HPV infection: its role in cervical and vulvar cancers

Philippe Delvenne
Dpt of Pathology
University Hospital of Liege
OUTLINES

1) HPV plays a major role in the development of cervical cancer

2) HPV is also involved in the development of other human cancers (including vulvar cancer)
Cervical cancer: general features

1) Important health problem (3rd cause of death in women by cancer worldwide)

Incidence of cervical cancer worldwide

N. AMERICA 14,670

C-S. AMERICA 71,862

EURÓPE 59,931

AFRICA 78,897

ASIA 265,884

< 87.3
< 32.6
< 26.2
< 16.2
< 9.3
Cervical cancer: general features

1) Important health problem (2nd cause of death in women by cancer worldwide)

2) Preneoplastic period (→ 2ary prevention of SCC by the detection & appropriate treatment of precancerous lesions)
Terminology of squamous preneoplastic lesions

Histological terminology

$\text{CIN} = \text{CIN (cervical intraepithelial neoplasia)}$

- **CIN 1** (= “slight dysplasia”)
- **CIN 2** (= “moderate dysplasia”)
- **CIN 3** (= “severe dysplasia” or “carcinoma in situ”)
### Recommendation

**SQUAMOUS INTRAEPITHELIAL LESIONS, WG2**

1. A unified histopathologic nomenclature with a single set of diagnostic terms is recommended for all HPV-associated preinvasive squamous lesions of the LAT.
2. A 2-tiered nomenclature is recommended for noninvasive HPV-associated squamous proliferations of the LAT, which may be further qualified with the appropriate -IN terminology.
3. The recommended terminology for HPV-associated squamous lesions of the LAT is LSIL and HSIL, which may be further classified by the applicable -IN subcategorization.

### Comment

-IN refers to the generic intraepithelial neoplasia terminology, without specifying the location. For a specific location, the appropriate complete term should be used. Thus, for an -IN 3 lesion: cervix = CIN 3, vagina = ValN 3, vulva = VIN 3, anus = AlN 3, perianus = PaIN 3, and penis = PeIN 3.

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2014 WHO Classification of tumours : Chapter 7 : Tumours of the uterine cervix
Cervical cancer: general features

1) Important health problem (2nd cause of death in women by cancer worldwide)

2) Preneoplastic period (→ 2ary prevention of SCC & clinical trials for diagnostic biomarkers or new treatments)

3) Tumour initiation in a small region of the uterine cervix ("transformation zone")
The transformation zone is located between the endocervix and the exocervix.

> 90% of HPV infections and SILs
Two main histological types of cervical cancers

- **Adeno-carcinoma**
- **Squamous cell carcinoma**
Cervical cancer: general features

1) Important health problem (2nd cause of death in women by cancer worldwide)

2) Preneoplastic period (→ 2ary prevention of SCC & clinical trials for diagnostic biomarkers or new treatments)

3) Tumour initiation in a small region of the uterine cervix (“transformation zone”)

4) 1 etiologic agent well identified (Human Papillomavirus; HPV)
HPV plays a major role in the development of cervical cancers

Do HPV-negative cervical carcinomas exist?

Walboomers JM, Meijer CJ. J Pathol 1997; 181: 253-254
HPV Testing Alone May Miss Cervical Cancer

HPV: virological features

* DNA sequences: > 130 HPV genotypes

- E1: DNA replication; episomal form; repression of transcription and immortalizing potential
- E2: DNA replication; episomal form; regulation of transcription and immortalizing/transforming potential
- E4: virus particle maturation and release (interactions with CK)
- E5: transformation (EGFR activation)
- E6: transformation (p53 inactivation)
- E7: transformation (Rb inactivation)
- L1: major capsid protein (VLP: conformational epitopes <- neutralizing Abs)
- L2: minor capsid protein (stabilization of the capsid structure)
Evidence for the etiological association between HPV & cervical cancer?

1) HPV DNA in the majority of cervical carcinoma and SILs

Walboomers JM, Meijer CJ. J Pathol 1997; 181: 253-254
Organotypic culture model

At confluency

Normal keratinocytes

HPV+ cell lines

Delvenne et al. Vaccine 2001; 19: 2557-2564
Evidence for the etiological association between HPV & cervical cancer?

1) HPV DNA in the majority of cervical carcinoma and SILs

2) Specific viral mechanisms during the neoplastic transformation:

* integration of viral genome into host DNA
* selective expression of viral oncoproteins (E6/E7):
  → inactivation of tumor suppressor proteins
    (E6<->p53; E7<->pRb)
Viral and cell tumor suppressor proteins interactions

-> No cell cycle arrest and no DNA damage repair in HPV-infected keratinocytes

E6 ⇔ p53 (Scheffner 1990)

- E6-BP (Chen 1995)
- paxillin (Tong 1997)
- hDLG (Kiyono 1997)
- human telomerase (Klingelhutz 1996)
- CBP/p300 (Patel 1999; Zimmermann 1999)
- hScrib (Nakagawa 2000)
- MAGI-1 (Glaunsinger 2000)
- MUPP1 (Lee 2000)
- ADA3 (Kumar 2002)
- ...

E7 ⇔ Rb (Dyson 1989)

- p107 (McIntyre 1996)
- cyclin A; p33cdk2 (Tommasino 1993)
- p21 (Jones 1997; Funk 1997)
- p27 (Zerfass-Thome 1996)
- smad (Lee 2002)
- ...
HPV & the cervical transformation zone
The increased cell proliferation after HPV infection may be assessed by IHC (Ki67)
Ki67 antigen

Normal exocervix

Squamous metaplasia

LSIL

HSIL
p16 protein (see Dr Colpaert presentation)
<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Diagnosis (D) /prognosis (P) Value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DNA methylation</strong></td>
<td></td>
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<tr>
<td>RASSF1</td>
<td>D and P</td>
<td>Mitra <em>et al.</em> 2012.</td>
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<tr>
<td>CDH1</td>
<td>P</td>
<td>Flatley <em>et al.</em> 2009.</td>
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<tr>
<td>CDKN2A/p16</td>
<td>P</td>
<td>Huang <em>et al.</em> 2011.</td>
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<tr>
<td>MGMT</td>
<td>D</td>
<td>Van der Zee <em>et al.</em> 2004.</td>
</tr>
<tr>
<td>CADM1</td>
<td>D and P</td>
<td>Hesselink <em>et al.</em> 2011 &amp; Bierkens <em>et al.</em> 2013</td>
</tr>
<tr>
<td>FHIT</td>
<td>D and P</td>
<td>Ren <em>et al.</em> 2006 &amp; Neyaz <em>et al.</em> 2008</td>
</tr>
<tr>
<td>TIMP3</td>
<td>D</td>
<td>Van der Zee <em>et al.</em> 2006</td>
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<tr>
<td>TERT</td>
<td>D</td>
<td>Eijsink <em>et al.</em> 2012</td>
</tr>
<tr>
<td>CDH13</td>
<td>D</td>
<td>Widschwendter <em>et al.</em> 2004</td>
</tr>
<tr>
<td>HPV16 DNA</td>
<td>D and P</td>
<td>Mirabello <em>et al.</em> 2012, 2013</td>
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### Other biomarkers?

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Diagnosis (D) / prognosis (P)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stathmin-1</strong></td>
<td>D</td>
<td>Howitt <em>et al.</em> 2013</td>
</tr>
<tr>
<td><strong>IMP3</strong></td>
<td>P</td>
<td>Lu <em>et al.</em> 2011</td>
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<tr>
<td><strong>HLA-DR</strong></td>
<td>D</td>
<td>Zhou <em>et al.</em> 2006</td>
</tr>
<tr>
<td><strong>CD99</strong></td>
<td>D</td>
<td>Zhou <em>et al.</em> 2006</td>
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<tr>
<td>« Junction Cells »</td>
<td></td>
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<tr>
<td>Krt7</td>
<td>P</td>
<td>Herfs <em>et al.</em> 2012 &amp; 2013</td>
</tr>
<tr>
<td>AGR2</td>
<td>P</td>
<td>Herfs <em>et al.</em> 2012 &amp; 2013</td>
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<tr>
<td>MMP7</td>
<td>P</td>
<td>Herfs <em>et al.</em> 2012 &amp; 2013</td>
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<tr>
<td>GDA</td>
<td>P</td>
<td>Herfs <em>et al.</em> 2012 &amp; 2013</td>
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*Herfs et al. PNAS 2012*
Junction cells: location & morphology

Herfs et al. PNAS 2012

Squamous (ectocervix/TZ) Cuboidal (SC junction) Columnar (endocervix)
Biomarkers of junction cells: discovery

Laser microdissection + DNA microarray
(columnar vs junctional vs squamous cells)

Herfs et al. PNAS 2012
Biomarkers of junction cells: detection by IHC in normal cervical biopsies

<table>
<thead>
<tr>
<th>Detection in junction cells</th>
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<tr>
<td><strong>Krt 7</strong></td>
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<tr>
<td>B</td>
</tr>
<tr>
<td>SC Junction</td>
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</tbody>
</table>

| **AGR2**                    |
| Endocervix                  |

| **CD63**                    |
|                             |
Biomarkers of junction cells: detection by IHC in pathological cervical biopsies

- Detection in the majority (95%) of HSIL / SCC and in all adenocarcinoma

Biomarkers of junction cells: new management of LSIL?

Ectocervical/TZ LSIL
- SC junction markers: negative
  - Diffuse full-thickness p16 staining: ~30%
  - High-risk HPV infection: ~50%
  - Over-diagnosed as HSIL: rare (~0%)
- HSIL outcome: extremely low (~0%)
  - Monitored less closely

SC junction LSIL or «QSIL»
- SC junction markers: positive
  - Diffuse full-thickness p16 staining: ~90%
  - High risk HPV infection: virtually all
  - Over-diagnosed as HSIL: frequent (~50%)
- HSIL outcome: high (~25%)
  - Monitored more closely

HSIL
- SC junction markers: positive
  - Diffuse full-thickness p16 staining: ~90%
  - High risk HPV infection: virtually all
  - Under-diagnosed as LSIL: rare (~0%)

Conization

Herfs et Crum
2013
Squamocolumnar junction ablation—tying up loose ends?

Michael Herfs and Christopher P. Crum

Despite the commercialization of HPV vaccines, cervical cancer remains a major cause of death, especially in developing countries. Recent data implicate a discrete population of cells within the cervical squamocolumnar junction in the pathogenesis of cervical precancerous lesions, indicating that ablation of these cells might reduce the rate of cervical cancer in high-risk populations.
HPV is also involved in the development of other human cancers

Evidence for a causal role of HPV in other cancers (IARC)

1) strong: vulva, vagina, penis, anus, oral cavity, oropharynx, skin (in patients with epidermodysplasia verruciformis)

2) limited: larynx, periungal skin, conjunctiva, skin (in the general population)

3) Not sufficient: esophagus, lung, colon, ovary, breast, prostate, urinary bladder, nasal and sinonasal cavities

The incidence of vulvar cancer is lower compared to cervical cancer and less correlated with HPV infection.
HPV DNA is detected only in a subset of vulvar invasive carcinoma

Figure 2: Pathogenesis of squamous cell carcinoma of the vulva. (Distinct pathways for carcinogenesis of keratinizing and warty/basaloid types of vulvar SCC from normal epithelium through precursor lesions are demonstrated.)

Ueda et al. J Skin Cancer 2010
HPV DNA is detected only in a subset of vulvar preneoplastic lesions (uVIN/HSIL) (see presentation of Dr Bosse)

<table>
<thead>
<tr>
<th></th>
<th>uVIN/HSIL</th>
<th>dVIN</th>
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<tbody>
<tr>
<td><strong>AGE</strong></td>
<td>YOUNGER</td>
<td>OLDER</td>
</tr>
<tr>
<td><strong>INCIDENCE</strong></td>
<td>INCREASING</td>
<td>STABLE</td>
</tr>
<tr>
<td><strong>SMOKING</strong></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><strong>PRESENTATION</strong></td>
<td>MULTIFOCAL</td>
<td>UNIFOCAL</td>
</tr>
<tr>
<td><strong>ASSOCIATION WITH CIN, VaIN,...</strong></td>
<td>+++</td>
<td>±</td>
</tr>
<tr>
<td><strong>VIROLOGY</strong></td>
<td>HPV 16/18</td>
<td>/</td>
</tr>
<tr>
<td><strong>IMMUNOHISTOLOGY</strong></td>
<td>p16</td>
<td>p53</td>
</tr>
<tr>
<td><strong>ASSOCIATION WITH CHRONIC VULVAR DYSTROPHIES</strong></td>
<td>±</td>
<td>+++</td>
</tr>
<tr>
<td><strong>RISK OF INVASION</strong></td>
<td>VARIABLE (0-15%)</td>
<td>~ 15 %</td>
</tr>
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Bornstein, Obstet Gynecol, 2016
Why these differences between cervical and vulvar cancers??