Review Article

Current concepts in diagnosis of unusual salivary gland tumors

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ABSTRACT

Salivary gland tumors are relatively uncommon and account for approximately 3-6% of all neoplasms of the head and neck. Tumors mostly involve the major salivary glands, 42.9-90% of which occur in the parotid glands and 8-19.5% in the sub-mandibular glands; tumors in the sub-lingual glands being uncommon. Despite the plethora of different malignant salivary gland tumor presented to pathologists for diagnosis, there is consensus on a limited number of pathologic observations that determine treatment and outcome. There are few absolutes in salivary gland tumor diagnosis given the marked spectrum and overlap of differentiated cell types that participate in the numerous benign and malignant tumors. Thus, there are enumerating antibodies that may be helpful in resolving difficult differential diagnoses when applied with astute morphologic correlation. In general, immunohistochemistry as an ancillary diagnostic tool should be used sparingly and wisely as a morphologic adjunct because of the lack of specificity of many markers for specific histologic tumor types. The aim of this review is to discuss the molecular profiling of salivary gland neoplasms and correlate this with histogenesis of salivary gland neoplasms. We have elected to discuss and illustrate some of the unusual salivary gland tumors that the practicing pathologist find difficult to diagnose. These have been selected because they readily simulate each other but have very different clinical therapies and, therefore, should be included routinely in differential diagnosis.

Key Words: Histogenesis, IHC, unusual salivary gland

INTRODUCTION

Salivary gland tumors are relatively uncommon and account for approximately 3-6% of all neoplasms of the head and neck.[1-3] Tumors commonly involve the major salivary glands; 42.9-90% (parotid glands), 8-19.5% (sub-mandibular glands).[1-6] Only around 14-22% of tumors affect minor salivary glands, mainly appearing in the palate.[1,2,6] Despite the plethora of malignant salivary gland tumor types presented to

pathologists for diagnosis, there is consensus on a limited number of pathologic observations that determine treatment and outcome. There are few absolutes in salivary gland tumor diagnosis given the marked spectrum and overlap of differentiated cell types that participate in the numerous benign and malignant tumors. Having said that, numerous antibodies that may be helpful in resolving difficult differential diagnoses have evolved.[7-15] Many immunohistochemical investigations have pursued differentiation markers, especially of myoepithelium, to assist in classification. The first antibodies applied, directed to S-100 protein, vimentin and GFAP, were to be non-specific in their reactivity. However, there is promise for some of the newer myoepithelial smooth muscle markers like α-smooth muscle actin (SMA), smooth muscle myosin heavy chain, calponin, and p63 in select diagnostic situations.[10,13,14,16,17] In general, immunohistochemistry

should be used as an ancillary diagnostic tool for identification of specific histologic tumor types.

The aim of this review is to discuss the molecular profiling of salivary gland neoplasms and correlate this with histogenesis of salivary gland neoplasms [Table 1]. We have elected to discuss and illustrate some of the unusual salivary gland tumors that the practicing pathologist find difficult to diagnose.

BENIGN TUMORS: MYOEPITHELIOMA

Myoepithelioma was first described by Sheldon in 1943. Many authors consider myoepithelioma to be a one-sided variant at the opposite end of spectrum of pleomorphic adenoma. The tumor is composed exclusively of neoplastic myoepithelial cells in different forms, as either spindle-shaped or plasmacytoid. Either a single cell type predominates in a tumor or there may be a combination of cell types.[18] Neoplastic myoepithelial cells show immunoreactivity for S-100 protein, GFAP, vimentin, actin and CK 14 are generally positive or focally positive, but the pattern frequency of positivity is highly variable. S-100 is a reliable marker, but it lacks specificity.[19] [Table 2]. Thus, the histogenesis of myoepithelioma is from reserve cell of intercalated duct.

BASAL CELL ADENOMA

Basal cell adenoma is a neoplasm of a uniform population of basaloid epithelial cells arranged in a solid, trabecular, tubular, or membranous pattern. It was first reported as a distinct entity by Kleinsasser and Klein in 1967. These basaloid cells have two or more morphologic forms. One is a small cell with scanty cytoplasm and a round, deeply basophilic nucleus arranged in palisading forms. The other is a larger cell with amphophilic to eosinophilic nucleus that is more ovoid and paler staining.[18]

Arujo, *et al.* observed that luminal ductal cells from the basal cell adenomas, express CK 7, 8, 14, and 19 while the non-luminal cells were rarely positive to CK 14. On the outside of the solid cell nests,

Table 1: Markers in normal salivary gland tissue

Markers	Acinar	Ductal	Myoepithelial	Basal
Pan-cytokeratin	Variable	+	+(weak)	+
Low molwt CK (CAM 5.2)	+	+	+	+
High mol wt. CK (CK14)	-	-	+	+
Amylase	+	-	-	-
EMA	+	+	-	-
CEA	+	+	-	-
S-100	-	+/-	+/-	-
Actin, Myosin, Calponin	-	-	+	-
Desmin	-	-	-	-
GFAP	-	-	+/-	-

Table 2: Taxonomy of salivary gland neoplasms and their markers[18,20,34,37]

Classification of neoplasms	Sub-classification of neoplasms	Benign	Malignant	IHC markers
Composed of luminal and modified myoepithelial cells	Histologically apparent with proteoglycan and basal lamina production Histologically lacking proteoglycan and basal lamina production	Basal cell adenoma Cellular pleomorphic adenoma Basal cell adenoma Warthin's tumor	Malignant mixed tumor Adenoid cystic Carcinoma (cribriform) Basal cell adenocarcinoma Adenoid cystic carcinoma (solid/tubular) Epithelial-myoepithelial carcinoma mucoepidermoid carcinoma polymorphous low grade adenocarcinoma	Cytokeratins (LMWK, HMWK) S100, GFAP Calponin, Maspin, Laminin, Collagen IV cytokeratins (LMWK, HMWK) S100, GFAP Calponin, Maspin
Composed of myoepithelial/ basal cells		Myoepithelioma	Malignant Myoepithelioma	S100, Calponin, Maspin
Composed of luminal acinar cells		Canalicularadenoma Ductal papilloma Cystadenoma Oncocytoma	Acinic cell carcinoma Salivary duct Carcinoma Adenocarcinoma (Not otherwise specified) Oncocytic carcinoma	Cytokeratins (LMWK, HMWK)
Composed of undifferentiated cells			Undifferentiated carcinoma small cell carcinoma	

there were smaller elongated myoepithelial-like cells, which expressed cytokeratin 14 and vimentin. A peri-cytoplasmic rim pattern of CEA immunostaining from ductal structures of basal cell adenomas is similar to that expressed by luminal columnar cells from striated ducts of normal salivary glands [Table 2]. A positive reaction to vimentin in both epithelial and stromal components basal cell adenomas has been observed[20] [Figures 1a and b].

CANALICULAR ADENOMA

It is an uncommon benign neoplasm that has a marked predilection for occurrence in the upper lip. Bauer and Bauer first used the term in 1953. It is composed of long columns and cords of cuboidal or columnar cells in a single layer. The supporting stroma is loose and fibrillar with delicate vascularity[18] [Figure 2].

Based on ultrastructural and histochemical aspects, some authors have suggested that possibly the canalicular adenoma has an excretory duct origin, although an intercalated duct origin has also been indicated by Arajou, *et al*. Most of these cells positively expressed AE1/AE3 cytokeratins and S100 protein [Table 2]. Ferreiro reported that the canalicular adenomas are CEA-negative and only infrequently EMA-positive. The epithelial nature was determined by the high positivity for related cytokeratins, and the absence of any reactivity for calponin rules-out a myoepithelial origin of this lesion. An absence of myoepithelial differentiation in canalicular adenoma, both via immunohistochemical and ultrastructural studies, is evident and consistent with this tumors putative ductal luminal cell differentiation.[21]

SEBACEOUS ADENOMA AND SEBACEOUS LYMPHADENOMA

These are a rare benign neoplasm of salivary glands.[18] Immunohistochemical results show strong staining for CD 68 in the giant cells and histiocytes, p63 in the basal layer of sebaceous cells, and for her-2/neu in luminal sebaceous cells. All sebaceous cells showed androgen receptor positivity. Negative stains were observed for calponin and membrane thyrosinase-kinase receptor (c-kit/CD 117) [Table 2].[22]

CYSTADENOMA

Cystadenoma is a rare, benign, and well-circumscribed tumor originating from the salivary glands, with the cystic cavities containing intra-luminal papillary projections.[18] Immunohistochemistry showed that the ductal cells were diffusely positive for CK7 with strong nuclear expression of androgen receptors. Focal but strong staining for CK19 was also present. EMA staining was seen in the apical surface of the ductal cells. S-100 was negative in the ductal cells. The myoepithelial cells demonstrated strong nuclear staining for p63 with diffuse expression of calponin, SMA, CK14, and S-100[23] [Table 2].

DUCTAL PAPILLOMA

Ductal papillomas of minor salivary gland are rare. The differential diagnosis rests between intra-ductal papilloma, inverted ductal papillomas, and sialoadenoma papilliferum. They arise from salivary gland duct systems.[18]

INVERTED DUCTAL PAPILLOMA

The term was first described by White *et al.* in 1982. Histologically, it consists of basaloid and squamous cells arranged in thick, bulbous papillary proliferations that project into the lumen.[24] Immunohistochemical study revealed that tumor cells displayed strongly positive reaction with cytokeratins 13 and 14, and less strong reactions with cytokeratins 7, 8, and 18 [Table 2], which leads to an origin from proximal portion of a salivary gland excretory duct.[25]

Intra-ductal papilloma
It is an ill-defined lesion exhibiting a unicystic dilated structure. The cyst wall is lined by a single or double row of cuboidal and columnar cells, extending into the cystic lumen as papillary projections having thin fibrovascular cores. It has been suggested that intra-ductal papilloma arise from the excretory duct reserve cell population.[25]

Sialadenoma papilliferum
It presents with a complex histology, showing a biphasic growth pattern, with both exophytic papillary and endophytic proliferation of ductal epithelium. Sialadenoma papilliferum comprises dilated ducts with intra-luminal foldings from intercalated ducts. It is presumed that it arises from intercalated ducts.[26]

SIALOBLASTOMA

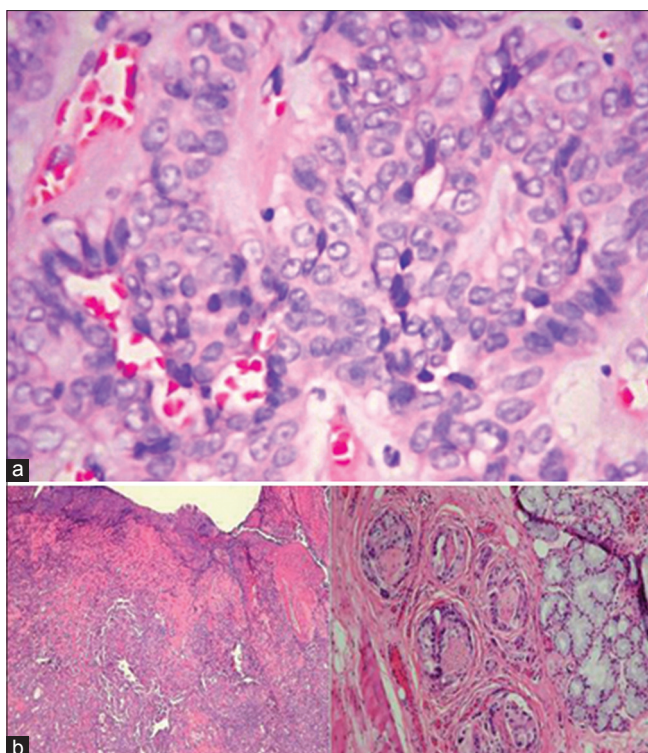Sialoblastoma are rare congenital epithelial tumors of the major salivary glands. To our best knowledge, only

Figure 1: (a) Shows the tumor is composed of isomorphic basaloid cells with a prominent basal cell layer, a distinct basement membrane-like structure, (b) Showing IHC for CK7

Figure 2: Typical histology of branching canaliculi of columnar cells forming double rows with intermittent "beading" set in a pervi-cellular, edematous, and finely vascular stroma
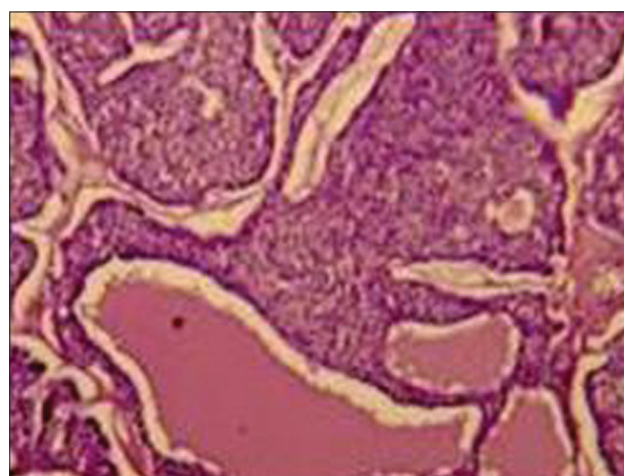
Figure 3: Microphotograph of sialoblastoma showing individual nests of basaloid epithelium separated by fibrous stroma

24 cases have been previously reported in literature. In 1966, Vawter and Tefft described the first cases of salivary gland tumor of epithelial origin, called as embryoma.[27] Since then, various terms have been used to describe this tumor, including congenital basal cell adenoma, basaloid adenoma, and congenital hybrid cell adenoma-adenoid cystic carcinoma[28] in 1988.

Sialoblastoma are locally invasive and have the propensity for re-occurrence, but no case of fatal metastasis has been reported.[29] Histologically, the epithelial cells may be basaloid with scant-to-normal cytoplasm and either solitary or very few nucleoli.[28,30,31] Acini have been reported to stain positive for mucin by the diastase/periodic acid-Schiff method.[32] Immunohistochemistry findings have shown cytokeratin in the ductal components, vimentin in both the ductal structures and solid nests, and S-100 protein [Table 2], which confirms the presence of myoepithelial cells [Figure 3].[33]

MALIGNANT TUMORS

Polymorphous low-grade adenocarcinoma
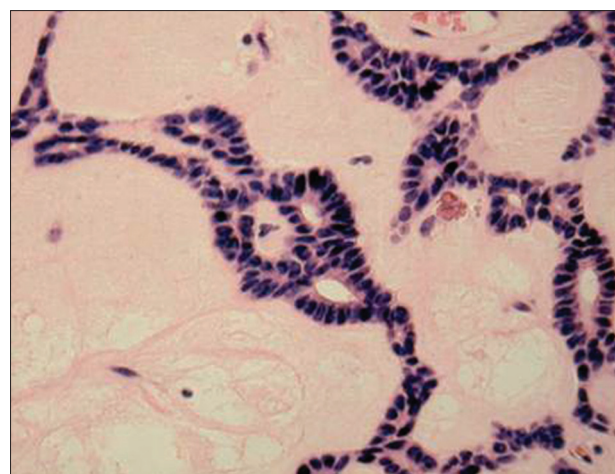Evans and Batsakis, in 1984, described a group of oral minor salivary gland neoplasms and used the term polymorphous low-grade adenocarcinoma, emphasizing the salient features of the tumor, namely, the varied histomorphology, malignant, and indolent behavior [Figure 4a].[18] The tumor is often well circumscribed but unencapsulated [Figure 4b]. It has varied growth patterns like- solid, glandular, cribriform, ductular, tubular, trabecular, or cystic lesions.[34] PLGA is composed of luminal and abluminal cells, which according to their relative distributions and proportions differentiate into a variety of different morphological patterns.[18,34]

The immunohistochemical features [Table 2] support the presence of luminal and abluminal cells. The luminal cells exhibit moderate to strong expression for low molecular weight keratin (LMWK), vimentin, and S-100. Abluminal cells show a phenotype consistent

with basal cell differentiation and less commonly with myoepithelial cells demonstrating immunoreactivity to high molecular weight keratin (HMWK), S-100, low molecular weight keratin (LMWK), vimentin, and SMA[27] [Figure 4].

Salivary duct carcinoma

It is a rare, high-grade malignant neoplasm composed of structures that resembled salivary gland ducts. Kleinsasser *et al.* coined the term in 1968 [Figure 5]. Histopathological characteristic of this tumor is intra-ductal or circumscribed nests of dysplastic ductal cells that grow in solid, cribriform, and papillary configurations. Central, comedo-type necrosis of the tumor nests is a distinctive feature.[18] Cellular and nuclear pleomorphism varies from mild to severe. Mitotic figures are nearly always present. Invasion of nerves and blood vessels is frequently seen.[34] Immunohistochemically, [Table 2] majority of salivary duct carcinomas exhibit epithelial membrane antigen, cytokeratin, and carcinoembryonic antigen expression. Araojo *et al.* studied the expression of cytokeratins 7, 8, 13, 14, 19 [Figure 4a] vimentin, and alpha-smooth muscle actin. All tumor cells were positive for cytokeratins 7 and 8. Few neoplastic structures expressed cytokeratin 14 in cells surrounding tumor islands. Staining for S-100 is inconsistent and often focal. It also expresses BRST-2 and c-erb-2, as in its mammary counterpart. Kapadia and Barnes have demonstrated overexpression of CD44, a molecule involved in cell-cell and cell-matrix interactions.[30] The immunohistochemical studies identify the ductal cells but no myoepithelial
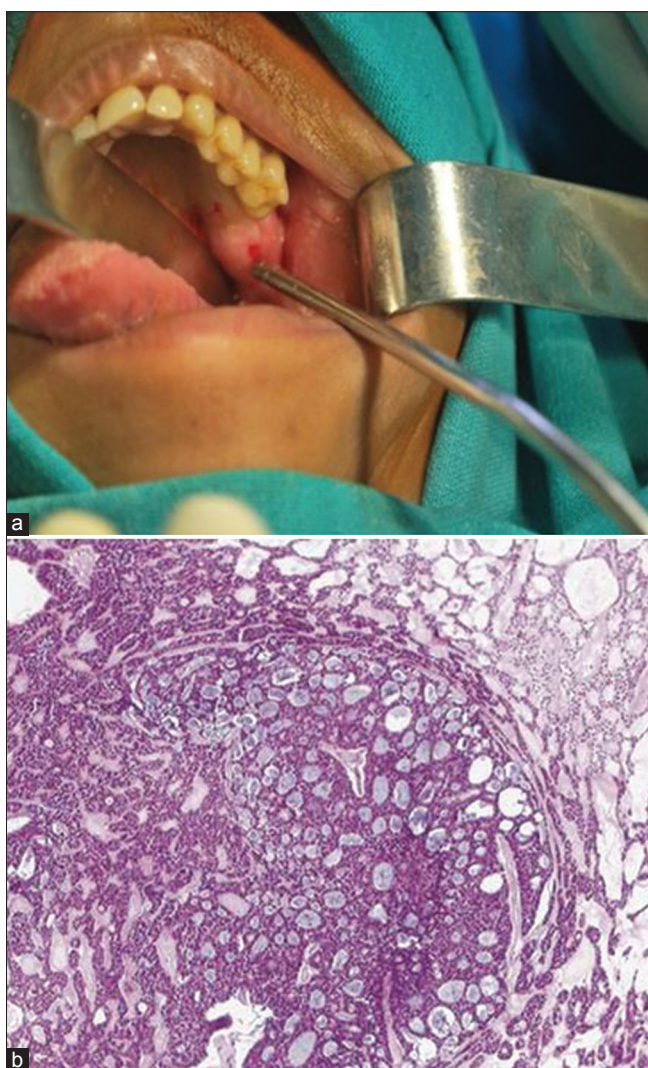

Figure 4: (a) Polymorphous Low Grade Adenocarcinoma: As a swelling on the hard palate extending to retro molar region, distal to 26. (b) H and E-stained section of PLGA showing many irregular dilated glandular spaces and focal papillarity

Figure 5: (a) Growth in the left upper posterior region of the maxilla. The swelling was dome-shaped, ulcerated swelling was seen extending from the upper left second premolar on the buccal side to the first molar region. (b) Showing (A) Diffuse and focal immunoreactivity in salivary duct carcinoma (cytokeratin (AE1/AE3), ×400

cells. Therefore, the tumor takes origin from the ductal system.

Epithelial-myoepithelial carcinoma

It is an uncommon, biphasic, low-grade neoplasm composed of variable proportions of ductal and large, clear-staining, differentiated myoepithelial cells. In 1972, Donath and co-workers described cases and introduced the term epithelial-myoepithelial carcinoma of salivary glands.[18] Most tumors show a multi-nodular growth pattern The islands of tumor cells are composed of small ducts lined by cuboidal epithelium that is surrounded by clear cells that interface with a thickened hyaline-like basement membrane. The tumor's growth pattern varies from solid lobules to irregular, papillary cystic arrangements with tumor cells that partially or completely fill cyst-like spaces.[34] Immunohistochemistry expression of different cell proteins is essential in identifying the two cell types demonstrated in this tumor [Table 2]. The outer basal or myoepithelial cell layer expresses S-100, calponin, low molecular weight cytokeratins (CK5/6) and variably expresses other cytokeratin subclasses. The inner luminal layer is typical of all epithelial cells in expressing cytokeratin but not smooth muscle proteins [Figure 6].[35]

Basal cell adenocarcinoma

It is believed to be a malignant counterpart of basal cell adenoma.[18] It is divided into four subtypes: (1) Solid, (2) Ductal, (3) Trabecular, and (4) Membranous. It predominantly shows solid growth, characterized by a lobular pattern with palisading of cells at the periphery of tumor islands.[34] Immunohistochemically, cytokeratin (AE1/AE3) stains all tumors, more peripherally in the solid pattern and usually centrally in the trabecular areas; vimentin shows a diffuse expression; SMA is mainly confined to peripheral tumor cells in both the solid and the trabecular growth patterns; EMA and CEA stains some of the tumors, predominantly in the luminal cells; p53 oncoprotein is focally positive in some tumors; Ki-67 stains less than 5% of the tumor cells [Table 2]. Staining patterns of cytokeratin and actin varies with the architecture of the tumor. Therefore, neither ultrastructural characteristics nor immunohistochemistry findings appear to distinguish basal cell adenocarcinoma from basal cell adenoma.[36]

Cystadenocarcinoma

A rare, malignant epithelial neoplasm characterized histopathologically by prominent cystic and frequently papillary growth. The tumor comprises of numerous irregular cysts with variation in size and frequent intra-luminal papillary processes. The stroma shows fibrosis with areas of sclerosis and hyalinization.[37]

Immunohistochemistry [Table 2] showed that the ductal cells were diffusely positive for CK7 with strong nuclear expression of androgen Receptors. Focal but strong staining for CK19 was present. EMA staining was seen in the apical surface of the ductal cells. S-100 was negative in the ductal cells. The myoepithelial cells demonstrated strong nuclear staining for p63 with diffuse expression of calponin, SMA, CK14, and S-100. This suggested differentiation into both ductal and myoepithelial cells, arising from reserve cell of intercalated duct.[38]

Malignant mixed tumour

It include three distinct clinicopathologic entities-Carcinoma ex pleomorphic adenoma, Carcinosarcoma, Metastasizing mixed tumour [Table 2]. Microscopically malignant appearing cells appear adjacent to a typical appearing pleomorphic adenoma [Figures 7 and 8].[18] Carcinoma areas characterized by ductal structures containing both benign myoepithelial cells positive for alpha-smooth muscle actin (alpha-SMA), vimentin and CK 14 and proliferating atypical luminal cells reactive for CK7, CK8 and CK19. Tumours with a myoepithelial component were composed mainly or exclusively of cells that expressed vimentin and alpha-SMA. Immunostaining with CAM5.2 is detected only in the carcinomatous islands [Figure 9].[18,32]

DISCUSSION

Carcinomas of salivary gland origin represent an important subset of malignant epithelial tumors. These tumors can metastasize to distant sites and should be included in the differential diagnosis of metastatic tumors of unknown primary.[22] The improvement of immunohistochemical techniques [Table 3] has had an enormous impact on tumor diagnosis and classification. With regard to the pathology of salivary gland tumors, various antibodies have been employed to characterize the constituent tumor cells to establish differences among tumor types.

The application of immunohistochemistry to the salivary gland began in the early 1980s with studies of the intermediate filaments keratin, vimentin, and desmin.[1-12] Since then, a major concern has been

Table 3: Antibodies that may be helpful in resolving difficult differential diagnoses[18,19,39,40-50]

| Nonspecific markers of luminal/acinar epithelial differentiation	Markers of muscle differentiation (myoepithelium)	Markers of cell organelles/secretions
Epithelial membrane antigen (EMA) Carcinoembryonic antigen (CEA) Low molecular weight keratins (CK 8, 18, 19) Non-specific markers of myoepithelium (also seen in some ductal epithelial phenotypes) S-100 GFAP High molecular weight keratins (CK14) Vimentin	Alpha-smooth muscle actin Smooth muscle myosin heavy chain Calponin p63	Mitochondria (oncocytic metaplasia and oncocytictumors) Amylase, lactoferrin, lysozyme, secretory component (acinar differentiation) Type IV collagen, laminin, maspin
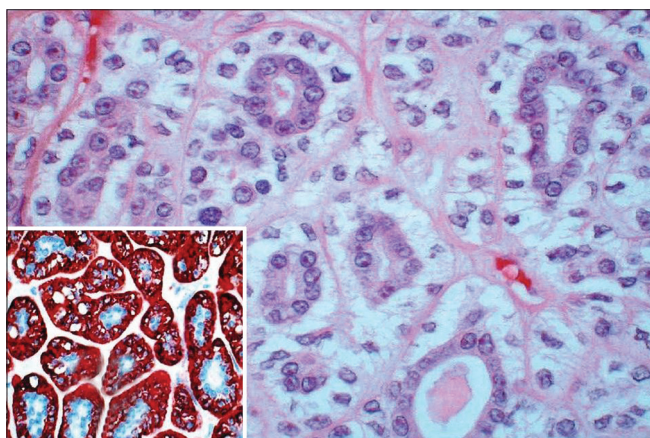
Figure 6: Classic form is a double cell proliferation composed of a darker inner layer of ductal cells associated with a margins prominent outer layer of clear myoepithelial cells accentuated by immunostain for calponin (inset)
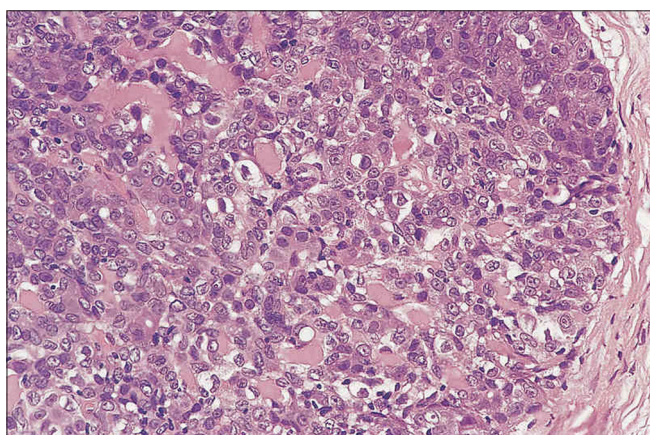

Figure 7: Showing nuclear and cellular pleomorphism (×10)

Figure 8: (a) Shows a large palatal pleomorphic adenoma developing into a carcinoma ex pleomorphic adenoma, as evidenced by its surface ulceration. It has been presented for 15 years. (b) The carcinoma ex pleomorphic adenoma consisting benign pleomorphic adenoma and poorly differentiated carcinoma with the ulcerative epithelial surface. Tumor cells presented the perineural invasion (H and E, ×40)

the identification of myoepithelial cells. Thus, the S100 protein was detected in the normal salivary gland and has become the most popular antibody for the identification of myoepithelial-tumoral cells as myoepithelioma.[39-41] Around the same time, glial fibrillary acidic protein (GFAP) was demonstrated in the myoepithelial cells of the pleomorphic adenoma, as well as vimentin was expressed in the
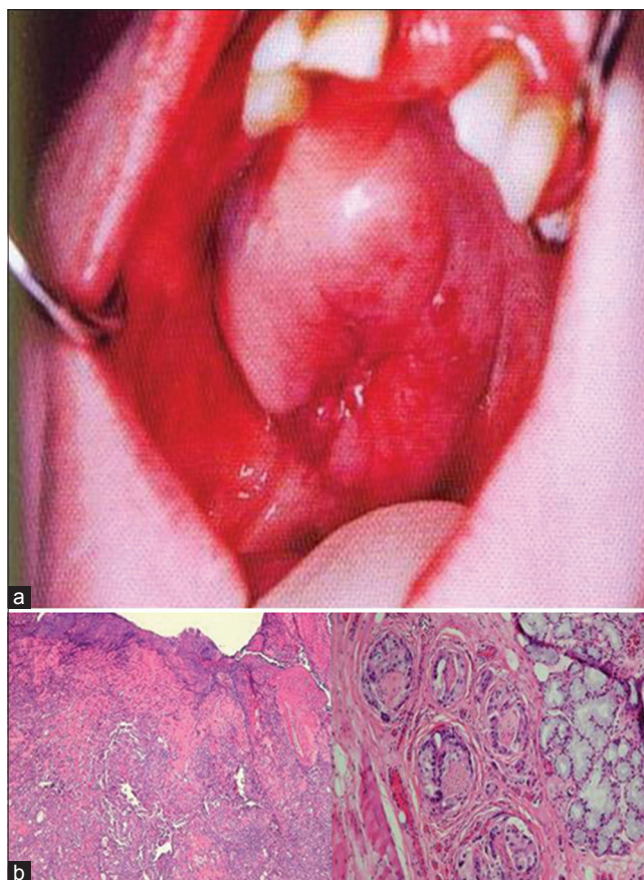
tumoral-myoepithelial cells.[40,42,43] Early attempts to identify myoepithelial cells in normal salivary glands included immunohistochemical procedures with anti-smooth muscle myosin, although fixation was considered a critical step in this reaction. Later, muscle-specific actin (MSA) was demonstrated in normal myoepithelial cells and became the most

important marker of the tumor myoepithelial cell. More recently, calponin, a protein isolated from smooth muscle and non-muscle cells, has been used to identify myoepithelial cells in salivary gland tumors.[37,38,44] Epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA), a tumor marker from the oncofetal group of antigens, have been used as indicators of luminal or acinar differentiation in both benign and malignant salivary gland tumors.[43,45,46] However, a diffuse staining pattern for EMA and CEA was described in polymorphous low-grade adenocarcinoma of minor salivary gland.[47,48]

The IHC studies have revealed [Table 3] that luminal cells of intercalated duct-like structures, such as those seen in pleomorphic adenoma, basal cell adenoma, adenoid cystic carcinoma, and epithelial-myoepithelial carcinoma, expressed CKs 7, 8, 14, and 19. The outer cells of these structures exhibited vimentin or vimentin plus muscle-specific actin, but rarely CK14, which is seen particularly in pleomorphic adenoma,
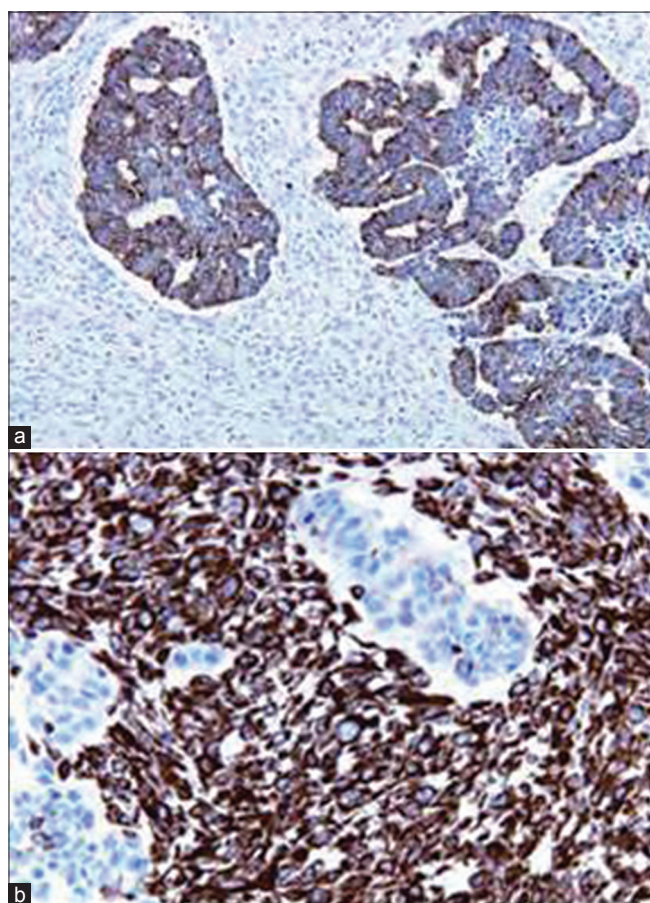

Figure 9: (a) Immunostaining with CAM5.2 is only detected in the carcinomatous islands ×10. (b) Strong vimentin and staining of the mesenchymal cells (×40)

in the tubular type of basal cell adenoma, and seldom in the tubular type of adenoid cystic carcinoma. Modified myoepithelial cells of pleomorphic adenoma and myoepithelioma exhibited a variable immunoprofile. CKs 7 and 8 were also observed in acinar cell adenocarcinoma and polymorphous low-grade adenocarcinoma with vimentin in the latter. CK13 was expressed only by canalicular adenoma and mucoepidermoid carcinoma cells. This study showed that the panel of antibodies employed was effective in distinguishing among salivary gland tumors. Studies have shown the presence of CK14 in normal salivary myoepithelial cells and in basal cells of excretory ducts. CKs 13 and 16 in basal cells of excretory ducts; isolated CK13 in excretory ducts; CK19 in luminal ductal cells and in myoepithelial cells; CK7 and 19 in ductal luminal cell; CK18 in acinar and luminal ductal cells, and CK8 in acinar cells. Thus, on the basis of the expression of these cytokeratinsacinar cell carcinoma, adenoid cystic carcinoma, salivary duct carcinoma, basal cell adenocarcinoma can be differentiated from each other. As in salivary gland tumors, the presence of CK8 in acinar cell carcinoma; CKs 7, 8, 13, 14, 18, and 19 in mucoepidermoid carcinoma; CKs 7 and 14 in myoepitheliomas; CK8 in polymorphous low-grade adenocarcinoma, adenoid cystic carcinoma, and monomorphic adenoma (25); CKs 7, 8, 14, 18, and 19 in adenoid cystic carcinoma; and the expression of a variety of CK subtypes in the modified myoepithelial cells of pleomorphic adenoma have been observed.[40-43]

In addition, antibodies to cell cycle–associated antigens, such as proliferating cell nuclear antigen and Ki-67, have been used in immunohistochemical studies of salivary gland tumors to evaluate the proliferating fraction of neoplastic cell populations and their implications in histogenesis. These markers may be valuable either as prognostic indicators in the same tumor type or as an additional diagnostic method to distinguish among different tumor types. Antibodies against p53 nuclear phosphoprotein, a product of tumor suppressor gene p53, have also been used in salivary gland tumors to detect the accumulation of mutated protein in neoplastic cells.[44,49]

Myoepithelioma [Figure 10] can be differentially diagnosed from the myoepithelial carcinoma with the latter showing much more intense staining for p53, ki67, and PCNA as compared to the benign counterpart though the staining with other makers is also positive as that of S-100, vimentin, calponin,

keratin, SMA, and GFAP. Basal cell adenoma may have areas, which closely simulate adenoid cystic carcinoma. The diagnosis in such cases is based on the typical solid pattern areas as well as non-invasive growth with lack of perinural invasion, which is supported by the IHC staining pattern of keratins, GFAP which is less intense in BCA. Separation from basal cell adenocarcinoma as shown by Nagao *et al.* is based on the high proliferative rates of Ki67 in basal cell adenocarcinoma as compared to BCA. In canalicular adenoma, immunohistochemical studies confirm the ductal nature of the tumor cells. As BCA, unlike canalicular adenoma frequently has myoepithelial cells that stain for keratins, calponin, actin, and myosin. The faint staining for GFAP differentiates it from PLGA.[49,50]

Gnepp *et al.* published a small series suggesting differences in staining patterns of EMA and polyclonal CEA, which helped in D/D of PLGA and ACC. The staining patterns of these antigens were almost identical and limited to true luminal staining in ACC. However, they were different in PLGA with focal luminal staining with CEA, whereas EMA stained both luminal and non-luminal cells. In addition, there have been two studies which indicate proliferative rates of Ki-67 for PLGA [Figures 11a and b] (showing less intense staining patterns) and ACC (showing more intense staining patterns).[46] Also, studies carried out by Baltren *et al.* used a series of markers and found that PLGA gives a significantly weaker immunohistochemical expression of c-kit compared with ACC.[51] Other immunohistochemical studies have shown that PLGA expresses big amounts of vimentin, which is absent in canalicular adenoma. These two tumors share similar histological characteristics, so vimentin is a useful marker for
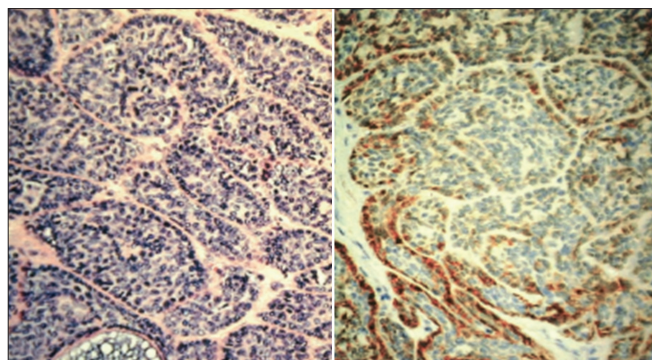
differential diagnosis.[52] Also, the GFAP staining was seen in focal areas of PLGA and was localized only to the epithelial component. In contrast, 93% of mixed tumors expressed.[50]

CONCLUSION

Latest developments in the field of immunohistochemistry have led to the discovery of newly recognized tumors in salivary glands and new variants of existing tumor entities. Hence, there is further scope in understanding and deciphering the origin and histogenesis of salivary gland neoplasms. The future will see definition of the genetic and
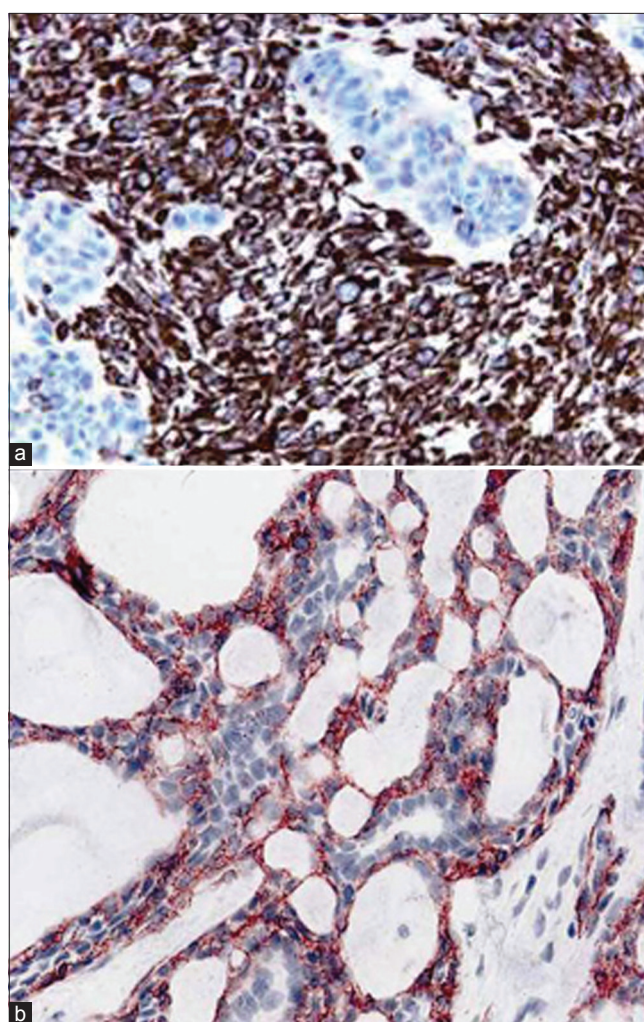

Figure 11: (a) Photomicrograph of polymorphous low-grade adenocarcinoma demonstrating no staining, indicative of an absent myoepithelial cells component. The noted area of positive staining (arrow) represents SMA uptake in arterial wall smooth muscle (×400 is pronounced). (b) Photomicrograph showing adenoid cystic carcinoma showing a strong SMA staining pattern in the para-luminal SMA where the myoepithelial component is pronounced component (×40)

Figure 10: Myoepithelial differentiation identified by immunostaining for calponin in tubular form of basal cell adenoma

proteomic underpinnings of many of the morphologic and biologic distinctions we currently recognize. Hopefully, this will translate into more effective therapies for prevention, local control, and cure for many of the salivary gland malignancies currently associated with notoriously protracted but lethal courses.

ACKNOWLEDGMENT

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