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Special Issue Histochemistry of Salivary Glands

Review

Immunohistochemical Analysis of Salivary Gland Tumors: Application for Surgical Pathology Practice

Toshitaka Nagao[1], Eiichi Sato[1], Rie Inoue[1], Hisashi Oshiro[1], Reisuke H. Takahashi[1], Takeshi Nagai[1], Maki Yoshida[1], Fumie Suzuki[1], Hiyo Obikane[1], Mitsumasa Yamashina[1] and Jun Matsubayashi[1]

[1]*Department of Anatomic Pathology, Tokyo Medical University, Japan*

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Salivary gland tumors are relatively uncommon and there exists a considerable diagnostic difficulty owing to their diverse histological features in individual lesions and the presence of a number of types and variants, in addition to overlapping histological patterns similar to those observed in different tumor entities. The classification is complex, but is closely relevant to the prognostic and therapeutic aspects. Although hematoxylin-eosin staining is still the gold standard method used for the diagnosis, immunohistochemistry (IHC) can enhance the accuracy and be a helpful tool when in cases to investigate the subjects that cannot be assessed by histological examination, such as the cell nature and differentiation status, cell proliferation, and tumor protein expression. This review depicts on the practical diagnostic utility of IHC in salivary gland tumor pathology under the following issues: assessment of cell differentiation, focusing on neoplastic myoepithelial cells; discrimination of histologically mimic tumor groups; diagnosis of specific tumor types, *e.g.*, pleomorphic adenoma, adenoid cystic carcinoma, and salivary duct carcinoma; and evaluation of malignancy and prognostic factors. IHC plays a limited, even though important, role in the diagnosis of salivary gland tumors, but is often useful to support the histological assessment. However, unfortunately few tumor type-specific markers are still currently available. For these reasons, IHC should be considered a method that can be used to assist the final diagnosis, and its results themselves do not directly indicate a definitive diagnosis.

Key words: salivary gland tumor, immunohistochemistry, pathology, diagnosis

I. Introduction

The salivary glands are exocrine organs comprising ducto-acinar units that produce and secrete saliva. They are divided into the major and minor salivary glands. The major salivary glands consist of three pairs of glands: the parotid, submandibular, and sublingual. The minor salivary glands are widely distributed throughout the mouth, and similar seromucous glands are present in the oropharynx, upper respiratory and sinonasal tracts, and paranasal sinuses. Tumors uncommonly arise in the salivary glands, and these comprise approximately 1% of all neoplasms in the whole body. A pathological diagnosis of common types of salivary gland tumors, such as pleomorphic adenoma, Warthin tumor, mucoepidermoid carcinoma, and adenoid cystic carcinoma, are generally not difficult in typical cases, even for general surgical pathologists. However, they are known to have diverse histomorphological features in individual lesions, and there are a number of types and variants, in addition to histological patterns similar to those observed

Correspondence to: Toshitaka Nagao, M.D., Department of Anatomic Pathology, Tokyo Medical University, 6–7–1 Nishishinjuku, Shinjuku-ku, Tokyo 160–0023, Japan.
E-mail: nagao-t@tokyo-med.ac.jp

Abbreviations: HE, Hematoxylin-eosin; IHC, immunohistochemistry or immunohistochemical; CK, cytokeratin; EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; SMA, smooth muscle actin; MSA, muscle-specific actin; GFAP, glial fibrillary acidic protein; AR, androgen receptor.

in different tumor entities. Therefore, these tumors may present a considerable diagnostic challenge.

In general, salivary gland tumors are pathologically diagnosed according to the WHO classification, in which 10 and 23 specific benign and malignant epithelial tumor entities have been listed, respectively [4]. Although this classification is complex, it has advantages with regard to the prognostic and therapeutic aspects, because the biological behavior of each tumor type is considerably different [4, 19, 24].

In clinical practice, the histopathological diagnosis of salivary gland tumors is made carefully through the assessment of the growth pattern of the tumor borders, histological architecture, cellular structure and differentiation, and components of the tumor stroma, along with the clinical information. Although hematoxylin-eosin (HE) staining is still the gold standard method used for diagnosing the salivary gland tumor, immunohistochemistry (IHC) can enhance the accuracy of such analysis, while its role may be limited. IHC can be a helpful tool when in cases to investigate the subjects that cannot be assessed by histological examination, such as the cell nature and differentiation status, cell proliferation, and tumor protein expression. We herein depict the utility of immunohistochemical (IHC) assessment of salivary gland tumors in general surgical pathology practice. A summary of the useful IHC markers is shown in Table 1. In this review, we described the staining properties of the markers according to generally-used standard procedures on the formalin-fixed paraffin-embedded tissue sections; hence they might different under specific conditions in individual instances.

II. Assessment of Cell Differentiation

Although salivary gland tumors show diverse histological appearances, they still exhibit differentiation toward the cells that morphologically constitute the normal salivary gland [15]. Histologically, the salivary gland basically comprises ducts and acini (ducto-acinar units) (Fig. 1A), which consist of four types of cells: ductal, acinar, myoepithelial, and basal cells. Both ductal and acinar cells are present at the luminal side of the duct system, and are thus called

Table 1. *Summary of the useful immunohistochemical markers of salivary gland tumors in general surgical pathology practice*

Markers [antibodies]	Positivity in normal salivary gland parenchymal cells	Uses and significance for salivary gland tumors
Pan-cytokeratin (CK) [AE1/AE3]	Both luminal and abluminal cells	Epithelial marker; differential diagnosis between myoepithelioma/myoepithelial carcinoma or "undifferentiated carcinoma" and non-epithelial tumors
Epithelial membrane antigen (EMA)	Luminal cells	Ductal (luminal) cell marker; apical staining pattern; bubbly positive in sebaceous cells
Carcinoembryonic antigen (CEA)	Luminal cells	Ductal (luminal) cell marker
α-Smooth muscle actin (SMA)	Myoepithelial cells	Myoepithelial marker (high specificity, very useful)
Calponin	Myoepithelial cells	Myoepithelial marker (high specificity, very useful)
Muscle-specific actin (MSA) [HHF35]	Myoepithelial cells	Myoepithelial marker (high specificity)
p63	Myoepithelial and basal cells	Myoepithelial marker (note: also positive for basal and squamous epithelial cells)
CK14	Myoepithelial and basal cells	Myoepithelial marker (note: also positive for basal and squamous epithelial cells)
Glial fibrillary acidic protein (GFAP)	Myoepithelial cells (variable)	Myoepithelial marker (low sensitivity); highly positive in pleomorphic adenoma and myoepithelioma
S-100 protein	Variable	Myoepithelial marker (good for screening, low specificity)
Vimentin	Myoepithelial cells	Myoepithelial marker (good for screening, low specificity)
Ki-67 [MIB-1]	Few cells	Cell proliferation marker; differential diagnosis between benign and malignant tumors; prognostic factor
p53	Negative	Differential diagnosis between benign and malignant tumors; prognostic factor
HER2/*neu*	Negative to weakly positive in ductal cells	Highly overexpressed in salivary duct carcinoma; diagnosis of non-invasive carcinoma ex pleomorphic adenoma; expected use for molecular targeted therapy
α-Amylase	Acinar cells	Positive in acinic cell carcinoma (low sensitivity)
Androgen receptor (AR)	Negative	Often positive in salivary duct carcinoma; diagnosis of non-invasive carcinoma ex pleomorphic adenoma; expected use for molecular targeted therapy
Gross cystic disease fluid protein-15	Luminal cells	Often positive in salivary duct carcinoma (low specificity)
Mitochondria	Striated duct cells	Strongly positive in oncocytic cells
Renal cell carcinoma/CD10	Negative	Diagnosis for metastatic renal cell carcinoma
Melan A	Negative	Diagnosis for metastatic malignant melanoma
Lymphoid cell markers	Negative	Diagnosis for malignant lymphoma
EBER *in situ* hybridization	Negative	Positive in lymphoepithelial carcinoma

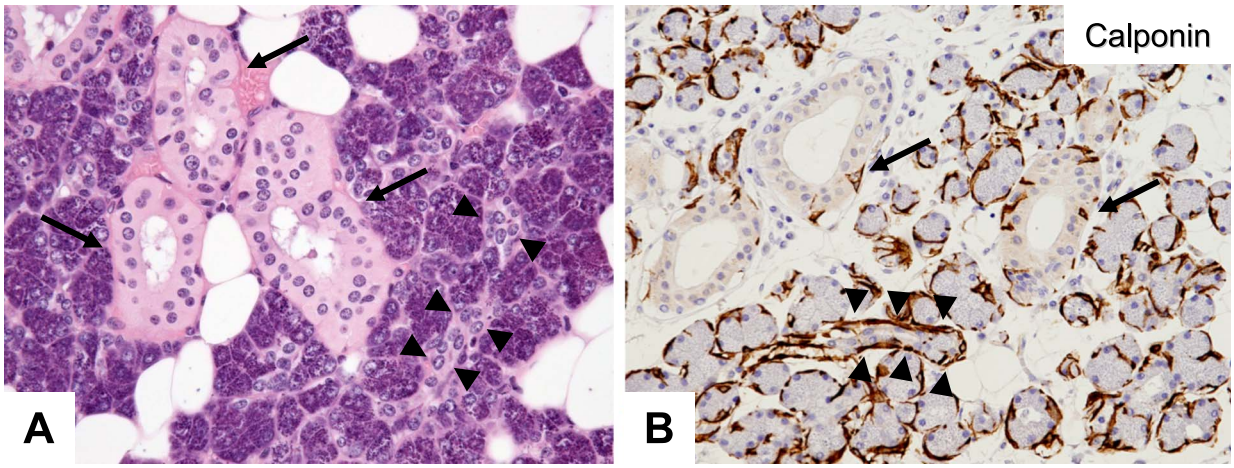

Fig. 1. Normal parotid gland. **A**: Parenchyma contains serous acini and striated (arrows) and intercalated (arrowheads) ducts. Myoepithelial cells are inconspicuous. HE staining. **B**: Calponin highlights myoepithelial cells at the periphery of the acini, intercalated ducts (arrowheads), and some striated ducts (arrows). Immunohistochemistry.

luminal cells. In contrast, myoepithelial and basal cells are located on the basement membrane side surrounding the luminal cells, and are thus called abluminal cells. All four types of cells are usually pan-cytokeratin (CK) [AE1/AE3]-positive; both duct and acinar cells are epithelial membrane antigen (EMA)- and carcinoembryonic antigen (CEA)-positive, while only acinar cells are α-amylase-positive (Table 1). Both myoepithelial and basal cells are CK14- and p63-positive, and are EMA- and CEA-negative; the expression of α-smooth muscle actin (SMA), muscle-specific actin (MSA), calponin (Fig. 1B), podoplanin [30], and vimentin are only observed in myoepithelial cells; and S-100 protein staining is variable for all four cell types (Table 1). Immunoreactivity for S-100 protein in the parenchymal cells is sometimes observed adjacent to the tumors. A recent report indicated that α-SMA, calponin, S-100 protein, and p63 are present from the earliest stages of salivary gland maturation [32].

With regard to cell differentiation, the most important role of IHC for the differential diagnosis of salivary gland tumors would be to discriminate whether the neoplastic myoepithelial cells are participating in the tumor or not (Table 2) [15, 63, 81]. Approximately 70% of the salivary gland tumors exhibit myoepithelial cell differentiation, and are further classified based on the presence or absence of luminal cell differentiation; the tumors that do not reveal luminal cell differentiation are myoepithelioma or myoepithelial carcinoma. The tumors that do not differentiate into myoepithelial cells, but display acinar cell differentiation, are considered to be acinic cell carcinoma.

a. Ductal/acinar (luminar) cell differentiation

Neoplastic ductal cells form glands, and their luminal surface (apical portion) shows EMA- (Fig. 2B) and CEA-positive reactions. The identification of ductal cell differentiation is necessary for the differential diagnosis between pleomorphic adenoma and myoepithelioma, and between epithelial-myoepithelial carcinoma and myoepithelial carcinoma. However, neoplastic myoepithelial cells often form gland-like structures, and they are often confused with true ductal cells. Although EMA can be detected along the whole cell membrane of neoplastic myoepithelial cells, an EMA-positive signal in the apical portion at the gland-luminal surface suggests ductal cell differentiation and is helpful in the differential diagnosis.

It is necessary to identify serous acinar differentiation for the diagnosis of acinic cell carcinoma. However, a positive signal for α-amylase, a specific marker of normal acinar cells, is not detected in many acinic cell carcinoma cases, so it is not always useful for the diagnosis [12, 33]. Although α1-antichymotrypsin, α1-antitrypsin, transferrin, lactoferrin, secretory component, and lysozyme have been applied as markers of ductal and acinar cells, they are currently not generally used. A recent paper reported that DOG1 staining is a marker of salivary acinar cells, and strong staining can be applied to support the diagnosis of acinic cell carcinoma [9].

b. Myoepithelial (abluminar) cell differentiation

The tumor cells that differentiate into myoepithelial cells (neoplastic myoepithelial cells) are one of the unique pathological features of salivary gland tumors. Neoplastic myoepithelial cells by themselves do not demonstrate glandular formation, but are located around the ductal cells in the gland-forming tumors (Fig. 2C–H). The cells show various morphologies, such as epitheloid, spindle, plasmacytoid and clear cell features, and frequently produce a mucinous or basement membrane-like extracellular matrix. Neoplastic myoepithelial cells can sometimes be identified by HE staining, but an IHC analysis is often necessary for a more accurate identification.

Representative markers used for the IHC identification of the differentiation toward myoepithelial cells in clinical practice are listed in Table 1. Among them, calponin, as

Table 2. *Classification of salivary gland tumors based on the presence or absence of myoepithelial differentiation*

Presence of myoepithelial differentiation	Absence of myoepithelial differentiation
Benign tumor	Benign tumor
- Pleomorphic adenoma	- Warthin tumor
- Myoepithelioma	- Oncocytoma
- Basal cell adenoma	- Canalicular adenoma
	- Sebaceous adenoma
	- Lymphadenoma
	- Ductal adenomas
	- Cystadenoma
	- Keratocystoma
	- Striated duct adenoma
Malignant tumor	Malignant tumor
- Adenoid cystic carcinoma	- Acinic cell carcinoma
- Polymorphous low-grade adenocarcinoma*	- Mucoepidermoid carcinoma
- Epithelial-myoepithelial carcinoma	- Polymorphous low-grade adenocarcinoma**
- Basal cell adenocarcinoma	- Clear cell carcinoma, NOS
- Adenocarcinoma, NOS (minority)	- Malignant sebaceous tumors
- Myoepithelial carcinoma	- Cystadenocarcinoma
- Carcinoma ex pleomorphic adenoma	- Low-grade cribriform cystadenocarcinoma
- Metastasizing pleomorphic adenoma	- Mucious adenocarcinoma
- Sialoblastoma	- Oncocytic carcinoma
	- Salivary duct carcinoma
	- Adenocarcinoma, NOS (majority)
	- Carcinosarcoma
	- Squamous cell carcinoma
	- Small cell carcinoma
	- Large cell carcinoma
	- Lymphoepithelial carcinoma
	- Mammary analogue secretory carcinoma

*, minority of cases, **: majority of cases, NOS: not otherwise specified.

well as α-SMA, are highly specific markers (Fig. 2) [27, 63], while a weak non-specific signal for calponin is occasionally observed in ductal cells. Although the S-100 protein (Fig. 2E) and vimentin are highly sensitive markers for neoplastic myoepithelial cells, they are also often detected in ductal cells. Therefore, these markers are not sufficiently specific, and are only appropriate to use for an initial screen for myoepithelial differentiation.

Care should be taken when evaluating the expression of CK14 and p63, since they are positive not only in neoplastic myoepithelial cells, but also in basal and squamous epithelial cells, including epidermoid cell, one of the fundamental elements of mucoepidermoid carcinoma [10]. However, there are some advantages to evaluating these markers, because they are not present in vascular smooth muscle cells and myofibroblasts, both of which are positive for α-SMA and calponin, and only p63 is stained in a nuclear pattern (Fig. 2F). Glial fibrillary acidic protein (GFAP) generally has low sensitivity as a myoepithelial marker, but is frequently detected in pleomorphic adenoma (Fig. 2G) and myoepithelioma, it may therefore be useful for distinguishing them from polymorphous low-grade adenocarcinoma or adenoid cystic carcinoma [13, 14, 66]. In addition to the markers listed in Table 1, h-caldesmon and smooth muscle myosin heavy chain have been reported to be markers of neoplastic myoepithelial cells [32, 63], however, they have poor sensitivity, and thus cannot generally be used, although their specificities may be sufficient. Maspin [55], CD10 [56], and podoplanin [39] also exhibit low specificity, and thus are not appropriate for diagnostic use. Recently, WT1 was reported as a sensitive marker of the neoplastic myoepithelial cells in pleomorphic adenomas (Fig. 2H), and it is not expressed in normal myoepithelial cells [41].

The staining properties of the myoepithelial markers greatly depend on the antibodies and cell types. Staining for α-SMA, MSA, and calponin is usually observed diffusely in spindle cells, whereas positive cells are focally detected in epithelioid and clear cell types. Plasmacytoid cells are usually calponin-positive, but are negative for α-SMA and MSA [27]. Since neoplastic myoepithelial cells, especially spindle cells and plasmacytoid cells in myoepithelioma and myoepithelial carcinoma, are pan-CK-positive, positive staining results can rule out soft tissue tumors and plasmacytoma, respectively. However, clear neoplastic myoepithelial cells in epithelial-myoepithelial carcinoma are often pan-CK-negative. For example, myoepithelial carcinomas are almost always positive for pan-CK, S-100 protein,

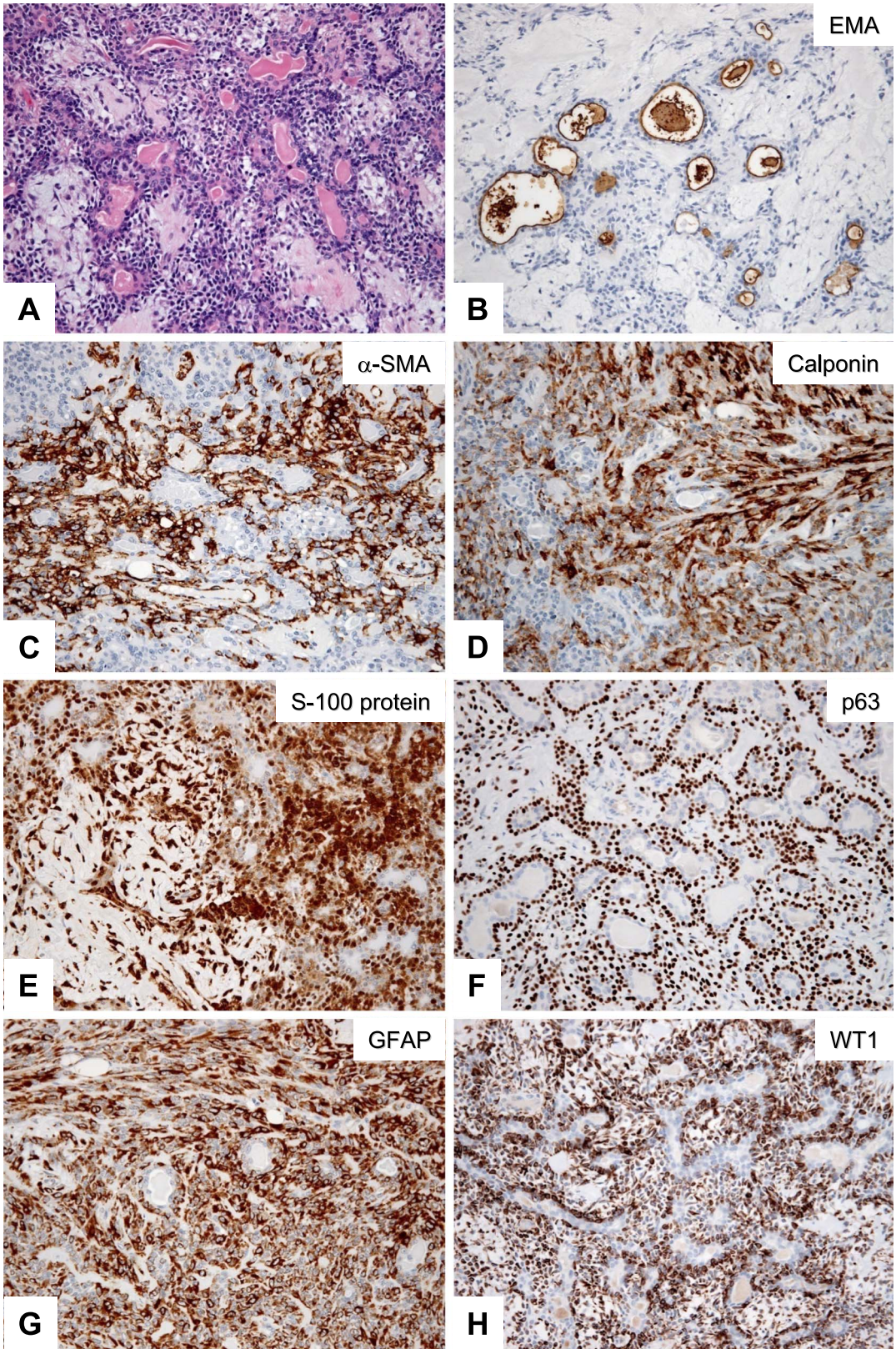

Fig. 2. Pleomorphic adenoma. **A**: Glandular structures composed of luminal cells and several layers of abluminal cells, the latter being merged into surrounding myxoid stromal components. HE staining. **B**: Epithelial membrane antigen (EMA)-positive signal in the apical portion at the duct-luminal surface. **C–H**: Abluminal cells are intensely positive for α-smooth muscle actin (SMA) (**C**), calponin (**D**), S-100 protein (**E**), p63 (**F**), glial fibrillary acidic protein (GFAP) (**G**), and WT1 (**H**). **B–H**: immunohistochemistry.

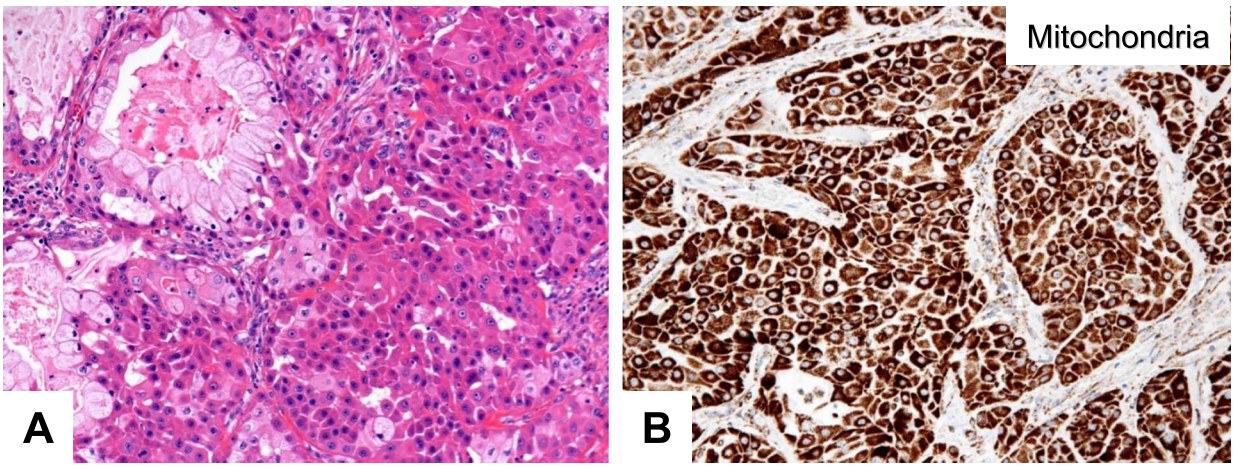

Fig. 3. Oncocytic mucoepidermoid carcinoma. **A**: Cystic structures lined by mucous cells and solid nests of epidermoid cells, characteristic of mucoepidermoid carcinoma, accompanied by extensive oncocytic differentiation. HE staining. **B**: Tumor cells are diffusely and intensely positive for anti-mitochondria antibody. Immunohistochemistry.

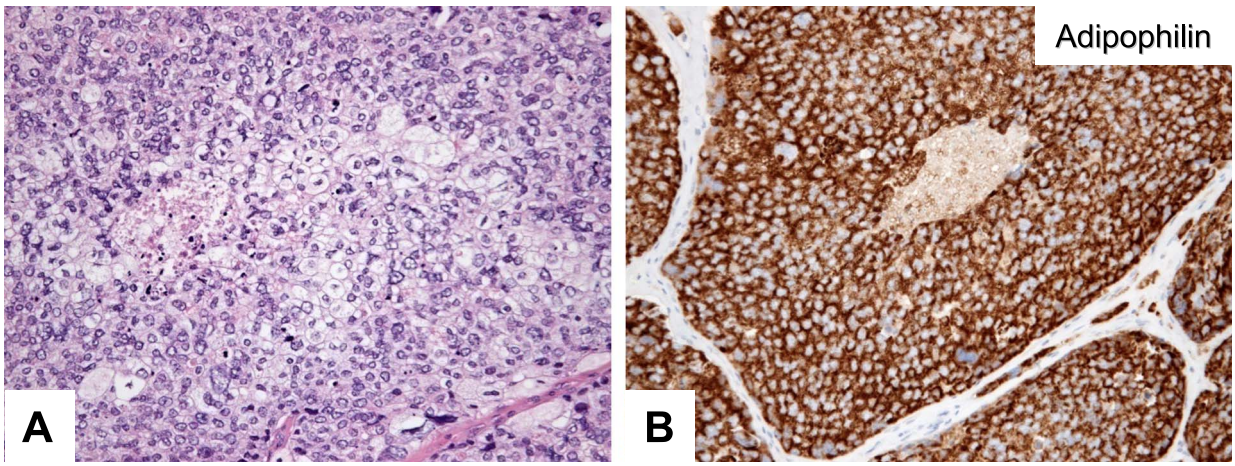

Fig. 4. Sebaceous carcinoma. **A**: Sheets of atypical tumor cells exhibiting clear cytoplasm with focal necrosis. HE staining. **B**: Diffuse and strong positivity for adipophilin. Immunohistochemistry.

vimentin, and p63, whereas the tumor cells are variably immunoreactive for calponin (75–100%), α-SMA (35–80%), CK14 (53–80%), caldesmon (50%), MSA (31–70%), GFAP (31–50%), smooth muscle myosin heavy chain (30%), and EMA (20–100%) [38, 48, 62]. Consequently, a panel or a battery of as many antibodies as possible is necessary to investigate neoplastic myoepithelial differentiation. In cases with a limited number of sections, screening with pan-CK, calponin, α-SMA, p63 (or CK14), and S-100 protein is the best in terms of their specificity. Since normal myoepithelial cells serve as a good internal control for the detection of myoepithelial markers, IHC should be performed with a section that includes normal salivary gland tissue, if possible.

c. Oncocytic and sebaceous differentiation

Oncocytic differentiation can be observed not only in Warthin tumor, oncocytoma, and oncocytic carcinoma, but also in various other tumor entities, such as mucoepidermoid carcinoma (Fig. 3A), pleomorphic adenoma, myoepithelioma, and acinic cell carcinoma. An oncocyte is an acidophilic cell filled with abundant mitochondria in the entire cytoplasm, which is consistent with intense positivity for anti-mitochondria antibodies (Fig. 3B) [68]. In addition, sebaceous differentiation is observed in sebaceous adenoma and sebaceous carcinoma (Fig. 4A), as well as various types of salivary gland tumors. Sebaceous cells are immunohistochemically intensely positive for EMA (with a characteristic bubbly pattern), adipophilin (Fig. 4B), and perilipin [67].

III. Differential Diagnosis of Problematic Histologically Similar Salivary Gland Tumors

As mentioned at the beginning of this review, one characteristic pathological feature of salivary gland tumors is that they display a variety of histological architectures/

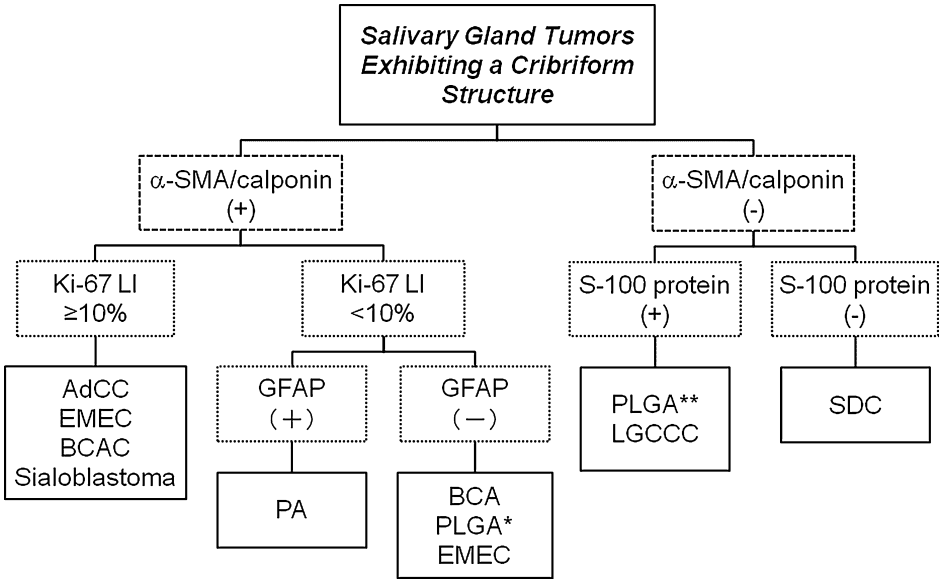

Fig. 5. An immunohistochemistry-based differential diagnosis of salivary gland tumors with a cribriform structure. SMA, smooth muscle actin; LI, labeling index; GFAP, glial fibrillary acidic protein; AdCC, adenoid cystic carcinoma; EMEC, epithelial-myoepithelial carcinoma; BCAC, basal cell adenocarcinoma; PA, pleomorphic adenoma; PLGA, polymorphous low-grade adenocarcinoma; LGCCC, low-grade cribriform cystadenocarcinoma; SDC, salivary duct carcinoma; *, minority of cases; **, majority of cases.

structural patterns. Moreover, despite different tumor entities, their histological architectures/structural patterns and cell types can be partially, or rarely mostly, identical. Therefore, it may be difficult to differentially diagnose such tumors based on only by the histological observation. In this instance, an IHC examination is often useful. Below, I describe the cribriform structure and clear cells as examples of the histological architecture and cell type, respectively. Discrimination between the benign and malignant counterparts of salivary gland tumors is also discussed.

a. Tumors exhibiting a cribriform structure

Adenoid cystic carcinoma and salivary duct carcinoma are representative examples of tumors that exhibit a cribriform structure. In addition, basal cell adenoma, pleomorphic adenoma, epithelial-myoepithelial carcinoma, polymorphous low-grade adenocarcinoma, low-grade cribriform cystadenocarcinoma, basal cell adenocarcinoma, and sialoblastoma also need to be considered in the differential diagnosis. The IHC-based differential diagnosis is shown in Figure 5, whereas only the clinical and morphological assessments can adjudicate the final diagnosis for some of the tumors [11, 13, 14, 49, 59, 66, 70]. For example, among α-SMA/calponin-positive tumors, distinction between adenoid cystic carcinoma (Fig. 6A) and basal cell adenoma (Fig. 6C) is sometimes challenging based on histological examination alone. The Ki-67 labeling index in adenoid cystic carcinoma (≥10%, Fig. 6B) is reported to be different from that in basal cell adenoma (<10%, Fig. 6D) [49, 70]. Furthermore, presence of strongly S-100 protein-positive spindle shaped "stromal" cells supports the diagnosis of basal cell adenoma (Fig. 6E). Sialoblastoma can be easily distinguished based on the age of onset, since this tumor develops almost exclusively in neonates. Polymorphous low-grade adenocarcinoma mainly arises in the minor salivary glands with a few exceptions [50], thus this would be excluded for the diagnosis of tumors of major salivary gland origin.

b. Tumors consisting of clear cells

Epithelial-myoepithelial carcinoma, mucoepidermoid carcinoma, myoepithelioma, myoepithelial carcinoma (Fig. 7A), acinic cell carcinoma, oncocytoma, sebaceous carcinoma, clear cell carcinoma, not otherwise specified (Fig. 7C), and metastatic renal cell carcinoma and malignant melanoma are all included in the category of tumors consisting of clear cells [18, 45, 76]. For the first step of the differential diagnosis, it is recommended to distinguish the type of tumor using myoepithelial markers. α-SMA- and calponin-positive immunoreactions are seen in epithelial-myoepithelial carcinoma, myoepithelioma, and myoepithelial carcinoma (Fig. 7B), but not in the other tumors (Fig. 7D). Identification of an EMA-positive signal in the apical portion at the gland-luminal surface further distinguishes epithelial-myoepithelial carcinoma from myoepithelioma or myoepithelial carcinoma. Diagnosis of metastatic renal cell carcinoma or malignant melanoma is confirmed by immunopositive for RCC and CD10 or Melan-A, respectively [58]. Strong and diffuse immunoreactivity for mitochondria can help diagnosis of oncocytoma, and positive immunostaining for p63 is a feature of this tumor but not of metastatic renal cell carcinoma

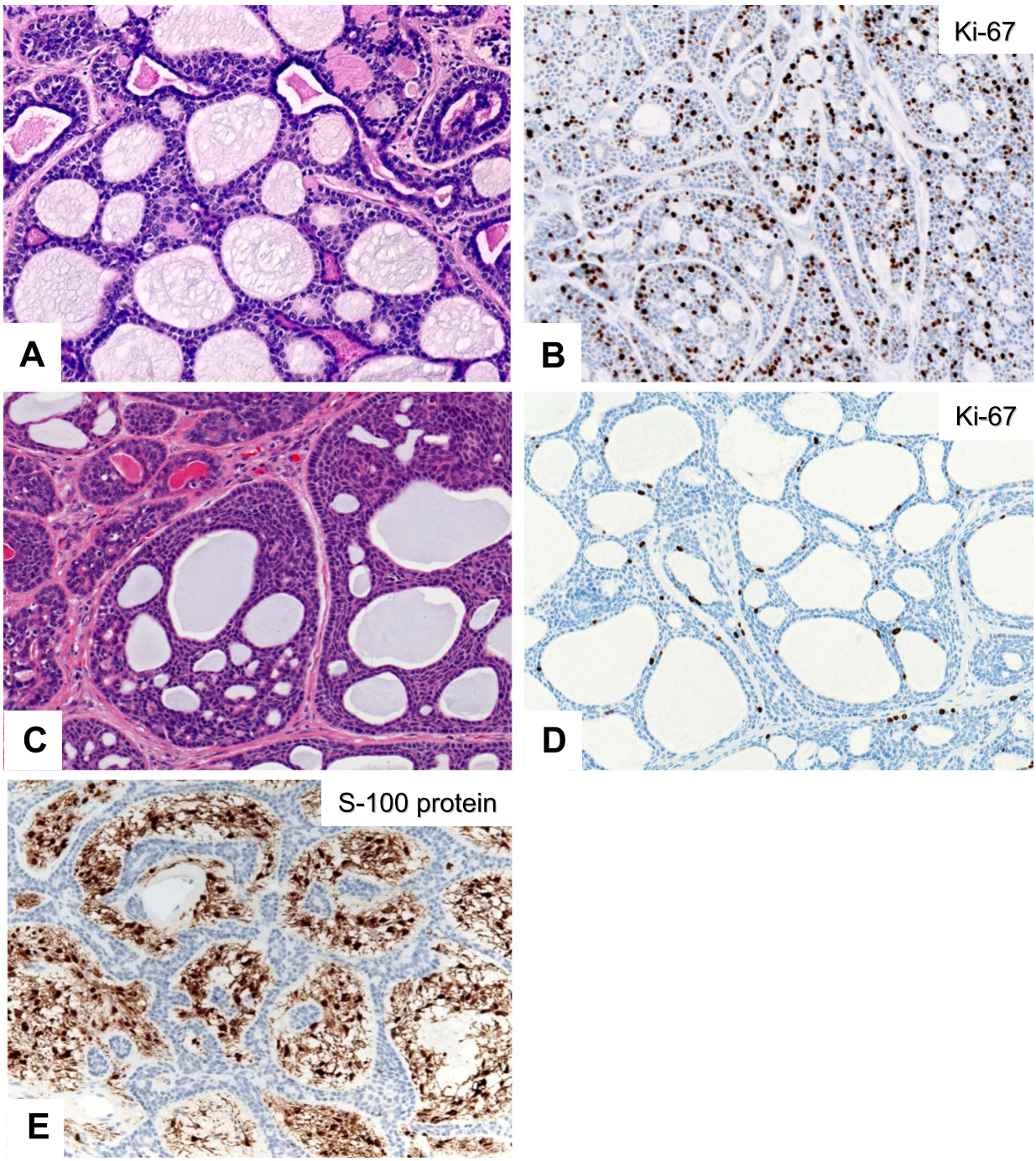

Fig. 6. Adenoid cystic carcinoma (**A, B**). **A**: Cribriform structures with multiple pseudocysts. HE staining. **B**: Higher rate of Ki-67 labeling index (15%). Immunohistochemistry (IHC). Basal cell adenoma (**C–E**). **C**: Multiple pseudocysts form cribriform structures mimicking adenoid cystic carcinoma. The peripherally located cells in the basaloid cell nests show a palisading arrangement. HE staining. **D**: Lower rate of Ki-67 labeling index (3%). IHC. **E**: Strongly S-100 protein-positive spindle shaped "stromal" cells. IHC.

[44]. However, some of the clear cell salivary gland neoplasms are unfortunately difficult to differentially diagnose by an IHC analysis alone. Histologically, it is a key for the diagnosis to determine the tumor-specific appearance at a site other than the areas of clear cell proliferation.

c. Tumors of benign and malignant counterparts

The benign and malignant counterparts of salivary gland tumors, such as myoepithelioma and myoepithelial carcinoma, basal cell adenoma and basal cell adenocarcinoma, oncocytoma and oncocytic carcinoma, sebaceous adenoma and sebaceous carcinoma, and cystadenoma

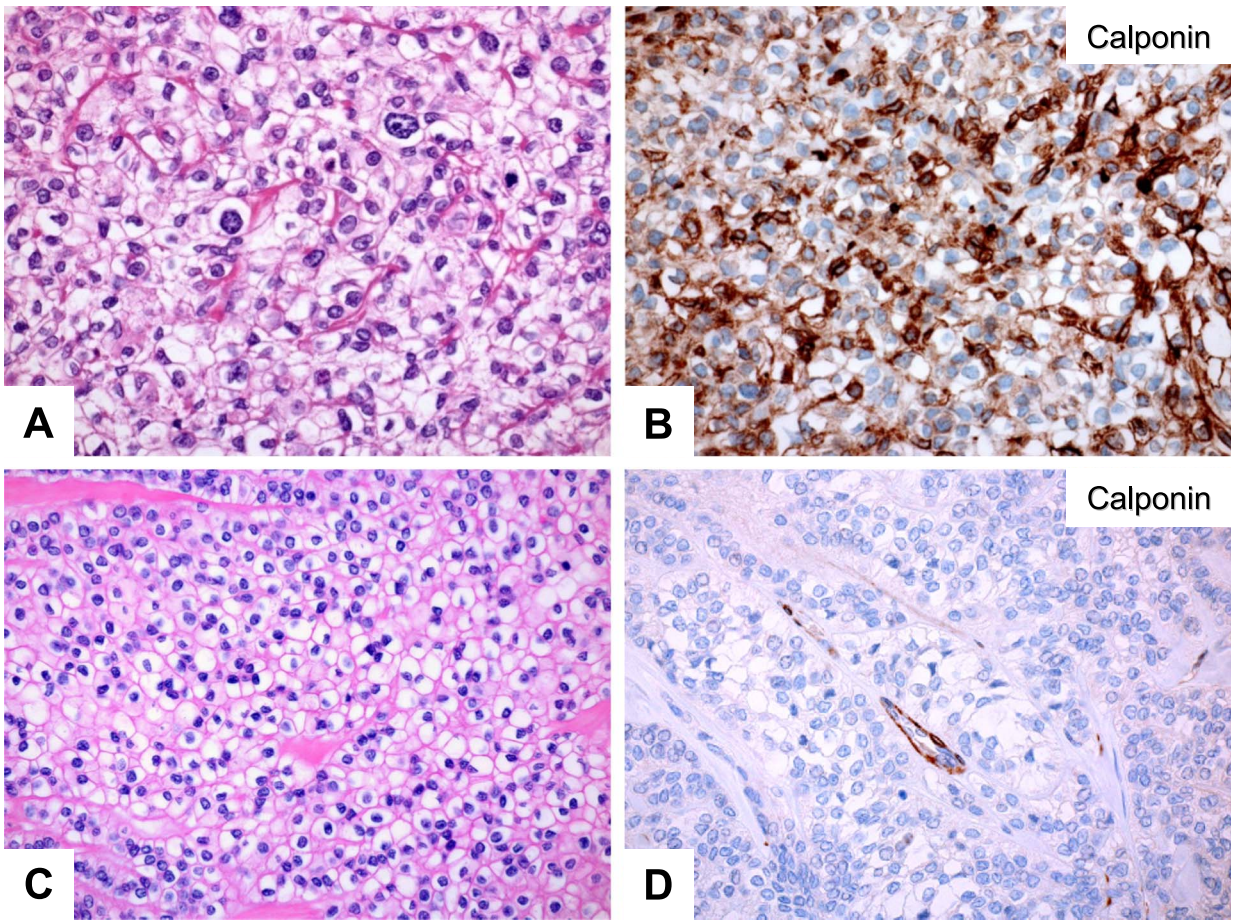

Fig. 7. Myoepithelial carcinoma, clear cell variant (**A, B**). **A**: HE staining. **B**: Many tumor cells are positive for α-smooth muscle actin (SMA). Immunohistochemistry (IHC). Clear cell carcinoma, not otherwise specified (**C, D**). **C**: HE staining. **D**: Negative reaction for α- SMA. IHC.

and cystadenocarcinoma, share similar basic histological appearances to each other in terms of the structures/patterns and cellular features. Because of the frequent bland cytology of the malignant tumors, they are often distinguished from their benign counterpart by other histological hallmarks, such as their invasive outgrowth (being the most important diagnostic feature), perineural and vascular invasion, necrosis, and mitosis. However, in cases with limited samples, the morphological appearance is not sufficient to provide a differential diagnosis between the two.

The IHC assessment of the Ki-67 labeling index is helpful in the differential diagnosis between myoepithelioma (<10%) and myoepithelial carcinoma (>10%) [48]. On the other hand, IHC markers that demonstrate evidence of ductal and myoepithelial cell differentiation display a striking similarity in basal cell adenomas and basal cell adenocarcinomas, so that they are of little value in the differential diagnosis. However, a higher rate of cell proliferation (a Ki-67 labeling index >5%) and apoptosis (an apoptotic index of >0.4% as determined by the TUNEL method), along with strong expression of p53 and EGFR, and loss of bcl-2 expression may be diagnostic for basal cell adenocarcinomas rather than basal cell adenomas [49]. In cases of other types of tumors, there has been no such large scale analysis, perhaps because of their low incidence.

IV. Differential Diagnosis of So-Called Undifferentiated Carcinoma and Malignant Lymphoma

Salivary gland tumors, which were previously called "undifferentiated carcinoma", are currently classified into three different entities: small cell carcinoma, large cell carcinoma, and lymphoepithelial carcinoma. All of them may sometimes be histologically confused with malignant lymphoma. The "undifferentiated carcinomas" are immunopositive for pan-CK and negative for leukocyte common antigen, while malignant lymphoma shows the opposite immunostaining results. Additionally, small cell carcinoma and some large cell carcinomas exhibit neuroendocrine differentiation: positive staining for chromogranin A, synaptophysin, and CD56 [51], and lymphoepithelial carcinomas are often labeled with EBER *in situ* hybridization (Fig. 8) [47].

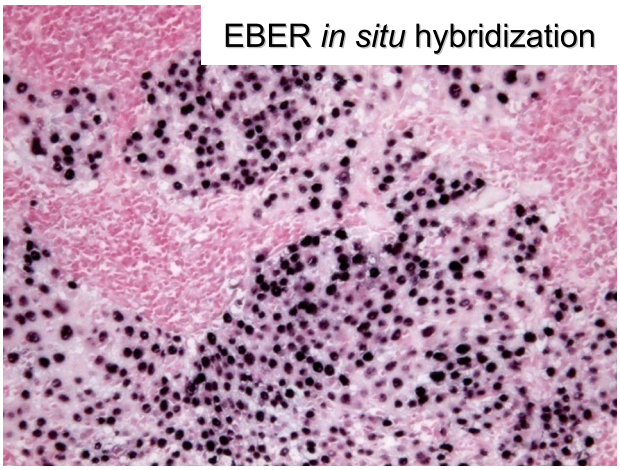

Fig. 8. Lymphoepithelial carcinoma. *In situ* hybridization for EBER. Almost all of the carcinoma cells express strong nuclear EBER hybridization signals. Note complete absence of signal in the surrounding lymphoid stroma.

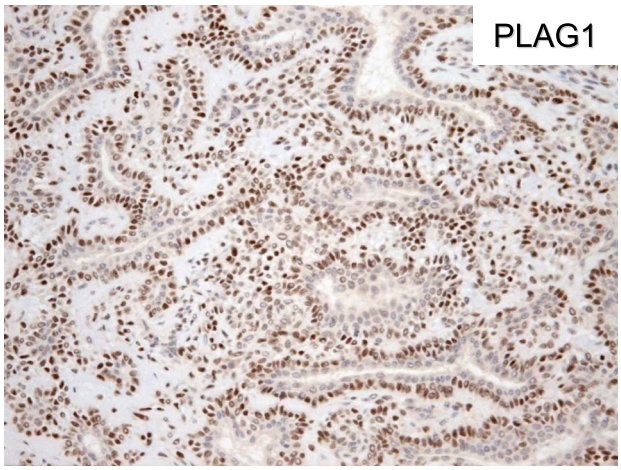

Fig. 9. Pleomorphic adenoma. Nuclear staining for PLAG1 in abluminal tumor cells. Immunohistochemistry.

V. Differential Diagnosis of Lymphoproliferative Disorders

Low-grade lymphoma, especially MALT lymphoma (extranodal marginal zone B-cell lymphoma), frequently arises in the salivary gland in the setting of autoimmune diseases such as Sjögren syndrome. A salivary gland lesion associated with Sjögren syndrome is called lymphoepithelial sialadenitis. A differential diagnosis between this benign condition and MALT lymphoma may sometimes be difficult based only on the histological observation, although the diagnostic criteria are still controversial and no consensus exists among experts. Immunohistochemically, diffuse staining of B-cell markers such as CD20 and CD79a, clonality of immunoglobulin light chain (κ and λ chains) (light chain restriction), or abnormal expression of CD43 in B-cells suggests MALT lymphoma [1]. Clonality is indicated by a more than five- or ten-fold higher expression of the κ chain as opposed to the λ chain. However, the immunoreactivity of immunoglobulin light chains is sometimes reduced in lymphomas, so this diagnostic criterion is often unreliable [1]. Since MALT lymphoma is almost always CD5-negative (positive in small cell lymphoma and mantle cell lymphoma; note that a few CD5-positive MALT lymphoma have been described [35]), CD10-negative (positive in follicular lymphoma) and cyclin D1-negative (positive in mantle cell lymphoma), it can be distinguished from these other B-cell lymphomas based on the IHC staining results.

VI. Diagnosis of Specific Tumor Types

Pleomorphic adenoma

A recent study revealed that tumor cells in all 45 pleomorphic adenomas were immunopositive for PLAG1, irrespective of PLAG1 rearrangements; tumor cells displaying myoepithelial or cartilaginous differentiation were almost constantly positive for PLAG1, whereas a limited expression was observed in glandular or keratinizing cells (Fig. 9). While, among the 46 tumors other than pleomorphic adenoma, 4 carcinomatous components of carcinomas ex pleomorphic adenoma were positive for PLAG1, the other 39 were negative for PLAG1, and the remaining 3 were only faintly and/or focally stained, indicating that the IHC detection of PLAG1 is diagnostically useful [43]. Another study indicated that PLAG1 immunostain was specific for carcinoma ex pleomorphic adenoma against other carcinomas, its application as a standalone discriminatory test was limited by variable expression [3].

Adenoid cystic carcinoma

Although c-kit was previously reported to specifically show a diffuse expression pattern in adenoid cystic carcinoma, it was recently indicated that its specificity is questionable [2]. Strong Myb immunostaining is a specific and useful diagnostic marker for adenoid cystic carcinomas, but is only present in 65–82% of all cases [5, 8, 78]. Moreover, focal Myb immunoreactivity is observed in some non-adenoid cystic carcinoma neoplasms [8].

Salivary duct carcinoma

This is of a high-grade malignancy, which displays a similar histological appearance to ductal breast carcinoma (Fig. 10A). Gross cystic disease fluid protein-15 and androgen receptor (AR) (Fig. 10B) are frequently positive in this tumor, and their staining can thus help in the diagnosis [25, 40, 54, 79], but it was reported that they are not sufficiently specific [16, 54]. The estrogen receptor and progesterone receptor are not detected in most salivary duct carcinomas. This fact is sometimes useful for distinguishing this tumor

from a breast cancer metastasis when noted in conjunction with AR-positive staining. Prostate-specific antigen, which is a marker for prostate cancer, is occasionally detected in this tumor and thus should be used carefully. More than 20% of tumors show diffuse and strong membranous staining for HER2/*neu* [34, 79]. AR and HER2/*neu* are expected use for molecular targeted therapy [36, 77].

Non-invasive carcinoma ex pleomorphic adenoma

Among the carcinomas arising in pleomorphic adenoma, the one in which the cancer cells are confined within the capsule of a preexisting pleomorphic adenoma component basement is called non-invasive carcinoma ex pleomorphic adenoma (Fig. 11A). This type of tumor contains cancer cells that often show pleomorphism, coarse nuclear chromatin, conspicuous nucleoli, abnormal mitoses, and necrosis, and the observation of such features generally lead to the correct diagnosis. However, immunohistochemically strong positivity for AR, p53, and HER2/*neu* (Fig. 11B) and a high Ki-67 labeling index increase the accuracy of the diagnosis [17, 29, 53]. Additionally, a recent report indicated that S100P may play an important role in the malignant transformation of ductal cells of pleomorphic adenoma, and that IHC staining for S-100P, a member of the S-100 protein family, would be a useful diagnostic marker for identifying the early phase of carcinoma ex pleomorphic adenoma [29].

VII. Evaluation of Malignancy and Prognostic Factors

Ki-67 is the most frequently reported prognostic factor in mucoepidermoid carcinoma, adenoid cystic carcinoma, acinic cell carcinoma, carcinoma ex pleomorphic adenoma, and salivary duct carcinoma, as well as many other cancers [69, 71]. For example, in mucoepidermoid carcinoma and

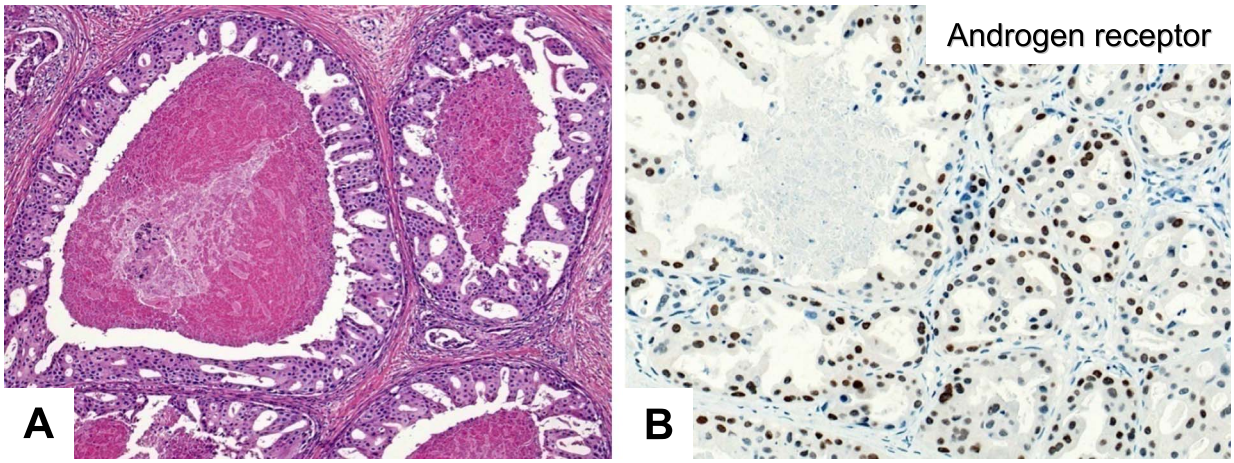

Fig. 10. Salivary duct carcinoma. **A**: Dilated ductal structures with a cribriform growth pattern and "Roman-bridge" architecture. Comedo-type necrosis is evident. HE staining. **B**: Carcinoma cells are diffusely positive for androgen receptor in their nuclei. Immunohistochemistry.

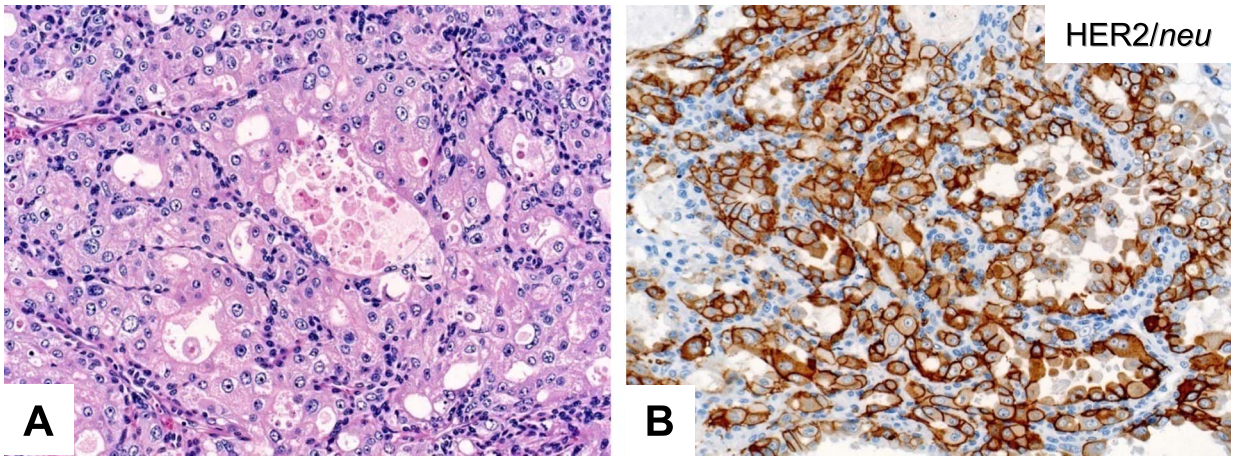

Fig. 11. Non-invasive carcinoma ex pleomorphic adenoma. **A**: Glandular structures composed of carcinoma cells rimming with benign neoplastic myoepithelial cells. HE staining. **B**: Diffuse and strong membranous staining for HER-2/*neu* in carcinoma cells. Immunohistochemistry.

acinic cell carcinoma, there were no recurrences when the Ki-67 index was less than 5%, and it was also reported that cases with an index above 10% were often associated with poor outcomes [69]. p53 (for adenoid cystic carcinoma and salivary duct carcinoma) and HER2/*neu* (for salivary duct carcinoma) are also considered prognostic factors [34, 65]. It was reported that, in small cell carcinomas, CK20-negative cases have a worse prognosis than the positive cases [51]. Bcl-2 (for adenoid cystic carcinoma) [37], E-cadherin (for adenoid cystic carcinoma) [26], p27 (for adenoid cystic carcinoma and mucoepidermoid carcinoma) [57, 73], MUC1 (for mucoepidermoid carcinoma) [28], glucose transporter type 1 (for salivary gland carcinoma) [46], heparanase [6], pRb2/p130 [61], vascular endothelial growth factor (for adenoid cystic carcinoma) [82], survivin [23, 72], RB1-inducible coiled-coil 1 [74], geminin [80], p63 (for adenoid cystic carcinoma) [60], Skp2 [7], EGFR [20, 21], c-kit [20], RUNX3 (for adenoid cystic carcinoma) [31], Cks1 [52], topoisomerase IIα [42], maspin [64], PI3K/AKT/mTOR [22, 75], and PTEN [21] were also reported to be significant IHC markers for evaluating the malignancy of the salivary gland carcinoma and for their prognostic estimation [65, 71].

VIII. Conclusion

IHC plays a limited, albeit important, role in the diagnosis of salivary gland tumors, but is often useful to support the histological assessment. However, few tumor type-specific markers are currently available. It is necessary to fully understand that IHC should be considered a method that can be used to assist the final diagnosis, and not that can change the HE-based diagnosis. It should also be recognized that exceptional and unexpected results are often obtained by IHC. An IHC analysis must be performed after approximate identification of the particular tumor type by HE staining. For these reasons, the IHC findings do not directly indicate a definitive diagnosis, and it is always necessary to diagnose tumors after comparing both the IHC and morphological findings.

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