Detailed description of high-risk HPV RNA in situ hybridization patterns in endocervical adenocarcinoma and correlation with p16 immunohistochemistry

Maryam Shahi, MD

Maryam Shahi, Johns Hopkins Medical Institutions (Primary Presenter)
Lynn Hoang, University of British Columbia
Takako Kiyokawa, The Jikei University School of Medicine
Simona Stolnicu, University of Medicine
Robert Soslow, Memorial Sloan Kettering Cancer Center
Kay J. Park, Memorial Sloan Kettering Cancer Center
Conflict of interest

No conflict of interest to declare.
HPV functions in two forms: Episomal and Integrated.

HPV infection (in nutshell)

- Access to basal epidermal layer
- Endocytosis and viral capsid disassembly (L2 mediated, episomal form)
- Viral genome replicates in synchrony with host cell DNA (E1, E2, E4, E5)
- E2 open reading frame disruption > viral genome integrates into the host DNA
- E2 no longer inhibits E6 and E7.

HPV E7 protein Hijacks normal function of pRB, leading to overexpression of p16 gene

- P16 is a tumor suppressor protein > can be overexpressed in different tumors
- HPV E7 protein binds to pRB, preventing inactivation of E2F > cell cycle activation > overexpression of p16 with no negative feedback

P16 IHC is being used as a surrogate marker for HPV-associated lesions


- HSIL vs mimickers
- CIN1 vs CIN2 (with the caveat that 40-50% LSILs are p16 positive)

In glandular lesions:

- HPV-related glandular lesions vs benign
- HPV-related glandular vs non-HPV related lesions
P16 IHC is not always cooperating!

- Rare cases of “null” p16 in squamous intraepithelial lesions, possibly due to CDKN2A gene methylation or allelic loss (Proc Natl Acad Sci U S A. 1999;96:1254–1259, Virchows Arch 2011 Feb;458(2):221-9)

- False positive cases > tubal metaplasia, endometrial carcinomas

Photo courtesy of Dr. Deyin Xing
High-risk HPV ribonucleic acid in situ hybridization (HR HPV RNA-ISH): a very sensitive tool for detection of HPV infected epithelia.

❖ mRNA E6/E7 transcripts > integration of the viral genome in host’s > a decisive indicator of biological activity
❖ More robust than HR DNA ISH > 84% of HPV DNA ISH (-) HNSCC were RNA ISH (+) with 94% concordance with p16 (Bishop et al. Am J Surg Pathol. 2012 Dec; 36(12): 1874–1882.)
  ❖ HPV PCR is not necessarily an indicator of viral genome integration
  ❖ 40% of PCR invalid and 20% of PCR (-) had evidence of HPV infection
HR HPV RNA-ISH signal patterns has been studied in squamous intraepithelial lesions (PLoS One. 2014 Mar 13;9(3):e91142.)

❖ Two distinct patterns:
  - Diffuse nuclear: 81% of CIN1 and 63% of CIN2 (productive phase)
  - Punctuate (nuclear and cytoplasmic):
    (transformative phase)
❖ Diffuse nuclear pattern resistant to RNase treatment > suggests some DNA hybridization when DNA load is high
❖ CIN 1 with diffuse p16 showed punctate integrated signal pattern

CIN 1 cases with different p16 and RNA-ISH patterns
Does HR HPV RNA-ISH signal pattern in cervical adenocarcinoma correlate with p16 IHC?

Objectives:
❖ To study different pattern of p16 staining in cervical adenocarcinoma
❖ To evaluate any correlation of p16 and HR HPV RNA ISH pattern of staining

Material and Methods:
❖ Two TMAs composed of 200 cases of invasive endocervical adenocarcinoma (1994 -2015) including 6 cores for each case
❖ P16, Ki-67 IHC and HR HPV RNA-ISH (ACD RNAScope)
HPV RNA chromogenic in situ hybridization scoring:

- Overall viral load - apparent on high magnification (40x); apparent on medium magnification (10x); apparent on low power (4x)
- Nuclear signal coarseness - signal <10% of nuclear diameter; 10-20%; >20%
- Percentage of nuclei with coarse signal
- Pattern of nuclear signal - single, dual, multiple
- Amount of cytoplasmic signals - not present; rare or difficult to find; abundant or easy to find; 2- in between
- Presence of “reservoir-like cells” (cells with very dense load of cytoplasmic signals which covers the nucleus)
P16 IHC scoring:
- Positive: diffuse, strong, block-like
- weak/variable positive: diffuse but weak; strong but patchy (variable in different cores)
- Negative

Ki-67 scoring:
- Quantifying the positive malignant nuclear staining (percentage)
Various patterns of HPV-ISH staining

Viral load score 3 with multiple fine and coarse nuclear signals

Viral load score 2 with single coarse nuclear signals

Viral load score 1 with fine nuclear signals

Single/dual coarse nuclear signals with abundant cytoplasmic signals

Multiple coarse nuclear signals with scattered cytoplasmic signals

Reservoir-like signals
Patterns of p16 staining in HPV-ISH positive groups

- Strong and diffuse
- Weak and variable
- Negative

P16 IHC

HPV ISH
# P16 IHC and HR HPV RNA ISH concordance rate in cervical adenocarcinoma: 79.4%

<table>
<thead>
<tr>
<th></th>
<th>HR HPV RNA-ISH</th>
<th>P16</th>
<th># (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>G1</strong></td>
<td>Negative</td>
<td>Negative</td>
<td>32 (18.4%)</td>
</tr>
<tr>
<td><strong>G2</strong></td>
<td>Positive</td>
<td>Positive</td>
<td>106 (61%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block-like</td>
<td>69 (39.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variable/weak</td>
<td>37 (21.3%)</td>
</tr>
<tr>
<td><strong>G3</strong></td>
<td>Negative</td>
<td>Positive</td>
<td>8 (4.5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Block-like</td>
<td>2 (1.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variable/weak</td>
<td>6 (3.4%)</td>
</tr>
<tr>
<td><strong>G4</strong></td>
<td>Positive</td>
<td>Negative</td>
<td>28 (16.1%)</td>
</tr>
</tbody>
</table>

174 cases had conclusive p16 staining and HR HPV ISH results
Ki-67 proliferation index is higher in p16 (+) group regardless of HPV status * and in HPV (+) group regardless of p16 status **

<table>
<thead>
<tr>
<th>Ki-67 proliferation index median</th>
<th>P16</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>negative</td>
</tr>
<tr>
<td>HR HPV RNA ISH</td>
<td>negative</td>
</tr>
<tr>
<td>positive</td>
<td>G4: 43% (n=28)</td>
</tr>
</tbody>
</table>

Mann-Whitney U test: * p-value: 0.013, ** p-value: ;0.016
P16 status did not correlate with viral load (number of signals in the tumor cells)
Summary

❖ P16 is not entirely sensitive or specific for detecting HPV in cervical adenocarcinomas
  ❖ In a large scale study (n=174), more than a third of HPV-related endocervical adenocarcinoma were either totally negative (16%) or focally/weakly positive (21.3%) for p16.
  ❖ Rare cases of NHPVA showed diffuse p16 staining.
❖ HPV RNA-ISH patterns in endocervical adenocarcinoma are varied.
  ❖ Coarse, fine, single, multiple, nuclear, cytoplasmic
❖ P16 status does not necessarily correlate to the quantity and intensity of HR HPV RNA-ISH positivity.
❖ Diffuse strong p16 is associated with higher proliferation index.
❖ HR HPV RNA-ISH is recommended to distinguish HPV versus non-HPV related cervical adenocarcinoma.
Thank you for your attention.

Any questions?
<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>G1: HPV-/p16-</th>
<th>G2: HPV+/p16+ (wk/var)</th>
<th>G3: HPV-/p16+ (wk/var)</th>
<th>G4: HPV-/p16-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usual</td>
<td>14</td>
<td>56 (19)</td>
<td>3 (1)</td>
<td>20</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>4</td>
<td>12 (2)</td>
<td>2 (2)</td>
<td>1</td>
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<tr>
<td>Intestinal</td>
<td>3</td>
<td>4</td>
<td>0</td>
<td>1</td>
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<td>Gastric-type</td>
<td>1</td>
<td>2 (1)</td>
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<tr>
<td>Endometrioid</td>
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<td>iSMILE</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell</td>
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<td>3 (2)</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Serous</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NOS</td>
<td>2</td>
<td>1 (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>24 (11)</td>
<td>3 (3)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>106</td>
<td>8</td>
<td>28</td>
</tr>
</tbody>
</table>
HR HPV RNA-ISH signal patterns has been studied in squamous intraepithelial lesions


❖ Higher percentage of basal layer cells with punctate (integrated pattern) signals (versus diffuse “episomal” signal patterns) by HR HPV DNA ISH may predict progression to CIN2/3 (Gynecologic Oncology 112 (2009) 114–118).
