Chapter 3
Cellular and Molecular Pathology of Head and Neck Tumors

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Abstract Head and neck pathology encompasses a multitude of organs of diverse histogenesis. Malignances arising from head and neck sites accordingly are diverse in origins, morphogenesis, and biological behavior. Excluding connective tissue and vascular entities, the main entities that are presented in this chapter include squamous mucosal sites, salivary, thyroid and sinonasal, and skull base tumors. The histopathological classification remains the main reference to the diagnosis and to a large extent, malignancy grading. Advances in immunohistochemical techniques and the development of reagents to cellular intermediate filaments and lineage markers have led to better diagnosis and categorization of undifferentiated entities with overlapping morphologic features. More recently, major strides have been achieved in the molecular genetic characterization and understanding of head and neck tumorigenesis. Although clinically applicable and validated molecular biomarkers have yet to be realized, it is important to address the recent discoveries and their potential integration with the phenotypic and pathologic features.

This chapter concisely presents the relevant pathomorphologic and molecular features of the tumors of the major head and neck sites for clinical management.

Keywords Head and neck squamous carcinoma • Molecular genetics • Squamous tumorigenesis • Tumor heterogeneity

Squamous Mucosal Carcinogenesis

Head and neck squamous carcinoma (HNSC) is the fifth most common cancer worldwide with approximately 500,000 new cases per year. They develop from the squamous mucosal lining of the upper respiratory tract mainly in individuals with a history of abusing risk factors, including cigarette smoking, alcohol abuse, and human papillomavirus. Only 20% of individuals with these risk factors, however, develop squamous carcinoma [1, 2].

Head and neck mucosal sites are an ideal model of investigating the molecular genetic alterations leading to squamous carcinoma development because of their readily accessible location, association with known risk factors, and the presence of defined histopathologic progression stages. In contrast to other major cancer types, HNSC lacks familial inheritance, is difficult to cultivate and there are no faithful animal models to advance research and development in this field [1].

Squamous tumorigenesis is thought to result from successive accumulation of molecular genetic alterations in the squamous epithelium lining the upper aerodigestive tract [1, 2]. Although the temporal occurrence and the order of these events are largely unknown, some certainly precede the phenotypic changes associated with preinvasive dysplastic lesions. The progression of late stage dysplasia to invasive carcinoma is a complex one and comprised of both cellular and structural changes as a result of dysregulation of key pathways triggered by interaction of epithelium and the host stromal elements [3] (Fig. 3.1).

Histopathology

The diagnosis and management of head and neck mucosal lesions are based on the histopathologic assessment of biopsied or excised specimens.

Oral Premalignant Lesions

These lesions are recognized as grossly abnormal mucosa of no definitive etiology and can broadly be classified into leukoplakia (white) and erythroplakia (red). The risk of developing

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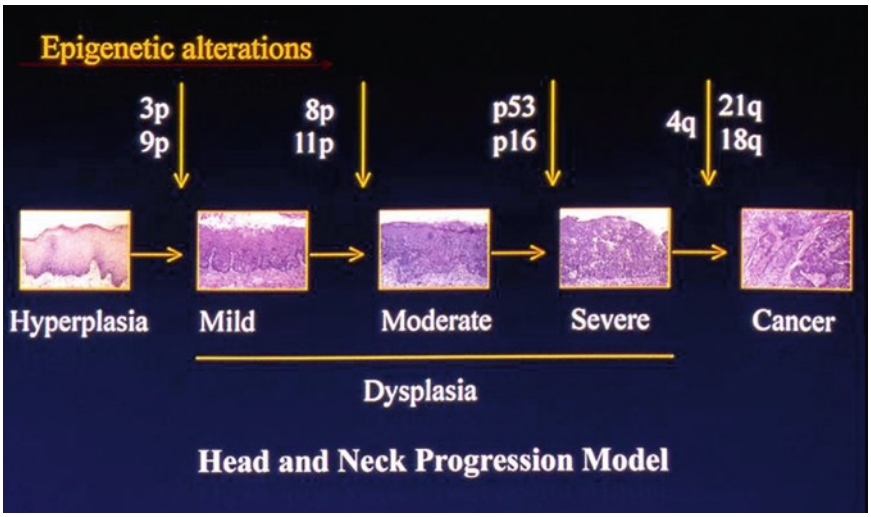

Fig. 3.1 Phenotypic and molecular progression model of head and neck squamous tumorigenesis

invasive carcinoma from these lesions varies greatly and range from 3 to 16% for leukoplakia and from 30 to 50% for erythroplakia [2].

Leukoplakia

Leukoplakia is defined as a persistent white area of unknown etiology. These lesions may present as either discrete homogenous or delimited nonhomogenous forms. Generally, the nonhomogenous lesions are associated with higher risk than their homogenous counterparts. The majority of leukoplakias develop in tobacco consuming individuals and their location and appearance varies according to the geographic location and the manner and nature of the tobacco consumption. A definitive diagnosis is based on the histopathological evaluation of lesional biopsy and serves to rule out mimics, such as Lichen Planus and to assess the presence or absence of dysplasia [2]. Histologically, leukoplakia is characterized by epithelial hyperplasia with hyperkeratosis and/or parakeratosis. The development of dysplasia in these lesions is heralded by progressive alteration of the squamous epithelium manifested by changes in basal cell polarity and cellular and nuclear features and is graded as mild, moderate, or severe based on the extent of the dysplastic cellular features.

Erythroplakia

Erythroplakia is defined as a grossly red squamous mucosa. They present as either homogenous or nonhomogenous red mucosa with and without leukoplakia association. Erythroplakia represents the end stage of dysplasia histologically and carries the highest risk of progression to invasive squamous carcinoma. Both severe dysplasia and microinvasive carcinoma (>3 mm) are generally treated with complete excision without neck dissection. Lesions with more than 5 mm invasion are eligible for neck dissection [2].

Squamous Carcinoma Variants

Squamous carcinoma manifests multiple, distinct phenotypes with variable site predilections and biological behaviors and include verrucous, papillary, basaloid, and sarcomatoid phenotypes [5].

Verrucous Hyperplasia

Verrucous hyperplasia grossly appears as a white, warty raised growth mainly in the oral cavity. Both verrucous hyperplasia and carcinoma share clinically and pathologically similar and overlapping features. Verrucous hyperplasia shows exophytic growth with minimal inward stromal involvement. A diagnosis can only be achieved by an excisional biopsy where the edges and the full depth of the lesion are represented. The histologic diagnosis, therefore, is generally arbitrary and the difference is essentially academic since both lesions should be completely excised [4].

Verrucous Carcinoma

This is a locally invasive squamous carcinoma with warty gross features and minimal cellular abnormalities. These lesions may frequently present in oral and laryngeal sites and in its pure form, has minimal metastatic potential.

Verrucous carcinoma typically affects the oral and laryngeal sites, is locally invasive and in pure form, rarely metastasizes. Histologically, these tumors are well differentiated and invade with broad pushing borders [5].

Conventional Squamous Carcinoma

This is the most common form of presentation and typically graded based on the degree of squamous epithelial alterations and state of keratinization into well, moderately and poorly differentiated carcinoma. The pattern of invasion of these lesions may also impact on the extent of invasion, metastasis, vascular, and perineural permeation. Generally, broad, invasive fronts are less ominous than finger-like invasive fronts [1].

Papillary Squamous Carcinoma

Papillary squamous carcinoma is typically laryngeal or nasal in origin and is exophytic in presentation with minimal tissue invasion. An association with HPV infection has been suggested, but remains uncertain. Papillary squamous carcinoma typically pursues less aggressive behavior than the other forms of squamous carcinoma, except the verrucous variant [5].

Basaloid Squamous Carcinoma

This is a unique high-grade variant of squamous carcinoma with a predilection for hypopharyngeal, tonsillar, and base of tongue sites. They are characterized by uniform, highly malignant basaloid cells with focal squamous differentiation and collagen-like deposition. Recently, an association with high-risk HPV infection has been reported. Morphologically, tumors are characterized by a proliferation of homogenous basaloid cells with necrosis and focal abrupt areas of luteinization. These tumors may be confused with solid adenoid cystic and neuroendocrine carcinomas [5, 6].

Sarcomatoid Squamous Carcinoma

Two forms of sarcomatoid squamous carcinoma are recognized: the exophytic form and the ulcerative invasive forms. The exophytic are usually found in laryngeal and hypopharyngeal sites and may or may not manifest areas of conventional squamous carcinoma. The distinction between this entity and pure sarcoma is based on combined morphologic and immunohistochemical staining for keratin intermediate filaments. Patients with the exophytic form may pursue a relatively better clinical course than the endophytic counterpart [7].

Viral Associated Squamous Carcinoma Subtypes

Oropharyngeal Carcinoma

Increasing evidence links HPV as an etiologic agent in the development of a subset of HNSC. Current data indicate that the majority of these cases are oropharyngeal, including the tonsils. This is further supported by the high risk of oropharyngeal carcinoma in seropositive HPV-16 and high risk of anogenital cancer patients. The exact prevalence of HPV in HNSC is not accurately known with figures ranging from 5 to >70%. These variations are related to several factors, including differences in population, tumor sites, method of HPV detection, and histological subtypes. It is clear, however, that HPV-16 is dominantly present in more than 50% of patients with oropharyngeal SCC. Integration of viral DNA into the nuclear genome is a critical step in the malignant transformation. Subsequent to viral integration, detection of early genes (E2) occurs and upregulation of E6 and E7 genes is noted. The E6 of the HPV-16 bind to the p53 suppressor genes, consequently, and lead to uncontrolled proliferation of the oropharyngeal squamous mucosa. It has also been shown that elevated expression of p16 is a surrogate marker in HPV infection. Approximately 10–60%, dependent on the population and the site of infection of HNSCs, are reported to harbor HPV. Patients with this type of tumor respond better, do not have traditional risk factors, and have better survival. E7 leads to inactivation of Rb protein and the release of the transcription factor E2F and the upregulation of both p14 and p16 proteins. Evidence for viral integration, especially in tonsillar carcinoma, in tumor cells is critical to the diagnosis. Also, the detection of p16 overexpression as an alternative/complimentary to the detection of HPV infection may be helpful. The contribution of viral load to variations in reporting these markers remains to be addressed. In one study, high viral load of <60 copies/cells was found to correlate positively with survival; however, a later subsequent larger study failed to confirm this finding [6, 8–13].

The traditional risk factors associated with conventional squamous carcinoma may play a secondary, but deleterious role in this demographic population. Only certain oncogenic subtypes of the papilloma virus, especially HPV-16 and l8, have been identified as etiologic factors in tumorigenesis of HNSC. The E6 and E7 genes of the HPV-16 genes bind to the p53 and Rb suppressor genes and upregulation of the p16-IK4 inhibitor leading to dysregulation of the cell cycle and tumor development. Interestingly, these tumors are less aggressive and more sensitive to conventional therapy than conventional squamous carcinoma.

Nasopharyngeal Carcinoma

This is a unique form of HNSC that develops in the nasopharyngeal region. They are classified based on their histological appearance into differentiated squamous carcinoma (WHO I) and undifferentiated carcinoma with lymphoid stroma (WHO II or III). The histologic features of type I are similar to well-differentiated squamous carcinoma, while the types II and III are highly undifferentiated carcinoma with integral lymphoid components. Nasopharyngeal carcinoma (NPC) is associated with Epstein–Barr virus infection especially in patients from the Orient and Middle East but less likely in patients from the Western hemisphere. These tumors are highly sensitive to radiation therapy [14].

Adverse Pathologic Features of Clinical Relevance

The following histopathologic factors are considered features associated with high risk of recurrence and failure to therapy response:

1. Poor histologic differentiation
2. Finger-like and single cell invasive pattern
3. Perineural invasion
4. Close surgical margins (<5 mm)
5. Presence of high-grade dysplasia
6. Extra nodal extension of lymph node metastasis [15]

Molecular Pathology

Cellular Concept

The molecular and biological analysis and understanding of squamous tumorigenesis of the head and neck is largely based on the concept of field characterization conceived by Slaughter et al. in 1953 [16]. This concept assumes that risk factors render the entire aerodigestive mucosal surface susceptible to the squamous carcinoma development. In the small subset of patients with no history of risk factors, and/or short temporal exposure to these factors, an inherent genetic susceptibility may play a role [1, 17, 18]. The cellular concept's premise for squamous carcinoma development and progress is that HNSC carcinoma results from molecular and/or biological alterations in the squamous epithelial cells.

DNA-Based Studies

LOH Findings

Microsatellites are short tandem repeat DNA sequences scattered throughout the genome. The vast majority of these repeats are polymorphic, inherited differently from each parent among different populations. Using constitutional DNA extracted from fresh or archived specimens as a standard, loss or shift in mobility in tumor microsatellite bands on gel electrophoresis, determines the presence or lack of microsatellites of alterations. In general, frequent loss of loci on chromosomes 3p, 9p, and 17p has been detected in premalignant squamous lesions and many constitute any early alterations that may be used in screening of high risk individuals for early detection of cancer. Other chromosomal alterations, including 4q, 6p, 8p, 11q, 13q, and 18q are typically more frequent in invasive and advanced squamous carcinomas. Chromosomal gains, in contrast, are infrequent in squamous tumorigenesis and limited to chromosomes 3q26 and 11q13 amplicons and generally are late events [18–22].

Specific Gene Findings

p53 gene: p53 is a tumor suppressor gene on chromosome 17p. It is the most frequently mutated gene in HNSC in approximately 50% of the cases. Tumors from patients with long histories of risk factor exposure are more frequently mutated. Most of the p53 mutations are transversion in type (G:T), but missense mutations can also be found and clustered between exons 5 and 9.

p16 gene: p16 is another tumor suppressor gene on chromosome 9p21. Loss of p16, a potent inhibitor of cell cycle, leads to uncontrolled proliferation. In contrast to p53, mutations of p16 are infrequent events in HNSC. Instead, hypermethylation of the p16 promoter and the first exon is the major mechanism for loss of function [23–25].

FHIT gene (Fragile histidine triad): FHIT, on the short area of chromosome 3p14.2, has also been implicated in HNSC. However, the frequency and the temporal involvement of this gene in squamous tumorigenesis remain undefined [2].

Cyclin-D1 gene: Cyclin-D1, a critical cell cycle gene within chromosome 11p amplicon, has also been found to be highly amplified in advanced premalignant and invasive lesions. Polymorphism at this gene has been associated with high risk of developing squamous carcinoma [26].

p63 gene: P63 is a member of the p53 gene family and located on chromosome 3q29-29 region. P63 is a vital gene in normal epithelial development and has been implicated in

several epithelial tumor developments. P63 has two different promoters resulting in two different protein products, on retaining the transactivation domain (TA p63) and another lacking it (Δ(delta)Np63).

Both isotypes undergo alternative splicing at the carboxy terminal leading to six isoforms (three each) (α(alpha), β(beta), and ν(upsilon)). Studies of this gene and its main isotypes in HNSC indicate an important role in tumorigenesis, especially the ΔN isotypes. Overexpression of this isotype blocks differentiation and metastasis, promotes proliferation in HNS tumorigenesis, and may be an attractive target for therapeutic intervention in a subset of patients with these tumors [27, 28].

Epigenetic Alterations

Epigenetic alteration is the process of gene silencing by non-DNA alterations and includes cytosine methylation of the CpG islands at the promoter and/or chromatin modulation and histone acetylation. These epigenetic modifications are reversible and may be of future therapeutic value. Cytosine methylation of several tumor suppressor genes in HNSC has been the target of numerous studies. Genes that have been found to be highly methylated in HNSC include p16, MGMT, RARB, E-Cadherin, and DAPk [29, 30]. The diagnostic and therapeutic potential of these alterations remain to be achieved.

Genomic Studies

In genomic studies of HNSC using varied platforms, patient populations have recently been conducted. The inherent heterogeneity of these tumors complicates the interpretation and renders a clear conclusion difficult. Although results have shown evidence for segregating different responsive and aggressive behaviors, lymph node metastasis and tumor sites, the complexity of the analysis and the heterogeneity of tumors and biological behaviors limit the clinical utilization of these platforms [31–34].

MicroRNAs

MicroRNAs, highly conserved and ubiquitous short (18–22 nt) noncoding RNA sequences, were found to regulate gene expression posttranscriptionally by base pairing with 3′-UTR (untranslated region) of cognate RNA transcript. Dependent on the extent of base pairing with target RNA, miRNA may lead to translational regression of degradation. Because of the partial complementarity between miRNAs and their targets, each miRNA may regulate several genes. A few recent studies of these molecules have recently been published. Several miRNAs, including miR-375 and miR-221, have been found to be significantly altered in HNSC [35, 36]. Another study of squamous carcinoma of the tongue identified 24 upregulated and 13 downregulated miRNAs. Of the most significantly upregulated, miR-184 was identified. Inhibition of the miR-184 cell lines led to decreased proliferation, downregulation of C-Myc, and induction of apoptosis. Further analysis of these molecules is warranted for their potential therapeutic use [37].

Growth Factors and Signal Transduction Pathways

Understanding the signaling pathways, trafficking and regulation of fundamental, inter- and intracellular tumor/host interactions, will lead to understanding the biology of individual tumors and the development of effective targeted therapy in HNSC. Alterations in several growth factor receptor pathways play a critical role in the development and progression of HNSC. Several growth factors affecting signaling pathways in HNSC have been identified. These include the EGFR, Ras, NFkB, TGFβ, and PI3k/AkT/mammalian target of rapamycin (mTOR) pathways.

Epidermal Growth Factor

The epidermal growth factor (EGFR) gene is located on the short arm of chromosome 17 and encodes for a transmembrane tyrosine-kinase receptor expressed on several epithelial cells. EGFR activation is a critical early event in the development of squamous carcinoma. EGFR is a glycoprotein receptor with a cystin-rich ligand-binding domain with short sequence and intracellular tyrosine kinase and carboxy-terminal scaffolding domains. The activation of EGFR family members is either through ligand dependence or independence.

The independent activation is the result of mutation or overexpression-induced homodimerization or heterodimerization with other Grb family members. Ligand-independent activation of EGFR in HNSC has been linked to a transition mutation, EGFR variant III (EGFRvIII). Ligand binding to the EGFR initiates phosphorylation and triggers a signal transduction cascade that result in the activation of

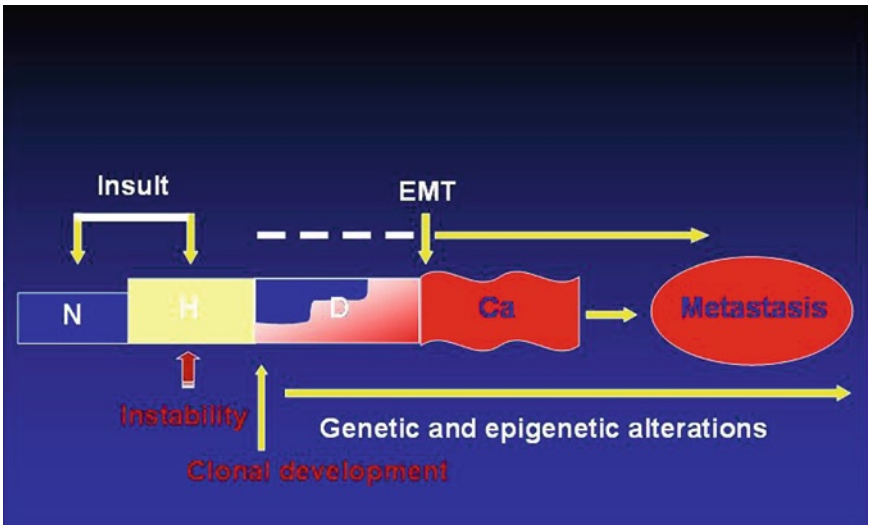

Fig. 3.2 Proposed model of epithelial to mesenchymal transition in squamous tumors

downstream molecules and increase in cell proliferation. Overexpression of EGFR has been amply reported in HNSC to be associated with aggressive behavior, poor progression and response to targeted anti-EGFR therapy [38–40]. Studies of mutations in the hot spot exons of this gene have yielded negative results. However, increased gene copy numbers have been reported in a subset of these tumors. Currently, immunohistochemical staining with anti-EGFR is the most commonly used method of assessment of this gene. It is unknown, however, whether the activated form (phosphorylated) or the total EGFR level correlates better with the activity and response to therapy in HNSC [41]. The interest and available data on EGFR have led to interest in the development of molecularly targeted small-molecule inhibitors in the treatment of HNSC. New anti-EGFR tyrosine-kinase activity has been used in clinical trials as single or multiple agents and modalities with limited success (response rate 10–15%). The binding by ligands (EGF, TGF2, amphiregulin, and heparin binding – EGF) leads to antiphosphorylation of multiple tyrosine residues at the carboxy terminus, where SRC and other proteins interact with transducer mitogenic signals [39].

VEGF and FGF

Elevated expression of VEGF and FGF and their receptors have been reported associated with angiogenesis and aggressive behavior in HNSC. The regulation of this growth factor is primarily through the hypoxia-inducible factor-1α (HIF-1α)-dependent and -independent processes and involves both PI3k and AkT pathways [42–47].

A humanized VEGF monoclonal antibody (Bevacizumab) has recently been tested and shown to inhibit angiogenesis [48, 49].

PI3k/AkT/mTOR Pathway Inhibitors

Activation of these pathways plays an important role in the development and progression of HNSC. Mutation of the PI3k gene leads to cellular transformation of HNSC. Restoration of this pathway may lead to inhibition of PI3k phosphorylation and expression, which is responsible for radio resistance in HNSCC [50]. Also, activation of the AkT pathway may lead to EGFR overexpression and enhance resistance to targeted treatment. The mTOR has been shown to regulate critical cellular processes, including motility, proliferation, survival, and transcription.

mTOR inhibition, however, may lead to negative feedback of the insulin-like growth factoral, which may lead to activation of PF3k and AkT and potentially counteracting the mTOR inhibitor [51]. Multiple agents or single agents targeting multiple pathways may be an ideal strategy.

The complexity of the aberrant signaling in HNSCC underlines the difficulties in treating these patients (Fig. 3.2).

Structural Concept

Mesenchymal Epithelial Transformation

In the last two decades, minimal attention has been paid to the role of epithelial/stromal interactions of invasion, progression, and metastasis in HNSC. Recent investigations in several solid tumor models have shown that invasion and metastasis are associated with alteration in cell to cell and cell to matrix adhesion altered epithelial cell polarity and increased motility. Several studies have shown that this process is initiated in response to extracellular stimuli

and factors. Growth factors and their receptors play a central role in the transduction of key events associated with this process. Among the most important of these are the Ras, SRC, PI3k, and the MAP kinase pathways. The activation of these pathways have been shown to lead to downregulation of adhesion molecules (e.g., E-Cadherin) and elevation of surrogate mesenchymal markers (e.g., Vimentin) [3, 52, 53]. This process is highly relevant to squamous carcinoma invasion and metastasis, where E-Cadherin is a key adhesion molecule in squamous epithelial cells. E-Cadherin not only is important in cell to cell and cell basement adhesion, but also in mediating cell to cell cross-talk through Ca-dependent homotypic interactions [38, 54, 55]. Several growth factors, including TGFB, lead to downregulation of E-Cadherin and other cellular features associated with EMT. However, the manifestation of EMT in HNSC may vary considerably from tumor to tumor and within a given tumor. Not infrequently, minimal EMT changes are observed in well differentiated with broad invasive fronts while complete mesenchymal transformations is found in the sarcomatoid form of these tumors. In addition to the semiquantitative changes in these molecules, qualitative changes may also occur. This is clearly manifested in the phenotypic distribution of E-Cadherin from membranous to cytoplasmic localization.

EMT, therefore, is a dynamic and heterogeneous process that underlies the biology of a squamous carcinoma and that the degree and extent of these changes reflect their aggressive nature.

Biomarker Applications in Head and Neck Tumorigenesis

Early diagnosis in high risk individuals for HNSC is key to improving treatment and prognosis of this disease. Similarly, predicting the biological behavior, response to nonsurgical therapy and toxicity is important in stratifying patients for treatment and targeted therapy. Therefore, the identification of sensitive and reproducible markers is critical to the success of these efforts. The application of tissue-based assay requires that they accurately and reproducibly reflect the underlying pathological and biological processes. These processes are dynamically varied in and between individuals. Quantitation of lesional variabilities and confounding non-neoplastic processes is necessary for accurate interpretation and the exclusion of false positive and negative results. Integrating tissue assessment and biomarker results might ultimately be the best model of risk assessment for head and neck cancer patients [2, 24, 56].

Salivary Gland Tumors

Salivary gland tumors are rare and remarkably heterogeneous neoplasms of an uncertain histogenesis. They constitute only 2–3% of all head and neck neoplasms, with an overall incidence of approximately 2.5–3 per 100,000 persons per year [57, 58]. Major salivary glands are the most commonly afflicted sites, with 80% of tumors occurring in the parotid, 10–15% in the submandibular gland, and 5–10% in the sublingual and minor glands [59]. Most tumors (80%) of parotid gland origin are benign, whereas those arising in submandibular, sublingual, and minor glands are more often malignant. Primary malignant salivary gland neoplasms compose approximately 5–10% of all the head and neck carcinomas and 0.3% of all cancers [57]. Generally, salivary neoplasms present in middle and older age (mean age 56 years), with only 2–3% occurring in children under 10 years of age, and more commonly in males than in females [57, 60].

Salivary Tumors in Children

The majority of salivary neoplasms in children are nonepithelial and mainly of vascular origin. The most common is mucoepidermoid followed by acinic cell carcinomas forming approximately 60% of malignant neoplasms in this category. The most common benign epithelial neoplasm in this age group is pleomorphic adenoma (PA). It is worth noting that a rare congenital tumor known as embryoma or sialoblastoma occur prenatally. Histologically, these tumors represent a neoplastic growth of embryonic, primitive, basaloid epithelial cell of salivary gland. These lesions are considered low-grade malignancy. The differential diagnosis is basal cell adenocarcinoma and adenoid cystic carcinoma (ACC) [61–63].

Fine Needle Aspiration in the Evaluation of Salivary Masses

Fine needle aspiration (FNA) may be used in the initial evaluation of a salivary mass. The main indications of this procedure is to exclude lymphoreticular disorder, inflammatory and granulocytic reactive lesions and metastasis. FNA may not be recommended in the diagnosis of primary salivary gland tumors and cystic lesions. Not uncommonly, FNA may induce neurosis, reactive inflammatory, and reparative manifestations that may obscure the underlying neoplastic conditions. Occasionally, however,

especially in the planning of the extent of the operation, surgeons may utilize this technique to obtain a malignant diagnosis.

Pathologic features of clinical importance:

1. Tumor size
2. Histologic diagnosis
3. Malignancy grade (when applicable)
4. Margin status
5. Perineural involvement

Histopathology

Table 3.1.

Benign Tumors

Pleomorphic Adenomas

PAs are the most common benign salivary tumors that primarily occur in the parotid. Clinically, these tumors pursue a benign clinical course with a tendency for local recurrence due to mainly nodular extension. Rarely, some PAs may metastasize while retaining their benign phenotypic features. Histologically they manifest varied cellular components, comprising epithelial and myoepithelial cells in variable background of myxoid and/or chondroid stroma [57, 64–66].

Karyotypic analyses have identified recurrent and specific cytogenetic abnormalities, with t(3;8) (p21;q12) reported in more than 40%, and a small subset manifesting rearrangements of the 12q14-15 region [67]. The latter include translocation involving 12q14-15 with chromosome 9p12 or different partners and/or inversion of both chromosomes at the same breakpoint. Random clonal abnormalities have also been detected in more than 20% of PAs [68, 69]. Molecular studies using microsatellite repeat markers reported frequent loss of heterozygosity at the long arm of chromosomes 8 and 12p loci [67, 70]. Two specific genetic markers have been consistently identified in PAs; the PLAG1 on chromosome 3p21 is the most frequent upregulated gene, but its biological significance in the development of pleomorphic adenoma remains uncertain [71].

The second recurrent and specific chromosomal alteration involving 12q14-15, leads to overexpression of the high mobility group A2 gene (HMGA2). The gene is an architectural factor that regulates transcription through binding to AT-rich DNA. Microarray analysis of PA and PLAG1-transfected cells have identified most of the unregulated genes to be growth factors, such as IGF, BDGF1, CRABP2, SMARCD1, and EFNB1 [72]. Together these findings indicate that the PLAG1 gene contributes to oncogenesis through the induction of growth factors [73].

Table 3.1 A simplified classification of salivary gland tumors

Myoepithelial/epithelial	Epithelial
Benign	
Myoepithelioma	Oncocytoma
Pleomorphic adenoma	Basal cell adenoma
Malignant (carcinoma)	
Myoepithelial	Mucoepidermoid
Epi-myoepithelial	Salivary duct
Basaloid salivary	Adenoid cystic, solid
Adenoid cystic	Basaloid salivary
Terminal duct	Acinic cell

Warthin's and Oncocytic Tumors

Warthin's tumor (WT) is the second most common benign salivary gland tumor. It arises almost exclusively in intra- or periparotid lymphoid stroma. Histopathologically, the tumor manifests oncotypic epithelial cell proliferation within lymphoid stroma with and without cystic formation. A spectrum of oncocytic tumors ranging from nodular oncocytic hyperplasia, adenoma, and carcinoma have been described and most likely related to Warthin's tumors [74]. Current molecular and cytogenetic studies indicate that the majority of these lesions manifest a normal karyotype [75], while approximately 10% have cytogenetic abnormalities; the most common cytogenetic alteration identified is the t(11;19)(q21-22;p13) [76, 77]. The same translocation and its fusion gene product *CRTC1/MAML2* were also found in mucoepidermoid carcinoma (MEC). The finding of this abnormality in both tumors, along with their reported simultaneous occurrence, indicates a genetic link between these lesions. Collectively, the data support a clonal origin in a subset of these tumors with a propensity to transformation to MEC or oncocytic carcinoma.

Basal Cell Tumors

Both basal cell adenomas and carcinomas are rare and constitute approximately 2–3% of all salivary gland tumors. These tumors may not infrequently pose diagnostic difficulties due to their cytomorphologic similarities. They are typically formed of bland basal cell proliferation in nests and/or cords formation with intercellular eosinophilic homogenous material deposition [78]. Because of the infrequency of these tumors, only small numbers have been genetically analyzed; a common cytogenetic alteration in few tumors was a trisomy

8, but other sporadic cytogenetic alterations, including t(7;13) translocation, have also been reported [79]. CGH analyses of examples of these tumors showed loss of chromosomes 2, 6, and 7, gains of chromosomes 1 and 8, and amplification of 12q region. Molecular analysis of these tumors has reported frequent loss of heterozygosity at chromosome 16q12-13, a region that houses the cylindromatosis gene (CYLD) [79].

Canalicular Adenoma

Canalicular adenoma is characterized by columnar epithelial cells forming anastomosing bilayered cellular formations including nests and is trabecular in a vascular stroma. The lesions are typically well circumscribed and encapsulated [57, 65]. Differential diagnosis of canalicular adenoma from basal cell adenoma and ACC may occasionally be difficult, especially on biopsy specimens. Because of their rarity and benign nature, molecular studies of this entity are very rare.

Myoepithelial Tumor

Myoepithelial tumors are formed almost exclusively of myoepithelial cells, which are rare and are less than 1% of all salivary gland neoplasms. Some tumors may show focal areas of pleomorphic adenoma. They may manifest a variety of phenotypic forms, including plasmacytoid, spindle, clear, and/or epithelial features. Current molecular genetic data on these lesions are sparse and preclude any definitive findings that contribute to either their development or biology. Cytogenetic analyses of a few examples have reported nonspecific chromosomal abnormalities and were insufficient for comment on their contribution to these tumors [80, 81]. Upregulation of the WT1 mRNA has been detected in some benign and malignant myoepithelial tumors, but the oncogenic role of this event in their development is unknown [82].

Malignant Tumors

Mucoepidermoid Carcinoma

MECs compose approximately 30% of malignant salivary neoplasms and are most common in children and adolescents. MEC manifests three distinctive phenotypic grades based on the cellularity and architectural features of the tumors. Of all salivary neoplasms, MEC is the only entity in which both cytogenetic and molecular analyses have led to the identification of consistent unique alteration that may constitute an initiating event in the development of a subset of these tumors. Several cytogenetic analyses of MEC have shown translocation t(11;19) (q21;p13) either alone or with other nonspecific alterations [75, 83–85].

Cloning of this translocation has identified a fusion oncogene composed of exon 1 of the *MECT1* (*CRTC1/WAMTP*) gene (Fig. 3.3) on chromosome 19p13 and exons 2–5 of the MAML2 gene on chromosome 11q21 regions [86]. MAML2, a member of the mastermind gene family, encodes a nuclear protein that binds to the CSL transcriptional factor and the intracellular domain of the Notch receptor to activate the Notch target gene. The fusion partner is the *CRTC1 (MECT1)*, a member of the highly conserved CREβ/cAMP coactivator gene family [87, 88]. Studies of this fusion transcript in a series of MEC have reported a correlation between fusion-positive tumors and low tumor grade and better behavior. Fusion-negative MEC may evolve from a different evolutionary pathway and may represent a biologically distinctive category. The results also suggest that tumors lacking the fusion transcript behave more aggressively. The finding of the fusion transcript in both sporadic Warthin's tumor and MEC and concomitant tumors supports an early or etiologic role in the development of a subset of these tumors. Epithelial ductal cells in heterotypic salivary tissue in intra- or parapharotid lymphoid stroma acquiring the t(11;19) fusion gene give rise to Warthin's tumor, while the same alteration in the salivary tissue gives rise to MEC in sporadic presentations.

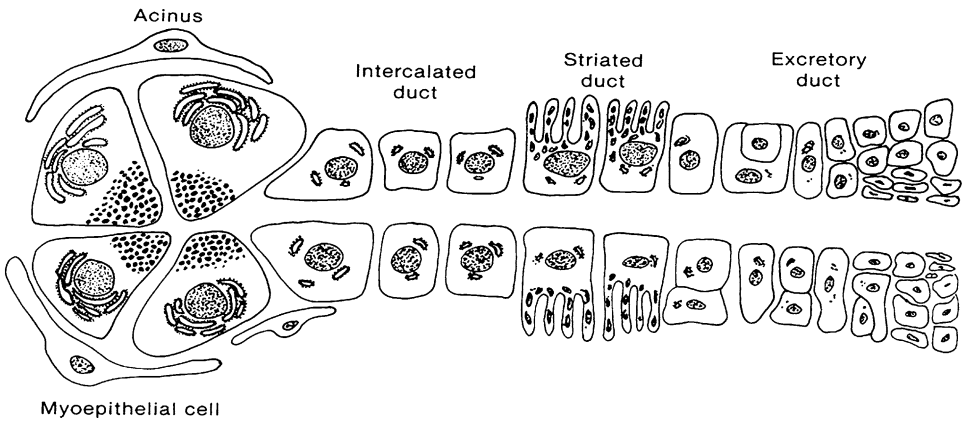

Fig. 3.3 Ductal structure and proposed origin of salivary gland tumors

The development of MEC in a Warthin's tumor may therefore result from metaplastic changes in ductal cells with the fusion transcript [89–92].

Salivary Duct Carcinoma and Adenocarcinoma Ex-Pleomorphic Adenoma

Salivary duct carcinoma (SDC) and adenocarcinomas present either de novo or in the setting of pleomorphic adenoma and manifest remarkable similarity to mammary duct carcinoma [93, 94]. Cytogenetic studies of some of these tumors have shown rearrangements of chromosome 8q12, alteration of chromosome 12q13-15 region, and amplification of both the HMG1C and MDM2 genes may be potentially associated with these tumors. Other studies have shown that translocations of chromosome 5(q22-23, q32-33) and t(10;12)(p15;q14-15) resulted in transportations of the entire HMG1C gene to chromosome 10 marker [57, 95–97].

Using microsatellite markers on microdissected benign and matching malignant components of salivary gland carcinoma ex-pleomorphic adenoma (Ca ExPA), have shown alterations at 8q and/or 12q in both components and restricted alterations at chromosome 17p loci in the malignant component [95, 96]. These findings suggest that alterations at 8q and 12q regions represent early events, whereas alteration at 17p is associated or coincident with the malignant transformation. Studies of specific genes and loci have also reported homozygous deletion of the p16 gene on chromosome 9p21 [98, 99], and p53 alterations and loss of heterozygosity at different loci on chromosome [73]. A subset of SDC, as in mammary ductal carcinoma express hormonal and growth factor overexpression that may be used in their biological and therapeutic stratification [98]. Overexpression of HER-2, EGFR, and androgen receptors are found in more than one-third of these tumors [100, 101].

Adenoid Cystic Carcinoma

ACC is the second most common malignant salivary gland tumor and the most clinically relentless malignancy. ACC is known for its indolent and persistent clinical behavior and propensity for perineural invasion. ACC manifests three phenotypic subtypes, which they nearly always present in the majority of tumors but with variable proportions [102]. These include tubular, cribriform, and the solid morphologic variants. In both the tubular and the cribriform phenotypes, the tumor units consist of myoepithelial and ductal epithelial cells. Cytogenetic studies of these tumors have reported frequent alterations at chromosomes 6p, 9p, and 17p, with the most consistent alteration at the 6q regions (Table 3.2) [99, 103].

Table 3.2 Adenoid cystic carcinoma (ACC)

Acinic cell carcinoma	Pleomorphic adenoma	Warthin's	Mucoepidermoid carcinoma
	Adenoid cystic carcinoma	Oncocytoma	Adenocarcinoma
	Monomorphic adenomas		
	Epithelial myoepithelial carcinomas		

Studies of ACC found a high frequency of loss of heterozygosity at 6q23-25, and this correlated with histologic grade and clinical behavior. Studies using microsatellite markers have also reported frequent loss at chromosomes 12q, 6q23-qter, 13q21-33, and 19q regions. These regions house two genes, PLAGL1 and LATS, that were not mutated in any of these tumors. A recent comparative genomic hybridization of ACCs identified a novel gain at chromosome 22q13 region in 30% of the tumors in addition to the loss of chromosome 6q and gains of chromosomes 16p and 17q regions [104–106]. Microarray analysis of a few examples of these tumors have shown amplification of MDM2, HMG1C, MYC, and other genes located on chromosomes 8q and 12q14 [107–109]. A frequent finding in these tumors is the overexpression of the C-Kit protein. C-Kit (CD117) is a transmembrane tyrosine kinase receptor encoded by the C-Kit gene on chromosome 4. The C-Kit ligand, a stem cell factor (also known as steel factor and mast cell growth factor) induces signal transduction pathways affecting development, cell growth, and migration of different cell functions [110–112]. The role and the cellular distribution of this gene product in the biology and as a target in these tumors remain to be determined.

Acinic Cell Carcinomas

Acinic cell carcinoma is a distinctive salivary malignancy that develops almost exclusively in the parotid gland. These tumors arise from acinar cells and manifest granular serous cellular features with variable and overlapping morphologic subtypes [113]. They are generally low-grade indolent carcinomas, occasionally presenting as high-grade carcinomas with high mitotic figures, necrosis, and lymph node metastasis [114]. In addition, several examples of transformation into dedifferentiation or anaplastic carcinomas have been reported. Cytogenetic and molecular studies of these tumors are few and inconclusive. One study cites evidence for a frequent loss of heterozygosity at limited chromosomal regions [115], including 4p15-16, 6p25-qter, and 17p11, suggesting that these regions may contain critical genes related to their development. In another study of multiple samples of an ACC, variable clonal alterations were obtained, suggesting

multiclonal origin [116]. Studies of dedifferentiated acinic cell carcinoma have shown an association of such transformation with Cyclin D_1 upregulation. The lack of confirmatory and validation follow-up studies precludes any speculation on the role of these findings in this entity.

Polymorphous Salivary Adenocarcinoma (Terminal Duct Carcinoma)

This entity is characterized by intratumoral growth pattern variabilities and uniformed monotonous cellular composition. The hard palate is the most frequent site but they may rarely occur in major salivary glands. The tumor constitutes 19.6% of malignant minor gland tumors. Because of the lack of encapsulations, these tumors typically infiltrate adjacent tissue and are prone to perineural invasions. The recurrence rate for these tumors is approximately 17% and regional metastasis occurs in approximately 9% [117].

Epi-Myoepithelial Carcinoma

This rare entity represents a malignancy of low grade and indolent course that is composed of dual myoepithelial and ductal tumor cells. Histopathologically, the tumor forms duct and tubular formations of relatively prominent clear myoepithelial cells and inner cuboidal and uniform duct cells.

Rare Salivary Gland Neoplasms and Subjects

Squamous Carcinoma

Rarely squamous carcinoma may arise de novo in major salivary glands and if presented not underlined. The exclusion of metastasis from other sites must be proved. Rare carcinomas reported to be of primary origin include small cell and lymphoepithelial carcinoma.

Nonepithelial Neoplasms

Nonepithelial neoplasms form less than 5% of all salivary gland tumors. They represent lesions arising from salivary gland supporting connective tissue. The most common lesions are angioma, lipoma, neurofibroma, and hemangiopericytoma. The growth and microscopic features of these lesions are identical to those encountered in other sites.

Primary Lymphoma

Lymphomas are very rare and mainly found in the parotid gland. The majority of primary lymphomas are of the MALT type. They may arise in either intraparotid lymph nodes or the parenchyma. The vast majority are of the follicular B-cell derivation with rare instances of T-cell origin.

Metastasis to Salivary Glands

The most common metastasis to major salivary glands, especially the parotid, is squamous carcinoma followed by melanoma of the skin. This is largely due to the lymphatic drainage of skin of the face. Hematogenous spread to the parotid originates primarily from kidney, breast, and lung carcinomas. Metastasis to the submandibular gland is very rare due to the lack of intraglandular lymph nodes. Epithelial neoplasms are rarer and disproportionately malignant [57].

Genomic and Proteomics of Salivary Gland Tumors

Proteomic analysis of solid tumors remains limited and difficult to execute. There is only a single study of ACC xenografts by fluorescent two-dimensional gel, electrophoresis, and matrix-assisted laser-desorption/ionization techniques. This study identified four upregulated and five downregulated proteins. Of these proteins, maspin and stathmin were confirmed to be highly expressed in human ACC. Similar attempts have been made in some salivary gland tumors. The results, however, should be considered preliminary or suspect until verified [72, 93, 118, 119].

Thyroid and Parathyroid Tumors

Thyroid

Thyroid nodules are one of the most common clinical conditions. The vast majority of these are reactive lesions or benign tumors and only 10% are malignant. Approximately 14,000 new cases of thyroid carcinomas are diagnosed per year in the USA [120]. The histologic subtypes of thyroid malignancies include papillary, follicular, poorly differentiated, anaplastic, and medullary carcinomas. Broadly, these tumors can be categorized into differentiated (papillary, follicular, and medullary) and undifferentiated (poorly differentiated and anaplastic) carcinomas [121, 122]. The papillary, follicular, poorly differentiated (insular), and the majority of anaplastic carcinomas arise from the follicular epithelial cells while the MTC is derived from parafollicular calcitonin-producing c-cells [123–126].

Etiology

The etiology of thyroid malignancies is largely unknown, although exposure to radiation during childhood (papillary) and iodine deficiency (follicular) have been linked to the development of certain carcinoma subtypes. Papillary thyroid carcinoma may affect any age, but especially children, young adults, and females. Carcinomas typically present as an enlarged mass with or without ipsilateral nodal involvement [127–130].

Initial radioscintigraphy is helpful in distinguishing between hot (benign) and cold (malignant) nodules [131].

Pathology

Cytology

Fine needle aspiration (FNA) is the first line of diagnostic techniques for thyroid tumor diagnosis. In general, an accurate diagnosis of papillary and medullary thyroid carcinoma can be readily made on FNA. The sensitivity and the specificity of FNA in diagnosing follicular lesions, including follicular variant of papillary carcinoma, however, is low. It is estimated that up to 30% of FNA-based diagnosis of follicular neoplasms are indeterminate [132, 133].

Histology

Thyroid neoplasms are generally classified based on their histogenesis from epithelial (follicular cell) and neuroectodermal (C-cell) neoplasms. Epithelial neoplasms are broadly benign follicular adenomas and differentiated neoplasms and poorly differentiated and anaplastic carcinomas.

Follicular Adenoma

Adenomas are characterized by a well-circumscribe nodular growth with thin encapsulation. They may present as solitary or multiple nodules at any age and gender. Microscopically, they may manifest microfollicular, trabecular, and macrofollicular forms. The main differential diagnoses for adenomas are follicular hyperplasia (Goiter) and follicular carcinoma. Oncocytic changes due to the high content of mitochondria are most likely secondary to respiratory cellular demands. The biological behavior of these neoplasms is similar to those of corresponding follicular tumors [134–136].

Differentiated Carcinomas

- *Follicular type*
  Follicular carcinomas comprise approximately 5–10% of all thyroid malignancies. They generally afflict females in their middle age than males. A high incidence of these tumors is reported in iodine deficient regions, suggesting a role for continuous TSH stimulation in the genesis of this entity. The diagnosis of this entity is based on the findings of a thick fibrous capsule and the presence of capsular and/or vascular penetration [134]. These tumors can be further classified as minimally invasive or encapsulated, if invasion did not extend beyond the capsule.

  Follicular carcinoma is typically solitary and may present or be preceded by metastasis typical to bone, lung, and brain [125, 137–139].

  Patients present with a single palpable cold mass with a high propensity for radioactive iodine uptake [140, 141].
- *Papillary type*
  Papillary carcinoma is the most common of all thyroid carcinomas, accounting for more than 70% of these tumors. They may present at any age with peak incidence between 30 and 40 years of age. Females are far more affected than males, and young patients typically have a better and long protracted course than older patients, especially men. There is strong circumstantial evidence linking Hashimoto's thyroiditis to increased incidence of papillary thyroid carcinoma [142–144].

  Papillary thyroid carcinomas are multifocal in more than 75% of the cases and total thryoidectomy is generally the treatment of choice. Papillary thyroid carcinoma may present as a thyroid mass (80%) or as a lymph node metastasis (20%). The hallmark of papillary carcinoma is finding papillary structures lined by cuboidal or columnar cells with clear and/or cleaved nuclei. The nuclear features are especially helpful in the diagnosis of the follicular variant of this entity. Not uncommonly present (40%) is the concentric calcification associated with this tumor (psammoma bodies). Several histopathologic variants of this entity have been described with some being associated with a more aggressive clinical course. However, the lack of prospective studies with long-term follow-up render the significance of these subtypes tenuous. The clinical aggressiveness of papillary thyroid carcinoma varies depending on the gender, age, and size of the tumor with older males having a more aggressive course as well as patients with large invasive tumors [120, 126].
- *Undifferentiated carcinomas*

  (a) *Poorly differentiated*
  This histologic variant represents a tumor that lacks follicular or papillary differentiation and the cellular anaplasia of anaplastic carcinoma. Tumors typically manifest cell nests or cords with monotonous cellular features. The differential diagnosis is mostly with medullary thyroid carcinoma. Tumor cells react positively to antithyroglobulin antibodies and they are negative for calcitonin. Their behavior is considered more aggressive than the fully differentiated tumors [122, 145].

(b) *Anaplastic*
Anaplastic thyroid carcinoma (ATC) is the most clinically aggressive neoplasm and accounts for 4–10% of all thyroid malignancies. This entity afflicts elderly individuals and is more common in females than males (3:1) [146, 147].

Clinically, patients present with rapidly progressive local disease. The majority of these tumors arise from preexisting differentiated thyroid carcinoma, most commonly the papillary phenotype. In resected specimens of these tumors, evidence for a differentiated carcinoma can be found. The etiology of ATC is unknown, but previous radiation of thyroid lesions has been linked to the development of these tumors. Histopathologically, these tumors manifest highly malignant tumor cell composition with heterogeneous features and tumor necrosis. The most common pathologic phenotypes are sarcomatous, giant cell, and squamous variants. The differential diagnoses of these tumors include sarcomatoid carcinoma of the upper aerodigestive tract, sarcoma, and melanoma [121, 148–150]. Immunostaining assists in excluding sarcoma and melanoma. The prognosis of these patients is very poor.

- *Medullary carcinoma*
Medullary thyroid carcinoma arises from the C-cell, a neuroectodermally derived cell, and accounts for 3–10% of thyroid cancer. The tumors present in two forms: sporadic, the most common, which accounts for 70–80% and the familial form, which represents the remaining 20–30%. The tumors affect both genders equally and patients in middle age.

The familial and the sporadic forms have mutation in the RET gene, the frequency and the type of these mutations vary. Tumors in the sporadic form present with a solitary mass with/or without neck enlargement and paraneoplastic syndrome. Tumors in the familial form are generally multifocal and affect the younger age and children [151, 152].

The most common location of these tumors is the lateral aspect of the upper 2/3 of the thyroid lobes, where a high aggregation of C-cells can be found. Histopathologically, tumors consist of nests and cords and organized structures composed of small to medium-sized cells with uniform nuclei. Tumor clusters are encircled by delicate vessels and fibrous tissue. Not uncommonly, deposition of dense homogenous eosinophotic materials representing amyloid deposition is noted. The amyloid nature of these materials can be verified by either congo red staining or by light microscopic birefringence [52].

Immunostaining for calcitonin and other neuroendocrine markers may be used for confirmation. The most common sites of metastasis for MTC are regional lymph nodes, lung, liver, and bone. The prognosis of MTC depends on several factors, including age, gender, size, and stage. Generally, the young and females have better outcomes. Patients with MEN-2B have a worse outcome.

The differential diagnosis of these tumors include metastasis from neuroendocrine carcinoma, renal cell carcinoma, and microfollicular thyroid neoplasm.

- *Sclerosing mucoepidermoid carcinoma*
This is a rare malignancy of the thyroid gland, typically in association with Hashimoto's thyroiditis. It is characterized by infiltrating sclerotic stroma with infiltrating nests of squamoid cells with occasional mucinous cells. The stroma is characteristically infiltrated by numerous eosinophilis.

Molecular Analysis of Thyroid Neoplasms

Genetics

RAS gene mutations were frequently found not only in thyroid carcinomas but also in adenoma [153]. Point mutations in RAS have been linked to early thyroid tumorigenesis. Whether adenomas with RAS gene mutations represent a biologically malignant lesion remains unknown [154–157]. Rearrangements of the PPARγ/RAX8 translocation have also been reported in follicular carcinoma and adenomas suggesting that it may constitute an early event in their development [158–162].

Several studies have also shown mutation in the RAS gene RET/PTC rearrangements on chromosome 10 and BRAF oncogene mutations in thyroid carcinoma. The frequency and the biological significance of these events are the subject of debate and remain to be determined. The most frequent of these genetic alterations is the BRAF point mutation in Exon 15 at codon 600 [129, 163–166]. This mutation has been reported in up to 70% of PTC cases.

RET mutated MTC are characterized by early onset and metastasis to lymph nodes and distant organs [152, 167]. The RET proto-oncogene encodes a receptor tyrosine kinase (RTR) that is widely expressed in neuroendocrine cells. RET point mutation in the intracellular kinase domain or extracellular occur in medullary thyroid carcinoma [171, 172]. RET gene rearrangements; however, are associated with papillary thyroid carcinoma.

The common underlying denominator in tumor growth is the constitutive activation of the RET kinase [143, 168–170]. The molecular mechanisms that result in RET activation and the pathophysiology vary widely [87].

PTC with RET gene arrangements are heterogeneous and generally indolent and rarely present with metastasis. In these tumors, chromosomal rearrangements involving the RET gene fuse the 5′ end and a promoter of a gene upstream of the RET kinase domain leading to the expression of a chimeric product, a RET/PTC. RET/PTCs are localized to the

cytoplasm since they lack the NH2 terminal sequence and the transmembrane domain of the RET gene. All NH2-terminal fusion partners identified to date contain homodimerization domains that mediate dimerization and activation of the kinase region in RET/PTC oncoproteins [168, 169, 172–174]. Recent studies have also established the anaplastic phenotypic transformation from differentiated thyroid carcinoma through the analysis of RAS, BRAF genes [146, 147, 156, 175–177]. Galectin-3 is an antiapoptic molecule of the B-galactoside binding lectin family. Alteration in the expression of galactin-3 has been proposed as a diagnostic marker of thyroid malignancy [131, 133, 178, 179].

Genomics

Gene expression analysis of several thyroid neoplasms have been performed. Upregulation of MET, SGRPINA, FNI, CD44, and DPP4 and downregulation of TFF3 gene have been reported in some of these studies [160, 178, 180–183]. Genomic analysis although allowed for the identification of thyroid neoplasm and the biological categorization within carcinomas, the utilization of these assays in the clinical diagnosis is limited and impractical.

Parathyroid Lesions

Parathyroid glands are derived from the third and fourth pharyngeal pouches and are recognized by the fifth to the 6 weeks of gestation. The majority of humans have two pairs of parathyroid glands. Multiples up to 10 (13%), and as few as one, have been reported in humans.

Normal glands are encapsulated, small, soft, and tan to red-brown in color. Parathyroid cells are organized in lobules with fat cells and vascular stroma. The degree of fat in normal parathyroid varies but in general is approximately 60%. Although, literally a non-neoplastic process, evidence of clonality and evolution to adenoma and carcinoma based on clonality analysis has been documented [184–187].

Parathyroid Hyperplasia

Parathyroid hyperplasia is pathologically characterized by increased parathyroid cells with reduction of fat cells in parathyroid lobules. This may occur in all four glands with a variable degree. Generally, this may signify a systemic etiology such as calcium deficiency, vitamin D alterations, or kidney diseases. Hyperplasia of the parathyroid can also be a manifestation of MEN type I syndrome. Histopathologically, they manifest diffuse or nodular cellular proliferation. The cellular feature varies and may include clear and oncocytic cytoplasm [188].

Parathyroid Adenoma

Parathyroid adenoma is a benign parathyroid gland neoplasm and is the most common cause of hyperparathyroidism accounting for more than 80% of cases. Parathyroid adenoma affects more females than males in the middle age. These lesions are considered clonal in origin and present as a single well-circumscribed nodule with a peripheral rim of parathyroid tissue [127, 189]. Adenoma is typically homogenous and contains no adipose tissue cells. Although, they may arise in any gland, they are more frequently reported in the lower glands [120].

Locally Infiltrative Parathyroid Neoplasm (Atypical Parathyroid Adenoma)

Occasionally, parathyroid neoplasms with cytomorphologic features identical to those of hyperplasia or adenoma and infiltrative growth into surrounding soft tissue with intersecting fibrous bands may be encountered. The lack of high and abnormal mitotic figures, necrosis and marked cellular pleomorphism preclude a definitive malignant diagnosis. These lesions are typically prone to local recurrence because of the difficulties to completely excise them. These lesions may also be called atypical parathyroid adenoma.

Parathyroid Carcinoma

Parathyroid carcinoma is a rare, highly malignant neoplasm accounting for less than 5% of patients with hyperparathyroidism. This entity may be hormonally active or inactive [190]. The inactive carcinoma has reportedly been more aggressive. These tumors present as a solid mass that are difficult to excise due to its infiltrative nature. Histopathologically, these tumors are characterized by a proliferation of markedly pleomorphic cells, high and abnormally mitotic figures, broad intersecting fibrous bands, vascular and soft tissue invasion and necrosis. This is a surgically treated disease but more than a third of these patients experience metastasis.

Molecular Analysis of Parathyroid Lesions

Alterations in overexpression of Cyclin D and chromosome 11q13 regions have been shown to characterize parathyroid nodular hyperplasia and adenoma. Other clonal and molecular findings support a clonal basis for the development of at least a subset of these lesions. The Cyclin D and retinoblastoma glue have frequently been found in parathyroid carcinoma alterations [120, 191–193]. Mutation at the MEN1 gene on chromosome 11q13 region has been reported in up

to 50%. Genome-wide studies have also shown loss of 11q region in addition to other chromosomes [194–196].

Molecular alterations of parathyroid carcinoma are rare and inconclusive, but alterations of the retinoblastoma and the MEN1 genes have been reported. Proteins have reported to be limited to these tumors. Loss of heterozygosity and mutation of the HRPT2 gene, which encodes for the parafibromin has also been documented in parathyroid carcinoma and are believed to be restricted to malignancy. If validated, may have a diagnostic and therapeutic implication [197–199]. Somatic mutations as well as germline mutations of the HPRT2 have been implicated to underlie primary hyperparathyroidism [200].

Sinonasal and Skull Base Tumors

A wide spectrum of malignant neoplasms arises from the sinonasal and skull base regions. The majority of these tumors are poorly or undifferentiated malignancies and manifests overlapping features resulting in diagnostic challenges [201, 202]. Excluding tumor-like lesions like hamartomas and teratomas, the most commonly encountered benign neoplasms are Schneiderian papillomas.

Schneiderian Papillomas

Schneiderian papillomas account for 0.4–5.0% of all sinonasal tumors and are classified based on their growth and histological features into exophytic, inverted and cylindrical subtypes. The exophytic form arises predominantly in the nasal septum, but they may also occur in the nasal cavity and the maxillary sinus. They are usually solitary and rarely associated with malignant transformations. Histologically, they manifest a fibrovascular core lined by hyperplastic nonkeratinizing squamous and/or transitional epithelium. The main differential diagnosis is papillary squamous carcinoma. The latter exhibits cellular features of malignancy and stromal invasion. These lesions are prone to recurrence in up to 22–40% of the cases. Inverted papillomas comprise approximately 45% of all papillomas and are characterized by inward growth due to invagination of the epithelial components into the stroma. They commonly arise in the nasal cavity and paranasal sinuses and rarely in the septum. These lesions are also known for high recurrence rate and progression into carcinoma [203]. The epithelial lining of the inverted papilloma is commonly nonkeratinizing, stratified, squamous epithelium with vacuolation, intraepithelial microcysts, and acute inflammatory cells. Malignant transformation may present as differentiated or poorly differentiated squamous carcinoma with and without evidence of dysplasia. The presence of keratinization is always associated with carcinoma. The differential diagnosis of inverted papilloma includes other forms of Schneiderian papilloma. Recurrence rate is approximately 45–75%. Molecular studies of these lesions are rare. However, evidence for monoclonity has been reported, but no specific genetic alterations were linked to progression [203].

Salivary Type Neoplasms

Salivary tumors arising at these locations derived from minor glands and manifest identical morphologic features to those arising in major and minor salivary glands. The difference is their uncapsulated nature and the associated difficulties in assessing margin status. The most common benign tumor is pleomorphic adenoma and the most common malignancies are adenoid cystic, mucoepidermoid and acinic cell carcinomas in descending order and adenocarcinoma, not otherwise classified. The differential diagnosis is mainly from metastasis and nonsalivary seromucinous carcinoma [202].

Nonsalivary Type Adenocarcinoma

These adenocarcinomas are classified into seromucinous type and intestinal type. The seromucinous type most likely arise from the seromucinous glands lining, the respiratory epithelium of the nasal cavity. They are typically well-differentiated adenocarcinoma. The intestinal type is similar to adenocarcinoma of the colorectal sites. These tumors arise from the respiratory epithelium most likely due to intestinal metaplasia as a result of exposure to wood dust or leather chemical processing. These tumors affect middle and elderly individuals with the aforementioned risk factors. The tumors manifest identical phenotypic features to their intestinal counterparts, including mucinous production and signet-ring formation. The biological behavior of these tumors is generally aggressive with the majority of patients succumbing to their disease within 3 years. Molecular and phenotypic studies of this entity have shown evidence for shared molecular alterations with colonic adenocarcinoma [204–208].

Squamous Carcinoma

Carcinomas of the sinonasal cavity comprise approximately 3% of all malignant tumors. The majority (70%) are squamous in derivation. The vast majority occurs in the maxillary sinus

and a small subset occurs in other nasal sites. Several etiologic factors have been linked to the development of these tumors, among which nickel and thorotrast exposure were the most commonly incriminated. These tumors typically affect men in their 50s to 60s. Histopathologically, they may present as keratinizing squamous carcinoma or nonkeratinizing [201, 209].

Other forms of squamous carcinomas as verrucous and spindle cell and basaloid squamous carcinomas have been described. The differential diagnoses of these tumors include metastasis, ameloblastomas, and inverted papilloma. The biological behaviors of this entity depend on the site and degree of differentiation with the nasal carcinoma patients fairing better than those with paranasal tumors [202].

Undifferentiated Sinonasal Carcinoma

These tumors are characterized by their lack of differentiation and affects both males and females equally. Histologically, they manifest undifferentiated carcinoma similar to those of type III NPC. These tumors run an aggressive biological course and present in advanced stage. Because of the undifferentiated nature, they may be confused with a wide variety of undifferentiated neoplasms at these sites. These include poorly differentiated squamous carcinoma, NPC, neuroblastoma, melanoma, lymphoma, and small round cell tumors. Immunohistochemical and molecular markers are important in differentiating these tumors, especially on small pretreatment biopsies [202, 210, 211].

Neuroendocrine Carcinomas

Neuroendocrine carcinomas of the sinonasal region are uncommon relative to the larynx and are classified into typical (well-differentiated) and atypical carcinoid (moderately differentiated) and poorly differentiated (small and large cell) carcinoma. The most common subtype is the poorly differentiated subtype, which typically affects the nasal cavity with extension to the ethmoid and maxillary sinuses. They affect men and women equally with a wide range of age. The diagnosis and differential diagnosis is established by performing keratin and other neuroendocrine markers [212–214].

Small Round Cell Tumors and Neuroblastoma

A host of tumors that share a small rounded and basal-like tumor cell composition is not uncommonly presented at these sites. These include neuroblastoma, rhabdomyosarcoma, neuroendocrine carcinoma (small cell), and Ewing's/neuroectodermal tumors [215–217]. Although younger age groups are more frequently affected, older ages may also be presented with these tumors. They occur equally in both sexes. There are no known predisposing factors associated with the development of these tumors and most likely familial and genetic factors may underlie their development. The diagnosis of these tumors, especially on initial biopsy, is challenging and is largely aided by ancillary immunohistochemical and molecular markers [201, 217–221].

Sinonasal Melanoma

Primary sinonasal melanoma is very rare and accounts for 1% of all melanomas and 2.4% of nasal malignancies. The most common sites for this entity is nasal cavity and the paranasal sinuses with the most frequent sites being the nasal septum, lateral nasal wall, and the middle and inferior turbinates. Histologically, cells are small, rounded and undifferentiated and commonly manifest melanin pigment. These tumors are highly aggressive and prone to recurrence. They are typically presented at middle or older age, but they may present at any age. The differential diagnosis of this tumor includes all small round undifferentiated tumors at these locations (Fig. 3.4) [216, 222–225].

Fibrous and Vascular Neoplasms

These tumors are divided into a benign, low-grade category and include fibromatosis, fibroma, myxoma, hemangioma, Schwannoma and hemangiopericytoma and solitary fibrous tumor and low-grade fibrosarcoma. Their diagnosis is based on the histopathologic features and their treatment is largely surgical [202].

Odontogenic Tumors

Odontogenic lesions may also present in the sinonasal sites especially the maxillary sinus and includes calcifying odontogenic and tumor ameloblastoma. The most important differential diagnosis for these tumors is inverted squamous papilloma and squamous carcinoma. These tumors typically occur in young and middle age individuals and behave as benign or locally destructive tumors. Ameloblastoma may however, transform into more malignant ameloblastic carcinoma. Complete excision of these tumors is curative.

Teratocarcinosarcoma

Teratocarcinosarcoma is an extremely rare carcinoma that may lead to management difficulties. The histogenesis of

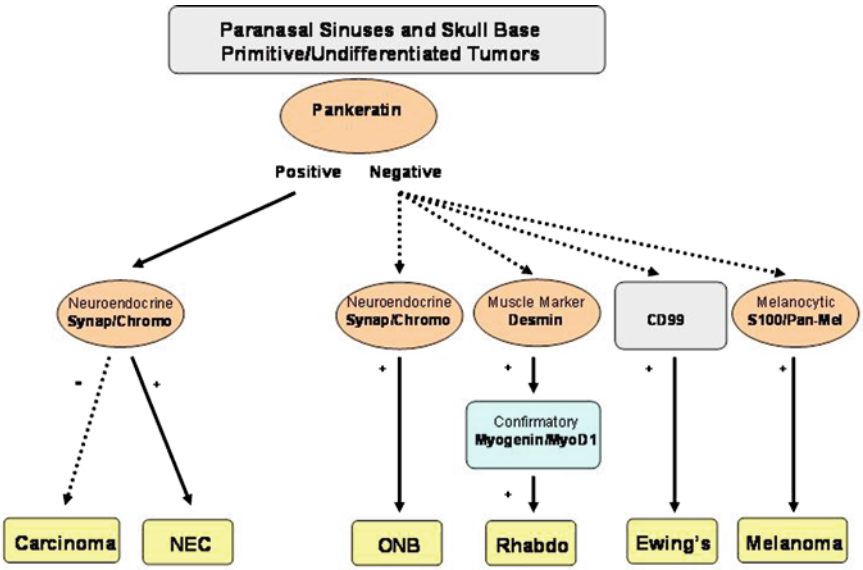

Fig. 3.4 Algorithmic marker applications for sinonasal undifferentiated neoplasms

this entity remains unsettled, but an origin from stem cell is possible. Histologically, these tumors are characterized by the presence of immature neural elements and malignant epithelial and mesenchymal tumors. The tumor affects mainly men in their middle and old age. These tumors are treated surgically with postoperative radiotherapy [226].

Lymphoproliferative Disorders

Non-Hodgkin lymphoma is the most common lymphoproliferative disease in the sinonasal tract. Of the different subtypes that represent this category, the Nk1 T-cell lymphoma is the dominant lymphoma at these sites.

T-cell lymphoma (natural killer) typically afflicts predominantly men in the middle or old age. The disease has been reported to be more common in Asians. The most common presentation is destructive mid-facial lesions with obstructive symptoms. The disease is strongly associated with EBV. Histologically, the disease is characterized by polymorphous cell infiltrate, including lymphocytes, plasma cells, histiocytes, and eosinophils with necrosis [227–231].

The differential diagnosis of this entity includes infectious conditions, especially fungal organisms, and especially Wegener's granulomatosis. The absence of EBV virus and antineutrophil cytoplasmic antibodies exclude the latter.

Molecular and Genetic

Advances in molecular genetic studies of skull-base neoplasms are limited to small round cell tumors, including Ewing's, synovial, and rhabdomyosarcomas. Specific translocation generating oncogenic fusion transcripts have been identified in some of these tumors and currently used in their diagnosis and management stratification. In Ewing's sarcoma and peripheral primitive neuroectodermal tumor, the EWS/FLI-1 gene resulting from the t(11;22) (q24;q12) is detected in 80% of tumors. The fusion gene has also been detected in neuroblastoma and rhabdomyosarcoma [220, 221, 232]. The PAX-FKHR fusion gene has also been used in the diagnosis and to guide treatments in alveolar rhabdomyosarcoma. Future identification of specific translocation will lead to better diagnosis and classification of other tumors.

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