express S100, vimentin, GDFP-15. No expression of MUC4 was present in acinic cell carcinoma.\(^{46}\)

Besides acinic cell carcinoma, the other entity occasionally considered in the differential diagnosis is adenoid cystic carcinoma. However, adenoid cystic carcinoma is rarely positive for mammaglobin, S100, vimentin, CK8, CK19, and GDFP-15. Mammary analogue secretory carcinoma can be...
Figure 1. Expression of myoepithelial/basal markers in normal salivary glands. A, Calponin expression in myoepithelial cells of acini. B, Smooth muscle actin expression in myoepithelial cells of acini and intercalated duct. C, Smooth muscle myosin heavy chain expression in myoepithelial cells of acini and intercalated duct. D, Cytokeratin 5/6 expression in myoepithelial cells of acini and basal cells of excretory duct. E, p63 expression in myoepithelial cells of acini and basal cells of excretory duct. F, S100 expression in myoepithelial cells of acini (original magnification ×400 [A through F]).
differentiated from mucoepidermoid carcinoma by its expression of S100 and lack of expression of p63.50,51

Although coexpression of both mammaglobin and S100 protein is characteristic of mammary analogue secretory carcinoma, polymorphous low-grade adenocarcinomas (60%) and adenoid cystic carcinomas (13.3%) showed significant coexpression of both markers without evidence of ETV6-NTRK3 fusion.50 Polymorphous low-grade adenocarcinomas and adenoid cystic carcinomas were positive for p63 and negative for GCDFP-15.25,53 In difficult cases, molecular testing for ETV6-NTRK3 fusion would be helpful for the differential diagnosis.46

**Mucoepidermoid Carcinoma**

Mucoepidermoid carcinoma is a malignant epithelial neoplasm composed of mucous, epidermoid, intermediate, columnar, clear, and oncocytic cells. It is usually positive for CK5, CK6, CK7, CK8, CK14, CK18, CK19, EMA, carcinoembryonic antigen (CEA), and p63 and is negative for CK20, SMA, muscle specific actin (MSA), and S100. However, focal expression of S100, c-KIT, glial fibrillary acidic protein (GFAP), and vimentin can be seen in some tumors (Figure 7, A and B).20,54–59

**Figure 2.** Keratin expression in normal salivary glands. A, CAM 5.2 expression in acini and ducts. B, Cytokeratin 7 expression in acini and ducts (original magnification ×400 [A and B]).

**Figure 3.** SOX10 expression in adenoid cystic carcinoma (original magnification ×400).

**Table 4.** SOX10 Expression in Salivary Gland Tumors

<table>
<thead>
<tr>
<th>SOX10-positive tumors</th>
<th>SOX10-negative tumors</th>
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<tbody>
<tr>
<td>Acinic cell carcinomas</td>
<td>Salivary duct carcinomas</td>
</tr>
<tr>
<td>Adenoid cystic carcinomas</td>
<td>Mucoepidermoid carcinomas</td>
</tr>
<tr>
<td>Epithelial-myoepithelial carcinomas</td>
<td>Squamous cell carcinomas</td>
</tr>
<tr>
<td>Myoepithelial carcinomas</td>
<td>Oncocytic carcinomas/oncocytomas</td>
</tr>
<tr>
<td>Pleomorphic adenomas</td>
<td>Warthin tumors</td>
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</table>

Abbreviation: SOX10, SRY-related HMG-box 10.

p63 is a useful marker to differentiate acinic cell carcinoma from mucoepidermoid carcinoma. Sams et al20 compared p63 expression among 31 cases of acinic cell carcinomas and 24 cases of mucoepidermoid carcinomas. They found that all acinic cell carcinomas were negative for p63, while all mucoepidermoid carcinomas were strongly positive for p63. p63 immunohistochemical expression pattern can be helpful in distinguishing low-grade mucoepidermoid carcinoma from mucous retention cysts and papillary cystadenoma of the salivary glands. Fonseca et al58 found that p63 immunostaining in mucous retention cysts and papillary cystadenomas was limited to the basal layers of the cystic spaces, whereas in low-grade mucoepidermoid carcinomas, positive staining was also found diffusely in the suprabasal layers of the epidermoid component of the tumor. Oncocytic mucoepidermoid carcinoma can also be differentiated from oncocytoma and oncocytic carcinoma by p63 staining pattern. It has been reported that in oncocytic mucoepidermoid carcinomas, more than 50% of the cells throughout the tumor nests were positive for p63, while only scant peripheral cells of the tumor nests in oncocytoma and oncocytic carcinoma were positive for p63.59

**Myoepithelial Carcinoma**

Myoepithelial carcinoma is a malignant tumor of the salivary glands, with tumor cells displaying exclusively myoepithelial differentiation. The tumor cells can be quite diverse, including spindled, stellate, epithelioid, plasmacytoid, or clear cells. They can resemble sarcoma, melanoma, or other tumors. Immunoreactivity for both keratins and at least 1 myoepithelial marker is required for the diagnosis of...
Figure 4. Discovered on gastrointestinal stromal tumor protein 1 (DOG1) expression in acinic cell carcinoma (original magnification ×400).

Figure 5. c-KIT expression in adenoid cystic carcinoma (original magnification ×400).

Figure 6. A, S100 expression in mammary analogue secretory carcinoma. B, Mammaglobin expression in mammary analogue secretory carcinoma (original magnification ×400 [A and B]).

Figure 7. A, Cytokeratin 5/6 expression in mucoepidermoid carcinoma. B, p63 expression in mucoepidermoid carcinoma (original magnification ×400 [A and B]).
myoepithelial carcinoma.60 Myoepithelial carcinomas frequently express vimentin (100%), calponin (75%–100%), S100 (82%–100%), CK AE1/3 (90%–100%), 34βE12 (92%), CAM 5.2 (89%), pancytokeratin (74%), and EMA (21%–100%); less frequently express caldesmon (50%), SMA (35%–50%), MSA (31%), GAFP (31%–50%), SMMHC (30%), p63 (28%), EMA (27%), and Ki-67 (labeling index, 4%–65%); occasionally express CD117 (6%) and desmin (10%); and do not express CEA.61–64 Calponin appears to be the most sensitive and specific marker to identify myoepithelial differentiation for myoepithelial carcinoma. A panel of multiple markers, including CK AE1/3, CAM 5.2, CK5/6, calponin, SMA, S100, and vimentin, can be helpful to make an accurate diagnosis.

**Polymorphous Low-Grade Adenocarcinoma**

Polymorphous low-grade adenocarcinoma is a low-grade malignant epithelial carcinoma almost exclusively arising in minor salivary glands with bland cytologic features. This tumor typically has diverse growth patterns, including lobular, papillary or papillary-cystic, cribriform, trabecular, and ductal or tubular areas. The tumor frequently expresses CK AE1/3, CAM 5.2, 34βE12, EMA, p53, p63, vimentin, bcl-2, S100, and it infrequently expresses SMA and GAFP.65–69 Carcinoembryonic antigen immunoreactivity is present in up to 54% of tumors. Polymorphous low-grade adenocarcinoma has overlapping histologic features with pleomorphic adenoma/canalicular adenoma and adenoid cystic carcinoma. In practice, it is sometimes difficult to distinguish polymorphous low-grade adenocarcinoma from these tumors, especially in small biopsy specimens. Most pleomorphic adenomas show strong GAFP expression, while only rare polymorphous low-grade adenocarcinomas show faint patchy reactivity for GAFP.65–69 Curran et al70 reported that 96% of canalicular adenomas demonstrated weak to strong cytoplasmic staining for GAFP, which was confined to a row of cells at the tumor/connective interface. All pleomorphic adenomas demonstrated weak to strong diffuse cytoplasmic staining for GAFP in the ductal and myoepithelial cells, while 2 cases also showed a weakly staining linear row of immunoreactive cells at the tumor/connective tissue. All polymorphous low-grade adenocarcinomas showed little or no intralobional reactivity and no peripheral linear immunoreactivity for GAFP. GAFP can be helpful to distinguish pleomorphic low-grade adenocarcinoma from pleomorphic adenoma/canalicular adenoma (Table 5).

**Salivary Duct Carcinoma**

Salivary duct carcinoma is an aggressive malignant epithelial tumor arising from intralobular and interlobular excretory ducts. It resembles high-grade breast ductal carcinoma and demonstrates ductal, papillary, solid, and cribriform with comedo necrosis growth patterns.

Salivary duct carcinoma usually expresses AR, GCDFP-15, CK AE1/3, CK7, 34βE12, CEA, and EMA (Figure 8, A through C). Occasionally, it can be positive for ER, PR, and S100.71–73 The Ki-67 expression markedly increases and the indices are higher than 25%.72,73,77 Androgen receptor is expressed significantly more often in salivary duct carcinomas of men than in salivary duct carcinomas of women.78

The immunohistochemistry of AR/ER/PR/GCDFP* is characteristic of salivary duct carcinoma, but it does not completely exclude metastasis from the breast, which might also be AR* and ER/PR* in a lesser proportion of cases.74,80–82 GATA3, a new marker for breast carcinoma, was detected in all salivary duct carcinomas.83

More than 80% of salivary duct carcinomas show HER2/neu and p53 overexpression, which was correlated to poor prognosis.77,81–83 However, tumors with HER2/neu expression may respond to trastuzumab-based therapy.84,85

Prostatic acid phosphatase and prostate-specific antigen expression can rarely be detected in salivary duct carcinomas from men and women.86,87 In men with unknown prostatic acid phosphatase–positive and prostate-specific antigen–positive metastatic carcinoma, salivary duct carcinoma should be included in the differential diagnosis in addition to prostatic adenocarcinoma. Expression of CK7 and HMWks support the diagnosis of salivary duct carcinoma.88

**Pleomorphic Adenoma and Carcinoma Ex Pleomorphic Adenoma**

Pleomorphic adenoma is a benign epithelial neoplasm demonstrating both epithelial and modified myoepithelial differentiation with diversified histologic features. The tumor expresses CK7, CK14, CEA, SMA, MSA, SMMHC, calponin, p63, S100, vimentin, Wilms tumor 1 (WT1), and GAFP.6,10,13,71,86–91

Pleomorphic adenoma gene 1 (PLAG1) is a proto-oncogene that is activated by recurrent chromosome rearrangements in pleomorphic adenoma, resulting in PLAG1 protein overexpression.92–94 Rotellini et al95 examined 101 benign and malignant salivary gland tumors, including 36 pleomorphic adenomas, 8 myoepitheliomas, 5 basal cell adenomas, and 1 canalicular adenoma among the benign tumors; 16 mucoepidermoid carcinomas, 8 acinic cell carcinomas, 8 polymorphous low-grade adenocarcinomas, 7 salivary duct carcinomas, and 4 epithelial-myoepithelial carcinomas among malignant tumors. They found that PLAG1 protein showed diffuse positivity in 94.4% of pleomorphic adenomas and weak positivity in all myoepitheliomas. Among malignant tumors, only 2 polymorphous low-grade adenocarcinomas and 1 salivary duct carcinoma ex pleomorphic adenoma were positive; other tumors were negative. The authors concluded that PLAG1 protein is a sensitive and specific marker for pleomorphic adenoma and can be useful to differentiate pleomorphic adenoma

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**Table 5. The Differentiation of Adenoid Cystic Carcinoma, Polymorphous Low-Grade Adenocarcinoma, and Pleomorphic Adenoma**

<table>
<thead>
<tr>
<th></th>
<th>c-KIT</th>
<th>Calponin/SMA/SMMHC</th>
<th>CK7</th>
<th>MIB-1</th>
<th>Myb</th>
<th>PLGA1</th>
<th>GFAP</th>
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<tbody>
<tr>
<td>AdCC</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>&gt;10%</td>
<td>+ or -</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PLGA</td>
<td>- or +</td>
<td>- or +</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>PA</td>
<td>+</td>
<td>or +</td>
<td>+</td>
<td>&lt;5%</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Abbreviations: AdCC, adenoid cystic carcinoma; CK, cytokeratin; GFAP, glial fibrillary acidic protein; PA, pleomorphic adenoma; PLGA, polymorphous low-grade adenocarcinoma; SMA, smooth muscle actin; SMMHC, smooth muscle myosin heavy chain.
from adenoid cystic carcinoma, mucoepidermoid carcinoma, epithelial-myoepithelial carcinoma, and acinic cell carcinoma.

Carcinoma ex pleomorphic adenoma is a malignant epithelial neoplasm arising from benign pleomorphic adenoma. Bahrami et al96 studied PLAG1 expression by immunostaining and PLAG1 gene rearrangement by fluorescence in situ hybridization (FISH) in 22 carcinoma ex pleomorphic adenomas along with 39 cases representing various benign and malignant salivary gland neoplasms: adenocarcinomas, not otherwise specified (4 cases); mucoepidermoid carcinomas (8 cases); adenoid cystic carcinomas (7 cases); salivary duct carcinomas (4 cases); epithelial-myoepithelial carcinomas (2 cases); polymorphous low-grade adenocarcinoma (1 case); myoepithelial carcinoma (1 case); adenoid cystic carcinoma (1 case); basaloïd carcinoma (1 case); pleomorphic adenomas (5 cases); basal cell adenomas (4 cases); and low-grade salivary gland neoplasm, not otherwise specified (1 case). They found that all 5 pleomorphic adenomas were positive for PLAG1 (2+ to 3+); 17 of 22 carcinomas ex pleomorphic adenomas were positive for PLAG1 (1+ to 3+), while 5 of 22 carcinomas ex pleomorphic adenomas scored as rare (1%–4% positive cells) or negative. All other tumors scored as rare (1%–4% positive cells) or negative. Fluorescence in situ hybridization study showed that 12 of 19 carcinomas ex pleomorphic adenoma (63%) were positive for gene rearrangement, 2 showed only a trisomy/polysomy profile, and 5 had a normal pattern. One FISH-positive tumor showed amplification of PLAG1. One of 3 cases analyzed for high-mobility-group AT-hook 2 gene (HMGA2) by FISH was positive for gene rearrangement. The authors suggested that a diffuse and unequivocal expression for PLAG1 in a carcinoma is a strong indication for the diagnosis of carcinoma ex pleomorphic adenoma in the proper clinicopathologic setting. Immunostaining for PLAG1 protein and FISH for PLAG1, particularly in combination, may be helpful ancillary studies for carcinoma ex pleomorphic adenoma.

**Nuclear Protein in Testis Midline Carcinomas**

Nuclear protein in testis (NUT) midline carcinomas are rare, aggressive malignant epithelial tumors arising from midline structures, including upper aerodigestive tract,
thymus, mediastinum, lung, and bladder.97–100 Approximately half of these tumors occur in the head and neck regions. The NUT midline carcinomas affect patients from childhood to old age, though they many occur in children and young adults. The tumors present as poorly or undifferentiated carcinomas that frequently contain aberrant and abrupt squamous differentiation. These tumors may be mistaken for poorly differentiated or undifferentiated carcinoma, squamous cell carcinoma, Ewing sarcoma, sinonasal undifferentiated carcinoma, thymic carcinoma, or neuroblastoma. It is important to recognize this type of malignancy because the patients with NUT midline carcinomas have much poorer prognosis than those with non-NUT midline carcinomas. The NUT midline carcinomas have characteristic chromosomal rearrangements of the NUT gene at 15q14. The most common translocation involving the NUT gene is the t(15;19)(q14;p13.1), resulting in BRD4–NUT fusions.100

The NUT midline carcinomas are positive for CK7, p40, p63, and EMA and are negative or only focally positive for CK20. Approximately one-third of NUT midline carcinomas are positive for CD34. Rare tumors may show focal weak immunoreactivity for synaptophysin, chromogranin, and S100.99,101,102

The diagnosis of NUT midline carcinomas can be made by demonstration of NUT rearrangement by FISH or by demonstration of a BRD4–NUT fusion transcript by reverse transcription–polymerase chain reaction (PCR).103 Recently, Haack et al104 studied NUT expression in a panel of 1068 tissues, including 30 FISH-positive NUT midline carcinomas and 876 other carcinomas, by using C52, a rabbit monoclonal antibody against a recombinant NUT polypeptide (Cell Signaling Technologies, Inc, Danvers, Massachussets). They found that the C52 immunostain achieved a sensitivity of 87% and a specificity of 100% in NUT midline carcinomas. Some germ cell tumors, including 6% of seminomas, 8.6% of embryonal carcinomas, and 64% of dysgerminomas, showed weak, focal nuclear staining. Among normal tissues, weak cytoplasmic staining was seen in hepatocytes and rare renal tubular cells. No other carcinomas showed immunoreactivity to C52. The study confirmed that C52 immunostain is a new sensitive and highly specific marker for NUT midline carcinomas. Owing to possible false-negative NUT expression by immunostaining, the authors suggested that FISH for NUT rearrangements should be performed when C52 immunostaining is negative and NUT midline carcinoma remains highly likely in the differential diagnosis (Table 6).

Table 6. Differentiation of Nuclear Protein in Testis (NUC) Midline Carcinomas, Nasopharyngeal Carcinoma, Small Cell Neuroendocrine Carcinoma, and Sinonasal Undifferentiated Carcinoma

<table>
<thead>
<tr>
<th>NMC</th>
<th>SNUC</th>
<th>NPC</th>
<th>SCN</th>
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<tbody>
<tr>
<td>EBV</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NUT (C52)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>p63</td>
<td>–</td>
<td>+</td>
<td>or +</td>
</tr>
<tr>
<td>Synaptophysin, chromogranin</td>
<td>–</td>
<td>–</td>
<td>or –</td>
</tr>
</tbody>
</table>

Abbreviations: EBV, Epstein-Barr virus; NMC, NUT midline carcinoma; NPC, nasopharyngeal carcinoma; SCN, small cell neuroendocrine carcinoma; SNUC, sinonasal undifferentiated carcinoma.

Human Papillomavirus–Positive Carcinomas of Oropharynx and Oral Cavity

Human papillomavirus (HPV), predominantly HPV 16, infection has been identified in a unique subset of patients with head and neck squamous cell carcinomas especially carcinomas of oropharynx and base of tongue. There is extensive evidence to support a causal role of HPV in oral carcinomas.105–108 The prevalence of HPV infection in oropharyngeal carcinoma has increased significantly over time, from 40.5% before 2000 to 72.2% between 2005 and 2009, while the HPV prevalence in nonoropharyngeal carcinoma has not increased (18.9%–25.1%).109,110 The patients with HPV-positive head and neck squamous cell carcinomas are younger than patients with HPV-negative tumors. More often these tumors are seen in nonsmokers and nondrinkers, are less likely to harbor p53 mutations, and often have a favorable prognosis.111–114 It is important to identify the HPV-positive carcinomas of oropharynx and oral cavity owing to their unique biological and clinical behaviors. Personalized therapy may provide the maximal benefit for patients, depending on their HPV status.

In the HPV-infected tumor cells, the expression of HPV E2 viral transcription/replication factor is disrupted, resulting in dysregulation of expression of viral E6 and E7 oncoproteins. The viral E6 and E7 oncoproteins inactivate p53 and retinoblastoma (Rb) proteins, which lead to the overexpression of p16, a cyclin-dependent kinase inhibitor.115–117 Direct detection of HPV messenger RNA by reverse transcription–PCR, and HPV DNA by PCR and FISH, is laborious, expensive, technically challenging, and not available in many laboratories. However, immunohistochemistry is a relatively simple and inexpensive method widely used in most laboratories. p16 overexpression was highly correlated with HPV-positive tumors (detected in up to 98% of HPV-positive carcinomas) (Figure 9). Patients with p16-positive squamous cell carcinomas of oropharynx and oral cavity showed improved local tumor control, improved disease-free survival, and better overall survival, compared to patients with p16-negative squamous cell carcinomas.118–129 p16 immunostain was considered a very sensitive surrogate biomarker for HPV infection, although p16 positivity is not 100% specific for HPV infection. Lewis et al127 analyzed p16 overexpression and HPV status in 239 cases of oropharyngeal carcinoma. They found 78% of cases were positive for p16; of these, 74% were positive for HPV by in situ hybridization (ISH). In 45 p16-positive, HPV ISH–negative cases, 19 were positive for HPV by PCR. In the p16-positive cohort, there was no difference in survival between HPV–ISH positive and HPV ISH–negative cases. Comparing the HPV ISH–positive and HPV ISH– and PCR–negative carcinomas, there was again no difference in survival. p16-positive, HPV-negative carcinomas were associated with significantly better survival than p16-negative carcinomas. The authors concluded that p16 immunostaining is the best test for risk stratification in oropharyngeal squamous cell carcinoma.

The 2011 National Comprehensive Cancer Network guidelines130 recommended p16 immunostain alone as a valuable prognostic marker for patients with oropharyngeal cancers.

For metastatic squamous cell carcinoma of the cervical lymph nodes of unknown primary, p16 immunostaining as well as HPV tests can be helpful in identifying a hidden oropharyngeal primary tumor.131–133 El-Moffy et al133 re-
ported that among 93 cases of head and neck squamous cell carcinoma with cervical lymph node metastasis, 23 cases were positive for HPV by ISH; of these, 22 of 23 were of oropharyngeal origin. Twenty-one HPV-positive oropharyngeal carcinomas were nonkeratinizing and strongly and diffusely positive for p16 immunostain. Begum et al. found that in 68 cases of head and neck squamous cell carcinoma with cervical lymph node metastasis, HPV 16 was detected by ISH in 22 of 31 metastases from the oropharynx (71%) but in none of the 37 metastases from other sites (0%). p16 expression was detected in 24 of 31 metastases from the oropharynx, and only 1 of 37 nonoropharyngeal carcinomas was p16 positive.

CONCLUSIONS

In summary, immunohistochemistry plays an important role in the diagnosis of certain salivary gland and head and neck tumors. The keratin markers (CK7, CAM 5.2, CK5/6, and 34BE12) and myoid/basal cell markers (calponin, SMA, SMMHC, and p63) can be used to identify the cellular differentiation of most salivary gland tumors. Other markers, including c-KIT, GFAP, Myb, AR, GCDFP-15, mammoglobin, SOX10, DOG1, and PLGA1, are useful in the differential diagnosis of salivary gland tumors. The NUT midline carcinomas can be detected by the highly specific and sensitive marker C52. H3K9me3, H3K9ac, MIB-1, and p53 may be used to identify some aggressive tumors. Finally, p16, a sensitive surrogate marker for HPV infection, can provide prognostic information for HPV-positive oropharyngeal carcinomas and is helpful for identifying oropharyngeal primary tumor of metastatic cervical lymph node.

References


Selected Salivary Cland and Head and Neck Tumors—Zhu et al

64 Arch Pathol Lab Med—Vol 139, January 2015


