When Immunostains Can Get You in Trouble: Gynecologic Pathology

*p16: Panacea or Pandora’s Box*

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*p16 in Gynecologic Pathology: Panacea or Pandora’s Box?*

**p16**

- Cervix
- Vulva
- Uterine corpus

**p16 in Cervical Squamous Lesions**

- Tumor-suppressor p16 is overexpressed in cervical carcinomas
- p16 expression is altered by the effect of HPV on the retinoblastoma protein
- IHC staining for p16 has become standard practice in the evaluation of cervical lesions
- Although considered a surrogate marker for HPV infection \textit{in the appropriate setting}, p16 does not, in general, act as a surrogate marker for HPV infection

**Stimulation of cell-cycle progression by high-risk HPV**

p16 Immunohistochemistry

Positive stain
• Diffuse (>80%) strong block positive nuclear or nuclear and cytoplasmic staining of basal layer and extending up at least 1/3 of epithelial thickness:
  • Correlates with presence of HR-HPV and diagnosis of dysplasia
  • Grading of dysplasia MUST be based on histology


Negative stain
• Cytoplasmic only staining
• Focal or patchy staining
• Discontinuous staining of basal layer
• Staining of upper layers but not basal layer


Cytoplasmic only – interpret as negative

Discontinuous basal p16 – interpret as negative
p16 Recommendations

**USE:**
- HSIL vs. benign mimic
- Confirm diagnosis of CIN2
- Disagreement about diagnosis of HSIL
- Negative biopsy with prior high-risk cytology: HSIL, ASC-H, AGC-NOS, ASC-US/HPV16+

*J Low Genit Tract Dis. 2012;16:205-42*

**DO NOT USE:**
- LSIL vs. benign mimic
- Morphology unequivocally diagnostic of:
  - LSIL (CIN1)
  - HSIL (CIN3)
- Negative for dysplasia

*J Low Genit Tract Dis. 2012;16:205-42*

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**Case #1**

- 44 year old with vaginal bleeding. History of HR-HPV
3/23/2017

Case #2

- 42 year old with vaginal bleeding. History of HR-HPV.
Squamous cell carcinoma

Neuroendocrine carcinoma
Cervical Neuroendocrine Carcinoma
- <5% of all cervical cancer
- Most in mid 50's
- Clinically aggressive
- Most bulky & deeply invasive with necrosis
- 50% high stage (FIGO III/IV)

Cervical Neuroendocrine Carcinoma
- Most express one or more neuroendocrine markers
- Often associated with AIS, HSIL, and conventional invasive cervical adenocarcinoma
- Both small and large cell types may be p16-positive and harbor high-risk HPV (esp HPV 18)
- TREATED like neuroendocrine carcinoma elsewhere – NOT like cervical cancer

Endometrial Neuroendocrine Carcinoma
- Most express one or more neuroendocrine markers
- Often associated with conventional endometrioid carcinoma
- Predominantly large cell type followed by mixed, and small cell
- Often PAX-8 negative
- May exhibit MMR deficiency (MLH1/PMS2 most common)
- May be p16-positive (24%)


Summary: p16 in Cervical Squamous Lesions
- Should be strong, diffuse block-positive staining
- Many other lesions can have focal strong staining patterns & some may have extensive p16 (neuroendocrine carcinoma)
- Do not use on LSIL lesions – significance unknown
- Location matters – know where you are

p16 in Cervical Glandular Lesions
- Since AIS is associated w/ high-risk HPV, AIS demonstrates diffuse, strong expression of p16
- Ki-67 is also elevated & can be used as complimentary marker
- This can be used in confirming diagnosis of AIS in biopsy or curettage samples, but requires experience
p16 Predicament

- p16 can be misleading in small samples
- Focal, strong, diffuse p16 in up to 10% of uterine corpus cancers – extensive in uterine serous
- Minimal deviation adenocarcinoma & other cervical special variant carcinomas are p16-negative

Summary: p16 in Cervical Glandular Lesions

- Should be strong, diffuse staining
- Many other cervical lesions can have focal strong staining patterns (endometriosis)
- Caution on limited sampling
- Be clear about what you’re looking at – is it cervical or endometrial?
p16 in Vulvar Squamous Lesions

Exceptions:
• Differentiated (simplex) VIN
• Verrucous carcinoma

Differentiated (Simplex) VIN

• 10% of vulvar intraepithelial neoplasia
• Post-menopausal women
• Associated with lichen sclerosus, not HPV-related
• Typically identified adjacent to well-differentiated keratinizing carcinoma
• No counterpart in the cervix (or anal canal)

Are There “Hybrid” Types?

• Usual HPV-related VIN in patients with lichen sclerosus
• Usual HPV-related VIN and non-HPV-related (differentiated [simplex]) VIN in same patient
• Usual “basaloid” non-HPV related VIN

Case #3

- 54 year old with vulvar mass
Anogenital Basal Cell Carcinoma
- No squamous intraepithelial component
- Stromal retraction – but may not be present
- CK5/6-pos, p63-pos
- BerEp4-pos, BCL2-pos
- p16 may be focally strong positive – limited (small) sampling problem

Vulvar Verrucous Carcinoma
- Rare, special variant of SCC
- Slow-growing, minimal metastatic potential
- Typically low-risk HPV (6, 11)
- Histology:
  - Exophytic, hyperkeratotic fronds
  - Cytologically bland squamous epithelium
  - Well-circumscribed pushing border with chronic inflammation

Case #4
- 51 year old with raised vulvar plaque
High-grade squamous intraepithelial lesion???

CK7

p16
Anogenital Paget’s Disease

• Squamous hyperplasia with hyperkeratosis and parakeratosis in 90% of anal extra-mammary Paget’s
  ➢ Pseudoepitheliomatous hyperplasia
  ➢ Fibroepithelioma-like
  ➢ Papillomatous (mimics HPV)
• Paget cells may be unapparent
• Paget cells may strongly express p16


Anogenital Paget’s disease

• Primary (may rarely invade)
  ➢ CK7+/CK20-/HER2
• Secondary to anorectal or bladder cancer
  ➢ CK7+/CK20+/GATA3+
  ➢ CK7+/+CDX2+

Anogenital Paget’s & HSIL

• Rare, but may coexist
• Paget cells are HPV-negative
• HSIL is HPV-positive

Summary: Use of p16 in Vulvar Lesions

• LAST recommendations only apply to HPV-VIN
• VIN lesions may change over time (dVIN vs HPV-VIN)
• Be clear about the question you are asking and be clear that you are using the right bioassay for that question
• Consider panel to diagnose unusual vulvar lesions (CK7, p16, S100, BerEp4)
Use of p16 in Metastatic Lesions

Exceptions:
- Metastatic neuroendocrine carcinoma
- Urothelial carcinoma

Use of p16 in Bladder Neoplasms

- Use p16 in conjunction with HPV ISH in the evaluation of cervical cancer vs urinary tract cancer
- Not all metastatic lower genital tract squamous cancer express p16 in strong diffuse pattern

Summary: Use of p16 in Metastatic Lesions

- In general, cannot be relied on for determining primary site
- Requires careful clinicopathologic correlation
- Consider HPV testing (in situ hybridization)

Use of p16 in Endometrial Glandular Lesions

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>p16 (%)</th>
<th>p53 (%)</th>
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| HPV Endocervical adenocarcinoma | Diffuse, mod-strong (60-100%) | ???
| Serous adenocarcinoma      | Diffuse, mod-strong (60-100%) | Strong, diffuse (>90%) or null – 45/49 |
| Endometrioid adenocarcinoma| Variable, weak-strong (10-90%) | ???

Case #5

- 62 year old with vaginal bleeding
Papillary Syncytial Change (Metaplasia)
- Papillary syncytial change associated with stromal breakdown, atrophy, karyorrhectic debris
- Papillary syncytial metaplasia occurs over surface of endometrium, may be extensive, often mixed epithelial types
- May overlie atrophy, hyperplasia or carcinoma

Papillary Syncytial Change (Metaplasia)
- Decreased expression of ER
- Increased expression of p53 (although still wild-type staining) and p16, the latter marker typically being diffusely positive
- Low MIB1 proliferation index
- In problematic cases, IHC may result in a misdiagnosis

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Use of p16 in Uterine Mesenchymal Lesions

- p16 is overexpressed in uterine leiomyosarcomas by gene expression studies
- p16 (as well as p53, Ki-67, and other cell cycle regulatory markers) have been utilized in the distinction between leiomyomas with unusual features (mostly leiomyomas with bizarre nuclei) and leiomyosarcoma


Leiomyoma with Bizarre Nuclei vs LMS

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<thead>
<tr>
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<th>LBN</th>
<th>LMS</th>
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<tbody>
<tr>
<td>Mitotic index</td>
<td>( \leq 10 )</td>
<td>( &gt; 10 )</td>
</tr>
<tr>
<td>Tumor cell necrosis</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Ki-67 (MIB-1)</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>p16</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>p53</td>
<td>Negative</td>
<td>Positive</td>
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Summary

- p16 has transformed the diagnosis and treatment of lower genital tract squamous & glandular lesions
- p16 is only a surrogate and so there are many caveats & limitations to its utility
- HPV in situ or PCR may be required to establish definitive diagnosis in difficult cases
- Panels should always be utilized in the appropriate clinical context

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