HPV in Tumors of the Head and Neck

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Overview

- Role of HPV in pathogenesis of head and neck tumors
  - Laryngeal papillomas
  - Head & neck squamous cell carcinoma (HNSCC)
- HPV-related HNSCC:
  - Histology & Terminology
  - Prognosis
- HPV assays and diagnostic applications

Head and Neck SCC

Oral cavity & oropharynx carcinomas:
- 8th most common new cancer among men and 14th most common among women in U.S. (1975-2002)
- Significant increase in incidence of base of tongue and tonsil SCC from 1973-2001 in younger whites ages 20-44 yrs, specifically men
- Overall declining incidence of SCC in non-HPV related sites from 1983-2004

Chaturvedi, J Clin Oncol 2008; Shiboski, Cancer, 2005
HPV in Head & Neck Tumors

Mucosal HPV types infect upper respiratory tract (similar to cervix and anogenital area):

- Low-risk types 6 and 11:
  - Laryngeal papillomas
- High-risk types:
  - Squamous cell carcinoma

Laryngeal Papillomas

<table>
<thead>
<tr>
<th></th>
<th>Juvenile-onset</th>
<th>Adult-onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset</td>
<td>&lt; 5 yrs</td>
<td>&gt; 20 yrs</td>
</tr>
<tr>
<td>Gender</td>
<td>M=F</td>
<td>M &gt;&gt; F</td>
</tr>
<tr>
<td>Number</td>
<td>Multiple</td>
<td>Usually single</td>
</tr>
<tr>
<td>Recurrence</td>
<td>Frequent</td>
<td>Only if multiple</td>
</tr>
</tbody>
</table>
HPV in Laryngeal Papillomas

- Typically low-risk HPV types 6 and 11
  - HPV 6: 50-84% juvenile-onset
  - HPV 11: 25-37% juvenile-onset
- HPV 11 associated with more aggressive course
  - Higher incidence of malignant transformation
  - Higher incidence of tracheotomy
  - Higher mortality rate

Gerein, Otolaryngol HNS 2005; Cook, Mod Pathol 2000

Head & Neck SCC: *pathogenesis*

Two Pathways:
- Non-HPV related
  - Associated with tobacco smoking, alcohol use and poor dentition
- HPV-related
  - Associated with infection by high-risk HPV

Head & Neck SCC: *HPV prevalence*

- Published estimates of HPV prevalence in HNSCC vary from 8-100%
- International Agency for Research on Cancer (IARC) review of literature through February 2004
  - Overall prevalence of HPV: 25.9%
  - 5,035 HNSCC cases with HPV PCR from 60 studies and 26 countries

Kreimer, 2005

Prevalence of HPV Types by Site

<table>
<thead>
<tr>
<th>Site</th>
<th>All Types</th>
<th>HPV 16</th>
<th>HPV 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharynx</td>
<td>35.6%</td>
<td>86.7%</td>
<td>2.8%</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>23.5%</td>
<td>68.2%</td>
<td>34.1%</td>
</tr>
<tr>
<td>Larynx</td>
<td>24.0%</td>
<td>69.2%</td>
<td>17.0%</td>
</tr>
</tbody>
</table>

Kreimer, 2005
Prevalence of HPV by Country

<table>
<thead>
<tr>
<th>Location</th>
<th>North America</th>
<th>Europe</th>
<th>Asia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oropharynx</td>
<td>47.0%</td>
<td>28.2%</td>
<td>46.3%</td>
</tr>
<tr>
<td>Oral Cavity</td>
<td>16.1%</td>
<td>16.0%</td>
<td>33.0%</td>
</tr>
<tr>
<td>Larynx</td>
<td>13.8%</td>
<td>21.3%</td>
<td>38.2%</td>
</tr>
</tbody>
</table>

Kreimer, 2005

Other HPV Types Detected

- High-risk types:
  - HPV 31, 33, 35, 45, 51, 52, 56, 58, 59, 68
- Non-oncogenic types
  - HPV 6
  - Less commonly HPV 11, 32, 44, 53, 57, 81
- Infection by multiple types in 3.6% cases
  - Most cases coinfected with HPV 16

Kreimer, 2005

HPV-related HNSCC: risk factors

- Increased numbers of vaginal or oral sex partners & infrequent use of barriers
- History of sexually transmitted disease
- Marijuana use
- Immunosuppression
  - HIV(+), transplant patients

Gillison, JNCI 2008; Fakhry, J Clin Oncol 2006

HPV-related HNSCC: risk factors

<table>
<thead>
<tr>
<th></th>
<th>HPV16+ Odds Ratio</th>
<th>Control Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifetime # vaginal sex partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>2-10</td>
<td>2.5</td>
<td>0.61</td>
</tr>
<tr>
<td>≥ 11</td>
<td>6.4</td>
<td>0.74</td>
</tr>
<tr>
<td>Lifetime # oral sex partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>1-5</td>
<td>2.1</td>
<td>0.93</td>
</tr>
<tr>
<td>≥ 6</td>
<td>4.3</td>
<td>0.70</td>
</tr>
<tr>
<td>History of STD</td>
<td>2.6</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Gillison, JNCI 2008
HPV-related HNSCC

role of marijuana

• Insufficient evidence to determine if marijuana is carcinogenic
• Some studies show strong association of marijuana use with HPV16(+) HNSCC
• Possible pathogenic role:
  – Presence of carcinogens similar to those in tobacco smoke
  – Immune-modulatory effects

Gillison, JNCI 2008

HPV-related HNSCC

role of marijuana

• Cannabinoids suppress humoral and cell-mediated immune response
  – Bind to receptors on B-cells, T-cells, NK-cells, macrophages and dendritic cells
• Reduce host cell resistance to viral and intracellular pathogens
• May suppress anti-tumor immunity

Gillison, JNCI 2008

HPV-related HNSCC: risk factors

• Fanconi anemia:
  – Increased risk (500-700x) of HNSCC but conflicting results regarding role of HPV
• Tobacco and alcohol:
  – Conflicting results regarding synergistic effects

Gillison, JNCI 2008; Fakhry, J Chin Oncol 2006

HPV-related Head & Neck SCC
Head & Neck SCC: *histology*

- Non-HPV related:
  - Moderately differentiated keratinizing morphology
- HPV-related:
  - Basaloid morphology: Nests of high N:C ratio cells with dense, hyperchromatic nuclei and central necrosis

[Images of Keratinizing SCC and SCC with basaloid morphology]
Basaloid morphology can be seen with both non-HPV related and HPV-related SCC

- “Basaloid squamous cell carcinoma”
  - Clinically aggressive subtype
  - 2/3 develop LN mets, 35-50% distant mets
- HPV-related
  - Better prognosis
- Use HPV 16 ISH to subdivide

Begum, AJSP 2008
Head & Neck SCC: terminology

• Basaloid SCC:
  – Implies clinically aggressive subtype
• SCC with basaloid features:
  – May be confused with “basaloid SCC”
• Poorly differentiated SCC:
  – May also imply aggressive behavior due to grade

Begum, AJSP 2008

Head & Neck SCC: terminology

Non-keratinizing squamous cell carcinoma (see comment)

• Comment: The tumor cells exhibit “basaloid” features. In the oropharynx, this phenotype is often associated with HPV 16.

Begum, AJSP 2008

Head & Neck SCC: terminology

Non-keratinizing HPV-related squamous cell carcinoma (see comment)

• Comment: The tumor is notable for basaloid morphology and diffuse strong reactivity for p16 which is associated with the presence of high-risk HPV.

Head & Neck SCC: prognostic factors

• Locoregional metastasis
• Lymphatic/vascular invasion
• Margin status
• Extracapsular extension of lymph node metastasis
• HPV status
• EGFR status
HPV-related HNSCC: *prognosis*

Based primarily on retrospective studies:
- Increased sensitivity to chemotherapy and chemoradiation
- Higher disease-specific survival

Fakhry, JCO 2006 & JNCI 2008

Prospective study of 96 patients with stage III or IV HNSCC (oropharynx or larynx)
- Higher response rate to therapy:
  - Induction chemo: 82% vs. 55%
  - Chemoradiation: 84% vs. 57%
- Improved overall survival
  - 95% vs. 62%
  - Median follow-up: 39.1 months

Fakhry, JNCI 2008

Possible reasons for improved survival:
- Improved response to treatment
- Intact apoptotic response to treatment due to non-mutated p53
- Absence of field cancerization effect in non-smokers
- Immune surveillance to viral antigens

Fakhry, JNCI 2008

EGFR overexpression in HNSCC correlates with worse prognosis
- Conflicting results regarding relationship between HPV and EGFR status
- 82 HNSCC patients at Stanford:
  - Small subset with HPV(+)/EGFR(+) tumors had significantly lower overall 5-year survival than HPV(+)/EGFR(-) tumors

Ang, Cancer Res 2000
Kong, Int J Rad Oncol 2009
HPV assays and diagnostic applications

HPV Assays

- In situ hybridization (ISH):
  - Dako GenPoint: HPV 16/18, 6/11
  - Ventana Benchmark: HPV 16
- PCR:
  - Home-brew assays
  - Roche Linear Array & Amplicor
- \( p16^{INK4A} \) immunohistochemistry

DAKO GenPoint™ HPV

- Catalyzed signal amplified ISH
- Chromogenic detection
- Performed on GenPoint™ system
- Probes:
  - High-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68
  - Wide-spectrum: 6, 11, 16, 18, 31, 33, 35, 45, 51, 52
  - Cocktails: 6/11, 16/18, 31/33
  - Type specific (16, 18) no longer commercially available

DAKO GenPoint™ HPV ISH

- Disadvantages
  - System does not have multiple testing capabilities
  - Semi-automated system, not easy to optimize
Ventana Inform HPV®

- Slide-based non-amplified in situ hybridization
- Chromogenic detection
- Performed on Ventana Benchmark (multiple testing capabilities)
- HPV Family 16 probe
- High-risk and low-risk cocktail probes no longer available

HPV PCR

- Assay: Type-specific vs. Consensus
- Portion of HPV genome targeted, i.e. L1, E1, E6/E7
- Fresh vs. FFPE tissue
- Extraction method
- Detection method
HPV PCR

- Home-brew assays
- Roche Diagnostics (*pending FDA approval*)
  - LinearArray: Type-specific detection of 37 high- and low-risk HPV types
  - Amplicor: Consensus assay – detection of 13 high-risk HPV types

Disadvantages
- Highly sensitive:
  - May detect clinically insignificant infection
- Equipment costs
- Dedicated lab space required
- Potential for contamination

p16\textsuperscript{INK4A} Immunohistochemistry

- mtm: Clone E6H4 (CINtec kit)
- Surrogate marker for high-risk HPV:
  - Diffuse strong (nuclear or nuclear & cytoplasmic) reactivity correlates with HPV DNA integration into host genome
  - Definition of “diffuse” varies among studies
  - Indicate in report whether staining is focal (5-80%) or diffuse (>80%)
Diagnostic Applications of HPV Assays

- Prognostic marker
- Determination of primary site
- Evaluation of squamous dysplasia
- Utility in FNA specimens

HPV Assays: *prognostic marker*

- $p16^{\text{INK4a}}$ correlates with survival:
  - Freedom from relapse (5 yr): 58% vs. 28%
  - Disease specific survival: 72% vs. 34%
  - Overall survival: 62% vs. 26%
- Strong independent prognostic factor
- Performed on all new cases of HNSCC at Stanford

Smeets, Int J Cancer 2007

Lassen, J Clin Oncol 2009

*p16^{\text{INK4a}}* Immunohistochemistry

- Overexpression can be seen in absence of HPV (true false positive)
- Suggested algorithm for detecting clinically relevant HPV infections:
  - Perform p16 stain
  - If positive, confirm with HPV PCR or ISH
HPV Assays: *determine primary site*

Metastatic carcinoma of unknown primary in a cervical lymph node:
- Histologic clues to HPV-related HNSCC:
  - Non-keratinizing “basaloid” morphology
  - Cystic lymph node metastasis
- Perform p16 stain:
  - Positive points to oropharyngeal primary (base of tongue, palatine tonsil)

43 year old with enlarged cervical lymph node
HPV Assays: determine primary site

Squamous cell carcinoma involving lung: Primary or metastasis?
- HPV typing by PCR or ISH
- Concordant HPV types in lung tumor and primary HNSCC favors metastasis
- Discordant HPV types favors two primaries

Weichert, AJSP 2009
Lung SCC: Primary vs. metastasis

HPV Assays
evaluation of squamous dysplasia

60 biopsies stained with p16:

- Benign keratoses:
  - Negative (12/12)
- Chronic ulcer:
  - Negative (4/10) or minimal basal staining (6/10)

Gologan, AJSP 2005

HPV Assays
evaluation of squamous dysplasia

60 biopsies stained with p16:

- Mild-moderate dysplasia:
  - 85% with lower 2/3 staining (17/20)
  - 15% with upper 1/3 staining (3/20)
- Severe dysplasia/CIS:
  - Upper-third (69%) staining (9/13)
  - 2 cases (15%) each with lower or mid-third staining

Gologan, AJSP 2005
HPV Assays
*utility in FNA specimens*

Metastatic carcinoma of unknown primary:
- Strong correlation with oropharyngeal primary:
  - HPV 16 ISH (+) or p16 (+)
  - Non-keratinizing “basaloid” morphology
- Perform EBV ISH, if HPV markers negative

Branchial cleft cyst vs. metastatic SCC:

Begum, Clin Cancer Res 2007

HPV Assays
*utility in FNA specimens*

Branchial cleft cyst vs. cystic SCC:
- Diffuse, strong staining with p16 supports metastatic SCC from oropharynx
- Focal, strong p16 staining: equivocal
- Negative p16: not helpful

Pai, Cancer Cytopathol 2009
Immunostains on Cytologic Material

- Formalin-fixed, paraffin-embedded cell blocks
  - Best for performing immunostains
  - Mirrors standard histology material
- Direct smears, cytospin, ThinPrep®:
  - High background and non-specific staining due to protein-rich fluid
  - Cell clusters may lead to false positive due to trapping of stain

Preparation of Cell Blocks

- Histogel™ (Thermo Scientific)
  - Similar to agarose gel
  - Specimen can be formalin fixed or viscous
- Fibrin clot
  - Uses thromboplastin and plasma
  - Specimen cannot be formalin fixed or contain bile
- Collodion bags

Summary

- Role of HPV in pathogenesis:
  - Laryngeal papillomas: HPV 6, 11
  - HNSCC: Primarily HPV 16
- HPV-related HNSCC:
  - Diagnose as “Non-keratinizing SCC (see comment)”
  - Avoid terms “basaloid” or “poorly differentiated” in diagnostic line

<table>
<thead>
<tr>
<th></th>
<th>HPV-related</th>
<th>Non-HPV related</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Younger</td>
<td>Older</td>
</tr>
<tr>
<td><strong>Location</strong></td>
<td>Oral cavity</td>
<td>Oral cavity</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>Basaloid</td>
<td>Keratinizing</td>
</tr>
<tr>
<td><strong>HPV status</strong></td>
<td>HPV 16</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Increased sex</strong></td>
<td>+++</td>
<td>N/A</td>
</tr>
<tr>
<td>partners/oral sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol/smoking</strong></td>
<td>N/A</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Marijuana</strong></td>
<td>+</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Immunosuppressed</strong></td>
<td>+++</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>p53</strong></td>
<td>Wild-type</td>
<td>Mutated</td>
</tr>
<tr>
<td><strong>p16</strong></td>
<td>Overexpressed</td>
<td>Loss</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>Better</td>
<td>Worse</td>
</tr>
</tbody>
</table>
Summary

- HPV assays and diagnostic applications:
  - p16 stain is the easiest to perform and interpret
  - Diagnostic applications of p16:
    - Prognostic marker, site of origin, diagnosis of squamous dysplasias, evaluate FNA specimens
    - HPV ISH and HPV PCR can be helpful as second line assays in p16(+) cases