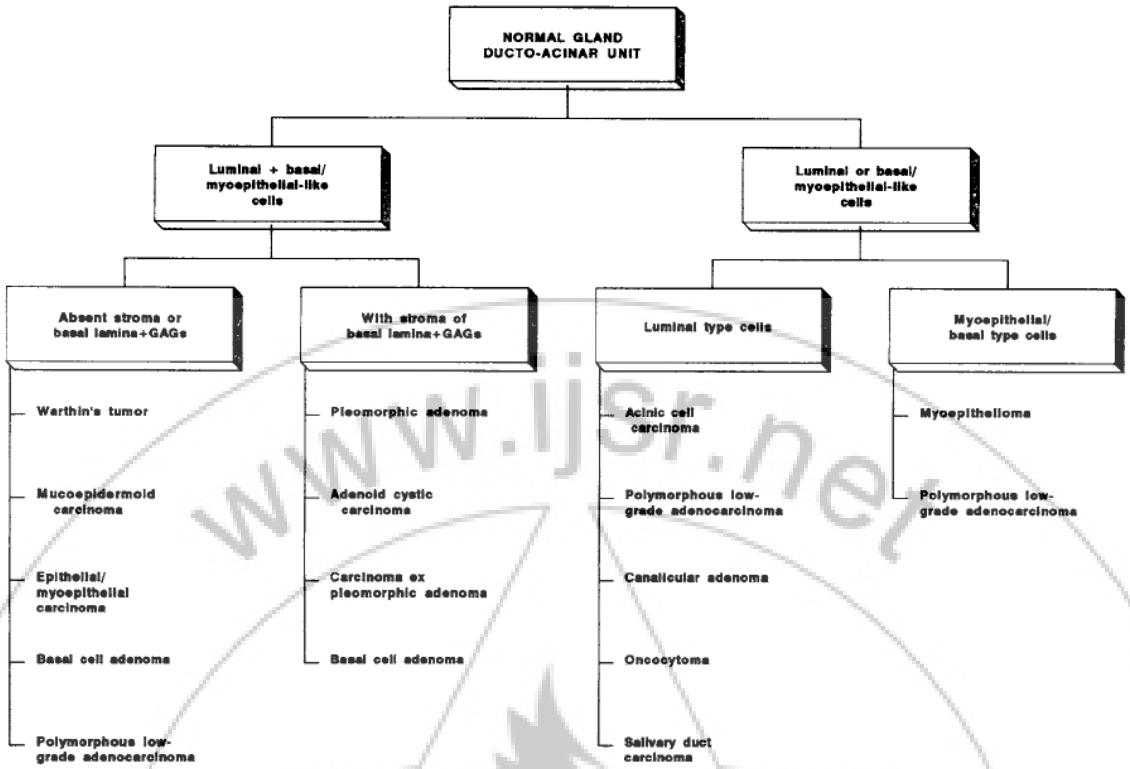

-Neoplasms composed of undifferentiated cells- Undifferentiated carcinoma, small cell carcinoma

Figure 3: Potential interrelationship between salivary gland tumors within the various taxonomic categories

Although the majority of salivary gland tumor subtypes are considered separate entities on the basis of their distinctive histology, many are interrelated. Awareness of this interrelationship helps to explain the overlap in histologies between some subtypes and the differential diagnostic and classification problems that all too frequently arise, as well as the particularly broad spectrum of histology within any individual subtype that itself poses diagnostic problems for the pathologist.

The cell types differentiating in salivary gland tumors, their organizational structure and the effects on tumor morphology of the extracellular matrix and basal lamina synthesized by the myoepithelial/ basal cells assists in appreciating how these factors influence the final histology. The knowledge of cellular differentiation, architectural organization, and synthetic products even within the subtypes of salivary gland tumors allows us to establish more tightly defined classification criteria.

5.1 Genetics in salivary gland neoplasms:

The goal of the molecular biological studies of salivary gland tumours is to define objective markers that may supplant the subjective phenotypic evaluation in the diagnosis, biological assessment and therapeutic stratification of patients with these tumours. The following molecular genetic events tentatively characterize some of these tumours:

1) Chromosomes 3p21, 8q12 and 12q13-15 rearrangements and the PLAG-1 and HMGI-C genes in pleomorphic adenomas
2) Translocations of chromosomes 11q21 and 19p13 in both Warthin tumour and mucoepidermoid carcinoma.
3) Structural and molecular alterations at 6q, 8q, 12q in adenoid cystic and carcinoma ex-pleomorphic adenoma.
4) Elevated HER-2 gene expression and gene amplification in mucoepidermoid, salivary duct and adenocarcinomas.

Oncogenes:

Oncogenes may be defined as genes whose function becomes enhanced in carcinogenesis, which usually play a role in controlling cell proliferation and which commonly encode growth factors and their receptors, transcription factors, signal transducers and apoptosis regulators.The complex mechanisms of tumor induction and progression in salivary gland tumors are likely to be best illustrated by investigating the chromosomal aberrations and oncogene expression.

EGFR

Several studies have shown high expression of EGFR/HER-2/neu family members in mucoepidermoid and adenoid cystic carcinoma. The data suggest a biological role for members of this pathway in these tumours and their potential use as a target for therapy.

C-erbB-2/HER-2/neu

This is an oncogene that encodes for a transmembrane glycoprotein receptor involved in cell growth and differentiation. The gene is a member of the EGFR signal transduction family and has been shown to be overexpressed in aggressive breast cancer. Studies in salivary gland adenocarcinoma, including salivary duct and mucoepidermoid carcinoma, point to a general consensus on the association of HER-2 overexpression and adverse clinicopathologic features.

C-Kit

This is a proto-oncogene that encodes a transmembrane receptor type tyrosine kinase that belongs to the colony-stimulating factor-1 (CSF-1) and plateletderived growth factors (PDGF;4-6). Upon binding to its ligand, a signalling cascade is initiated to stimulate growth and differentiation of haematopoietic cells . Studies of C-kit in salivary gland tumours have largely focused on adenoid cystic carcinoma and findings vary considerably. C-kit expression appears to be restricted to adenoid cystic carcinoma and myoepithelial carcinomas but absent in polymorphous low-grade adenocarcinoma and other types of salivary gland tumours.

None of the highly expressed tumours manifested genetic mutations at exons 11 & 17 which underscore that a mechanism for gene activation and other genetic alterations may play a role. A more recent study of this gene indicates high expression in other types of salivary gland neoplasms as well. (adenoid cystic carcinoma, polymorphous low-grade adenocarcinoma and monomorphic types of adenoma).

PLAG1

The pleomorphic adenoma gene 1 (PLAG1) encodes a zinc finger protein, which recognizes a specific bipartite DNA consensus sequence and acts on a wide range of target genes. Most significantly upregulated by PLAG1 are growth factors such as insulin-like growth factors IGF-II and IGF-IR. The most frequent chromosomal translocation to occur in human salivary gland pleomorphic adenomas (PAs) is t(3;8)(p21;q12). This involves 'promoter swapping', whereby the CTNNB1 promoter from the CTNNB1 gene (which codes for the ubiquitously present β-catenin protein involved in cell-to-cell adhesion and WG/WNT signalling pathway) is used to drive the PLAG1 gene. Similarly, in the t(5;8)(p13;q12) translocation, the leukaemia inhibitory factor receptor takes on the role of promoter.

A number of studies have shown down-regulation of WNT inhibitory factor 1 (WIF1), an inhibitor of the Wnt signalling pathway with an expected up-regulation of β-catenin. The Wnt signalling pathway essentially leads to an increase in free β-catenin and translocation of this to the nucleus, to regulate expression of target genes. There is some evidence to suggest that PLAG1 may bypass the Wnt pathway to directly activate binding sites in the β-catenin promoter region.

***Mect1–Maml2* fusion oncogene**

Tonon *et al* in 2003 first described a novel fusion product from a t(11;19)(q21;p13) chromosomal translocation that disrupted the Notch signalling pathway and could be implicated in salivary gland tumourigenesis. The intracellular domain of the Notch protein regulates gene expression in the nucleus via activation of the transcription factor, CBF1/*suppressor of hairless/Lag-1* (CSL).

The t(11;19)(q14-21;p12-13) chromosomal translocation is characteristic of mucoepidermoid carcinomas (MECs) of the salivary glands, which fuses exon1 from the mucoepidermoid carcinoma translocated 1 (MECT1) gene with exons 2–5 of the Mastermind-like gene family member, MAML2. Studies have consistently shown the association of the MECT1–MAML2 fusion transcript with MECs, but its absence in Warthin's tumour, polymorphous low-grade adenocarcinoma and acinic cell carcinomas makes detection of the fusion gene of diagnostic value.

HMGI-C/HMGA2 fusion oncogenes

Around 12% of PAs display chromosomal aberrations involving the 12q13-15 segment, which was shown to code for HMGIC or HMGA2. HMGIC is a member of the high mobility group (HMG) gene family that codes for non-histone components of chromatin and, therefore, has a role in transcription regulation. A number of fusion partners have been demonstrated to alter expression of HMGIC, most notably FHIT and NFIB. Analysis has shown that certain exons of HMGIC are expressed more than others in tumours with activation of the gene, further stressing that rearrangements and fusions are key to overexpression, which may be implicated in malignant transformation to carcinoma ex PA (CXPA).

ras

RAS is a G protein or GTPase that oscillates between activated (RAS-GTP) and inactivated states (RAS-GDP) in response to a variety of ligands, including epidermal growth factor receptor and interleukin 2 (IL-2). There are three human *ras* genes, *H-Ras*, *N-Ras* and *K-Ras,* with the latter having two splicing variants, *K-Ras4A* and *K-Ras4B*. Inactivation of RAS is accelerated by GTPase-activating proteins (GAPs) and increased release of bound GDP triggered by guanine nucleotide release proteins.
Mutations of *H-Ras* have been shown in 35% of salivary gland PAs, 23% of adenocarcinomas and 45% of MECs. A number of specific mutations have been identified, including a missense mutation at codon 61 of the *H-Ras* gene, identified as bypassing normal growth factor-dependent *ras* signalling, and transversion mutations at codons 12 and 13.

c-fos

The product of the *c-fos* oncogene is a transcription factor up-regulated in response to ligands such as epidermal growth factor, which dimerizes with *c-jun* to act as transcription factor AP-1that binds to the TPA-response element in a variety of genes concerned with growth and cellular differentiation.

It was shown that lower degrees of staining with the *c-fos* oncogene correlated very strongly with poorer cellular differentiation across a broad spectrum of salivary gland tumour types. In the poorly differentiated adenocarcinoma group, for instance, 96.8% of tumour specimens were associated with paucity of staining.

Whereas *c-fos* has been found to be overexpressed in osteosarcomas, its underexpression in poorly differentiated salivary gland tumours relative to normal salivary gland tissue is a reflection of its role in inducing cellular differentiation.

Sox-4

Sry-related HMG box 4 (*Sox4*) is a transcription factor which has been implicated in tumourigenesis, possibly via actions on Wnt pathway signalling or via up-regulation of src tyrosine kinases, such as $p56^{lck}$. The most significantly

overexpressed oncogene in ACCs relative to normal salivary gland was *Sox4*. **A summary of the oncogenes mentioned throughout the text, their role in salivary gland tumourigenesis (Table 1)**

Oncogene	*Salivary gland tumour*
Maml2	MEC, Warthin's tumour
c-kit/CD117	ACC, lymphoepithelioma -like carcinoma, myoepithelial carcinoma
HER2/*neu*	SDC, ACC, MEC, CXPA
H-ras	Pleomorphic adenoma, adenocarcinomas, MEC, CXPA
PLAG1	Pleomorphic adenoma, CXPA
WNT1	Pleomorphic adenoma, CXPA, ACC, MEC epithelial-myoepithelial carcinoma
HMGIC/HMGA2	Pleomorphic adenoma
Mdm2	Pleomorphic adenoma, myoepithelial carcinoma, ACC, CXPA
c-fos	Underexpression correlates with poorer differentiation in a wide variety of tumour types
Sox4	ACC

Tumour suppressor genes

TP53

TP53 is a tumour suppressor gene located at the short arm of chromosome 17. The protein product acts as a transcription factor for cell differentiation, proliferation and death. The role of this gene in salivary gland tumorigenesis remains unknown. Studies of different tumours have yielded variable results . The incidence of p53 expression in other benign, malignant and hybrid tumours is low and does not correlate with recurrence. At present there is insufficient information on the correlation between p53 and outcome[6].

6. Summary

Histogenetic terminology is an integral part of the classification of human tumors. Evidence, however, that in each case a particular tumor type arises from a cell within the tissue specified by the diagnostic terminology is lacking. Much of the evidence shows that all parts of salivary gland parenchyma, whether acinar or duct-and both luminal and basal-cells, can proliferate under a variety of circumstances and, therefore, cannot be excluded as potential cellular sites for neoplastic induction. Hence histogenetic concepts offer little advantage to the diagnostician.

Origin from the many cell forms composing salivary glands does not necessarily imply that the resulting histopathology and cellular differentiation of the tumor will reflect the parent cell, however, or even guarantee a consistent tumor morphology. In fact, the plasticity of the normal salivary gland for cellular alterations at all levels under various experimental and nonneoplastic conditions suggests the contrary. Current histogenetic classification of salivary gland tumors is based on the hypothesis that repair and replacement of terminally differentiated components of salivary gland such as duct epithelium and acinar cells are totally dependent on reserve or stem cells. However, there is mounting direct evidence that cell renewal can result from duct luminal and acinar cells that rapidly re-enter the cycling cell pool. Frequently cycling cells are generally considered the more obvious target for neoplastic transformation. However, in adults the weight of evidence indicates that cell renewal and gland regeneration are functions of each of the various cell types in salivary gland; acinar cells, as they form the bulk of the gland parenchyma, present the greatest proportion of cycling cells in rat and human salivary glands.

Salivary gland tumors, like tumors arising in other tissues, are classed on the basis of the differentiation properties of the tumor cells. For the pathologist, it is this differentiation process and the architectural arrangement of the tumor cells that are the keys to classifying a particular salivary gland tumor. On this basis, it becomes immaterial to attempt to predict from what segment of the duct system a particular tumor originates. This is the reason for stressing investigation of morphological processes as central to developing appropriate and consistent diagnostic criteria for the subtypes of salivary gland tumors.

Perhaps in the salivary gland, oncogenetic events that follow initiation of the multistage process that results in neoplastic transformation are partially governed by the type of cell in which neoplastic transformation has occurred, influencing both the biology of the tumor and the pattern of cellular differentiation within it.

Further research is necessary to obtain reliable information regarding the histogenesis and morphogenesis of salivary gland tumors and thus allow study of the processes that govern the final histological characteristics of the tumor, as well as the exact relationships between the various subtypes of salivary gland tumors. Such research will eventually improve the lot of pathologists burdened with the problems involved in classifying these challenging human neoplasms.

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