Molecular Pathology of Head and Neck Cancer

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Squamous Cell Carcinoma

Overview of Head and Neck Squamous Cell Carcinoma

- Head and neck cancer includes diverse tumor types arising from various structures: craniofacial bones, soft tissues, salivary glands, skin, and mucosal membranes
  - 90% are head and neck squamous cell carcinomas (HNSCC)
- HNSCC is the sixth most common cancer in the world with more than 560,000 new cases diagnosed annually and over 300,000 deaths per year
  - HNSCC arises from the mucosal lining of the upper aerodigestive tract and demonstrates squamous differentiation
  - Historically, the most commonly affected patients are older males with a long history of cigarette smoking and alcohol consumption
- Surgery, chemotherapy, and radiation are the currently available treatments
- 5-year survival rate in head and neck cancer has remained unchanged at 50% over the past 20 years; novel methods of cancer detection and therapeutic options need to be developed
- Genetic, epigenetic, and viral agents have been identified as etiologic factors in the

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development of head and neck cancer, which is now considered a heterogeneous group of cancers based on molecular studies of tumor biology
  - HNSCC tumors now have distinct genetic profiles that differ in their risk factors, pathogenesis, and clinical behavior
  - Specific molecular alterations and molecular therapy have been associated with improved treatment response and prognosis
  - Biomarker refers to an objectively measured characteristic which may provide an indication of an outcome in question, such as response to treatment, metastasis, or survival
  - Various biomarkers and their application for diagnosing, staging, monitoring, and prognosticating HNSCC are being developed
  - Human papilloma virus (HPV) and epidermal growth factor receptor (EGFR) are the two most well studied and frequently used biomarkers in HNSCC
- Improved molecular characterization of primary tumors and surgical margins will soon allow clinicians to detect and treat earlier lesions, predict response to treatment, and tailor specific therapy

Risk Factors and Molecular Pathology in HNSCC

- Tobacco smoking is a well-known risk factor for HNSCC; the risk of developing HNSCC correlates to the duration and amount of smoking
  - Increased risk of developing HNSCC is attributed to the nitrosamines and polycyclic hydrocarbons in tobacco smoke
  - The molecular targets of cigarette smoke and tobacco-induced mutations are currently being studied
  - One example is TP53 mutations in HNSCC, which occur more frequently in patients who smoke than in those who do not smoke, but are not present in all patients who smoke
  - Individual differences in carcinogen metabolizing enzymes may modify individuals' risk of HNSCC development
- Alcohol consumption is an independent risk factor for developing HNSCC, especially for hypopharyngeal squamous cell carcinoma
  - Alcohol consumption and tobacco smoke synergistically magnify the risk of developing HNSCC
  - Acetaldehyde, the metabolite in alcohol, interferes with DNA synthesis and repair
  - Alcohol likely potentiates the effects of smoking because it is a chemical solvent and therefore prolongs mucosal exposure to the carcinogens in tobacco smoke
- HPV type 16 is the causative agent in up to 70% of oropharyngeal cancers (20–25% of all HNSCC), which is shifting the demographics of HNSCC toward younger patients with no alcohol or tobacco risk factors
  - HPV-18 and other subtypes can also be found in up to 5–10% of HNSCC
  - HPV-associated HNSCC is associated with oral HPV infection and sexual practices which increase oral viral exposure (these include early age of initial sexual activity, a high number of oral and vaginal sexual partners, frequent oral-genital and oral-anal contact, and decreased use of barriers during intercourse)
  - HIV positivity increases the frequency of oral HPV detection
  - Marijuana is an independent risk factor for HPV-positive HNSCC; the risk increases with intensity, duration, and cumulative years of marijuana use
  - Cannabinoids from marijuana bind the CB2 receptor on B cells, T cells, NK cells, macrophages, and dendritic cells in human tonsillar tissue, which suppresses immune responses to HPV infection inducing tumorigenesis
  - Tonsillar anatomy consists of crypts lined by reticulated squamous epithelium, which serves to transport foreign antigens from the external environment to the tonsillar lymphoid tissue allowing for the

direct passage of lymphocytes and antigen-presenting cells, which also allows for HPV deposition
  - The microanatomy of the crypt epithelium likely contributes to the clinical observation that small oropharyngeal carcinomas often present with advanced regional metastases

Histopathology of HNSCC

- Squamous dysplasia refers to alterations in the surface epithelium prior to invasion beyond the epithelial basement membrane
  - Changes include abnormal cellular organization, increased mitotic activity, and nuclear enlargement with pleomorphism
  - Dysplasia is graded 1–3 based on the severity of atypia (grade 3 dysplasia is also known as carcinoma in situ)
  - Progression beyond the basement membrane is invasive squamous cell carcinoma (SCCA)
- HNSCC has different pathological variants based on tumor differentiation; the most common type of HNSCC is moderately differentiated SCCA
  - Spindle cell variant consists of a proliferation of noncohesive spindle cells resembling a sarcoma more than a carcinoma
  - Verrucous carcinoma is an exophytic mass with markedly thickened squamous epithelium with "church spires" of parakeratotic squamous cells and broad pushing borders without atypia and no potential for metastasis
  - Papillary variant of HNSCC has a prominent exophytic component of papillary growth; papillary fronds are lined by malignant squamous cells
  - Basaloid squamous variant is highly aggressive and consists of solid lobules of cells with peripheral palisading, scant cytoplasm, and dark nuclei
  - A subtype of basaloid SCCA consists of HPV-16 positive tumors which have a significantly increased overall survival despite being more likely to present with lymph node metastases

Genetic Model of HNSCC

- A multistep process of genetic and epigenetic alterations results in the transformation of normal mucosa or epithelium into HNSCC
  - A tumor progression model hypothesis was developed for other tumors and has been applied to HNSCC
  - Neoplasms are the result of tumor suppressor gene inactivation and/or protooncogene activation
  - There is a defined order of genetic events leading to a specific tumor phenotype
  - The net accumulation of genetic alterations determines the tumor phenotype
- Allelic loss appears to be more common than allelic gain
- A tumor progression model has been proposed for head and neck cancer based on microsatellite analysis for allelic loss at ten major chromosomal loci in benign hyperplasia, dysplasia, carcinoma in situ, and invasive cancer
  - The accumulation and not necessarily the order of genetic events determines tumor progression into invasive carcinoma
  - The accumulation of genetic alterations tends to follow a sequential order
  - There is an increased rate of allelic loss in areas of apparently benign mucosa adjacent to premalignant lesions lending support to the concept of "field cancerization" derived from a common clone
  - Importantly for future screening possibilities, genetic damage often precedes microscopic changes
- Field cancerization was proposed by Slaughter 40 years ago to explain multifocal tumor origin as 10–40% of patients with HNSCC will develop a second tumor of the aerodigestive tract
  - Field cancerization refers to a large area of mucosa or epithelium surrounding the

primary tumor which possesses an increased potential to develop malignancy
  - Contemporary molecular biology using microsatellite analysis and X chromosome inactivation has shown synchronous and metachronous lesions in head and neck cancer that originate from a common clone
  - Lesions separated by time or distance may have a common clonal origin which evolve into cancer through an accumulation of successive genetic alterations
  - Therefore, genetically damaged cells may be present in surrounding mucosa without any histopathologic evidence of dysplasia, accounting for local recurrence despite surgical resection with negative margins determined histopathologically
- Identifying early events within a tumor progression model will allow for the clinician to observe or treat lesions which may appear histopathologically normal, but have a high risk for progression to malignancy

Common Genetic Alterations in Head and Neck Cancer

- The p53 and retinoblastoma (Rb) tumor suppressor pathways are the most common pathways altered in HNSCC
- Loss of p53 in chromosome region 17p13 is almost universal in HNSCC, as in other malignancies
  - p53 mutation frequency is directly proportional to worsened HNSCC histologic appearance
  - Loss of function of p53 is seen in the transformation from a preinvasive to invasive phenotype; further genetic alterations subsequently continue through the cell cycle without repair
  - A mutation rate of 50–79% has been observed in HNSCC tumors
  - p53 mutations may be an obligatory genetic alteration in HNSCC
  - p53 expression has been associated with poor prognosis and progression of disease
  - HPV 16 viral products (E6, E7) inhibit the p53 pathway by degrading wild type p53 protein
  - Therefore, HPV-positive HNSCCs are less likely to contain a p53 mutation than HPV-negative HNSCCs
- The disrupted Rb pathway occurs most commonly from a loss of the p16/p14^{ARF} genes in chromosome region 9p21–22
  - This is the most common genetic change in HNSCC and occurs early in the progression of head and neck tumors
  - p16 prohibits cells from entering the cell cycle by inhibiting cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), disrupting Rb phosphorylation and leading to G1 cell cycle arrest
  - p14ARF inhibits MDM2 from inhibiting p53 and therefore causes an antiproliferative effect in wild type cells
  - Disruption of these tumor suppressor genes in HNSCC is from genetic mutations, loss of heterozygosity, or inactivation by promoter hypermethylation
  - The frequency of loss of heterozygosity at 9p21–22 in HNSCC is 70%
  - In HPV-positive cancer, wild type p16 protein is consistently expressed and immunohistochemistry of p16 protein can be used as a marker of HPV status in HNSCCs
- Epidermal growth factor receptor (EGFR) overexpression is a predictor of increased locoregional recurrence and decreased overall and disease-free survival in HNSCC
  - EGF induces cell division, migration, adhesion, differentiation, and apoptosis via a tyrosine kinase-dependent pathway
  - More than 90% of HNSCC tumors overexpress EGFR
  - Overexpression of EGFR increases with increasing severity of dysplasia in premalignant lesions
  - EGFR downstream effectors also regulate cellular growth properties
  - Signal transducer and activator of transcription (STAT3) is a downstream effector of EGFR, which has antiapoptotic

properties in a head and neck cancer xenograft model
  - EGFR has been identified as a potential target for cancer therapeutics; adenoviral E1A gene products downregulate EGFR expression in HNSCC cell lines and induce apoptosis, which is reversible with EGFR expression
- Loss of chromosome 3p heterozygosity is another early genetic alteration observed with a 60% frequency in HNSCC
  - There is no consensus on the specific locus responsible for the tumor suppressor phenotype of chromosome 3p
  - Three distinct regions of loss have been mapped
- HER2 is an oncogene with homology to EGF and is overexpressed in 40% of oral squamous cell carcinomas
  - HER2 overexpression in breast cancers is related to a poor prognosis
  - Trastuzumab (Herceptin) is a monoclonal antibody which binds and blocks the growth factor receptor of HER2, resulting in greater survival rates
- Amplification of Cyclin D1 in the chromosome region 11q13 is reported in 33% of HNSCC tumors
  - Cyclin D1 is a protooncogene, also known as PRAD1 and CCD1
  - Cyclin D1 activates Rb via phosphorylation, causing the cell to progress from the G_1 phase to the S phase
  - Constitutive activation causes increased proliferation and therefore tumorigenesis
  - Both amplification of cyclin D1 and inactivation of p16 result in increased phosphorylation of Rb and movement of cells from G_1 to the S phase in the cell cycle
- p12 is a tumor suppressor gene implicated in S phase-associated growth suppression, through binding with DNA polymerase α-primase and/or CDK2
  - Delivery of a p12 murine gene suppresses tumor cell growth in vivo in an orthotopic mouse model of HNSCC
  - Upon delivery of p12, an antitumor effect of p12 is noted
  - Delivery of a p12 murine gene suppresses tumor cell growth in vivo in an orthotopic mouse model of HNSCC
  - Upon delivery of p12: increases in TUNEL labeling and apoptotic indices and decreases in Ki67 cell proliferation labeling indices as compared with controls are noted, resulting in an antitumor effect consistent with the role of p12 as a tumor growth suppressor in vivo
  - p12 inhibits cell turnover and tumor growth and as such, may be a potent therapeutic agent suitable for further development in cancer gene therapy
- Matrix metalloproteinase (MMP) molecules are zinc-dependent endopeptidases, which degrade the extracellular matrix (ECM) and are involved in tumor invasion and metastasis in HNSCC
  - Tumor invasiveness has a direct correlation to MMP-13 expression in HNSCC tumors
  - MMP-2 is expressed more in metastatic cancer cells than in nonmetastatic cancer cells and correlates with more aggressive tumor growth and poor prognosis
  - MMP-9 is associated with poor prognosis
  - Antibodies against MMP-9 showed a decrease in invasive properties of HNSCC cell lines
  - The expression of MMP-2, MMP-9, and MMP-13 may serve as valuable markers of tumor progression because of their role in tumor invasiveness
  - Specific cancer treatments targeting MMPs include chelators that bind zinc ions, MMP signaling pathway inhibitors, and MMP antibodies

Practical Molecular Pathology Applications in HNSCC

Current Practical Applications

- Biomarkers can be objectively measured to evaluate the presence and progression of disease
  - As molecular pathways in HNSCC become more understood, there is an increasing number of potential biomarkers

  - Biomarkers have thus far been used mostly as prognostic indicators in HNSCC, though none has consistently proved reliable across multiple studies and none are currently used in routine surgical pathology practice
  - Their role is expanding to include early cancer detection, more accurate tumor staging, selection for targeted therapies, and post treatment cancer surveillance
- HPV types 16 and 18 are associated with an increased risk of development of oropharyngeal and to a lesser degree oral cavity squamous cell carcinoma
- The E6 and E7 viral proteins of HPV degrades p53 and Rb, which inactivates tumor suppressive mechanisms
- HPV-positive tumors are almost always associated with wild type p53 and overexpression of the p16 protein
- p16 immunohistochemical staining can be used alone as a surrogate marker for the presence of HPV
- HPV-positive HNSCC tumors are more common in younger patients without a significant alcohol or smoking history
- HPV-positive tumors are more likely to be poorly differentiated with higher cervical lymph node metastases, but a better overall prognosis and response to therapy, regardless of therapeutic regimen
- HPV-positive tumors also have a lower risk of second primary tumors
  - Mechanisms underlying the improved clinical outcome in HPV-positive tumors may include the combined effects of immune surveillance to viral-specific tumor antigens, an intact apoptotic response to radiation, and the absence of widespread genetic alterations (field cancerization)
  - Mechanisms to detect HPV include polymerase chain reaction (PCR) based assays, real-time or quantitative PCR, and in situ hybridization
- EGFR expression is a consistent marker of poor prognosis, but its utility in predicting response to EGFR-targeted therapies is not yet completely elucidated
  - Cetuximab, an EGFR monoclonal antibody, is the only currently approved EGFR targeting drug for use
  - Cetuximab with RT has been shown to be effective in locoregionally advanced HNSCC; Cetuximab with platinum-based chemotherapy has shown a survival benefit in recurrent and metastatic HNS
  - There is no correlation between the effectiveness of EGFR-inhibitor therapies and molecular or immunohistochemical testing for EGFR in tumor samples

Future Practical Applications

- Tumor localization in cases of regional metastases with an unknown primary can be aided by the detection of certain oncogenic viruses that target specific regions of the aerodigestive tract
  - Epstein-Barr virus (EBV) in a neck metastasis reliably suggests the nasopharynx as the tumor origin
- The presence of HPV in a cervical lymph node metastasis reliably points to an oropharyngeal primary tumor
- The application of this approach is currently limited because the majority of HNSCC tumors are not linked to an oncogenic virus
- Detecting genetic alterations early in the tumor progression cycle may result in earlier diagnosis when cure is more attainable
  - Identifying premalignant lesions (dysplasias) that are more at risk for transformation into invasive carcinoma is difficult by histopathology alone
  - Among the studied biomarkers, loss of heterozygosity at defined chromosomal loci may be the most promising technique to identify high risk premalignant lesions
- Several studies have shown that dual loss of heterozygosity at 3p and 9p can distinguish lesions which are likely to progress to invasive carcinoma from those that will not
- The detection of undiagnosed HNSCC is currently under investigation by using saliva as a substrate for biomarker assessment

  - Saliva has been used to assess HPV status, promoter hypermethylation profiles, p53 gene mutations, telomerase activity, and gene expression profiles
  - More recently, saliva has been shown to harbor clinical discriminatory transcriptomic (IL1B, IL8, SAT1, S100P, DUSP1, and OAZ1) and proteomic (IL1B, IL8, CD59, Profilin, MRP-14, Catalase, and M2BP) biomarkers
  - miRNAs have also been identified in both whole saliva and supernatant saliva. Two of these miRNAs, miR-125a and miR-200a, are differentially expressed in the saliva of HNSCC patients compared with that of healthy controls. miRNAs in saliva can be used as a noninvasive and rapid diagnostic tool for the diagnosis of oral cancer.
  - A nationwide oral cancer saliva biomarker validation study is currently ongoing in a prospective specimen collection, to meet the guidelines of the early detection network (EDRN) of the National Cancer Institute
- HPV 16/18 status may be useful in screening at risk patients for development of HNSCC
  - A large double-blind, randomized clinical trial showed that the HPV 16 vaccine in young women significantly reduced the incidence of HPV-16 related cervical intraepithelial neoplasia; a similar vaccine may prevent HPV-associated HNSCC
  - There is a potential to select a high risk population such as heavy drinkers and smokers, and screen them for potential molecular alterations, such as HPV DNA
  - Patients with concerning molecular alterations could undergo closer observation
- Microarray analysis distinguishes gene expression differences between normal and malignant tissue and has been used more recently in characterizing gene sets that identify subgroups of HNSCC
  - The unique "molecular signatures" are being studied in predicting disease course, response to therapy, and survival
  - Gene sets and microarray analysis have been used to predict response to chemoradiation (CRT) and radiation therapy (RT)
  - Probe sets have differed between responders and nonresponders to CRT and RT
  - Microarray analysis and genetic expression profiling have the potential to be used as markers of prognosis and response to CRT and RT in the future
  - Proteomic analysis of normal epithelia and well-differentiated, moderately, and poorly differentiated HNSCC has shown differences at each stage and evaluation of histologically normal mucosa in HNSCC may be able to predict the development of second primaries and local recurrence
  - Proteomics accounts for posttranscriptional and translational modifications and may be more accurate in understanding tumor biology
  - Methods in detecting protein expression include two-dimensional differential in gel electrophoresis, surface-enhanced laser desorption/ionization time of flight mass spectrometry, and matrix-assisted laser desorption ionization time of flight mass spectrometry
- Hypermethylation of CpG-rich promotor regions leads to tumor suppressor gene inactivation and can be detected on molecular detection assays
  - Physiologic promoter hypermethylation occurs in X chromosome inactivation and genetic imprinting, but has been implicated in various cancers by inactivating tumor suppressor genes
  - The promoter hypermethylation status in HNSCC patients has been assessed using a panel of four tumor suppressor genes: p16, O6-methylguanine-DNA methyltransferase (MGMT), GST-π, and death-associated protein kinase (DAPK)
  - At least one of the four genes exhibited promoter hypermethylation in 42–56% of head and neck squamous cell tumors
  - Creating an assay sensitive enough for clinic use has been difficult because not all

tumors have currently identifiable methylated genes in HNSCC

- Determination of telomerase activity in patients with HNSCC tumors could select patients likely to benefit more from chemotherapeutic agents which induce DNA double-strand breaks
  - Telomeres are tandem repeats of DNA capping the ends of chromosomes; telomere shortening during cell cycles leads to natural cellular senescence
  - Telomerase maintains telomere length in pluripotent germline cells and is absent in somatic cells
  - Telomerase activity has been reported in 90% of invasive HNSCC cell lines and in 100% of premalignant lesions
  - Nonsteroidal antiinflammatory drugs possibly inhibit telomerase in HNSCC treated with indomethacin and ibuprofen
- Molecular analysis of surgical margins (using p53 and eIF4E) may supersede conventional histopathologic criteria, which is currently associated with a high rate of local recurrence
  - Unique single-stranded DNA probes complementary to each type of p53 mutation have been used in analysis of surgical margins in HNSCC
  - In one study, patients whose margins were negative by molecular mutation analysis had a statistically significant decrease in local recurrence compared to margins with p53 mutations and histologically negative margins
  - eIF4E is a protooncogene which initiates translation and has also been investigated by molecular margin analysis
  - eIF4E overexpression was present in 98% of primary tumors and 52% of histologically negative margins; patients with clear histologic margins positive for eIF4E had significantly higher local recurrence rates than margins without eIF4E overexpression
- Predictive molecular pathology identifying patterns of genetic alteration could be used to predict the behavior and tumorigenic potential of premalignant head and neck lesions and determine which tumors would be more amenable to certain therapeutic interventions
  - HPV(+) and p16(+) are highly predictive for poorly differentiated tumors and basaloid SCCA. Additionally, HPV and p16 positivity demonstrate superior predictive value for lymph node metastasis above standard H&E histopathologic features
- COX2 activation increases the expression of SNAIL, a transcription factor. SNAIL in turn binds to the promoter region of cell adhesion molecule E-cadherin, blocking its expression. This pathway is thought to be critical in epithelial–mesenchymal transition and subsequent aggressive HNSCC behavior
- SNAIL positivity is significantly predictive of poorly differentiated, lymphovascular invasive, as well as regionally metastatic tumors. Because SNAIL positivity appears independent of HPV, p16, and EGFR expression, SNAIL can improve upon these markers' predictive limitations.
  - Analysis of p53 status has shown improved survival and response rates with wild type p53, but also with absent p53 expression
  - In the future, analysis of p53 function may be more important in predicting tumor response because wild type p53 can be inactivated by methods other than genetic alterations, such as HPV product inactivation
  - Increased pretreatment IL-6 levels were found to be an independent predictor of both recurrence and poor survival in a large longitudinal, prospective cohort study
- Several molecular targets for molecular therapy are under investigation
  - Targeted therapy toward EGFR has been studied most extensively and consists of monoclonal antibodies, tyrosine kinase inhibitors (inhibit the phosphorylation function of the cellular domain of EGFR), antisense oligonucleotides, and small interfering RNAs (attacks EGFR in extracellular and intracellular domains and at the translational stage)
  - EGFR expression status in primary laryngeal HNSCC was shown to positively correlate with the development of secondary tumors
  - Hyperphosphorylation of EGFR independently has been shown to be associated with increased lymph node metastases and higher nodal stage of disease

  - STAT proteins, specifically STAT3 is overexpressed in HNSCC tumors; in vitro and in vivo studies targeting STAT3 using oligonucleotide decoys show antitumor effects
  - Therapeutic HPV vaccines are being studied targeting the antigens E6 and E7 via viral vector vaccines, bacterial vector vaccines, peptide/protein vaccines, DNA vaccines, and cell-based vaccines
  - Gene therapy targeting replacement of the mutated tumor suppressor gene p53 has been difficult due to creating adequate delivery systems to all affected cells

Nonsquamous Cell Carcinomas

Nasopharyngeal Carcinoma

- Clinical
  - Nasopharyngeal cancer (NPC) is rare throughout the world, but more common in certain geographic areas such as southern Asia
  - NPC is a squamous cell carcinoma that develops around the ostium of the Eustachian tube in the lateral wall of the nasopharynx
  - Both environmental and genetic factors play roles in the development of NPC
  - Environmental factors include nitrosamine exposure in salted and pickled foods
  - The strong association of NPC with EBV makes NPC unique from other head and neck cancers
  - Treatment consists of radiotherapy with or without concurrent chemotherapy
- Histopathology
  - The World Health Organization classifies NPC into three subtypes
    - Type 1: Keratinizing squamous carcinoma
    - Type 2: Nonkeratinizing squamous carcinoma
    - Type 3: Poorly differentiated with highly variable cell types
  - Types 2 and 3 are EBV-associated and have better prognoses than type 1
- Molecular pathology
  - EBV has been implicated in the molecular abnormalities leading to NPC, which consist of a large variety of pathways and the alteration in expression of numerous proteins
  - EBV has tumorigenic potential due to a unique set of latent genes: latent membrane proteins (LMP1, LMP2A, LMP2B) and EBV-determined nuclear antigens (EBNA1 and EBNA2)
  - LMP1 is the principal oncogene of NPC and is present in 80–90% of NPC tumors and activates a number of signaling pathways
  - LMP1 is also involved in suppressing immunogenic responses against NPC
  - There is upregulation of cellular proliferation pathways such as the Akt pathway, mitogen-activated protein kinases, and the Wnt pathway
  - Dysfunctional cell adhesion results from abnormal E-cadherin and β (beta)-catenin function
  - There is dysregulation of p16, cyclin D1, and cyclin E resulting in aberrations in the cell cycle
  - NPC also contains antiapoptotic mechanisms with upregulated antiapoptotic factors bcl-2, survivin, and telomerase
  - High levels of p53 are also found in NPC
    - Most head and neck cancers contain low levels of p53 due to mutations
    - High LMP1 levels correlate with higher p53 expression, which fail to induce apoptosis because of p53 inactivation
    - It is unclear why p53 levels in NPC are high
  - p16 levels are decreased in NPC in two thirds of NPCs
  - There are ongoing studies to develop molecularly based treatments against NPC

Sinonasal Malignancies

- Malignancies of the nasal cavity and paranasal sinuses comprise only 0.2–0.8% of all malignant neoplasms
- Sinonasal malignancies most commonly arise in the maxillary sinus, followed by the nasal

cavity, the ethmoid sinus, and the sphenoid and frontal sinuses
- The most common malignancy of the sinonasal tract is SCCA, followed by adenocarcinoma, minor salivary gland tumors, and undifferentiated small cell tumors (olfactory neuroblastoma (ONB), melanoma, and sinonasal undifferentiated carcinoma)

Sinonasal Adenocarcinoma

- Sinonasal intestinal type adenocarcinoma (ITAC) is morphologically similar to colorectal adenocarcinoma and is associated with occupational exposures to wood or leather dusts
- ITAC has a range of microscopic features
  - Some tumors are indistinguishable from colonic adenocarcinoma
  - Other tumors resemble mucinous or signet ring cell carcinoma of the colon
- The immunophenotypical and genetic profile suggest that ITAC tumorigenesis may have some distinct molecular mechanisms from colonic adenocarcinoma
- The most commonly altered oncogenes are p53 (18–40% of ITACs) and p16 (60% of cases)
  - p53 mutations were detected in 86% of adenocarcinomas
  - Overexpression of p53 has been observed in 60% of cases
- There are frequent losses at 18q, which is the chromosome region which contains genes implicated in colorectal tumorigenesis, such as deleted in colon cancer gene (DDC)
- Mucinous ITACs follow a distinct molecular pathway from nonmucinous variants and pursue an aggressive clinical behavior based on microarray analysis
  - p53 expression greater than 20% was statistically significantly higher in nonmucinous ITAC
  - DDC was significantly lower in mucinous versus nonmucinous ITAC
  - The absence of E-cadherin was present significantly more in nonmucinous ITAC
- Research examining the molecular basis for sinonasal adenocarcinoma is ongoing to further develop diagnostic, therapeutic, and prognostic biomarkers

Small Round Blue Cell Tumors of the Sinonasal Area

- These tumors represent diverse malignancies of epithelial, hematolymphoid, neuroectodermal, and mesenchymal origin which are challenging to differentiate because of overlapping cytomorphologic features
- Small round blue cell tumors are a monotonous population of undifferentiated tumor cells with small-sized nuclei and scant neoplasm
- Immunohistochemistry is a technique which aids in diagnosis
- The discovery in recent years of chromosomal alterations in certain small round blue cell tumors is becoming an invaluable tool in pathologic diagnosis of these tumors

Poorly Differentiated, Nonkeratinizing Squamous Cell Carcinoma

- Poorly differentiated SCCA has histopathologic features which overlap with other small round blue cell tumors
- Immunohistochemistry is useful in diagnosis
  - Cytokeratin immunoreactivity helps distinguish poorly differentiated SCCA from ONB
  - The lack of immunoreactivity for synaptophysin, chromogranin, and CD56 distinguishes poorly differentiated SCCA from neuroendocrine type carcinoma
- Recently, an undifferentiated carcinoma with focal squamous differentiation arising in the midline of the sinonasal cavity has been described called NUT midline carcinoma (NMC), which is an aggressive lesion with a mean survival of 9 months
  - They are thought to arise from primitive neural crest-derived cells
  - NMC lack definitive clinical and histologic features, but are grouped together based on molecular rearrangements of the NUT gene on chromosome 15q14
  - Two-thirds of these tumors contain a t(15;19)(q14;p13.1) translocation resulting in a chimeric fusion oncoprotein encoding BRD4-NUT

  - Diagnosis is by FISH or RT-PCR to detect NUT rearrangement
  - There are ongoing studies to develop antibodies and inhibitors against MYB-NFIB
  - This tumor is unique because it is defined molecularly

Sinonasal Undifferentiated Carcinoma (SNUC)

- SNUC is a rare, highly aggressive carcinoma that presents with locally extensive disease
- This tumor tends to grow along the mucosal surface, extending into superficial mucosal glands, and into lymphovascular spaces
- Immunohistochemistry is not very useful in establishing a diagnosis as they are immunoreactive for pancytokeratin and simple keratins
- There are no known molecular diagnostic tests to establish the diagnosis of SNUC

Small Cell Carcinoma, Neuroendocrine Type (SCCNET)

- SCCNET is a high grade neoplasm that most frequently arises from the superior or posterior nasal cavity with frequent sinonasal extension
- Most are positive for cytokeratin and CD56; EBV-RNA is negative
- Cytogenetic studies for SCCNET have not yet been discovered

Olfactory Neuroblastoma (ONB)

- ONB originates from the olfactory bulb in the region of the cribiform plate
- The presence of fibrillary cell processes, Homer Wright rosettes, and S100-positive sustentacular cells when present are useful in diagnosis
- Recently, array comparative genomic hybridization (aCGH) studies have shown complex gene copy number profiles with a gain of 13q, 20q, and loss of Xp in high stage tumors

Sinonasal Mucosal Malignant Melanoma

- The amelanotic variant of mucosal melanoma can be especially difficult to accurately diagnose
- Diffuse immunostaining for S100, HMB45, and vimentin are useful in distinguishing this malignancy
- Diagnostic molecular studies are still being developed

Extraskeletal Ewing Sarcoma/Primitive Neuroectodermal Tumor

- CD99 immunoreactivity can be useful in distinguishing this tumor from other small round blue cell tumors, but is also positive in desmoplastic small round cell tumors, synovial sarcoma, and lymphoma
- Primitive neuroectodermal tumor has a characteristic EWSRI-FLII fusion transcript or t(11;22)(q24;q12)
  - Identifying this fusion transcript is diagnostic of this tumor

Desmoplastic Small Round Blue Cell Tumor

- Only a single case has been reported in the sinonasal tract
- Recognition of the t(11;22)(p13;q12) and associated EWSR1-WT1 fusion transcript is necessary for diagnosis

Rhabdomyosarcoma

- Embryonal and alveolar rhabdomyosarcoma occur in the sinonasal cavity
- The identification of the 2;13 and 1;13 translocations or respective PAX3-FOXO1 and PAX3 variant translocations is extremely useful in diagnosis as the immunohistochemical profile of rhabdomyosarcoma can often be misleading
  - These translocations create a chimeric oncogene

Extramedullary Plasmacytoma

- This tumor affects adults over the age of 65 and involves the sinonasal cavity in 75% of cases
- Diagnostic confirmation is based on immunohistochemistry or in situ hybridization for immunoglobulin mRNA with the identification of light chain restriction

Extranodal NK/T Cell Lymphoma

- NK/T cell lymphoma is an aggressive disease known for necrosis, vascular invasion, and destruction
- In situ hybridization demonstrating the presence of EBV virus by detecting EBV-encoded early RNAs in addition to an NK-cell immunophenotype is diagnostic

Salivary Gland Malignancies

Mucoepidermoid Carcinoma (MEC)

- Clinical
  - MEC is the most common salivary gland malignancy and appears in both minor and major salivary gland locations
  - MEC most commonly presents as a painless salivary gland mass (parotid gland is the most common location)
  - MEC occurs more commonly after radiation exposure
  - Clinical aggressiveness and rate of regional metastasis depend on the histologic grade
  - Treatment for low and intermediate grade MEC is wide local excision
  - Treatment for high grade MEC is wide local excision, elective neck dissection, and postoperative radiation therapy
- Histopathology
  - Three cell types are required for diagnosis: epidermoid, intermediate, and mucinous cells
  - Grading schemes rely on the percentage of the tumor composed of cystic spaces and more recently on point systems
- Molecular pathology
  - A translocation between the MECT1 gene and the MAML2 gene (t(11;19)(q12;p13)) has been identified, which initially appeared more prevalent in low and intermediate grade MEC than in high grade MEC; however, a recent study showed higher rates of this translocation in high grade MEC
  - The MECT1–MAML2 translocation can be identified using fluorescent in situ hybridization or assays based on reverse transcription PCR
  - Some studies have demonstrated this translocation in Warthin tumor
  - No other malignant salivary gland tumors have shown this translocation

Adenoid Cystic Carcinoma

- Clinical
  - Presents most commonly as a painless salivary gland mass which can occur in both major and minor salivary glands
  - Adenoid cystic carcinoma (ACC) is known for its propensity for perineural invasion and late recurrences both locoregionally and in the form of distant metastases
  - Treatment consists of wide local excision of the involved salivary gland and postoperative radiation for positive margins and/or perineural invasion
- Histopathology
  - ACC is a biphasic salivary gland tumor, containing both epithelial and myoepithelial cell components
  - Growth patterns fall into three groups: tubular, cribiform, and solid types
  - The presence of a significant solid component >30% is considered high grade and has a worse prognosis
- Molecular pathology
  - Interest in the molecular pathology of ACC began with the identification of c-kit (CD117) overexpression in ACC, but most studies have found no evidence of c-kit mutations
  - Imatinib, a monoclonal antibody against c-kit, has had low efficacy in ACC
  - c-kit is seen in other tumors (polymorphous low-grade adenocarcinoma and epithelial–myoepithelial carcinoma) and therefore cannot be used as a diagnostic marker in ACC
  - Recent studies have described a translocation fusing the MYB gene and the NFIB gene into a chimeric transcript (t(6;9)(q22–23;p23–24)) causing overexpression of MYB, which is specific to ACC and can be potentially used not only as a biomarker for ACC, but also to determine potential downstream therapeutic targets

  - Comparative genomic array technology and loss of heterozygosity studies have not shown widespread loss and gain mutations, which are more common in more aggressive tumors
  - Isolated genomic losses have been detected at individual genomic loci, including loss of heterozygosity of 12q, 1p, and 9p
  - p53 is likely involved in high grade transformation of ACC, as these tumors overexpress p53 and contain p53 gene mutations

Mammary Analog Secretary Carcinoma of the Salivary Gland

- Clinical
  - Newly described salivary gland tumor that histologically resembles secretory carcinoma of the breast and is an aggressive tumor
  - Most of these tumors have likely been misdiagnosed as acinic cell carcinomas because of histologic overlap
- Histopathology
  - The tumor grows in an infiltrative pattern with microcystic and tubular patterns
  - Secretary material within luminal spaces is PAS positive and diastase resistant
  - Low-grade, bland, monomorphic nuclei with vesicular type chromatin with prominent centrally placed nucleoli
  - Positive for CK7, vimentin, and S100 on immunohistochemistry
- Molecular pathology
  - Salivary gland mammary analog secretory carcinoma contains a translocation between ETV6 and NTRK3 genes, similar to the tumor version in the breast
  - The translocation can be detected using RT-PCR or fluorescent in situ hybridization

Thyroid Carcinoma

- The rapidly expanding knowledge of molecular genetics of thyroid cancer is beginning to translate into clinical practice to improve accuracy in preoperative diagnosis and prognostication

Papillary Thyroid Carcinoma (PTC)

- PTC is the most common type of thyroid carcinoma, accounting for 80% of all thyroid malignancies
- PTC carries point mutations of the BRAF and RAS genes and rearrangements of RET/PTC and TRK
  - All of these genetic alterations are able to activate the mitogen-activated protein kinase (MAPK) pathway
  - These mutually exclusive mutations are found in over 70% of PTCs
- BRAF is a serine threonine kinase belonging to the family of RAF proteins, which are intracellular effectors of the MAPK signaling cascade
  - Point mutations of the BRAF gene are the most common genetic alteration in PTC, occurring in 40–45% of tumors (the most common point mutation is Valine to Glutamate substitution at residue 600, V600E)
  - Point mutations in BRAF lead to constitutive activation of BRAF kinase and chronic stimulation of the MAPK pathway
  - BRAF V600E is found in PTC with classic pathology as well as the tall cell variant
  - BRAF V600E can also be seen in poorly differentiated and anaplastic carcinomas, especially those containing areas of well-differentiated PTC
  - BRAF V600E has not been found in follicular carcinomas and benign thyroid nodules, making it a specific marker of PTC and its related types
  - Studies have shown that BRAF V600E testing in fine needle aspiration (FNA) samples of thyroid nodules improves the accuracy of cytologic diagnosis (one large study showed a rate of malignancy of 99% in BRAF-positive nodules)
  - Molecular testing can be done using probe-specific real-time PCR, real-time allele-specific PCR, direct sequencing, and colorimetric assay
  - BRAF V600E may also be a good prognostic marker for PTC; It is associated with more aggressive tumor characteristics such as extrathyroidal extension, advanced

tumor stage at presentation, and lymph node, or distant metastases
  - BRAF V600E has also been shown to be an independent predictor of treatment failure, tumor recurrence, and tumor-related death
  - The prognostication of BRAF V600E has also been shown in T1 PTC tumors and papillary microcarcinomas (tumors less than 1 cm)
- The RET protooncogene encodes a cell membrane receptor tyrosine kinase and is highly expressed in thyroid parafollicular or C cells, but not in follicular cells; it is activated by the RET/PTC chromosomal rearrangement
  - 11 types of RET/PTC rearrangements have been reported with the two most common being RET/PTC1 and RET/PTC3
  - All fusions contain the intact tyrosine kinase domain of the RET receptor, enabling the RET/PTC protein to activate the MAPK signaling pathway
  - RET/PTC has been detected in some adenomas and benign lesions, but clonal RET/PTC (found in a significant portion of tumor cells) is specific for PTC
  - For frozen or freshly collected tumor tissue or FNA sample, RT-PCR is an adequate detection method
  - For formalin-fixed and paraffin-embedded tissue, FISH is the assay of choice; the assay should be set up to detect no fewer than 8–12% of cells with the rearrangement patterns
  - Clonal RET/PTC rearrangements are found in 10–20% of adult sporadic PTCs; RET/PTC occur in 50–80% of patients with a history of radiation exposure and in 40–70% of PTCs in children and young adults
  - Testing for RET/PTC rearrangements is of limited use in surgical specimens since PTC architecture is usually obvious, but they can be useful in preoperative diagnosis of thyroid nodules
  - Correlation between the RET/PTC rearrangement and prognosis remains unclear
- RAS genes encode G proteins located at the inner surface of the cell membrane, which send signals from tyrosine kinase receptors and G-protein coupled receptors along the MAPK, PI3K/AKT, and other pathways
  - In thyroid tumors, the most frequent mutations in RAS are NRAS codon 61 and HRAS codon 61
  - RAS mutations are found in all types of thyroid follicular cell-derived tumors
  - RAS mutations are in 10–20% of PTCs; almost all PTCs with a RAS mutation have a follicular variant histology
  - RAS mutations are found in 40–50% of follicular thyroid carcinomas (FTC) and in 20–40% of follicular adenomas
  - The RAS mutation cannot be used as a universal prognostic marker, though a predisposition for dedifferentiation and more aggressive behavior has been suggested in tumors with RAS mutations
  - Detection of a RAS mutation in a thyroid nodule is strong evidence for neoplasia, but does not establish that it is a malignancy
  - Its importance is as a marker for the follicular variant of PTC which is difficult to diagnose, especially on FNA

Follicular Thyroid Carcinoma

- FTC is the second most common type of thyroid malignancy (15%)
  - FTC is divided into conventional type and oncocytic (Hurthle cell) type
- FTCs contain RAS mutations or PAX8/PPARγ (gamma) rearrangements
  - These mutations are mutually exclusive and occur in 70–75% of FTCs
- The PAX8/PPARγ (gamma) rearrangement is a result of a t(2;3)(q13;p25) translocation that leads to the fusion between the PAX8 gene (encoding a paired domain transcription factor) and the peroxisome proliferator-activated receptor (PPARγ [gamma]) gene
  - PAX8/PPARg (gamma) is found in 30–40% of follicular carcinomas
  - The rearrangement results in overexpression of the PPARg (gamma) protein
  - Tumors with this rearrangement are usually in younger patients, smaller and more often have vascular invasion

  - The rearrangement is also found in as many as 38% of follicular variant PTCs
  - The PAX8/PPARg (gamma) rearrangement is also found in 2–13% of follicular adenomas
  - PAX8/PPARg (gamma) rearrangements and RAS point mutations rarely occur in the same tumor, suggesting two different molecular pathways in the development of FTC
  - Detection of PAX8/PPARγ (gamma) should prompt a further investigation into vascular or capsular invasion, though it is not fully diagnostic for a malignancy
  - PAX8/PPARγ (gamma) rearrangement appears to be useful in preoperative FNA diagnosis, but more studies confirming this are needed

Medullary Thyroid Carcinoma (MTC)

- MTC originates from thyroid parafollicular or C cells and accounts for 3% of all thyroid cancers
- Both familial and sporadic MTC frequently possess point mutations in the RET gene

Poorly Differentiated and Anaplastic Thyroid Carcinomas (ATC)

- Poorly differentiated and ATC arise de novo or from preexisting well-differentiated PTC or FTC
- Genetic alterations involving the PI3K/AKT pathway have a higher prevalence in poorly differentiated thyroid tumors
- Mutations in the TP53 and CTNNB1 genes are also common

Summary of Key Points in the Molecular Pathology of Head and Neck Cancer

HNSCC

- The most common cancer of the head and neck is by far SCCA
- HNSCCs arise from the epithelium lining the sinonasal tract, oral cavity, pharynx, and larynx with evidence of squamous differentiation
- Head and neck tumorigenesis is a multistep process resulting from the accumulation of multiple genetic and epigenetic alterations.
- Field cancerization refers to clones of phenotypically intact but genetically damaged cells which populate extended tracts of mucosa and give rise to secondary tumors
- There has been an increase in incidence of oropharyngeal SCCA in a population of younger patients without an alcohol and smoking history attributed to HPV infection
- HPV, especially type 16, is a causative agent in 70% of oropharyngeal cancers and result in different clinical and pathological tumor behavior
- HPV-positive tumors are more poorly differentiated and have a higher propensity for regional lymph node metastases, but have a better response to treatment with significantly improved overall survival
- The p53 and Rb tumor suppressor pathways are frequently disrupted in head and neck tumorigenesis with various mechanisms including genetic and epigenetic silencing (in the majority of HPV-negative HNSCC) and viral oncoprotein degradation by E6 and E7 in HPV-positive HNSCC
- Continuing research in the underlying molecular genetics of HNSCC will help further elucidate biomarkers to measure the presence, extent, and progress of disease
- Molecular targeted therapy against EGFR in HNSCC has been moderately successful; further research will assist in producing therapy inhibiting other signaling pathways involved in head and neck tumorigenesis
- The resolution of specific genetic profiles for each HNSCC is on the horizon with the goal of individualizing therapy targeting specific gene alterations and signaling pathways
- Early detection is the key to improve the survival of HNSCC patients
- Saliva, a local biofluid for oral cancer, has been shown to harbor clinical discriminatory proteomic and transcriptomic biomarkers, in addition to microRNAs

Nasopharyngeal Carcinoma

- The strong association between NPC and EBV distinguishes NPC from other squamous cell carcinomas of the head and neck
- The tumorigenic potential of EBV lies in its set of latent membrane proteins (LMP) and EBV-determined nuclear antigens, of which LMP1 is the principal oncogene
- The presence of EBV is necessary but not sufficient in the tumorigenesis of NPC in which multiple cell signaling pathways are implicated
- Studies are ongoing to develop molecularly based treatments against NPC

Sinonasal Malignancies

- Molecular cytogenetics is an ongoing field of study in malignancies of the sinonasal tract, which include SCCA, adenocarcinoma, minor salivary gland tumors, and the various small round blue cell tumors
- The molecular pathology of certain tumors in the sinonasal tract is currently most helpful in differentiating tumors which demonstrate the small round blue cell morphology
- Molecular markers for selected undifferentiated sinonasal tumors allows for identification of tumors in which other diagnostic criteria may not be sufficient, which include NMC, Ewing sarcoma, alveolar rhabdomyosarcoma, and desmoplastic small round cell tumor

Salivary Gland Malignancies

- The field of molecular biology in salivary gland malignancies remains in development for diagnostic, prognostic, and therapeutic biomarkers
- The MECT1–MAML2 translocation has recently been described in Mucoepidermoid Carcinoma and has not been found in other salivary gland tumors
- A translocation fusing the MYB and NFIB genes into a chimeric transcript has been discovered in ACC, which is a potential biomarker for diagnosis and developing downstream therapeutic targets

Thyroid Carcinoma

- Many genetic mutations and molecular alterations in PTC and FTC have been discovered
- The most clinical experience has been with the diagnostic use of the BRAF mutation, which is highly specific for malignancy
- Testing FNA samples for a panel of mutations which includes BRAF, RAS, RET/PTC, and PAX8/PPARγ (gamma) is suggestive of malignancy if any of these are present and helps to clarify clinical management in patients with indeterminate cytology

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Suggested Reading

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