Molecular Assessment of HPV in Patients with Head and Neck Tumors

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Agenda

• Detection of HPV in paraffin tissues
• HPV-related lesions of the head & neck
  – Oropharyngeal carcinomas
• Clinical value in HPV testing
Oncogenesis of HPV

- E7
  - p16
  - Cyclin D1
  - Rb
  - E2F
  - S-phase
  - p21
  - Apoptosis
  - ARF
  - p53
  - MDM2
  - E6
HPV Detection Methods

• Polymerase chain reaction

• In situ hybridization
  – Multiplexed (High risk vs. low risk)
  – Type specific probes

• Other methods
  – Hybrid capture (cytology samples)
  – Other technologies

• p16 immunohistochemistry
PCR for HPV

• Types of assays
  – Qualitative PCR assays
  – Quantitative PCR assays
  – PCR with sub-typing assays
    – line probe assay
## Normal Tissue with HPV

<table>
<thead>
<tr>
<th></th>
<th>HPV detection in normal</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ernster JA (2009)</td>
<td>0%</td>
<td>Normal paraffin embedded tonsils</td>
</tr>
<tr>
<td>Kreimer AR (2010)</td>
<td>3.5%</td>
<td>Meta-analysis, oral tissue</td>
</tr>
</tbody>
</table>

Kreimer AR, Sex Transm Dis, 2010
HPV Detection Methods

• In situ hybridization
  – Multiplexed (High risk vs. low risk)
  – Sub-type specific probes
HPV In Situ Hybridization
HPV positive tumor (*in situ* hybridization)
**Surrogate Marker: p16**

<table>
<thead>
<tr>
<th></th>
<th>HPV (+) (types 16/18)</th>
<th>HPV (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p16 (+)</td>
<td>100%</td>
<td>21%</td>
</tr>
<tr>
<td>p16 (-)</td>
<td>0%</td>
<td>79%</td>
</tr>
</tbody>
</table>

*Other Sub-types?*  
*P16 over-expression*

See also: Abstract 1236, JE Lewis

Kuo KT, Mod Path, 21:376, 2008
Tonsillar squamous carcinoma
P16, Tonsillar squamous carcinoma
Virus Detection: HPV

Better prognosis

Q-PCR  p16 IHC  in situ

Kuo KT, Mod Path, 21:376, 2008
Virus Detection: HPV

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  – Oropharyngeal carcinomas

• Clinical value in HPV testing
Human Papillomavirus

• Relationship between SCC and HPV
  - Increasing incidence of HPV-related tumors
    - Based on SEER data, annual percentage increase is ~3%
  - ~25% of all HNSCC
    - Oropharynx: >50% of tumors are positive for HPV
    - Up to 95% are HPV-16

Ryerson AB, Cancer 113:2901, 2008
Syrjanen S, J Clin Virol, 32:S59, 2005
Oropharyngeal Squamous Carcinoma

• Basaloid squamous cell carcinoma
• Non-keratinizing squamous carcinoma
• Lymphoepithelial carcinoma
Basaloid Squamous Carcinoma

• Histology
  – Rounded nests and sheets of cells
  – Basaloid morphology
    – High N:C ratio
    – Mitoses and apoptosis
    – Peripheral palisading
  – Comedo-type necrosis
Basaloid Squamous Carcinoma
Basaloid Squamous Carcinoma
Basaloid squamous cell carcinoma
Basaloid squamous cell carcinoma
Basaloid Squamous Cell Carcinoma

• IHC
  – Cytokeratins
  – Negative for myoepithelial markers
  – p63 positive
Basaloid squamous cell carcinoma, p63
Non-keratinizing Squamous Carcinoma

• Histology
  – Sheets of cells
  – Limited keratinization

• Cytology
  – High N:C ratio
  – Pleomorphism
  – Tracks along tonsillar crypts
Non-keratinizing Squamous Carcinoma
Non-keratinizing Squamous Carcinoma
## HPV and Tumor Characteristics

<table>
<thead>
<tr>
<th></th>
<th>HPV Positive</th>
<th>HPV Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td>• 5 years younger</td>
<td>• Typical ages</td>
</tr>
<tr>
<td></td>
<td>• Non-smokers/non-drinkers</td>
<td>• Tobacco and alcohol</td>
</tr>
<tr>
<td><strong>Site</strong></td>
<td>Tonsil &amp; Tongue base</td>
<td>All locations</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Poorly differentiated, non-keratinizing, basaloid</td>
<td>Keratinizing SCC</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td>• p53 inactivated by E6</td>
<td>• p53 inactivated by mutation</td>
</tr>
<tr>
<td></td>
<td>• Rb inactivated by E7</td>
<td>• Rb inactivated by cyclin D1 amplification</td>
</tr>
<tr>
<td></td>
<td>• p16 over-expressed</td>
<td>• Inactivation of p16</td>
</tr>
</tbody>
</table>
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• Clinical value in HPV testing
Why Test for HPV?

• Epidemiologic
• Diagnostic
• Prognostic
• Therapeutic
Cystic Metastasis and Unknown Primary

• **Histology**
  - Ribbon like epithelium
  - Thickness of tonsillar type epithelium
  - Can have endophytic or exophytic areas

• **Cytology**
  - Moderate N:C ratio
  - No maturation
  - Bland appearance
Cystic Metastatic Squamous Carcinoma
Cystic Metastatic Squamous Carcinoma
## Unknown Primary

<table>
<thead>
<tr>
<th>Site</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonsil/tongue base</td>
<td>63%</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>8%</td>
</tr>
<tr>
<td>Other</td>
<td>10%</td>
</tr>
<tr>
<td>None found</td>
<td>20%</td>
</tr>
</tbody>
</table>

Thompson L, Cancer 82:944, 1998
HPV as a Diagnostic Tool

• HPV in cystic lymph node metastases
  – Between 50 and 80% will be positive when originating from an oropharyngeal site
## Oral HPV-related Lymphoepithelial Carcinoma

<table>
<thead>
<tr>
<th></th>
<th>P16</th>
<th>HPV 16 ISH</th>
<th>HPV PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singhi (#1256)</td>
<td>22/22 (100%)</td>
<td>19/22 (86%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Carpenter (#1221)</td>
<td>14/15 (93%)</td>
<td>8/14 (57%)</td>
<td>6/6 (100%) (ISH -)</td>
</tr>
</tbody>
</table>
Oral HPV-related Lymphoepithelial Carcinoma

Courtesy of Dr. Ed Stelow
Why Test for HPV?

• Epidemiologic
• Diagnostic
• Prognostic
• Therapeutic
# Prognostic Value of HPV

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HPV Positive/TP53 wt</th>
<th>HPV Positive or Negative/TP53 or Mutated HPV Negative/TP53 wt</th>
<th>Total No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>15</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>% Survival, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>93</td>
<td>60</td>
<td>64</td>
</tr>
<tr>
<td>5</td>
<td>79</td>
<td>46</td>
<td>50</td>
</tr>
<tr>
<td>% Cumulative incidence of tumor relapse, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>47</td>
<td>42</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>53</td>
<td>48</td>
</tr>
<tr>
<td>% Cumulative incidence of second tumors, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

Abbreviations: HPV, human papillomavirus; wt, wild type.

Cumulative Incidence Relapse

- HPV-/ TP53wt
- HPV±/ TP53mut
- HPV+/ TP53wt

Survival Rates

HPV: Unanswered Questions

• What tumors should be tested?
• What test should be done?
• What value should be reported?
  – Qualitative: Positive vs. negative
  – Quantitative: Copy number
  – Sub-typing: High vs. low or exact subtype
Summary

• Detection of HPV in paraffin tissues

• HPV-related lesions of the head & neck
  – Oropharyngeal carcinomas

• Clinical value in HPV testing
Molecular Assessment of HPV in Patients with Head and Neck Tumors

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HPV Viral Oncogenesis in Head and Neck Tumors

There are a number of viruses that are known to be associated with tumorigenesis. The most common are Epstein-Barr Virus (EBV) and human Papillomavirus (HPV). Of course, HPV is particularly well known for its association with carcinomas of the uterine cervix. In fact, HPV testing of cervical smear specimens has become standard of care for the management of certain subsets and age groups of women.

Recent evidence has suggested that a subset of head and neck squamous cell carcinomas is also associated with HPV. These tumors are commonly in a younger population and often afflict patients who do not have traditional risk factors (non-smokers and non-drinkers) [1]. These tend to occur in the orpharynx, particularly in the tonsillar and tongue base regions [2, 3]. The tumors often have a characteristic morphology, including either a nonkeratinizing appearance or basaloid squamous cell carcinoma features [4]. These tumors, as with non-HPV associated tumors in these locations, can present with bulky lymph node metastases, and sometimes a primary tumor is not discovered despite very careful clinical workups. Detection of HPV virus DNA can be useful in directing the clinical to the oropharynx in search of a primary. Most HPV positive tumors of squamous origin are from these locations.

The most common subtype of HPV in oropharyngeal carcinomas is HPV 16 [5]. HPV 18 and HPV 33 can also be seen. HPV related oropharyngeal squamous cell carcinomas have a different prognosis [6] They have a decreased rate of second primary tumors, less local recurrence, and better survival rates [6]. They may also have different types of responses to chemotherapy and radiation therapy [7, 8].

Detecting HPV can be done with several different assays, including PCR based assays, in situ hybridization, and immunohistochemistry [9]. The
advantages of ISH are that the virus can be localized to the tumor and that it is not overly sensitive [10]. IHC is not very reliable, if the target is HPV. There is a surrogate marker that can be used to suggest HPV, and that is p16 by IHC. This correlates fairly well with the presence of HPV. Because p16 is also a tumor suppressor gene, alterations in expression will not always be associated with HPV. Tumors associated with HPV have a better prognosis and also have different response rates to traditional chemo and radiation therapy. Furthermore, there may be novel anti-viral therapies used in some HPV positive lesions, though perhaps not invasive carcinomas.

The reasons that HPV testing may be implemented in a clinical setting include diagnostic implications (i.e., helping to suggest site of origin for a neck metastasis with an unknown primary), therapeutic and prognostic reasons, since HPV-related tumors have a different behavior and prognosis.

References