Biomarkers in Head and Neck Carcinomas

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Head and neck region is a complex area and consists of various organs and tissues. Primary carcinomas may arise from numerous tissues including mucosal surfaces, salivary glands, seromucous glands, and odontogenic tissues. Squamous cell carcinomas (SCC) constitute the vast majority of carcinomas in head and neck region except for salivary glands, followed by neuroendocrine carcinomas and undifferentiated carcinomas which are observed rarely. Squamous cell carcinomas may have distinct characteristics by locations in head and neck, such as high-risk human papilloma virus (HPV) positivity in oropharyngeal SCC associated with favorable prognostic, and latent Epstein-Barr virus (EBV) infection is mostly related with nasopharyngeal SCC. Salivary gland tumors consist of various types of

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tumors which frequently have overlapping histological features. Genomic alterations, especially specific chromosomal rearrangements provide new insights into the pathogenesis of salivary gland neoplasms.

Histochemical, immunohistochemical, and molecular biomarkers mainly help as diagnostic tools in head and neck carcinoma. The prognostic and predictive importance of biomarkers is also increasing, and targeted therapy approaches in several cancers are gradually increasing.

4.1 Nasal Cavity, Paranasal Sinuses and Skull Base

4.1.1 Squamous Cell Carcinoma

Squamous cell carcinomas (SCC) constitute the majority of malignancies in the nasal cavity, paranasal sinuses, and skull bases. However, it is observed at a lower ratio (60–75%) when compared with SCC frequency in other head and neck sites. They generally present with nonspecific symptoms so that the majority of sinonasal SCCs present at an advanced stage and the prognosis is mostly poor. Tobacco; industrial exposures, such as leather dust, wood dust, and solvents; sinonasal papillomas; and high-risk HPV infection are risk factors for malignant transformation. Among them, the incidence of high-risk HPV-driven tumors has increased over the past 3 decades [1].

SCC has keratinizing, non-keratinizing, spindle cell, papillary, and basaloid subtypes. Keratinizing SCC is the most common sinonasal SCC type which exhibits histological features with irregular cords and nests of eosinophilic tumor cells demonstrating variable degrees of keratinization and stromal reaction. Non-keratinizing SCC consists of anastomosing ribbons and nest of tumor cells with minimal or no evidence of keratinization or stromal desmoplasia.

4.1.1.1 Diagnostic Biomarkers

SCC is positive for p63, p40 and high-molecular-weight cytokeratins, such as CK5/6.

Table 4.1 Frequencies of high-risk HPV positivity in squamous cell carcinoma by location

Tumor site	HPV positivity
Sinonasal site	9–32
Nasopharynx	10
Larynx	4–15
Hypopharynx	5
Oral cavity	5–15
Oropharynx	57–72

High-risk HPV positivity is detected in 9–32% of sinonasal SCCs (Table 4.1). HPV16 is the most common type, and less commonly HPV18, 31, 33 and rarely HPV35, 39, 45, and 82 are identified. Non-keratinizing, papillary, or basaloid histologic subtypes display a higher rate of HPV positivity. SCC in nasal cavity demonstrates slightly more HPV positivity than tumors in sinuses [1–9].

Nuclear p16 immunoexpression, frequently associated with cytoplasmic positivity in more than 70% of tumor cells, is seen in nearly 15% of sinonasal SCCs and is strongly associated with transcriptionally active high-risk HPV infection. In sinonasal SCC, the specificity and sensitivity of nuclear p16 expression in determining HPV positivity are 90–94% and 67–100%, respectively. Immunoexpression of p16 may be used as a reliable surrogate biomarker for demonstrating high-risk HPV status; nonetheless it should be noted that it has relatively weak positive predictive value and not yet approved by World Health Organization for sinonasal SCCs [2–4, 10].

HPV-negative SCCs are often associated with *TP53* mutation and loss of *CDKN2A*/B, while HPV-positive SCCs mostly contain *PIK3CA* and *PTEN* gene alterations [11]. *EGFR* copy number gains are detected in up to half of sinonasal SCCs and mostly correlate with EGFR overexpression [2, 12]. High-risk HPV positivity and *EGFR* copy number gains are mutually exclusive. *FGFR1* and *SOX2* amplifications are also identified in a small subgroup of sinonasal SCC [9].

Oncocytic sinonasal papilloma-associated SCC often harbors *KRAS* mutations in codon 12, whereas inverted sinonasal papilloma-associated

SCC contains *EGFR* mutations, mainly exon 20 insertions and occasionally exon 19 mutations [13–16].

4.1.1.2 Prognostic/Predictive Biomarkers

HPV positivity and p16 expression are favorable prognostic factors in sinonasal SCCs. HPV-positive SCC is mostly seen in patients who are younger and less likely to have a smoking history [2, 5, 6, 10, 17]. Routine HPV testing, as currently is recommended for oropharyngeal tumors, might be warranted in individuals with sinonasal SCC as well.

EGFR mutations may predict a response to therapy with EGFR tyrosine kinase inhibitors (TKI), while *KRAS* mutations are negative predictor of response to EGFR TKI. TKI treatment may become a potential option against sinonasal SCCs with EGFR gene alterations. However, inverted sinonasal papilloma-associated SCC displays mainly *EGFR* exon 20 mutations which are associated with reduced sensitivity to EGFR TKI [18].

Immune checkpoint inhibitors are approved by FDA as first-line treatment together with chemotherapy for advanced HNSCCs and monotherapy for HNSCCs which express PD-L1 with a combined positive score ≥ 1. Combined positive score is defined as the ratio of the number of PD-L1 positive cells including tumor and immune cells to the number of total tumor cells [19]. The membranous PD-L1 immunoexpression (>5%) is detected in 34% of sinonasal SCC tumor cells and 45% of non-tumoral immune cells. HPV-positive tumors display higher PD-L1 positivity than HPV-negative tumors, while expression of PD-L1 seems to be of no prognostic importance [20].

4.1.2 Lymphoepithelial Carcinoma

Lymphoepithelial carcinoma (LEC) is a squamous cell carcinoma variant which histomorphologically resembles non-keratinizing nasopharyngeal carcinoma, undifferentiated subtype (Fig. 4.1) [1].

4.1.2.1 Diagnostic Biomarkers

LECs in sinonasal region are usually positive for EBV-encoded small RNA (EBER) by in situ hybridization. LEC is diffusely positive for pancytokeratin and squamous markers like CK5/6, p63, and p40.

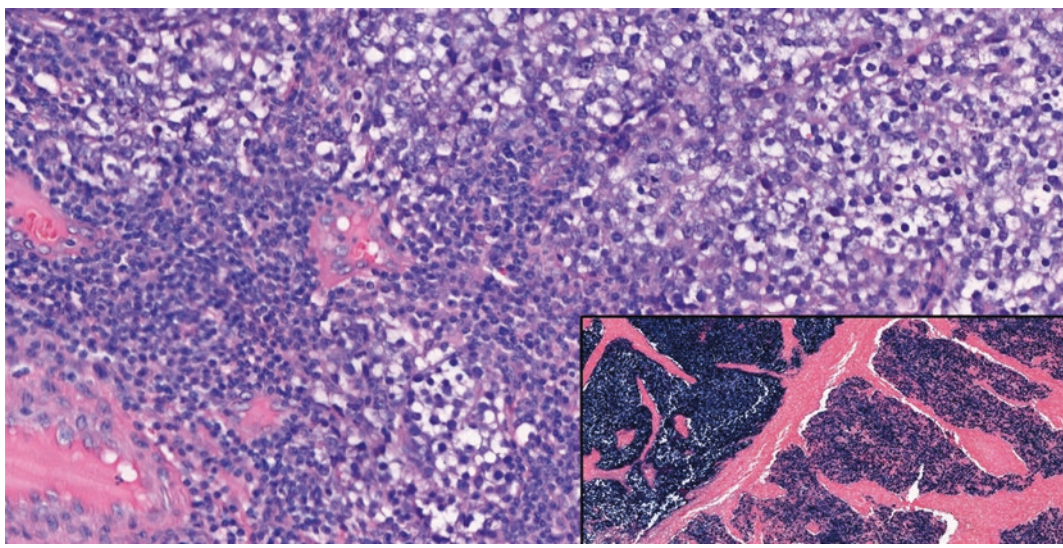

Fig. 4.1 Lymphoepithelial carcinoma of the nasal cavity with dense lymphocytic infiltration and EBER positivity (H&E and EBER, original magnification ×40)

4.1.3 NUT Carcinoma

NUT (nuclear protein in testis) carcinoma is an uncommon and aggressive midline tumor. Sinonasal region is the most common localization of these tumors in head and neck. Histological features of NUT carcinoma include poorly differentiated morphology with nests of monotonous tumor cells and "abrupt" keratinization focus [1].

4.1.3.1 Diagnostic Biomarkers

NUT carcinoma is often positive for p40, p63, and CK5/6 and occasionally positive for CD34, neuroendocrine markers, p16, or TTF-1.

NUT carcinoma is characterized by *NUTM1* gene translocations. The fusion partner is mostly *BRD4* gene (70%) and occasionally other BET family genes such as *BRD3*, *NSD3*, and *ZNF532*. These alterations can be detected by molecular methods including ISH, PCR, or NGS approaches. It can be confirmed with >50% nuclear NUT expression by immunohistochemistry which has a specificity of 100% and sensitivity of 87% and accepted as a diagnostic ancillary test. Focal NUT expression (less than 50% of tumor cells) can be observed in germ cell tumors or poorly differentiated carcinomas [21].

4.1.3.2 Prognostic/Predictive Biomarkers

Several studies indicate that tumors with *NUTM1-BRD4* translocation have worse clinical course than tumors with the fusion of *NUTM1* and non-*BRD4* partner genes [21]. Accordingly, molecular tests which reveal fusion partner of *NUTM1* gene may provide prognostic information [21].

Targeting chimeric fusion protein with BET inhibitors (extra-terminal bromodomain), histone deacetylase inhibitors, or p300/CBP inhibitors represents potential but not fully understood therapeutic approaches in NUT carcinoma [22–24].

4.1.4 Sinonasal Undifferentiated Carcinoma

Sinonasal undifferentiated carcinoma (SNUC) is defined as an undifferentiated carcinoma without glandular or squamous features. SNUC is characterized by high-grade undifferentiated cells without viral etiologies. Focal neuroendocrine differentiation may be observed in some of SNUCs. SNUC is typically thought as a diagnosis of exclusion; however, it has recently begun to be debated whether it is a distinct tumor type after certain molecular features of SNUC have been elucidated. *IDH2*-mutant sinonasal carcinoma is mostly described as a part of SNUC category, as SMARCB1-deficient sinonasal carcinoma and SMARCA4-deficient sinonasal carcinomas are emerging novel and distinct entities that are classified within the SNUC category for the present [25].

4.1.4.1 Diagnostic Biomarkers

Immunohistochemically, SNUC is positive for epithelial differentiation immunohistochemical biomarkers, such as PANCK and EMA, focally CK7 and CK8, but usually negative or occasionally positive for markers of any other specific squamous or neuroendocrine markers. These phenotypic features of SNUC can be confused with some high-grade mesenchymal tumors with focal cytokeratin expression, including Ewing family tumors, synovial sarcoma and alveolar rhabdomyosarcoma, and other sinonasal carcinomas with poorly differentiated/undifferentiated foci [25].

IDH2 R172X mutations, including R172S, R172T, R172M, and R172G, are detected in 55 to 82% of SNUCs which mostly display hypermethylator phenotype. IDH2 or IDH1/2 expression by immunohistochemistry is relatively specific to SNUC among head and neck carcinomas but has limited sensitivity, as *IDH2* R172T mutations cannot be detected with this method [25–29]. *TP53, CDKN2A/2B, KIT, MYC, SETD2*, and *SPEN* mutations are detected in *IDH2*-mutant SNUCs [28]. A small part of SCC, large-cell neuroendocrine carcinoma, and poorly differentiated carcinomas also display *IDH2* R172X mutation, but these tumors may represent phenotypic variants of the SNUC [28, 30]. Additionally, the metastasis of other IDH2-positive carcinomas such as solid papillary breast carcinoma with reverse polarity or intrahepatic cholangiocarcinoma may be considered in the differential diagnosis with IDH2-mutant SNUC despite histological dissimilarity [27].

4.1.4.2 Prognostic/Predictive Biomarkers

SNUCs with *IDH2* mutation have a trend of better prognosis than those without mutation, but it has not been fully validated yet [28, 30]. Targeting *IDH2* mutation is a potential treatment approach for SNUC patients. Promising results with IDH2 inhibitor are demonstrated in *IDH2*-mutated acute myeloid leukemia, and accordingly it may be predicted similar outcomes in SNUC as well; however, there are limited number of clinical trials on this subject yet [27].

4.1.5 Neuroendocrine Carcinoma

Sinonasal neuroendocrine carcinoma (SNEC) is a rare and aggressive malignancy which includes small-cell and large-cell sinonasal neuroendocrine carcinomas. Histological features of SNEC resemble neuroendocrine carcinomas in other sites such as lung.

4.1.5.1 Diagnostic Biomarkers

SNEC stains for cytokeratins, EMA, and at least one of the neuroendocrine markers including chromogranin, synaptophysin, CD56, and neuron-specific enolase; the latter two are less specific [31–33]. It has recently demonstrated that insulinoma-associated protein 1 (INSM1) expression is a promising biomarker for the diagnosis of NEC, especially differentiating it from SNUC [34–36]. SNEC may also express p16, ASCL1, occasionally p63, TTF-1, and calretinin [32].

Some small-cell neuroendocrine carcinomas harbor *ARID1A* mutation [28].

4.1.6 Adenocarcinoma

Sinonasal adenocarcinoma is a group of malignancy that includes intestinal-type and non-intestinal-type adenocarcinoma. Each tumor type displays distinct morphologic and immunohistochemical features. Intestinal-type adenocarcinoma (ITAC) is an aggressive neoplasm that is associated with industrial wood and leather dust exposures. ITAC is morphologically similar to primary intestinal adenocarcinoma. Non-intestinal-type sinonasal adenocarcinoma (non-ITAC) is a heterogeneous group of tumors including low-grade non-ITAC, high-grade non-ITAC, and recently proposed sinonasal renal cell carcinoma-like adenocarcinoma. Low-grade non-ITAC has papillary/tubular histomorphology and has particularly good prognosis, so that it is crucial to distinguish this type of tumor from others. High-grade non-ITAC is a group of histologically diverse tumors which mostly display high mitotic index, frequent necrosis, and solid pattern with moderate amounts of glandular structures.

4.1.6.1 Diagnostic Biomarkers

Intestinal-type adenocarcinoma is consistently stained with markers of intestinal differentiation, including cytokeratin 20, CDX2, villin, and MUC2.

TP53, *KRAS*, *BRAF* mutations, *EGFR* amplification, and loss of *CDKN2A* are detected in some intestinal-type adenocarcinomas [37].

4.1.7 Emerging Entities

4.1.7.1 HPV-Related Multiphenotypic Sinonasal Cancer

HPV-related multiphenotypic sinonasal cancer (HMSC) is a distinct entity which is previously termed as HPV-related carcinoma with adenoid cystic features and classified in non-keratinizing SCC. HMSC has indolent clinical behavior characterized by frequent local recurrences and rare distant metastasis, and disease-specific mortalities have not been reported yet [38]. HMSC displays high-grade histological features, basaloid cells including necrosis and high mitotic rates, and adenoid cystic carcinoma-like cribriform areas [39].

4.1.7.1.1 Diagnostic Biomarkers

HPV positivity is basically detected in all cases, and high incidence of HPV type 33 (%84) is more noticeable than others including HPV type 35, 16, 56, and 82 [38, 40]. p16 expression is also observed in majority of HMSCs. However, a small subset of solid type adenoid cystic carcinomas in sinonasal site may also express p16, with-

out HPV RNA ISH positivity. Accordingly, it is not recommended that p16 expression alone can be used as a surrogate for HPV testing to distinguish sinonasal adenoid cystic carcinoma from HMSC [41]. Unlike the characteristics of adenoid cystic carcinomas, *MYB/NFIB* or *MYBL1/NFIB* fusions are also not expected to be present in HMSCs.

HMSC is positive for PANCK, CK7, SOX-10, and LEF-1, p40, and a subset demonstrates expression of SMA, CK5/6, SMMS-1, and S100 [40].

4.1.7.2 Renal Cell-like Adenocarcinoma

Renal cell-like adenocarcinoma is a variant of non-intestinal-type adenocarcinoma (non-ITAC) that has similar features to clear cell renal cell carcinoma. A vast majority of these tumors are low-grade and display good clinical prognosis, but a rare case with lymph node metastasis has been reported [42].

Renal cell-like adenocarcinoma stains for CK7, CAIX (carbonic anhydrase IX), and EGFR and less consistently stains for SOX10 and S100, but it is negative for CK20, renal cell carcinoma antigen (RCCag), vimentin, and PAX8 [42].

4.1.7.3 SMARCB1-Deficient Sinonasal Carcinomas

SMARCB1-deficient sinonasal carcinoma is an uncommon and aggressive carcinoma which mostly involves paranasal sinuses and displays poorly differentiated histomorphology with monomorphic/basaloid cells. Tumor cells variably express p16, p40, CK5/6, synaptophysin, and chromogranin. Loss of nuclear expression of SMARCB1 (INI1) in tumor cells is useful for diagnosis [43, 44] (Fig. 4.2). A subset of sinonasal non-ITAC, renal medullary carcinoma and a subset of myoepithelial carcinoma of the soft tissue also display loss of SMARCB1 [45].

4.1.7.4 SMARCA4-Deficient Sinonasal Carcinoma

SMARCA4-deficient sinonasal carcinoma is a very rare poorly differentiated tumor in sinonasal region. The tumor consists of high-grade basaloid or epithelioid cells in nested and solid pattern with frequent necrosis. SMARCA4-deficient sinonasal carcinoma is positive for PANCK, focally neuroendocrine markers, and negative for CK5/6, p63, and p16. A distinguishing feature of these tumors is the loss of nuclear immunoexpression for SMARCA4 (BRG1), but it has been recently reported that majority of sinonasal teratocarcinosarcomas also show the loss of SMARCA4 [46–48].

4.1.8 Salivary Gland-Type Tumors of the Seromucous Glands

Minor salivary gland carcinomas of the sinonasal cavity are rare cancers. Adenoid cystic carcinoma, mucoepidermoid carcinoma, and polymorphous adenocarcinoma comprise the large majority of sinonasal malignant salivary gland tumors [49].

4.1.9 Metastatic Tumors to the Nasal Cavity

The sinonasal region is rare location for metastases. Metastatic tumors mostly originate from the kidney, breast, thyroid, and prostate. Renal cell-like adenocarcinoma may be confused with metastatic renal cell carcinoma, but the latter is positive for RCC-ab and PAX8 (Fig. 4.3). Differentiating ITAC from metastatic adenocarcinoma of the colon mostly depends on clinical history, colonoscopy, or imaging modalities. Colonic adenocarcinoma cases have lower expressions of CK7, MUC5, and chromogranin, and this feature may be helpful in differential diagnosis. Metastatic thyroid carcinoma expresses TTF-1, PAX8, and thyroglobulin, and metastatic prostatic carcinoma expresses PSA, PAP, EpCam, NKX3.1, and prostein. Metastatic breast carcinomas are positive for estrogen receptor, progesterone receptor, HER2, mammaglobin, gross cystic disease fluid protein-15 (GCDFP-15), and GATA-3. However, these biomarkers are also positive in salivary gland ductal carcinoma, a rare primary tumor at this location, and mostly expresses AR and Her2 [50]. Differential diagno-

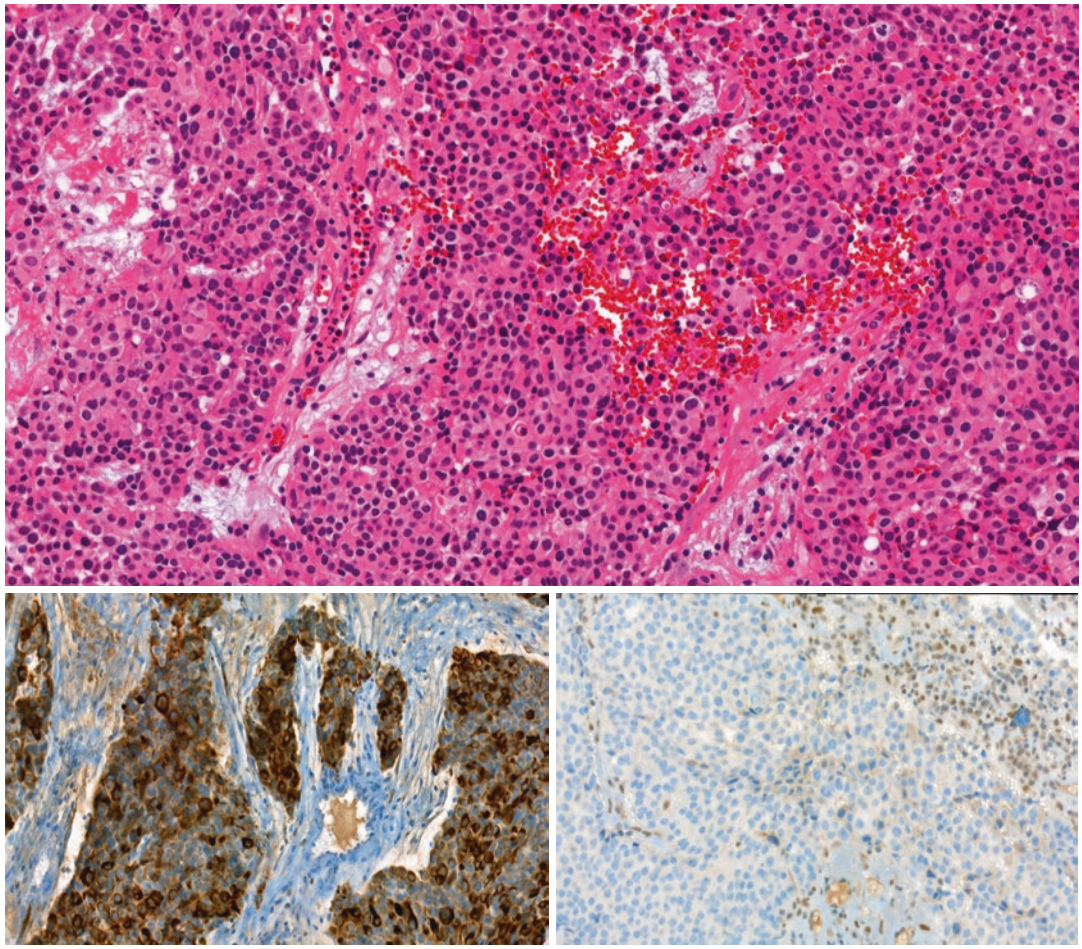

Fig. 4.2 SMARC1 (INI-1) deficient pan-keratin positive sheets of atypical epithelial cells with plasmacytoid morphology were negative for myoepithelial markers and SMARC1 (INI-1) (H&E, pan-keratin, INI-1, original magnification ×40)

sis from salivary gland-type tumors should be kept in mind; biomarkers of myoepithelial differentiation and GATA-3 positivity are in favor of primary sinonasal tumors [51–55].

4.2 Nasopharynx

4.2.1 Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a malignant tumor arising from the surface mucosa of nasopharynx associated with frequently associated with EBV infection. NPC has a distinctive ethnic and geographic distribution with a particularly high prevalence in the Southern China, Southeast Asia, North Africa, and Arctic. Host genetic susceptibility genes (*HLA-A*A2*, *B*46*, *B*17*, *A*0207* alleles and *CYP2E1*, *CYP2A6*, *GSTM1*, *GSTT1*, *OGG1*, *XRCC1* gene polymorphisms, etc.), consumption of tobacco, alcohol, and salted-fermented foods are likely contributing factors for the disease.

Three histological variants of NPC encompass keratinizing squamous, non-keratinizing, and basaloid squamous. Keratinizing NPC shows evidence of keratinocytic differentiation in the form of intercellular junctions and variable keratin. Non-keratinizing NPC histologically consists of malignant cells that form syncytial or cohesive

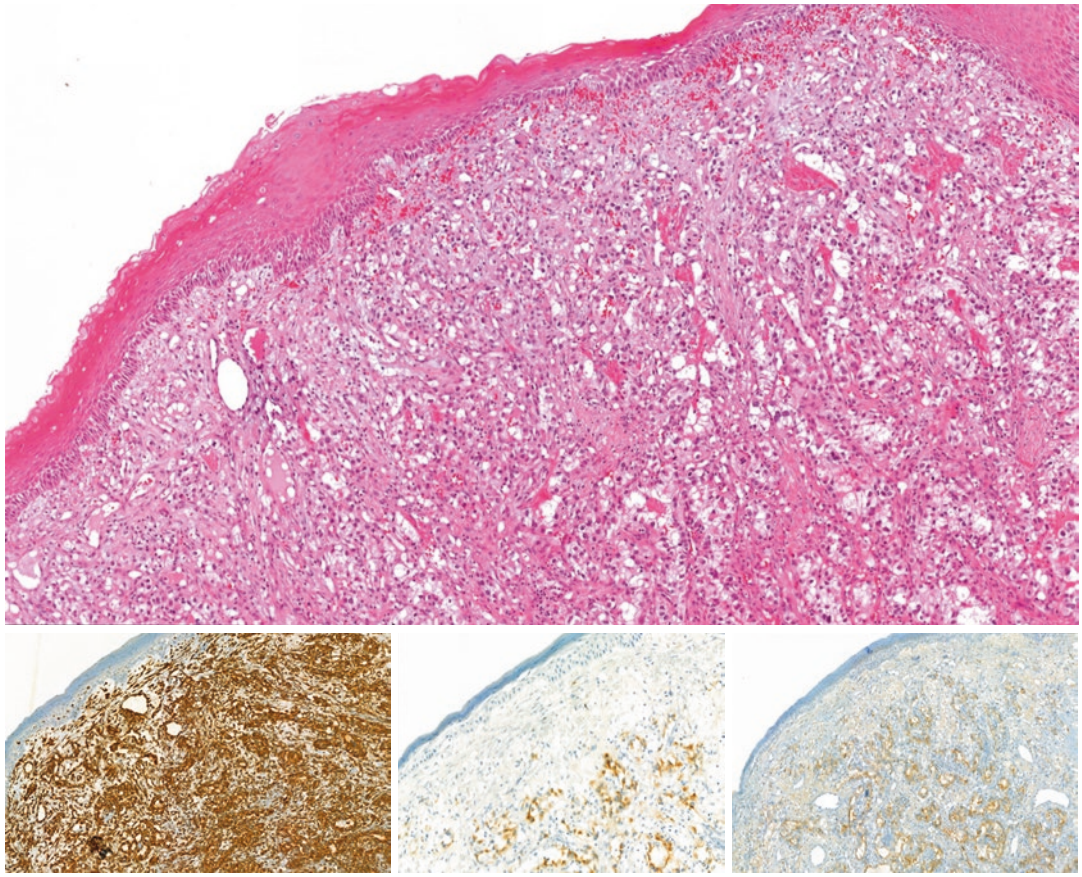

Fig. 4.3 Submucosal carcinoma at the nasal cavity; pan-keratin, vimentin, CD10, and RCC positive consistent with metastatic renal cell carcinoma (H&E, pan-keratin, CD10, RCC, original magnification ×10)

arranged irregular nests and are mixed with variable reactive lymphoplasmacytic infiltrate. Non-keratinizing NPC can exhibit differentiated and undifferentiated morphology. Other rare variant is basaloid type NPC that is histologically identical to other basaloid SCCs in other head and neck sites.

Non-keratinizing NPC is more common in endemic regions and closely related to EBV infection. EBV infection can be latent or lytic. In latent infection, EBV genome forms episomes and expresses EBV latent genes including LMP-1, LMP-2, EBNA1, EBNA2, EBERs, BARTs, and miR-BARTs. These genes are effectors of EBV-mediated malignant transformation and dysregulate mainly WNT/β-catenin, JAK/STAT, PI3K/Akt/mTOR, EGFR, MAPK, and NF-κB signaling pathways [56].

4.2.1.1 Diagnostic Biomarkers

Immunohistochemically, NPC expresses PANCK, p63, CK5/6, CK8, and CK19, and NPC is negative for CK7, CK14, and CK20 [57]. Undifferentiated morphology may require differential diagnosis with lymphoma, malignant melanoma, and neuroendocrine carcinoma leading to a larger panel of biomarkers. Loss of cytokeratin expression may be observed, and other epithelial markers should be applied. EBV infection is nearly detected in all non-keratinizing NPCs. Nuclear reaction for EBV EBER by in situ hybridization is the most reliable way to display EBV infection. Detecting LMP-1 expression by immunohistochemistry is also other method for it, but it has relatively low sensitivity and can detect only 50–80% of EBV infections in NPC [58]. High-risk HPV positivity is also detected in up to 10% of NPCs [59, 60].

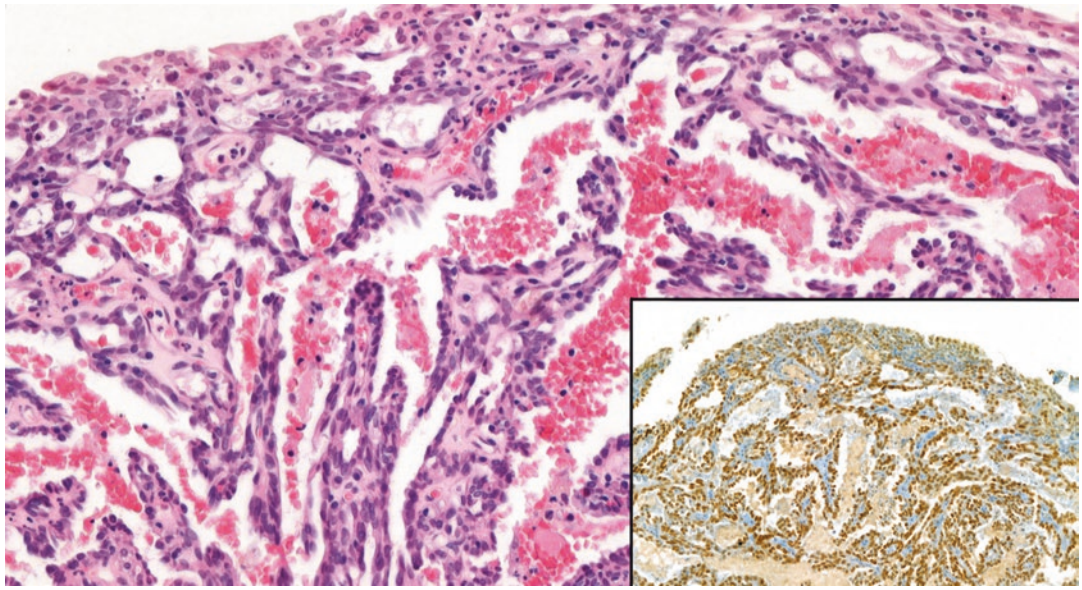

Fig. 4.4 TTF-1 positivity in thyroid-like low-grade papillary nasopharyngeal adenocarcinoma. Lack of thyroglobulin expression is valuable for differential diagnosis with metastatic thyroid papillary carcinoma (H&E and TTF-1, original magnification ×40)

Circulating cell-free EBV DNA in plasma is a useful tool for screening population for early asymptomatic NPC in endemic regions and for the early detection of tumor recurrence [61, 62].

4.2.1.2 Prognostic/Predictive Biomarkers

EBV infection in NPC is proposed as a favorable prognostic factor, although there are controversial results about the prognostic importance of EBV or HPV infections [59, 63, 64]. Several strategies targeting EBV are being developed such as DNAzymes against LMP-1 mRNA, adenovirus-based adoptive immunotherapy, and LMP-1-specific autologous CTLs [65]. Detectable circulating cell-free EBV DNA in plasma is also an indicator for unfavorable response to chemoradiotherapy/radiotherapy [66].

Nasopharyngeal carcinoma, especially undifferentiated non-keratinizing EBV positive type, is characterized by high PD-L1 expression in up to 90% of tumor cells and abundant infiltration of stromal lymphocytes that render it an attractive target for immunotherapy. Accordingly, in several early trials, immune checkpoint blockade therapies have showed promising clinical results, and phase III trials are still ongoing [67]. Thus far, the correlation between PD-L1 expression in tumor or immune cells and response rate to anti-PD-1 antibodies has not been found [68]. On the other hand, several studies show that high PD-L1 expression is associated with poor prognosis in NPC [69–71].

4.2.2 Nasopharyngeal Papillary Adenocarcinoma

Nasopharyngeal papillary adenocarcinoma (NPPA) is a low-grade adenocarcinoma which is histologically characterized by papillary structures and cellular features akin to seen in papillary carcinoma of thyroid gland.

4.2.2.1 Diagnostic Biomarkers

NPPA stains for EMA, CK5/6, CK7, and vimentin. A subset of NPPA which expresses TTF-1 and CK19 is called as thyroid-like low-grade NPAC (Fig. 4.4). These tumors are negative for EBER, thyroglobulin, and CK20 and do not harbor BRAF mutations [72].

4.2.3 Metastatic Tumors to the Nasopharynx

Metastatic carcinomas in the nasopharynx are quite rare. The metastasis of the lung, breast, and kidney carcinomas is reported [73–75].

4.3 Larynx, Hypopharynx, and Oral Cavity

4.3.1 Squamous Cell Carcinoma

Larynx, hypopharynx, and oral cavity are the most common locations for squamous cell carcinomas in head and neck area. Cigarette and alcohol are main risk factors for development of SCC. Other risk factors include gastroesophageal reflux, diet, social-economic status, poor-fitting oral dentures, and betel chewing in certain geographic regions. Transcriptionally active high-risk HPV infection is an etiologic factor in some of the SCC at these sites, but the frequency is less than oropharyngeal region [76, 77].

The Cancer Genome Atlas Network study demonstrated that head and neck SCCs which are mainly located in oral cavity, oropharynx, hypopharynx, and laryngeal sites are associated with distinct molecular backgrounds. Human papilloma virus-associated SCCs are mostly located in oropharynx and display *PIK3CA* mutation, the loss of *TRAF3*, and *E2F1* amplification. Smoking-related SCCs contain *TP53* inactivating mutation, *CDKN2A* inactivation, and 3q26/28 and 11q13/2 amplifications. A subset of oral cavity SCCs that demonstrate *HRAS* or *PIK3CA* activating mutations, *CASP8*, *NOTCH1*, and *TP53* inactivating mutations is associated with favorable clinical outcome. A subset of SCCs that are mostly at laryngeal sites displays *NSD1* (chromatin modifier), *AJUBA*, and *FAT1* (WNT pathway genes) loss-of-function alterations, and *NFE2L2*-activating mutations [78].

4.3.1.1 Diagnostic Biomarkers

Transcriptionally active high-risk HPV infection is identified in 4–15% of laryngeal SCCs, of 5% of hypopharyngeal SCCs, and 5–15% of oral cavity SCCs [76, 79–82]. While most of the HPV-positive SCCs in larynx, hypopharynx, and oral cavity share histologic features of oropharyngeal HPV-positive SCCs, a quarter of these tumors are recently called as warty variant SCCs which display exophytic growth, vigorous hyperkeratosis/parakeratosis, koilocytic atypia, and prominent nuclear pleomorphism. Warty variant SCC is similar to anogenital HPV-positive SCC and associated with good prognosis [83].

P16 expression is detected in 13–20% of laryngeal SCCs, 16–21% of hypopharyngeal SCCs, and 6–26% of oral cavity SCCs and is not well correlated with HPV status [82, 84, 85].

Laryngeal SCCs mainly harbor *NSD1*, *AJUBA*, *FAT1*, and *NFE2L2* mutations [86].

4.3.1.2 Prognostic/Predictive Biomarkers

The prognostic role of high-risk HPV infection status or p16 expression has not fully been established in SCCs of larynx, hypopharynx, and oral cavity, despite that several studies show that either high-risk HPV positivity or p16 expression is related with improved prognosis [79, 80, 84, 85, 87–89].

A subgroup of oral cavity SCCs which display *CASP8*, *NOTCH1*, and *TP53* mutations together with *HRAS* or *PIK3CA* mutations and infrequent copy number alterations demonstrates improved clinical courses [86]. In early glottic SCC, it has been reported that EGFR expression is an unfavorable prognostic factor [90].

Positive PD-L1 immunohistochemical expression defined by ≥1% tumor proportion score or ≥ 1 combined positive score renders head and neck SCCs potentially suitable for immunotherapies [19].

4.3.2 Neuroendocrine Carcinoma

The most common locations of neuroendocrine carcinomas in head and neck are the larynx, hypopharynx, and oral cavity [91]. Well-differentiated, moderately differentiated, and poorly differentiated neuroendocrine carcinomas are classified according to nuclear atypia of tumor cells and mitotic index.

4.3.2.1 Diagnostic Biomarkers

These tumors stain for cytokeratins, EMA, and neuroendocrine markers (e.g., chromogranin, synaptophysin, or CD56) and variably stain for TTF-1. Ki-67 proliferating index is not in routine for grading of neuroendocrine tumors of the head and neck region.

4.3.3 Metastatic Tumors to the Larynx, Hypopharynx, and Oral Cavity

Metastatic tumors which generally originate from the lung, breast, kidney, and skin may present in oral cavity, hypopharynx, and larynx. The gingiva and tongue are the most common locations for metastasis [92, 93].

The positivity of epithelial squamous cell differentiation markers, CK5/6, p40, p63, CK8, CK13, CK19, and EMA, is very frequent; besides CK4, CK7, CK10, CK14, P16, and NUT expressions may be observed in SCCs of the head and neck region [94]. These biomarkers are frequently positive in SCC of other sites, and in case of tumors without intramucosal carcinoma component rising suspicion of metastatic carcinomas, the biomarkers are not helpful about the primary site.

Minor salivary glands and seromucous glands give rise to salivary gland-type carcinomas. Oral cavity and oropharynx are the most frequent sites for this type of tumor if major salivary glands are excluded. p63 expression is an expected finding in salivary gland carcinomas with myoepithelial differentiation and mucoepidermoid carcinomas. p63 should be interpreted with caution as a squamous differentiation marker at the head and neck region, or other markers should be preferred. In cases with adenocarcinoma morphology without myoepithelial differentiation, metastatic tumors should be considered. The most frequent primary sites metastatic to the oral cavity are the lung, liver, breast, kidney, and colorectal in decreasing frequency, but rare sites like prostate should also be considered in the differential diagnosis (Fig. 4.5) [95].

4.4 Oropharynx

4.4.1 Squamous Cell Carcinoma

Oropharyngeal squamous cell carcinoma (OPSCC) mostly occurs in the base of tongue, tonsil, and adenoid of the oropharynx. OPSCC is classified according to high-risk HPV status, since it is a strong factor for favorable prognosis [96]. HPV-positive OPSCC generally occurs in young and nonsmoker patients and is presented as smaller primary tumors at advanced stage with nodal involvement. HPV-positive OPSCC arises from crypt epithelium and generally displays non-keratinizing morphology. HPV-negative OPSCC which is frequently associated with tobacco and alcohol consumption typically exhibits differentiated squamous features.

4.4.1.1 Diagnostic Biomarkers

HPV status can be detected by molecular methods including PCR and ISH or immunohistochemistry. p16 expression by immunohistochemistry is a surrogate biomarker for detecting transcriptionally active high-risk HPV. Overexpression of p16 occurs because of inactivation of tetinoblastoma (Rb) by HPV E7 early protein. p16 test may serve as a sufficient biomarker for determining HPV status in OPSCC. The nuclear and cytoplasmic strong immunoexpression of more than 70% of tumor cells is widely accepted as positive p16 staining result. The nuclear and cytoplasmic expression of more than 50% of tumor cells with >25% confluent staining may also be accepted. Molecular tests can be used following p16 test, if the clinical or histological features suggest otherwise. Among these molecular technics, HPV RNA-ISH seems to be the most effective method for determining transcriptionally active HPV status [97] (Fig. 4.6). The detection of cell-free HPV DNA in blood is recently described diagnostic tool for classifying OPSCC [98].

TRAF3, CYLD, PIK3CA, and E2F1 mutations are present in HPV-positive OPSCCs, while HPV-negative OPSCC displays CCND1, FADD, BIRC2, YAP1, and TP53 mutations [86].

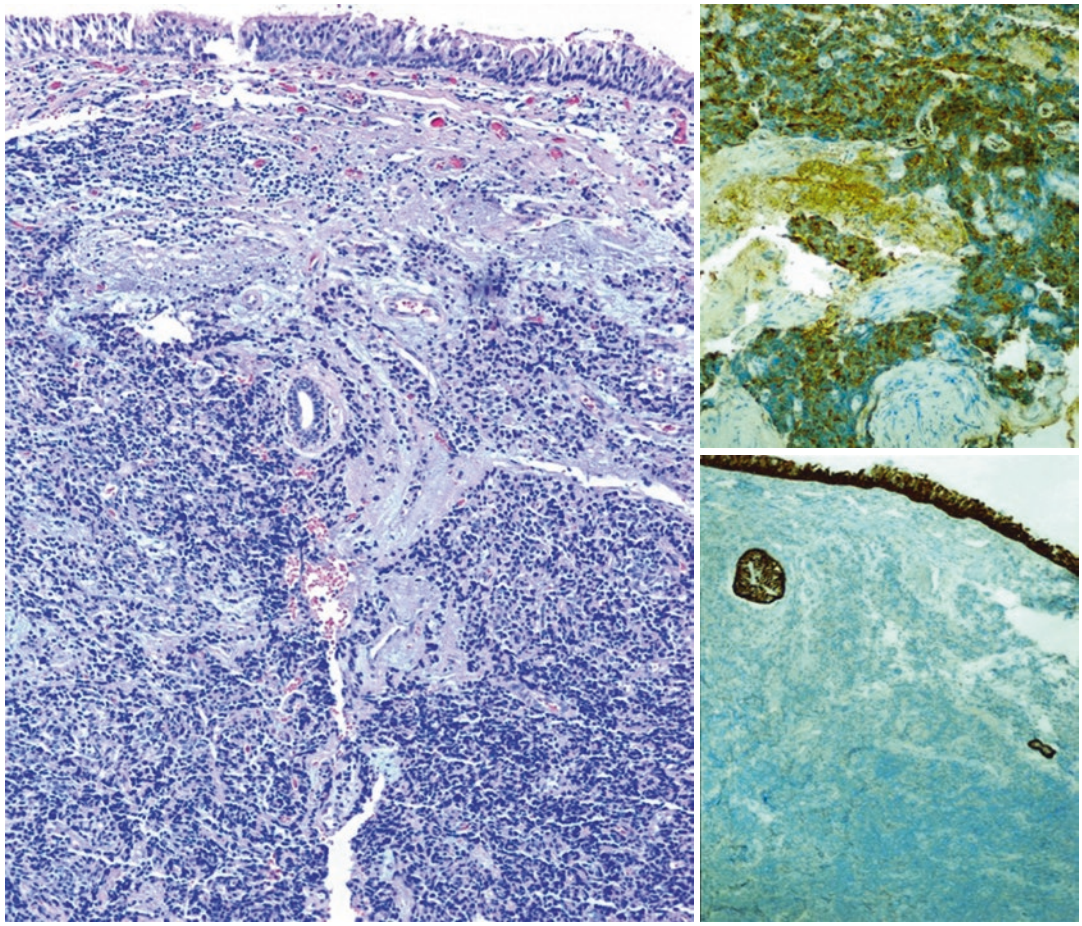

Fig. 4.5 Submucosal carcinoma at the laryngeal supraglottic region; PSAP is positive consistent with metastatic prostatic carcinoma, and CK5/6 is negative (H&E, PSAP, CK5/6, original magnification ×10)

4.4.1.2 Prognostic/Predictive Biomarkers

HPV status is an independent prognostic factor in OPSCC. At the same time, p16 expression in OPSCC is an independent prognostic factor regardless of the HPV status [99, 100]. *TRAF3/CYLD* mutations in HPV-positive OPSCCs are also associated with favorable clinical outcomes [101].

NOTCH1 mutation, *SOX2* amplification, and HER3 expression are associated with poorer overall survival, while expression of estrogen receptor-α is associated with improved overall survival in HPV-positive OPSCC [102–104].

The tumor-infiltrating lymphocytes and high immunoexpression of PD-L1 more than 5% of intratumoral immune cells are favorable prognostic factors [105].

4.4.2 Metastatic Tumors to the Oropharynx

The most common primary sites of oropharyngeal metastases are the lung, kidney, prostate, breast, and female genital organs [106, 107].

4.5 Salivary Glands

Immunohistochemical expressions and molecular alterations are summarized in Tables 4.2–4.4.

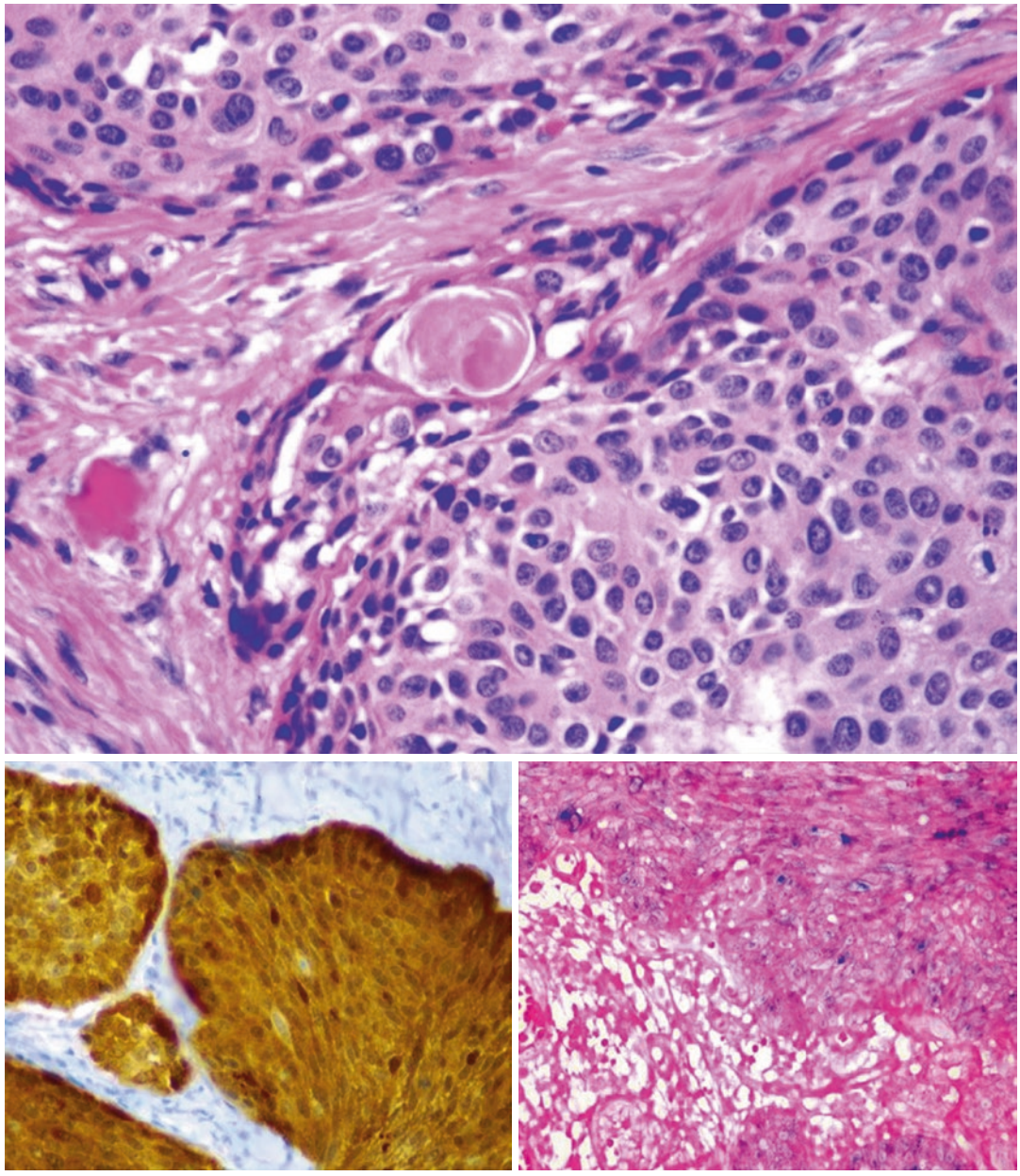

Fig. 4.6 Peripheral focal keratinization, strong p16 and high-risk HPV CISH positivity in tonsillary carcinoma (H&E, P16, high-risk HPV-CISH, original magnification ×40)

4.5.1 Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma (MEC) is the most common malignant salivary gland neoplasm. MEC is histologically composed of squamoid, mucin-producing, and intermediate cell types, which may form various patterns, including cystic and/or solid areas [108]. Several histopathological grading systems suggest stratifying MECs according to prognostic significance. At present, the WHO does not endorse a specific grading scheme. Currently, MECs are divided into three groups: low, intermediate, and high histological grade, with different clinical courses [109, 110].

Table 4.2 Immunohistochemical expressions in salivary gland carcinomas

Tumor type	Immunohistochemical biomarkers
Mucoepidermoid carcinoma	CK8, CK18, CEA, p63, CK5/6, CK8, AREG
Adenoid cystic carcinoma	Bcl-2, EMA, CK7, CK5/6, p63, p40, calponin, SMA, Myb, Sox-10, S100, vimentin, CD117, DOG-1
Acinic cell carcinoma	DOG-1, Sox-10, transferrin, laktoferrin α-amylase, EMA, CEA, NR4A3
Polymorphous adenocarcinoma	CK7, CK8, CK18, EMA, S100, GFAP, Bcl-2, galectin-3, DOG-1,
Clear cell carcinoma	CK5/6, CK14, p63, EMA, CEA
Basal cell adenocarcinoma	CK7, CK8, CK18, CEA, EMA, S100, GFAP, calponin, B-catenin
Salivary duct carcinoma	AR, HER-2, GATA-3, GCDFP-15, CK7
Myoepithelial carcinoma	S100, CK5/6, calponin, SOX10, vimentin
Epithelial myoepithelial carcinoma	CK8, CK18, EMA, S100, SMA, CK5/6, CK14, p63, SOX10, vimentin
Secretory carcinoma	MUC4, S100, mammaglobin, GCDFP-15, GATA-3, S100, CD117
Sebaceous carcinoma	Adipophilin, CK5, CK14 EMA

4.5.1.1 Diagnostic Biomarkers

Lack of expression of myoepithelial markers and p63 and intracellular mucin positivity are typical features. *CRTC1/3-MAML2* fusions are accepted as diagnostic markers for MECs. Molecular tests may especially help to diagnose variants of MECs, such as oncocytic or clear cell types or in cytology materials [110, 111].

4.5.2 Adenoid Cystic Carcinoma

Adenoid cystic carcinoma (AdCC) is a common, salivary gland malignancy. AdCC histologically consists of epithelial and myoepithelial cells. Various architectural morphologies, including cribriform, tubular, and solid patterns, can be seen. Local recurrences, metastases, and perineural invasion are eventually expected in most AdCCs [108, 112].

4.5.2.1 Diagnostic Biomarkers

MYB/*MYBL1* rearrangement is highly specific for AdCC among salivary gland tumors, and it has been described in 29–86% of AdCCs [113–116]. A similar rearrangement involving *MYBL1-NFIB* fusion is another major molecular alteration which is reported in 9–14% of AdCCs [115, 117, 118]. *MYB* FISH is a useful diagnostic biomarker to distinguish AdCC from other types of salivary gland tumors [115, 119, 120]. Increased nuclear *MYB* expression has been observed in up to 85% of cases [121, 122]. *MYB-NFIB* translocation is not always correlated; generally, MYB immunoexpression is higher in AdCCs with the *MYB-NFIB* fusion [110, 120, 123].

4.5.2.2 Prognostic/Predictive Biomarkers

Activating mutations in *NOTCH1* gene or loss-of-function mutations in *SPEN* gene seems to be associated with high-grade transformation [124].

MYB activation may represent a potential therapeutic target for AdCCs. Potential approaches include using short interfering RNA-mediated silencing of gene expression, targeting downstream effectors of *MYB*, and blocking protein-protein interactions in transcription complexes [125, 126]. Additionally, Notch signaling pathway inhibitors (including gamma-secretase inhibitors and Notch receptors targeting antibodies) and *PI3K*/*IGF*/*FGFR1* pathway inhibitors are potential therapeutic targets in a subset of AdCC. Partial response to Notch signaling pathway inhibitors has been reported in several case reports [127–129]. NICD (Notch intracellular domain) expression by immunohistochemistry is a sensitive tool for detecting *NOTCH1* mutations [127].

4.5.3 Acinic Cell Carcinoma

Acinic cell carcinoma (AciCC) is a low-grade carcinoma, composed of ductal and acinar cells with basophilic cytoplasm.

Table 4.3 Characteristic genetic changes in salivary gland neoplasms

Salivary gland neoplasms		Frequent molecular alterations and frequencies		Rare molecular alterations
Carcinoma ex-pleomorphic adenoma		*PLAG1* alterations *HMGA2* alterations	>50% 10–20%	
Mucoepidermoid carcinoma		*CRTC1-MAML2* and *CRTC3-MAML2* fusions	55–88% 5%	*EWSR1-POU5F1* fusion
Adenoid cystic carcinoma		*MYB-NFIB* and *MYBL1-NFIB* fusions	29–86% 9–14%	*MYB-PDCD1LG2*, *MYB-EFR3A*, *MYBL1-RAD51B*, *MYBL1-YTHDF3*, *NFIB-AIG1* fusions
Acinic cell carcinoma		*SCPP* gene cluster*- *NR4A3* fusions	84%	*HTN3-MSANTD3* fusion
Secretory carcinoma		*ETV6-NTRK3* fusion	*>95%*	*ETV6-RET*, *ETV6-MAML3*, *ETV6-MET* fusions
Polymorphous adenocarcinoma	Classic type	*PRKD1* somatic mutations	70%	
	Cribriform adenocarcinoma of minor salivary glands type	*ARID1A-PRKD1, ARID1A-DDX3X* fusion and variant *PRKD1, PRKD2*, and *PRKD3* fusions	80%	
Clear cell carcinoma		*EWSR1-ATF1* fusion	80–90%	*EWSR1-CREM* fusion
Intraductal carcinoma	Intercalated duct type	*NCOA4-RET* fusion	47%	*TUT1-ETV5*, *KIAA1217-RET* and *STRN-ALK* fusions *BRAF V600E* mutations
	Apocrine or hybrid type	*TRIM27-RET* fusion		
Salivary duct carcinoma		*AR* gene alterations *ERBB2* amplification *TP53*, *PIK3CA*, *H-RAS*, *KIT*, *EGFR*, *BRAF*, *N-RAS*, *AKT1*, *FBXW7*, *ATM, NF1* mutations Loss of heterozygosity of *CDKN2A/p16* and *PTEN*	40–70% 29–35%	*NCOA4-RET* *ETV6-NTRK3* *BCL6-TRADD* *HNRNPH3-ALK* *EML4-ALK* *ABL1-PPP2R2C* fusions
Myoepithelial carcinoma		*EWSR1* rearrangements	35%	*PIK3CA* and *HRAS mutations*
Epithelial myoepithelial carcinoma		*HRAS* mutations	33–83%	*PLAG1*, *TP53*, *FBXW7*, *PIK3CA*, *CTNNB1*, *AKT1* mutations

4.5.3.1 Diagnostic Biomarkers

AciCC displays PAS-positive, diastase-resistant zymogen granules, and it is positive for amylase, DOG-1, and CD117.

A recurrent translocation between the SCPP gene cluster and *NR4A3* fusion by t(4;9)(q13;q31) is recently reported in 84% of AciCCs [130]. SCPP gene cluster contains several genes, such as *STATH*, *HTN3*, and *HTN1*. The nuclear NR4A3 expression is a reliable biomarker with high specificity (100%) and sensitivity (98%) for the diagnosis of AciCC [130].

Additionally, *MSANTD3* rearrangements are described in 4–15% of AciCCs, and particularly *MSANTD3-HTN3* translocation has been identified in around 4–8% of AciCCs [131, 132].

4.5.4 Secretory Carcinoma

Secretory carcinoma (SC) (previously known as mammary analog secretory carcinoma) is a low-grade salivary gland carcinoma. This entity is recognized as SC due to different morphological,

Table 4.4 A summary of frequent genetic aberrations and potential related immunohistochemical biomarkers in malignant salivary gland tumors

Tumor type		Genetic aberrations	Related IHC marker
Mucoepidermoid carcinoma		*CRTC1* rearrangements	AREG
Adenoid cystic carcinoma		*MYB* rearrangements	Myb
		NOTCH1 mutations	NICD
Acinic cell carcinoma		*SCPP* gene cluster*-*NR4A3* fusions	NR4A3
Polymorphous adenocarcinoma	Classic type	*PRKD1* somatic mutations	–
	Cribriform adenocarcinoma of minor salivary glands type	*ARID1A-PRKD1*, *ARID1A-DDX3X* fusion and variant *PRKD1*, *PRKD2*, and *PRKD3* fusions	–
Clear cell carcinoma		*EWSR1-ATF1* fusion	–
Basal cell adenocarcinoma		*CYLD* mutation	–
Intraductal carcinoma	Intercalated duct type	*NCOA4-RET* fusion	–
	Apocrine or hybrid type	*TRIM27-RET* fusion	
Salivary duct carcinoma		*AR* gene alterations *ERBB2* amplification	AR HER2
Myoepithelial carcinoma		*EWSR1* rearrangements	–
Epithelial myoepithelial carcinoma		*HRAS* mutations	–
Carcinoma ex-pleomorphic adenoma		*PLAG1* rearrangements *HMGA2* rearrangements	Plag1 Hmga2
Secretory carcinoma		*NTRK* rearrangements	Pan-Trk

immunohistochemical, and molecular features from AciCC [108, 133, 134].

4.5.4.1 Diagnostic Biomarkers

MUC4, S100, mammaglobin, GCDFP-15, GATA-3, and CD117-positive SC harbors the *ETV6-NTRK3* fusion gene due to t (12;15) (p13;q25) translocation, unlike AciCC [133]. A small subset of SC harbors ETV6 translocations with unknown partners other than *NTRK3* designated *ETV6*-X fusions [135, 136].

4.5.4.2 Prognostic/Predictive Biomarkers

NTRK3 fusion is both a diagnostic and predictive biomarker. *ETV6-RET, ETV6-MAML3*, and *ETV6-MET* translocations have recently been reported in subsets of SCs and observed to be related with aggressive biological features [137–140].

4.5.5 Polymorphous Adenocarcinoma

Polymorphous adenocarcinoma (PAC) is a rare salivary gland tumor that generally has a good prognosis and is predominantly seen in minor salivary glands. The PAC-classic variant and the PAC- cribriform adenocarcinoma of minor salivary glands (CAMSG) variant are defined within the PAC spectrum in the new WHO classification [110, 141, 142].

4.5.5.1 Diagnostic Biomarkers

PRKD1 E710D hotspot point mutations have been identified in more than 70% of classic variant PACs [143, 144]. Rearrangements in *PRKD1*, *PRKD2*, or *PRKD3* genes rather than point mutations have been noted in about 80% of CAMSG-variant PACs [145]. *ARID1A* and *DDX3X* are identified as the partner genes of *PRKD1* rearrangement [142].

4.5.5.2 Prognostic/Predictive Biomarkers

Although the knowledge about the prognostic and clinical significance of *PRKD* mutation is limited, the *PRKD1* mutation may be associated with good, metastasis-free survival. Fusion-positive PACs are usually located in the base of the tongue and histologically show papillary pattern. They have a high risk of nodal metastasis [146].

4.5.6 Clear Cell Carcinoma

Clear cell carcinoma (CCC) is a low-grade salivary gland tumor, with clear cells, with or without hyalinization and typically arising in minor salivary glands, [108, 147].

4.5.6.1 Diagnostic Biomarkers

CCC is positive for p63 and PANCK and negative for S100, smooth muscle actin, calponin, and GFAP.

The *EWSR1-ATF1* fusion is a major molecular aberration in 80–90% of CCCs [148]. *EWSR1-ATF1* fusion is specific for CCCs among salivary gland malignancies; however, it should be noted that *EWSR* fusion with other genes may be detected in other salivary gland tumors.

4.5.7 Basal Cell Adenocarcinoma

Basal cell adenocarcinoma (BCAC) is a rare, low-grade, salivary gland malignancy with an indolent clinical course and is formed by nests and glandular structures, consisting of basal and myoepithelial neoplastic cells [1].

4.5.7.1 Diagnostic Biomarkers

Molecular alterations in the *CYLD* gene are identified in BCACs, notably the membranous type. These BCACs may present as a sporadic tumor or in the setting of a hereditary syndrome-related tumor, such as Brooke-Spiegler syndrome [149, 150].

Molecular alterations involving the *PIK3CA* and *NFKBIA* genes have also been reported in BCACs [151].

4.5.8 Intraductal Carcinoma

Intraductal carcinoma is (IC) a rare, low-to-intermediate-grade, recently described malignant salivary gland neoplasm which exhibits histologic features that are similar to mammary atypical ductal hyperplasia and ductal carcinoma in situ. IC tumors are histologically grouped in three patterns: apocrine, intercalated duct, and hybrid type [108, 152].

4.5.8.1 Diagnostic Biomarkers

ICs harbor RET gene rearrangements. *NCOA4-RET* fusion has been identified in 47% of intercalated duct type ICs, whereas *TRIM27-RET* fusion is generally observed in a subset of apocrine or hybrid type ICs [152–156].

4.5.9 Salivary Duct Carcinoma

SDC is a high-grade malignant neoplasm that mostly arises from the parotid gland. SDC is one of the most aggressive salivary gland tumors and shares similar microscopic features with high-grade mammary ductal carcinoma [108].

4.5.9.1 Diagnostic Biomarkers

The AR immunoexpression has been detected in more than 70% of SDCs and is seen in almost all apocrine variant SDCs [157, 158]. The amplification of *ERBB2* (also known as *HER2*) is identified in approximately one-third of SDCs [159]. The most frequent gene alterations in SDCs are observed in *TP53* (about two-thirds of SDCs), followed by the *PIK3CA* and H-*RAS* genes. Mutations of the KIT, *EGFR*, *BRAF*, *N-RAS*, *AKT1*, *FBXW7*, *ATM*, and *NF1* genes, loss of heterozygosity of *CDKN2A/p16*, and loss or mutation of PTEN have also been identified in a subset of SDC [159–169]. Gene fusions involving PLAG1 and HMGA2 have been detected in SDC ex-PA [110, 162].

4.5.9.2 Prognostic/Predictive Biomarkers

TP53 mutations and *ERBB2* amplification are related with an unfavorable prognosis in SDCs and are also more commonly observed in SDCs ex-PA than de novo counterparts [165].

AR immunoexpression appears to be both a diagnostic and predictive biomarker in SDCs [170]. Androgen deprivation therapy (ADT) seems to be an potential option with promising results for tumors with AR expression (Fig. 4.7) [171].

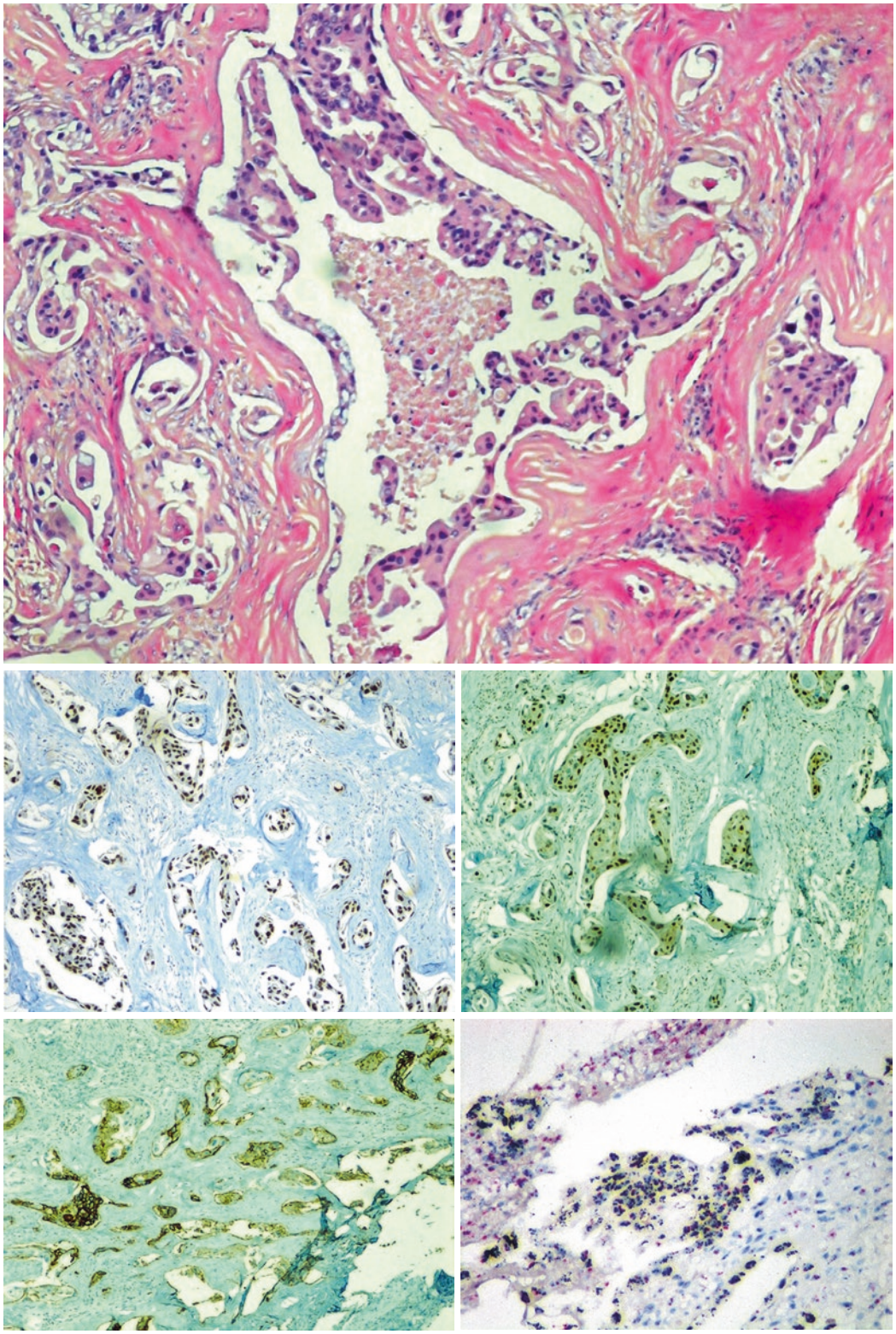

Fig. 4.7 Salivary duct carcinoma of the parotid gland; high ki67 expression, androgen receptor, cerbB2 positivity with HER2 amplification (SISH, CEP 17 red, HER2 black) (H&E, ki67, androgen receptor, c-erbB2, HER2 SISH, original magnification ×40)

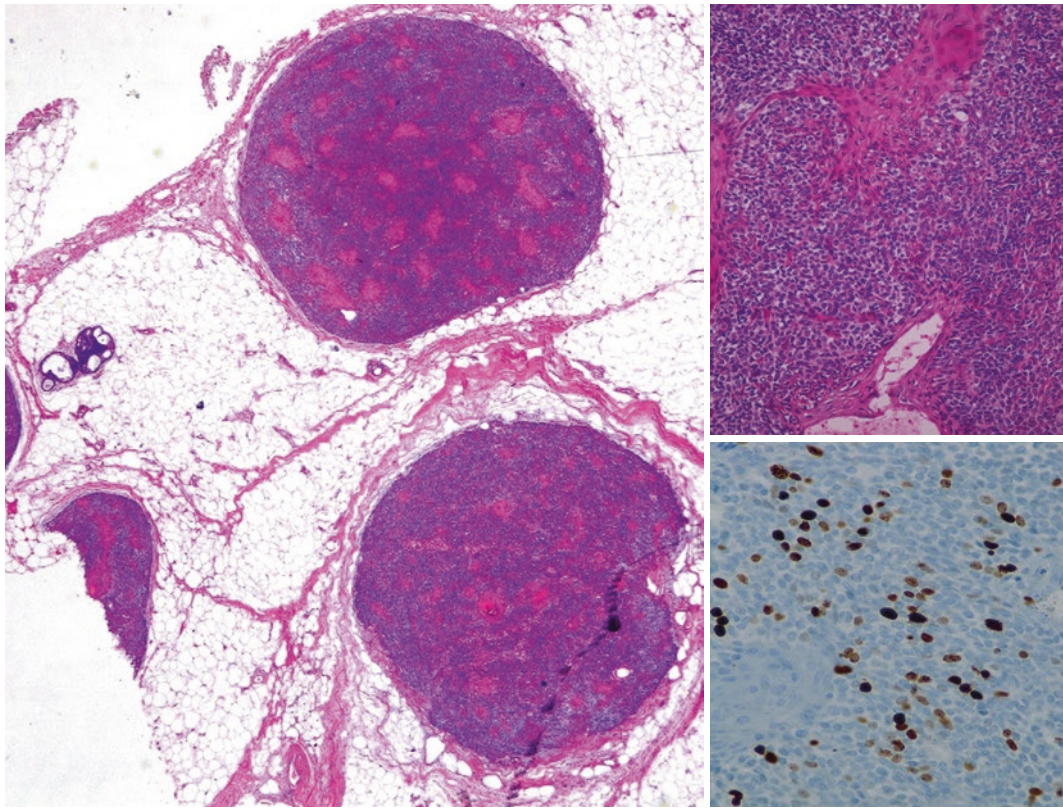

Fig. 4.8 Myoepithelial carcinoma following recurrent pleomorphic adenoma; note high ki-67 index (H&E, X2, H&EX40, IHC, ki67X40)

SDC cases with *ERBB2* amplification seems to have benefitted from anti-ERBB2 treatment [172].

4.5.10 Myoepithelial Carcinoma

MC is an uncommon malignant salivary gland tumor, fully composed of myoepithelial cells with an infiltrative pattern.

4.5.10.1 Diagnostic Biomarkers

Myoepithelial markers and increased ki67 expression may help in the diagnosis of malignant myoepithelioma (Fig. 4.8). *EWSR1* gene rearrangement has been identified in approximately one-third of MCs, which have predominantly clear cell morphology and aggressive clinical behavior [173, 174]. However, it is recently reported that none of MCs with *EWSR1* rearrangements identified with FISH had an *EWSR1* fusion transcript by sequencing methods, and this type of *EWSR1* abnormality in MCs may represent a passenger mutation with minor effect [175].

4.5.11 Epithelial-Myoepithelial Carcinoma

EMC is a rare, biphasic tumor with low malignant potential.

4.5.11.1 Diagnostic Biomarkers

HRAS mutations are the leading molecular aberrations in EMCs, including p.Q61R and p.Q61K, which have been reported in 33% to 83% of EMCs [176, 177]. In addition, *PIK3CA*, *CTNNB1*, and/or *AKT1* mutations may accompany HRAS mutations [176, 178, 179].

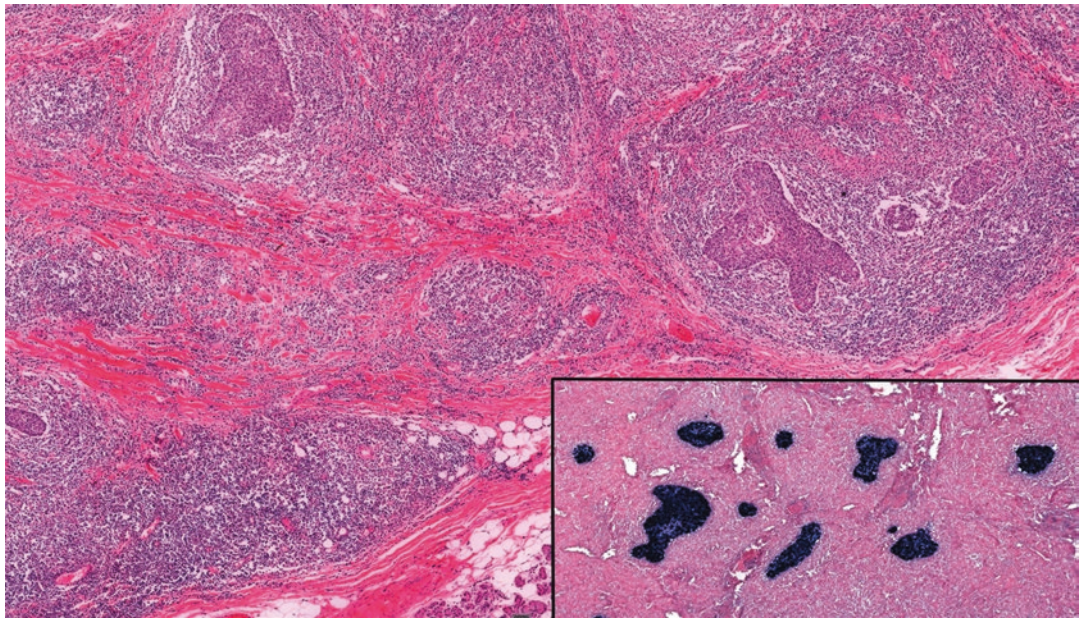

Fig. 4.9 Lymphoepithelial carcinoma of the parotid gland with dense lymphocytic infiltration and EBER positivity, mimicking myoepithelial sialadenitis (H&E, original magnification ×10, EBER CISH, original magnification ×4)

4.5.12 Sebaceous Carcinoma

Sebaceous adenocarcinoma (SAC) is a malignant tumor consisting of sebaceous cells.

4.5.12.1 Diagnostic Biomarkers

SAC is stained with EMA, antiadipophilin, CA15–3, and AR but is negative with BerEP4.

4.5.13 Lymphoepithelial Carcinoma

Lymphoepithelial carcinoma (LC) is an uncommon malignant tumor that occurs in salivary glands as well as in other organs. LCs are composed of undifferentiated malignant epithelial cells and non-tumoral lymphoid stroma [108].

4.5.13.1 Diagnostic Biomarkers

LCs are often associated with the Epstein-Barr virus (EBV). EBV can be detected using in situ hybridization for Epstein-Barr virus-encoded small RNA (EBER) and by PCR to detect latent membrane protein-1 (LMP-1) (Fig. 4.9) [180].

4.5.14 Oncocytic Carcinoma

Oncocytic changes may be seen in different types of salivary gland tumors like AciCC and MEC. Oncocytic carcinoma is a malignant epithelial neoplasm composed of oxyphilic cells and does not have histopathological features of other salivary gland tumor types. Mitochondrial antigen and BSND staining may help in identifying oncocytes [181, 182].

4.5.15 Carcinoma ex-Pleomorphic Adenoma

Carcinoma ex-pleomorphic adenoma (Ca-ex-PA) arises within a preexisting PA as a malignant proliferation that is often a high-grade carcinoma such as SDC but may also be any other malignant salivary gland tumor.

4.5.15.1 Diagnostic Biomarkers

The detection of *PLAG1* or HMGA2 rearrangements appears to be useful to distinguish Ca-ex-PA from its de novo counterparts [183,

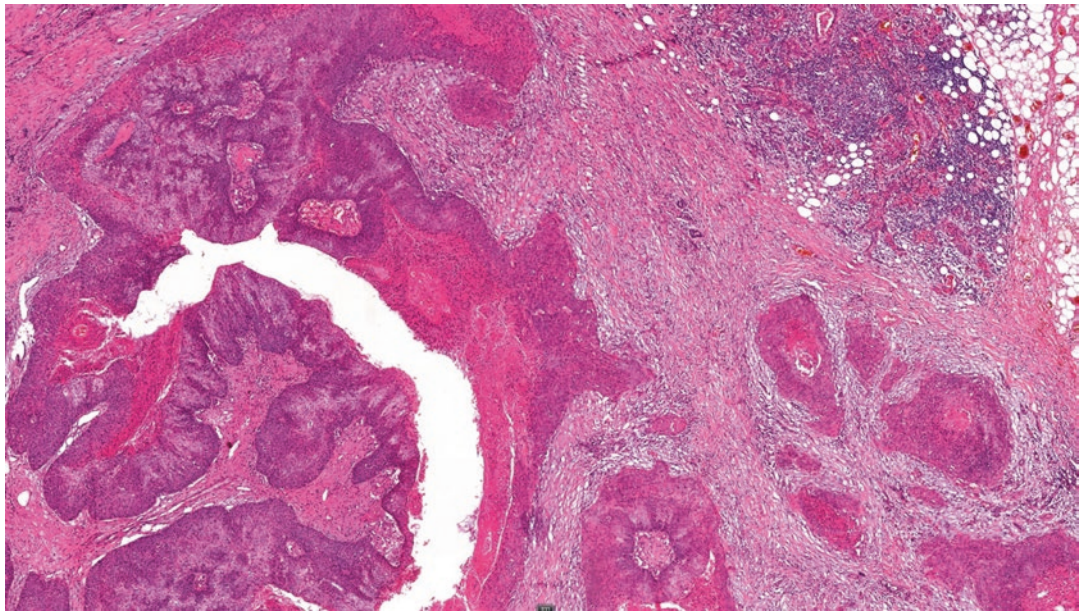

Fig. 4.10 Metastatic squamous cell carcinoma to the parotid gland (H&E, original magnification ×4)

184]. *TP53* mutations and/or *HER2* amplification may help to differentiate Ca-ex-PA from pleomorphic adenoma.

4.5.16 Emerging Entities

4.5.16.1 Microsecretory Carcinoma

Microsecretory carcinoma (MsC) is a recently proposed distinct low-grade salivary gland tumor type which harbors a novel gene fusion: *MEF2C-SS18*. MsCs display unique histologic features including intercalated duct-like cells, infiltrative microcysts, tubules and cords, intraluminal secretions, and fibromyxoid stroma. Tumor cells are S100 and p63 positive but p40 negative [185, 186].

4.5.17 Metastatic Tumors to the Salivary Glands

Salivary glands appear as one of the most frequently metastasized regions in head and neck. The most frequent salivary gland site is the parotid gland. Common primary sites for metastatic carcinomas include other head and neck regions, skin, lung, kidney, prostate, uterus, ovary, breast, and colorectum [187, 188]. Frequent histologic subtypes include squamous cell carcinomas arising from other head and neck regions or skin and malignant melanoma, renal cell carcinoma, lung carcinoma, and breast carcinoma [189, 190]. Lack of expression of myoepithelial markers in any tumor at the salivary gland may raise a suspicion of the possibility of metastatic carcinoma.

Primary or metastatic squamous cell carcinomas similarly express p63, p40, and CK5/6. Primary SCC of the salivary gland is quite rare. Primary SCC is a diagnosis of exclusion of metastatic tumors. The distinction between metastatic and primary salivary gland SCCs cannot be made by histology or immunohistochemistry. Before accepting as primary salivary gland SCC, any possible primary sites should be searched, and potential partially sampled mucoepidermoid carcinoma should be excluded (Fig. 4.10).

Malignant melanoma is positive for S100, HMB45, MART1, and MITF. SOX10 is also useful for diagnosis of malignant melanoma, but the latter is also a marker for salivary gland tumors arising from intercalated ducts or acini (Fig. 4.11).

Renal cell carcinoma expresses CD10, PAX8, and RCC Ag, while primary salivary gland carcinomas are stained with CK7. Clear cell renal cell

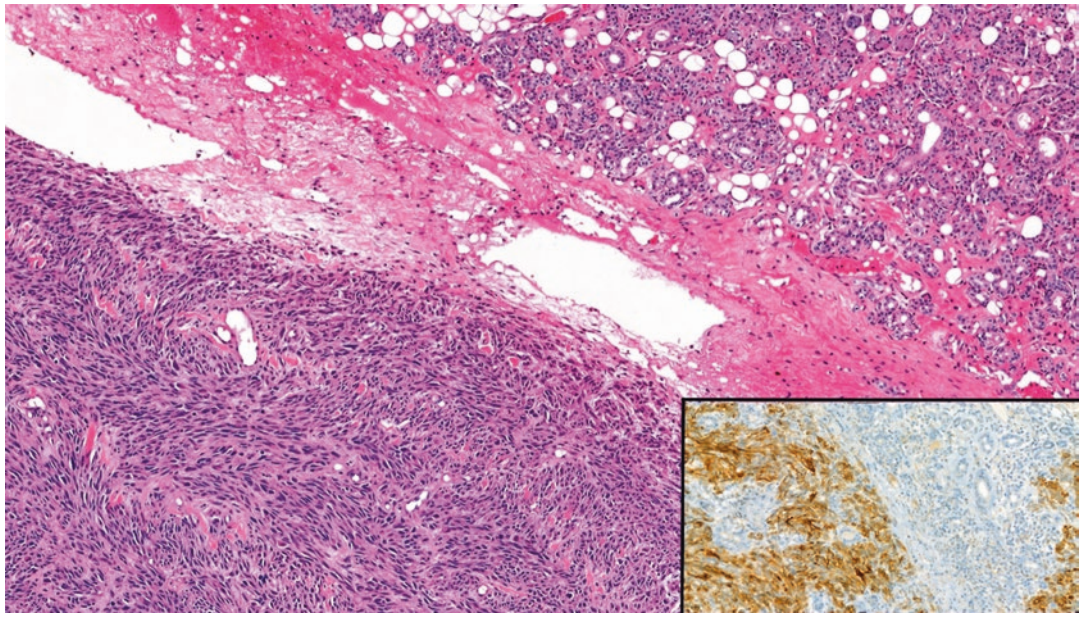

Fig. 4.11 Metastatic malignant melanoma to the parotid gland, MELAN-A positivity (H&E, original magnification ×4, IHC Melan-A, original magnification ×10)

carcinoma displays cytoplasmic clearing due to glycogen and lipid content and may mimic salivary gland tumors with clear cell components, such as mucoepidermoid carcinoma, clear cell carcinoma, and epithelial-myoepithelial carcinoma.

Estrogen receptor, progesterone receptor, GATA-3, mammaglobin, and GCDFP-15 are commonly utilized biomarkers in the diagnosis of metastatic carcinoma of the breast, but salivary gland tumors express GATA-3, while salivary duct carcinoma or secretory carcinoma of salivary glands may also express mammaglobin and GCDFP-15. Additionally, secretory carcinoma and salivary duct carcinoma share similar phenotypic and molecular features with counterparts in breast, secretory carcinoma of breast, and luminal androgen receptor type/molecular apocrine type of ductal carcinomas.

4.6 Odontogenic and Maxillofacial Bone Tumors

4.6.1 Ameloblastic Carcinoma

Ameloblastic carcinoma (AC) is an uncommon primary epithelial odontogenic malignant neoplasm which combines the histological features of ameloblastoma and cytologic atypia, poor differentiation, and high mitotic index.

4.6.1.1 Diagnostic Biomarkers

BRAF V600E mutations are detected in 40–60% of AC, as seen in other ameloblastic tumors, such as ameloblastomas, ameloblastic fibromas, and ameloblastic fibro-odontomas [191, 192].

SOX2 immunoexpression and higher Ki67 proliferative index in AC are more frequently detected in ameloblastic carcinoma than ameloblastoma.

4.6.1.2 Prognostic/Predictive Biomarkers

BRAF V600E mutation was associated with the aggressive behavior of AC [192].

4.6.2 Sclerosing Odontogenic Carcinoma

Sclerosing odontogenic carcinoma (SOC) is a locally aggressive and infiltrative odontogenic carcinoma.

4.6.2.1 Diagnostic Biomarkers

The tumor cells stain with PANCK, CK5/6, CK14, p63, and CK19 but are negative for CK7 and CK20 [193, 194].

4.6.3 Clear Cell Odontogenic Carcinoma

Clear cell odontogenic carcinoma (CCOC) is an odontogenic carcinoma which shares similar histological and immunohistochemical features with clear cell carcinoma of salivary glands. The tumor is composed of vacuolated and clear cells.

4.6.3.1 Diagnostic Biomarkers

CCOC harbors *EWSR1* translocations, mainly partner with *ATF1*; such tumors may be interpreted as an odontogenic analogue of clear cell carcinoma of salivary glands [195–197].

CCOC is positive for CK14, CK19, and pancytokeratin and negative for vimentin, S100, desmin, SMA, HMB45, alpha-1-antichymotrypsin, CD10, CD31, CD45, and GFAP.

4.6.4 Ghost Cell Odontogenic Carcinoma

Ghost cell odontogenic carcinoma is an odontogenic carcinoma which is composed of ghost cell keratinization and a dentinoid formation.

4.6.5 Metastatic Tumors to Maxillofacial Bone

The jawbones are frequently metastasized site due to their rich vascularization and high bone marrow content. The most common primary sites are the lung, kidney, prostate uterus, breast, colorectal, and ovary [198].

4.7 Ear

4.7.1 Squamous Cell Carcinoma

Squamous cell carcinoma is an external auditory canal or middle ear located malignant tumor which exhibit histological features that are no different from conventional SCC at any other site.

4.7.2 Ceruminous Adenocarcinoma

Ceruminous adenocarcinoma is a malignant tumor that arises from ceruminous glands of external auditory canal. Ceruminous adenocarcinoma is a heterogenous group of tumors involving adenocarcinoma, NOS, adenoid cystic carcinoma, and mucoepidermoid carcinoma. The latter two tumors show similar features those seen in salivary glands. Ceruminous adenocarcinoma has dual expression of luminal CK7 and CD117 and basal cell p63, S100, and CK5/6 expressions and low Ki67 index in most of the cases [199, 200].

4.7.3 Aggressive Papillary Tumor

Aggressive papillary tumor is a locally aggressive intermediate-grade tumor which composed of papillary pattern lined by two-layer cells. A subset of tumor is seemed to be related with von Hippel-Lindau syndrome.

4.7.4 Endolymphatic Sac Tumor

Endolymphatic sac tumor is a low-grade tumor that arises from endolymphatic sac of temporal bone. Tumor is composed of papillary or cystic architecture lined by usually one layer cell. This tumor shows a very high association with von Hippel-Lindau syndrome. Frequently CK5/6, CK8/18, EMA, synaptophysin, S100, and PAX8 positivity is observed [199, 201].

Questions

1. Which of the following squamous cell carcinoma location at head and neck area is the second highest for HPV positivity rate?
   (A) Oropharynx.
   (B) Larynx.
   (C) Oral cavity.
   (D) Sinonasal cavity.
   (E) Hypopharynx.

2. Which of the genetic alterations are characteristic for the sinonasal squamous cell carcinomas arising from inverted sinonasal papilloma and oncocytic sinonasal papilloma, respectively?
   (A) *KIT-NF1*.
   (B) *EGFR-KRAS*.
   (C) *KRAS-BRAF*.
   (D) *BRAF-KIT*.
   (E) *ALK-EGFR*.
3. Which of the tumor type shares phenotypic similarities with salivary duct carcinoma?
   (A) Mammary ductal carcinoma.
   (B) Clear cell renal cell carcinoma.
   (C) Colonic adenocarcinoma.
   (D) Pancreatic ductal adenocarcinoma.
   (E) Thyroid papillary carcinoma.
4. Which salivary gland tumor type shares histological and genetic similarities with clear cell odontogenic carcinoma?
   (A) Acinic cell carcinoma.
   (B) Secretory carcinoma.
   (C) Clear cell carcinoma.
   (D) Mucoepidermoid carcinoma.
   (E) Basal cell adenocarcinoma.
5. Which genetic aberration is frequently detected in ameloblastic carcinoma?
   (A) *EGFR* mutation.
   (B) *BRAF* mutation.
   (C) *KRAS* mutation.
   (D) *KIT* mutation.
   (E) *GNAQ* mutation.
6. Which of the following immunohistochemical marker is helpful for detecting Notch pathway mutations in adenoid cystic carcinoma?
   (A) LEF-1.
   (B) MYB.
   (C) PLAG1.
   (D) Pan-TRK.
   (E) NICD.
7. Which of the following immunohistochemical markers have predictive value in salivary duct carcinoma?
   (A) ER-PR.
   (B) EGFR-HER2.
   (C) AR-ER.
   (D) ER-HER2.
   (E) AR-HER2.
8. Which of the following salivary gland tumor harbors *NTRK* gene rearrangement?
   (A) Acinic cell carcinoma.
   (B) Secretory carcinoma.
   (C) Clear cell carcinoma.
   (D) Mucoepidermoid carcinoma.
   (E) Basal cell adenocarcinoma.
9. Which of the following gene is not often mutated in HPV-positive oropharyngeal squamous cell carcinoma?
   (A) *TRAF3*.
   (B) *CYLD*.
   (C) *YAP1*.
   (D) *PIK3CA*.
   (E) *E2F1*.
10. Which of the following gene rearrangements present in a salivary gland carcinoma expleomorphic adenoma?
    (A) *CTRC1, CRTC3*.
    (B) *MYB, NFIB*.
    (C) *PRKD1, PRKD2*.
    (D) *PLAG1, HMGA2*.
    (E) *RET, MET*.

Answers

1-D, 2-A, 3-B, 4-C, 5-B, 6-E, 7-E, 8-B, 9-C, 10-D.

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