Review Article

IMMUNOPROFILE OF COMMONLY ENCOUNTERED SALIVARY GLAND NEOPLASMS: A BRIEF REVIEW

Amandeep Kaur[1], Akshay Thakur[1], Harmanjit Singh[1], Avalpreet Singh[2], Bavneet Kaur[1], Buneet Kaur[1], Ramneek Kaur[1]

[1]BDS (Intern), Sri Guru Ram Das Institute of Dental sciences & Research, Amritsar, Punjab, [2]B.D.S.

ABSTRACT

Salivary glands tumors are one of the most complex and relatively rare group of lesions encountered in oral pathology practice. Their complexity is attributed to heterogeneity of the cells of origin of these cells. The problem is compounded by the ability of these cells to differentiate and modify into various morphological subtypes resulting in a myriad of histomorphological pattern. This also leads to a frequent overlap of microscopic features among various neoplasms. So there is a need of immunohistochemical analysis in order to classify various salivary gland tumors as well as determining the prognosis. The present article is aimed at reviewing and summarizing the concept regarding the histogenesis of salivary gland tumors and their relevance in immunohistochemical studies for differentiating between commonly occuring salivary gland tumors.

Key words: Histogenesis, immunohistochemistry, salivary gland tumors.

Corresponding Author: Dr. Amandeep Kaur, BDS (Intern), Sri Guru Ram Das Institute of Dental sciences & Research, Amritsar, Punjab. Email: guraman165@gmail.com

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INTRODUCTION

Salivary gland tumors are a morphologically and clinically diverse group of neoplasms which may present considerable diagnostic and management challenges to the pathologist or surgeon. Salivary gland tumors are rare with an overall incidence in western world of about 2.5-3.0 per 100000 per year. About 80% of all lesions are benign, hence salivary gland malignancies are particularly rare, comprising less than 0.5% of all malignancies and about 5% of cancers of head and neck.[1] Because of their rarity, individual clinicians are only frequently required to manage these lesions and most cancers are managed in specialist centres. This coupled with degree of morphological diversity makes this group of lesions, one of the most interesting and challenging entities in head and neck region.

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 in different tumor entities. Therefore these tumors may present a considerable diagnostic challenge.[2]

Although hematoxylin-eosin (HE) staining is still the gold standard method used for diagnosing the salivary gland tumor, immunochemistry (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 cell nature and differentiation status, cell proliferation, and tumor protein expression.[2] The present article aims to review the practicality of immunohistochemical markers in differentiating and diagnosing few salivary gland tumors laying emphasis on their histogenesis.

HISTOGENETIC PROSPECTIVE

(A) RESERVE CELL HYPOTHESIS:-

The existence of reserve cells in normal salivary gland was originally postulated from observations of embryonic developments of palatal minor salivary glands. These evolve as downgrowths of bilayered ducts and it was assumed that the inner or luminal layer derived from the outer or basal layer (Eversole 1971). As a result of these observations, basal cells were considered reserve cells based on the premise that they functioned as stem cells, particularly for generation of duct luminal and acinar cells with the maturation and development of salivary glands, it was suggested that such cells remained confined to the basal cell layer of excretory ducts. In this location, reserve cells associated with excretory ducts were presumed to be responsible for replacement of excretory duct luminal and basal cells and the progenitor cells for the intercalated ducts. The latter were responsible for development and replenishment of intercalated ducts, striated ducts, and acinar cells, hence this concept was labelled the semipleuripotential bicellular theory (Eversole, 1971).[3,4]

In 1977, the reserve cell concept was adopted and promoted as the underlying mechanism for the histogenesis of the salivary gland tumors (Regezi and Batsakis, 1977). In an attempt to explain the histogenesis of salivary gland tumors, a bicellular theory of origin was proposed.[5,6] The two cells, the excretory duct reserve cells and the intercalated duct reserve cell, were presented as the hypothetical cells of origin for salivary gland neoplasms. It was argued that the excretory duct reserve cells give rise to squamous cell carcinoma (SCC) and mucoepidermoid carcinomas, and the intercalated reserve cells give rise to all others. It was also shown that myoepithelial cells were responsible in part for the wide histologic variation of these neoplasms. As a highly and therefore, terminally, differentiated cell, the acinar secretory cell was said to play a minimal role in parenchymal renewal and thus, was incapable of a significant role in tumor induction.[5,6]

B) MULICELLULAR HISTOGENETIC CONCEPT:-It was proposed that differentiated cells at all the levels of the gland, including acinar and basal cells are capable of cell division.

PRINCIPAL CELL TYPES AND THEIR ORGANIZATION

Most salivary gland tumors originate from acinar/ductal epithelial cells (luminal cells) and/or myoepithelial/basal cells (abluminal cells). Monophasic tumors have only one 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. These 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.[7,8] Use of IHC to differentiate between luminal and abluminal cells can help in understanding the complex architecture of salivary gland tumors and aid in diagnosis.

MARKERS FOR LUMINAL CELLS

Luminal cells are readily highlighted by IHC for low molecular weight cytokeratin (CK) (such as CAM5.2), carcinoembryonic antigen (CEA) or epithelial membrane antigen (EMA) (Table 1). Interestingly, although CD117/ c-kit is negative in normal salivary gland cells, it is often positive in luminal cells of various types of salivary glands tumors and thus can be utilized to highlight the rudimentary or abortive glands in tumors.[7,9]

MARKERS FOR ABLUMINAL CELL

Abluminal cells are highlighted by IHC for high molecular weight CK (such as 34βE12 or CK 14) and myoepithelial cells, in addition are stained with antibodies against myoid proteins (such as muscle specific actin smooth muscle actin or calponin). p63 has recently become a popular marker for abluminal cells- both basal cells and myoepithelial cells, as well as their neoplastic counterpart, shows nuclear immunoreactivity.[7,10] (Table 1) CD10 can also be used as a myoepithelial marker but lacks specificity.[11] In salivary gland tumors with dual ductal cell - myoepithelial cell differentiation the myoepithelial or modified myoepithelial cell component generally strongly express maspin, whereas ductal cells are usually negative or show only weak focal immunoreactivity.[7]

Table 1: Expression of immunomarkers in normal salivary gland ells[2,8]

Markers	Interlobular/ Excretory duct luminal cells	Striated duct cells	Intercalated Duct luminal cell	Acinar Epithelial cells	Basal cells	Myoepithelial cells
Calponin/SMA	–	–	–	–	–	+
CK7/CAM 5.2	+	+	+	+ weak	+ weak/-	**+ weak**
CK5/6 /CK14	–	–	–	–	+	+
CK5/6	–	–	–	–	+	+
p63	–	–	–	–	+	+
S100	–	–	±	–	–	+
Vimentin	–	–	–	–	–	+
EMA/CEA	–	–	–	–	+	+
MSA	–	–	–	–	–	+
c-kit	±	–	–	–	–	–
GFAP	–	–	–	–	–	**+ (low sensitivity)**

SMA, Smooth muscle actin; CK, Cytokeratin; CEA, Carcino embryonic antigen; EMA, Epithelial membrane antigen; MSA, Muscle specific actin; GFAP, Glial fibrillary acidic protein + ,usually >70 % cases are positive; -, usually <5% cases are positive ; +weak, weakly positive; ±, weakly positive or negative

PLEOMORPHIC ADENOMA
(Benign mixed tumor)

It is the most common benign tumour of the salivary glands having a marked histological diversity with epithelial, myoepithelial and mesenchymal cell types arranged in variety of architectural and differentiation pattern.[8,12] Ultrastructurally two main types of cells, epithelial cell and myoepithelial cells have been described in mixed tumors.[13,14] Other cells such as mesenchymal cells and intermediate cells have also been reported.[15] The epithelial cells which vary from intercalated duct like cells to epidermoid cells, comprise the histological majority of most tumors. Myoepithelial and myoepithelial like cells have been described in varying numbers, depending upon the tumor studied. These cells are most commonly found in myxoid areas of mixed tumors.[13]

MOLECULAR GENETICS: Extensive cytogenetic studies of pleomorphic adenoma have shown that approximately 70% of the tumors are karyotypically abnormal.[16-18,12] Four major cytogenetic subgroups may be discerned:

--tumors with rearrangements involving 8q12 (39%)

-- tumors with rearrangements of 12q 13-15 (8%)

--tumors with sporadic, clonal changes not involving 8q12 or 12q 13-15 (23%)

--tumors with an apparently normal karyotype(30%).

A target gene in pleomorphic adenomas with 8q12 abnormalities is PLAG1, a developmentally regulated zinc finger gene.[12,19,20]

Table 2: The varying patterns of pleomorphic adenoma masquerades its resemblance to other tumor types as enumerated in following table[21]

Features	Resemblance
Bilayered ducts and cribriform pattern	Adenoid cystic carcinoma
Myoepithelial component	Myoepithelioma
Bilayered ducts with clear outer cells	Epithelial-myoepithelial carcinoma
Sheets of epitheloid or basaloid cells	Basal cell adenoma or adenocarcinoma
Myxoid stroma	Myxoma or neural tumors
Chondroid stroma	Chondrosarcoma
Plasmacytoid cells	Plasmacytoma
Squamous metaplasia	Squamous carcinoma
Oncocytic metaplasia	Oncocytoma

The major differential diagnosis is with Myoepithelioma, basal cell adenoma, ACC, and polymorphous low grade adenocarcinoma (PLGA). Myoepithelioma has no glandular component and lacks a chondromyxoid stroma. Basal cell adenoma is a monomorphic adenoma that shows a proliferation of basaloid cells encircled by a prominent basal lamina without a chondromyxoid stroma. ACC is invasive, shows a perineural invasion, and has a glycosaaminoglycan material and a reduplicated basement membrane surrounded small, uniform cells with an angulated shape and hyperchromatic nuclei. Small duct like structures can be seen in both tumors. GFAP tends to be absent, whereas CD117 highlights the central cells more strongly. PLGA develops only in the minor salivary glands. It shows prominent perineural invasion and more uniform, oval nuclei with delicate, fine vesicular nuclear chromatin; it shows weak GFAP and p63 expression.

IMMUNOPROFILE: The inner ductal cells in the tubulo-glandular structures are positive for CK 3, 6, 10, 11, 13 and 16 whereas the neoplastic myoepithelial cells are irregularly positive for cytokeratin 13, 16 and 14.[22] The neoplastic myoepithelial cells co-express vimentin and pan-cytokeratin and are variably positive for S-100 protein, a smooth muscle actin, GFAP, calponin, CD10 and muscle specific actin (HHF-35).[12, 23] (Table 3) Modified myoepithelial cells in these tumors are also reactive for p63.[24] The non-lacunar cells in the chondroid areas are positive for both vimentin and pan-cytokeratin, whereas the lacunar cells are positive only for vimentin.[12,25] (Table 3)

ADENOID CYSTIC CARCINOMA (ACC): ACC is a malignant biphasic epithelial tumor composed of modified epithelial and ductal cells. Overall ACC is a rare tumor accounting for only 1% of all malignant tumors of oral and maxillofacial region. It accounts for 22% of all salivary gland malignancies and is one of the most common malignant tumor of minor salivary glands and seromucinous gland.[27,28] ACC is well known for its prolonged clinical course and tendency for delayed onset of distant metastasis.

The microscopic appearance of tumor is heterogenous, consisting of varying amounts of 3 distinct growth patterns: cribriform, tubular and solid, frequently there are mixed growth patterns.[12] An inconsistently reported finding has been the presence of myoepithelial and myoepithelial like cells, suggesting to some that this is the cell of origin for the ACC. This apparent mixture of cells would be seen analogous to the mixture seen in the mixed tumor, even though it is quantitatively different. The bicellular theory of origin would account for the appearance of both ductal and myoepithelial cells through neoplastic transformaton of the intercalated duct reserve cell.[29,30]

MOLECULAR GENETICS: A recent study of 52 cases of ACC showed that detection of IP-32-36 was the most common genetic change in ACC and was significantly associated with solid tumor phenotype and decreased overall survival.[31] Past studies have shown that tumor aneuploidy correlates with more aggressive disease and a poor prognosis. A recent large scale micro-array analysis of 15 cases of ACC found the transcription factor-encoding gene SOX4 was significantly overexpressed in ACC relative to normal salivary gland tissue.[32]

Table 3: Immunprofile of pleomorphic adenoma with its comparison from commonly perplexed salivary gland tumors[26]

Markers	Pleomorphic adenoma	Basal cell adenoma	Canalicular adenoma	Polymorphous low grade adenocarcinoma	Adenoid cystic carcinoma
Vimentin	+	+	+	+	**+**
Pancytokeratin	+	+	+	+	**+**
CK7	+	+	+	+	**+**
CK18	+	+	N	+	**+**
CK5/6	+	+	NA	+	**+**
S100 Protein	+	+	+	+	**+**
GFAP	+	N	+(linear)	R (weak, few cells	**R (isolated cells)**
CD117	S	+	S (weak)	+ (weak)	**+ (inner cell layer)**
P63	+	+	N	weak	**+**
SMA	+(abluminal)	+	N	S(abluminal)	**+(luminal)**
MSA	+(abluminal)	+	N	+	**+ (abliminal, -50%)**
SMMHC	+ (abluminal)	+	N	+50%	**+ (abluminal)**
Calponin	+	+	N	S(20%)	**+(abluminal)**
Maspin	NA	NA	NA	+	**+**
CD117	S	+	S(weak)	+(weak)	**+(inner cell layer)**
Ki-67	**<5%**	**<2%**	**<2%**	**<5%**	**>20%**

+, almost always positive; N, negative; S, sometimes positive; R, rarely positive; NA, not applicable; CK,cytokeratin; GFAP, glial fibrilary acidic protein; SMA,Smooth musle actin; MSA, Muscle specific actin; SMMHC, Smooth muscle myosin heavy chain.

IMMUNOPROFILE: ACC expresses both ductal and myoepithelial /basal cell markers such as CK 7, CAM 5.2, Calponin, SMA, SMMHC, p63, SOX10, and S100 **(Table 3)**.[8] Use of immunochemical stains such as smooth muscle actin, S100 and smooth muscle myosin heavy chain will highlight cells showing myoepithelial differentiation surrounding the pseudocysts. The lumens of the pseudocysyts will stain positively for basement membrane components such as Type IV collagen and laminin.[33] ACC has also been shown to be strongly positive for C-kit (CD117) regardless of grade.[33,34] Strong C-kit expression is seen in all neoplastic cells in solid pattern, all cells surrounding pseudocysts in cribriform pattern, and all luminal cells in the tubular pattern (Table 1).[33]

Many markers have been studied as potential prognostic indicators in ACC. Increased expression of the cellular proliferation marker Ki-67 is seen with increasing amount of solid component and has been shown to correlate with worse prognosis.[35,36] Increased p53 expression may also be an independent marker of prognosis.

The differential diagnosis of ACC includes tumors that also exhibit tubular and cribriform structures such as PLGA and tumors with basaloid cellular morphology such as basal cell adenoma and adenocarcinoma and tumors with a dull population of ductal and myoepithelial cells such as pleomorphic adenoma. Pleomorphic adenoma can be identified by presence of mesenchymal, especially cartilaginous differentiation in the stroma. A recent study found that expression of GFAP and CD57 could be reliably used in cell block preparation to differentiate pleomorphic adenoma from ACC (Table 3).[37]

PLGA occurs almost exclusively in the minor salivary glands and may contain overlapping histopathologic features with ACC such as ductal, tubular and even cribriform growth. But PLGA lacks dual population of ductal and myoepithelial cells and typically has negative or low (50% of cells) expression of c–kit compared with high c -kit expression of ACC.[33]

Regarding Basal cell adenoma; Ki-67 labelling index in ACC (>20%) is reported to be different from that in basal cell adenoma (<2%) (Table no.3). Furthermore presence of strongly S-100 protein

positive spindle shaped stromal cells supports the diagnosis of basal cell adenoma.[38]

MUCOEPIDERMOID CARCINOMA (MEC)

Is the most common salivary gland malignancy representing between 2 - 16% of all salivary gland tumor.[26] It is basically a malignant epithelial neoplasm composed of mucus, epidermoid, intermediate, columnar, clear and oncocytic cells.[12] The cells of the excretory duct would be expected to have greatest potential to keratinize or become squamous in type under appropriate circumstances, because of their lack of specialization and their proximity to the gland orifice. Stimuli such as smoking or infection may result in mucous metaplasia of the excretory ducts. Neoplastic transformation of such metaplastic excretory duct may result in MEC. Alternatively, neoplasm can arise from direct neoplastic transformation of excretory duct reserve cell. Additional support for the excretory duct reserve cell as the cell of origin comes from observation of Eversole that mucoepidermoid carcinomas don't occur as intralobular lesions.[39]

MOLECULAR GENETICS: Molecular studies of these tumors are few and limited in number of cases. They show infrequent genetic loss at chromosomes 9p21, 8q, 5p, 16q and 12p. Studies of the H-ras gene in these tumors have reported 18% mutational at codon 12 and/or 13 and on mutation at codon 61. The mutation are mainly found in high grade tumors.[12]

IMMUNOPROFILE: It is usually positive for CK5, CK6, CK7, CK8, CK14, CK18, CK19, EMA, CEA and p63 and is negative for CK20, SMA, MSA and S100. However focal expression of S100, C-KIT, GFAP and Vimentin can be seen in some tumors.[8]

MEC needs to differentiated from acinic cell carcinoma, retention cyst and papillary cystadenoma and oncocytoma. The microcystic and follicular variants of Acinic Cell Carcinoma are most notable for being mistaken for MEC. p63 is a useful marker to differentiate these two. Sams et al[40] compared p63 expression among 31 cases of acinic cell carcinoma and 24 cases of mucoepidermoid carcinomas. They found that all ACC were negative for p63 while all MEC were strongly positive for p63. p63 immunostaining expression pattern can be helpful in distinguishing low grade MEC from retention cysts and papillary cystadenoma of salivary glands. Fonseca et al[41] found that p63 immunostaining in mucus retention cysts and papillary cystadenomas was limited to basal layers of the cystic spaces, whereas in low grade MEC's, positive staining was also found diffusely in the suprabasal layers of the epidermoid component of the tumor. MEC with prominent oncocytic population can also be differentiated from oncocytoma by p63 staining pattern. It has been reported that in oncocytic MEC, >50% of the cells throughout the tumor nests were positive for p63, while only scanty peripheral cells of the tumor nests in oncocytoma and oncocytic carcinoma were positive for p63.[42]

ACINIC CELL CARCINOMA

Acinic cell carcinoma is a malignant epithelial neoplasm of salivary glands in which atleast some of the neoplastic cells demonstrate serous acinar cells differentiation, which is characterised by cyoplasmic zymogen secretory granules. Salivary ductal cells are also component of this neoplasm. Ultrastructurally, these cells contain secretary granules similar to those seen in serous acinar cells and intercalated duct cells. Since secretory granules are found nowhere else in the salivary glands, this implies the acinar cell or intercalated duct cell as the cell of origin . Since the intercalated duct reserve cell gives rise to both these cells, it is this cell that is hyothetical source of these neoplasm.[12]

MOLECULAR GENETICS: Multiple structural and numerical abnormalities of these tumours have been reported but no consistent or specific alterations can be defined. Deletion of chromosome 6q, loss of Y, and trisomy 21 have been reported. The most frequently altered regions were noted at chromosome 4p, 5q, 6p, and 17p regions. Chromosomes 4p 15-16, 6p 25-qter and 17p11 showed the highest incidence of alterations. Another study of multiple spatially obtained samples from one tumor showed evidence for polyclonality suggesting different origins for this tumor.[12]

IMMUNOPROFILE: Although the immunoprofile is non-specific, acinic cell carcinomas are reactive for Cytokeratin, trasferrin, lactoferrin, alpha 1-antitrypsin, alpha 1-antichymotrypsin, CEA, Leu M1 antigen, COX2, vasoactive intestinal poly peptide, and amylase.[12,43,44] The zymogen granules in the neoplastic acinar cells are often non reactive with anti

alpha1 amylase immunostain, an enzyme in zymogen granules of normal serous acinar cells. Reactivity for oestrogen receptor, progesterone receptor, and prostate specific antigen has been described in some tumors. Approximately 10% tumors are positive for s-100 protein[45] (table 3). According to Weiler et al the presence of a diffuse distribution of basal cell component, stained by both p63 and CK 5/6 antibodies favors a diagnosis of oncocytoma, since acinic cell carcinoma is completely devoid of basal cells. Table 4 demonstrates the salivary gland neoplasms and their immunoprofile which need to be differentiated from acinic cell carcinoma.

Table 4: Differential diagnosis of acinic cell carcinoma and their immunoprofile

Markers	Mucoepidermoid carcinoma	Myoepithelial carcinoma	Clear cell carcinoma	Acinic cell carcinoma	Oncocytoma	Epithelial myoepthelial carcinoma
p 63	+	+	+	-	+(basal)	**+(outer layer)**
Calponin/SMA/ SMMHC	-	+	-	_	_	**+(outer layer)**
CK7/CAM5.2	-	+/-	+	+	+	**+(inner layer)**
S0X10	-	+	+	+	-	+

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 IHC based diagnosis. An IHC analysis must be performed after appropriate identification of the particular tumor type by HE staining to come to a definitive diagnosis.

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