Molecular Diagnosis in Head and Neck: What a Surgical Pathologist Must Know

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Bullet Points to highlight presentation

- Tumorigenesis in head and neck tumors can occur through a variety of pathways, including oncogenes, tumor suppressor genes, and viral etiology.

- Mucoepidermoid carcinoma has a translocation, particularly in the low and intermediate grade tumors.

- Epstein Barr Virus is associated with nasopharyngeal undifferentiated carcinoma.

- Parathyroid carcinoma occurs in sporadic and hereditary settings, frequently involving the HPRT2 gene which encodes for parafibromin.
Mucoepidermoid carcinoma (MEC): Illustration of a Translocation

Mucoepidermoid carcinoma is the most common salivary gland malignancy; it represents between 2 and 16% of all salivary gland tumors and up to 1/3 of malignant salivary gland tumors [1]. Histologically, MEC carcinoma has three cellular components: mucus cells, epidermoid cells, and intermediate cells. Grading is extremely important in MEC, as it correlates with prognosis in almost every study that has been done. The grading systems have varied over time. The first grading system suggested that only the amount of cyst content had to be examined. Tumors with more than 90% cyst content were considered to be low grade and those with less than 90% were considered to be high grade [2, 3]. Later grading systems converted this to a three-tiered system, with low, intermediate, and high grade tumors [4]. The three-tiered grading system has been validated by several additional series that consistently show correlation between tumor grade and prognosis [5-8].

MEC is treated primarily with surgery. Grade 1 tumors do not require additional therapy or lymph node dissection. High grade lesions, especially grade 3 tumors, may be treated with cervical lymph node dissection, and grade 2 tumors do not have standardized therapy. Adjuvant radiation therapy may be used for high grade tumors and for patients who have histologically positive surgical margins.

A very interesting finding has been the discovery of a translocation in MEC. The t(11;19)(q21-22;p13) translocation between WAMTP1-MAML2 disrupts the Notch signaling pathway [9-12]. Recent evidence suggests that this translocation may have prognostic relevance, since it is only seen in low and intermediate grade MEC and not in high grade tumors [10] (Table 3).

Table 3: Presence of translocation in MEC in comparison to grade of tumor. Data collated from [10-12].

<table>
<thead>
<tr>
<th>Grade</th>
<th>Positive</th>
<th>Negative</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>41</td>
<td>21</td>
<td>66%</td>
</tr>
<tr>
<td>Grade II</td>
<td>8</td>
<td>11</td>
<td>73%</td>
</tr>
<tr>
<td>Grade III</td>
<td>0</td>
<td>28</td>
<td>0%</td>
</tr>
</tbody>
</table>

Nasopharyngeal Carcinoma: Illustration of viral etiology

Lymphoepithelial carcinoma (LEC) is the descriptive name for the tumor that has also been referred to as “Nasopharyngeal undifferentiated carcinoma”. It has been variously named in the past as Regaud and Schmincke’s tumor, undifferentiated nasopharyngeal carcinoma (in the nasopharynx) and lymphoepithelioma. Nasopharyngeal LEC or those that involve Waldeyer’s ring
(base of tongue, palatine tonsils and adenoids) are more common in Southeast Asia and North Africa, where the incidence may be as high as 30-80/100,000 [13]. In the U.S. and Europe the incidence is well below 1/100,000. In Asia, there is a bimodal age distribution, with the largest peak in the 6th decade and the smaller peak between 10 and 25 years of age [14]. The most common presentation is a mass in the neck, with possible cranial nerve involvement and resulting neurologic symptoms.

In non-nasopharyngeal sites, LEC is quite rare. In several large series of all laryngeal carcinomas, LEC represented less than 0.2% of the cases studied. In our experience, the most common non-nasopharyngeal site is the larynx, and particularly the pyriform sinus.

The histologic features of LEC are unique. The cells in these tumors are large, and pleomorphic. The oval to round nuclei are vesicular and may contain large, distinct nucleoli. The cells grow in syncitial sheets that have prominent infiltrating lymphocytes. The lymphocytes in these lesions are polyclonal and therefore are considered to be reactive in nature. Two types of lesions have been described: pure LEC and mixed LEC with a component of conventional SCC. No differences in prognosis have been described, though this tumor is rare and large series have not been reported.

LEC in the nasopharynx is highly associated with Epstein-Barr Virus (EBV), particularly in endemic areas, such as Southeast Asia and North Africa [15]. In non-endemic areas, EBV is present in approximately 1/3 of cases [16, 17]. Tumors in non-endemic areas are more often smoking related. The age distribution in western countries is unimodal (6th decade predominance), which supports the fact that it parallels conventional squamous cell carcinoma.

Genetic predisposition to nasopharyngeal LEC has been suggested by correlation between development of tumors and certain HLA profiles [18, 19]. The at-risk HLA types appear to be different from region-to-region. Diet has also been implicated as having a role in the development of nasopharyngeal LEC. Salted fish and preserved foods containing nitrosamines and herbal teas are a few of the suspected agents [20-22].

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**Parathyroid Carcinoma: Illustration of a tumor suppressor gene**

The majority of patients with primary hyperparathyroidism present with incidental hypercalcemia, found at routine health screenings. A sestamibi scan can help the surgeon to pre-operatively locate the abnormal gland, but these may be nonspecific and thus exploration during surgery may still be needed.

Parathyroid adenoma will have typical histologic findings. The cellular composition may include show predominantly a single cell type, or can show mixed features [23]. Other morphologic features of parathyroid adenoma may include a rim of normal somewhat suppressed parathyroid tissue around the outside of the gland. This can be used as a diagnostic clue for the etiology of the
pathologic process. Mitoses are usually rare to absent in parathyroid adenomas [24, 25].

The differential diagnosis between benign adenoma and carcinoma cannot be reliably made just based on the histologic assessment alone (TABLE). The surgeon’s intraoperative opinion about adherence to surrounding structures is an important criterion in distinguishing benign from malignant parathyroid neoplasms. The surgeon who encounters a parathyroid carcinoma will describe the gland as “sticky,” “fibrotic,” “hypervascular,” or “adherent to local structures” [26, 27]. These descriptions should immediately alert the pathologist to the possibility of parathyroid carcinoma. The histologic features of malignancy may not all be seen in a given case [25, 28]. There are some histopathologic features which are associated with malignancy, though they are certainly not pathognomonic for carcinoma. Worrisome features include the presence of increased or atypical mitoses, broad bands of fibrosis, trabecular growth pattern, invasion of adjacent tissue, and perineural or angiolymphatic invasion [29]. These features usually correlate with malignancy, though these histologic features are not always present in every case of parathyroid carcinoma [25, 30, 31]. Parathyroid carcinomas tend to be locally invasive and invasion is most commonly seen into the thyroid gland, strap muscles, recurrent laryngeal nerve, esophagus or trachea [25, 32].

**TABLE**: Clinical and histologic features of parathyroid carcinoma

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>High calcium level (&gt;14 mg/dl)</td>
<td>Trabecular growth</td>
</tr>
<tr>
<td>Parathyroid hormone level &gt; 5 x normal</td>
<td>Broad intersecting fibrous bands</td>
</tr>
<tr>
<td>Palpable mass lesion</td>
<td>Increased mitoses</td>
</tr>
<tr>
<td>Bone symptoms</td>
<td>Stromal invasion</td>
</tr>
<tr>
<td>Operative findings of invasive growth (sticky, fibrotic, vascular gland)</td>
<td>Angiolymphatic or perineural invasion</td>
</tr>
</tbody>
</table>

There have been some studies of the molecular mutational findings in parathyroid neoplasia. One feature that is consistently noted is that parathyroid adenomas and carcinomas have a high rate of loss of the short arm of chromosome one (1p) [33-37]. This is not a feature that is generally seen in parathyroid hyperplasia. Other genes have also been implicated in parathyroid adenomas and carcinomas, including Retinoblastoma (RB, 13q14.3), the MEN gene (11q13), and the BRCA2 gene (13q12.3) [38-40].

Studies of a very interesting syndrome (hyperparathyroidism—jaw tumor syndrome) have provided insight into the pathogenesis of parathyroid neoplasia. The syndrome includes parathyroid cysts, parathyroid carcinomas, and fibro-osseous lesions of the jaw. Although it is reportedly rare, it is probably under-recognized. The syndrome is associated with mutations in a tumor suppressor gene mapping to 1q25-31 and designated as HPRT2. This gene harbors germline mutations in hereditary cases of this syndrome [34, 41]. Loss of
heterozygosity and somatic point mutations have been detected in sporadic parathyroid carcinomas, as well [34, 42].

Interestingly, the protein product for the HPRT2 gene, parafibromin, has been recently identified as a potential marker for parathyroid carcinoma. Expression of parafibromin is lost in between 70% and 90% of both syndromic and sporadic parathyroid carcinomas [43-45]. Parafibromin is almost always preserved in histologically and clinically benign parathyroid adenomas [46].

REFERENCES


Agenda

• Background
  – Oncogenes
  – Tumor suppressor genes

• Molecular Oncogenesis
  – Oncogene: Mucoepidermoid Carcinoma
  – Viruses: Nasopharyngeal Carcinoma
  – Tumor suppressor genes: Parathyroid carcinoma
Oncogenes

• Dominant
  – One copy mutated

• Mutation causes activation
Oncogenes

Proto-oncogene
Wild-type

Onco gene
Mutant

Normal Cell

Cancer Cell
Oncogenes
Oncogene Mutations

• **Amplification**
  – FISH

• **Translocations**
  – FISH
  – RT-PCR

• **Point Mutations**
  – Sequencing
# Oncogenes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>Lung</td>
</tr>
<tr>
<td>HER2/NEU</td>
<td>Breast</td>
</tr>
<tr>
<td>KRAS</td>
<td>Pancreatic &amp; Colon</td>
</tr>
<tr>
<td>BRAF</td>
<td>Melanoma &amp; Thyroid</td>
</tr>
</tbody>
</table>
Tumor Mutations

- Tumor suppressor genes
- Oncogenes
Tumor Suppressor Genes

• Recessive
  – Both copies mutated

• Mutation causes inactivation
Tumor Suppressor Genes

2 Functional copies --> Normal Cell --> Cancer Cell --> 0 Functional copies
Tumor Suppressor Genes
Tumor Suppressor Gene Mutations

• Deletion
  – Loss of heterozygosity

• Point mutation
  – Sequencing

• Methylation
  – PCR based assays
## Tumor Suppressor Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>Colon Cancer</td>
</tr>
<tr>
<td>p53</td>
<td>Many tumors</td>
</tr>
<tr>
<td>Rb</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>VHL</td>
<td>Renal tumors</td>
</tr>
</tbody>
</table>
Agenda

• Background
  – Oncogenes
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• Molecular Oncogenesis
  – Oncogene: Mucoepidermoid Carcinoma
  – Viruses: Nasopharyngeal Carcinoma
  – Tumor suppressor genes: Parathyroid carcinoma
Mucoepidermoid Carcinoma

• Incidence
  – Most common malignant salivary gland tumor
  – Major and minor salivary glands
  – Peak incidence 5th to 6th decades

• Clinical
  – Mass lesion
  – Surgical treatment with margins
Histology

• Mucus cells and cysts
• Epidermoid cells
• Intermediate cells
Mucoepidermoid Carcinoma
Translocation

• t(11;19)(q21;p13)

• MECT1-MAML2
  – MECT1: CREB (cAMP response element binding protein) coactivator
  – MAML2: Notch coactivator
MECT1-MAML2 Translocation

MECT1 Exon 1  \textcolor{teal}{Intron 1}  MECT1 Exon 2

MAML2 Exon 1  \textcolor{purple}{Intron 1}  MAML2 Exon 2

MECT1-MAML2
Detecting translocations

- Chromosomal level (cytogenetics)
- Genomic level (PCR, FISH)
- mRNA level (RT-PCR)
PCR From Template DNA

1000’s of basepairs
RT-PCR From mRNA
FISH for Translocations
FISH for Translocations
Translocations by FISH
Mucoepidermoid Carcinoma

Behboudi, Genes Chrom Canc 45:470, 2006
Okabe, Clin Cancer Res 12:3902, 2006
Martins, J Molec Diag 6:205, 2004
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• Molecular Oncogenesis
  – Oncogene: Mucoepidermoid Carcinoma
  – Viruses: Nasopharyngeal carcinoma
  – Tumor suppressor genes: Parathyroid carcinoma
Nasopharyngeal Carcinoma

• Clinical
  – Rare in Western hemisphere (1/100,000)
  – 30 times higher incidence in SE Asia
Histology

• Architectural
  – Syncitial sheets of cells or single cells
  – Infiltrating polyclonal lymphocytes

• Cytology
  – Large, pleomorphic cells
  – Round to oval nuclei
  – Vesicular to water-clear nuclei
  – Prominent single nucleoli

• Conventional squamous component
Lymphoepithelial Carcinoma
Lymphoepithelial Carcinoma
Nasopharyngeal Carcinoma

• Association with EBV
  — Asia & North Africa
  — Prevalent in nasopharyngeal lesions
  — Sometimes in Western countries
    — Particularly in NP

• Genetic association: HLA types

• Exposures
  — Salted fish
Virus Detection: HPV

• Detection methods
  – PCR
    – Highly sensitive
    – ? Too sensitive
  – ISH
    – Localization
    – Integrated vs. episomal
  – Other methods: Hybrid capture, combined techniques
ISH for Virus
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  – Tumor suppressor genes

• Molecular Oncogenesis
  – Oncogene: Mucoepidermoid Carcinoma
  – Viruses: Nasopharyngeal Carcinoma
  – Tumor suppressor genes: Parathyroid carcinoma
Parathyroid Carcinoma

• Incidence
  – Rare

• Clinical
  – High serum calcium (>14)
  – High parathyroid hormone (> 5 x normal)
  – Palpable mass
  – Bone and cardiac symptoms
  – Adherent gland intra-operatively
Parathyroid Carcinoma

• Histology
  – Trabecular growth
  – Broad intersecting fibrous bands
  – Increased or atypical mitoses
  – Stromal invasion
  – Perineural or angiolympathic invasion
  – Nuclear pleomorphism is scant
Case 1, Endothelial marker
HPT Jaw Tumor Syndrome

• Hyperparathyroidism jaw tumor syndrome
  – Parathyroid cysts
  – Parathyroid carcinomas
  – Fibro-osseous lesions of jaws
HPT Jaw Tumor Syndrome

• HPRT2 gene
  – Tumor suppressor gene
    – Point mutations in syndromic cases
    – Loss of heterozygosity in tumors
Molecular Diagnosis

Allele 1

Allele 2

Normal

Allele 1

Allele 2

Tumor
Parafibromin Immunohistochemistry

- Loss in parathyroid carcinoma (90%)
- Preserved in benign
Summary

• Oncogene
  – Mucoepidermoid translocation

• Viral etiology
  – Nasopharyngeal carcinoma
  – Basaloid squamous carcinoma

• Tumor suppressor gene
  – Parathyroid carcinoma
Thank You