HPV Infection and Invasive Cervical Carcinoma: Virus – Host interactions

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What HPVs are?

ds DNA virus with a 8000 pb genome

More than 100 types have been characterised, 1/3 are involved in genital infections

Based on their oncogenic power they are divided in HIGH and LOW risk types

HR-HPV associated tumours:
Squamous Cell Carcinoma
Adenocarcinoma
HPV morphology

HPV16 L1 VLPs
(J.Schiller, 2000)
HPV genomic structure
HPV-16 variants and human population migration
(Ho et al Journal of Virology 1993)
HR-HPV infection is the necessary cause for the genesis of high grade cervical lesions and invasive carcinoma. HPV type 16 and 18 are Class I human carcinogens.

Prospective studies demonstrated that persistent HPV infection precedes the development of lesions and represents an elevated risk for disease.

The level of association between HR-HPV infection and tumour development (RR:500) is much greater than cigarette smoking and pulmonary cancer (RR:10).

The great majority of infections is transient. Only persistent ones represent an oncologic risk.
ANNUAL AGE-STANDARDIZED RATE PER 100,000

Incidence and mortality rates for different regions of the world.
Age-specific prevalence of HPV infection

50%

HPV infection

precancerous lesions

normal cervix

viral persistence or clearance

cancer

< 15
15-25
26-35
36-40
41-45
46-50
51-55
>55

HPV (any type)
HR-HPV
LR-HPV
Host factors

E2 product:
DNA linking protein able to block E6 and E7 transcription

E1 product:
protein linking the replication origin (LCR) and induces episomial DNA replication
virus-cell interaction and oncogenetic effect

- High risk types have a marked tendency to integrate in the host genome.
- The integration process causes the interruption of E2 gene with the consequence of loss of its control over E6 and E7 expression.
- E6 and E7 products interact with and inhibit the function of cellular anti-oncogenic proteins (p53 e pRB).
E6 and E7 have a synergistic effect in the transformation process.
Virus shedding with superficial cells desquamation

BASAL LAYER CELL INFECTION

Oncogenesis

Integration

Suppressor genes inactivation

BASAL MEMBRANE INVASION

Lateral expansion

Episomial DNA replication

Transcription

"LATE" proteins synthesis

Viral particle assembly

Oncosuppressor genes inactivation

Integration
VIRAL FACTORS

- VIRAL TYPE
  - INTEGRATION CAPACITY
  - ONCOGENES EXPRESSION

- VIRAL LOAD
  - SINERGY
  - ANTAGONISM

PERSISTENCE
ENVIRONMENTAL FACTORS

✓ CIGARETTE SOKING
✓ HORMONS
✓ SEXUAL BEHAVIOUR
✓ PARITY
MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES
an ecologic analysis od 121 DC

Cervical cancer incidence (/100000 women)

Lowest Muslim tertile
Middle Muslim tertile
Highest Muslim tertile
Lowest Christian Tertile
Middle Christian Tertile
Highest Christian Tertile

P=0.041
P=0.018
P=0.54
P=0.45
P=0.0008
P=0.053

Drain PK et al, BMC inf. Dis nov 2006
MALE CIRCUMCISION, RELIGION AND INFECTIOUS DISEASES
an ecologic analysis od 121 DC

Drain PK et al, BMC inf. Dis nov 2006
HOST FACTORS

- IMMUNE RESPONSE
  - HUMORAL
  - CELL-MEDIATED

- GENETIC FACTORS
  - HLA
  - p53

NOT PRODUCTIVE
NO INFLAMMATION
**Innate response**

**Function:** to identify highly conserved antigenic structures (pathogen associated molecules-PAM) through superficial molecules (es. Toll-like receptors), system priming toward adaptative response

**Activation mechanisms:** PAM recognition, cell damage and death

**Response:** pro-inflammatory cytokine release

**Consequence:** local effectors recall (APC- dendritic cells), APC migration in the regional lymph-nodes, naive T cells activation, differentiation, migration to the infection site, recognition and disruption of the damaged cell
Adaptative response

T lymphocytes

CD4+
- Recognize non self peptides
  - MHC II presented
  - Th 2
  - IL-4 IL-10
  - Naive B cell
  - Memory B
  - Plasma cell

CD8+
- Recognize self peptides
  - MHC I presented
  - Th 1
  - INF-γ
  - Cytotoxic effectors
  - Macrophages
  - Natural killer
  - CD8 citox

APC
- Cytotoxic response
Why the immune response is scarce and inefficacious

1. The keratinocyte is programmed to die of 'natural death'
2. HPV as other DNA viruses interferes with IFN synthesis (natural antivirals, anti-proliferative, anti-angiogenetic, immuno-stimulators, pro-inflammatory)
3. LC do not respond to HPV capsid Ag
HPV integration associated events

✓ The level of chromosomal instability is related to the level of E7 expression

✓ Loss of heterozygosity at FHIT (fragile histidine triad)

✓ Disease progression toward invasive cancer correlate with the accumulation of genetic mutation and justify the latency period between precancerous lesion and invasive cancer

✓ The greater number of mutations accumulate at the fragile sites 8q24 e 3p14, close to c-myc and FHIT tumour suppressor gene
HPV integration associated events

Loss of heterozygosity (LOH): determines chromosomal instability; is involved in the ‘tumour suppressor genes’ activation. In a specific chromosomal region the first allelic site is lost, the second is mutated.

Microsatellite instability (MSI): diffuse mutations of small repeated DNA sequences (microsatellites). Originate from the inactivation of the DNA mismatch repair gene and from secondary mutations of genes coding for ms as the transforming growth factor receptor II.
HIV and HPV
BACKGROUND

Immunosuppression of any origin (genetic, iatrogenic, infectious etc) has been demonstrated to be an additional risk for HPV infection and related pathologies.

Cervical HPV infection and CIN are clearly increased in HIV+ women when compared with risk-matched HIV- women.

There are few data to suggest a direct role for HIV in the pathogenesis of HPV-associated neoplasia, but HIV-associated attenuation of HPV-specific immune responses may allow for persistence of high-grade CIN and sufficient time for accumulation of genetic changes that are important in progression to cancer.

The effect of HAART on HR-HPV infection and CIN has not been established.
IDENTIFICATION
STUDIED POPULATION

580 HIV-1 positive women (median 5.1yy)

<table>
<thead>
<tr>
<th></th>
<th>HR-HPV</th>
<th>HR+LR</th>
<th>LR-HPV</th>
<th>NO HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>26.8%</td>
<td>31.4%</td>
<td>9.4%</td>
<td>32.4%</td>
</tr>
</tbody>
</table>

\{ 58.2% \} \{ \text{58.2}\% \}

\{ 85.7\% \}  \{ \text{85.7}\% \}

<table>
<thead>
<tr>
<th></th>
<th>Persistent</th>
<th>Sporadic</th>
<th>NEVER HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39.0%</td>
<td>35.5%</td>
<td>25.5%</td>
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</tbody>
</table>

OVERALL PAP-HYSTO AGREEMENT

K=0.379

HGSIL PAP-HYSTO AGREEMENT

K=0.874
HPV TYPES PREVALENCE ACCORDING TO IMMUNE SUPPRESSION

Lillo et al JID 2001;184:547-51
CYTOLOGICAL AND HISTOLOGICAL DIAGNOSIS ACCORDING TO IMMUNE SUPPRESSION

**Cytology**

- > 500
- 200-500
- < 200

**Histology**

- > 500
- 200-500
- < 200

CD4+ cells /μl
TYPING
## Overall Agreement for Major Oncogenic Types

<table>
<thead>
<tr>
<th>HYSTO/load</th>
<th>ROCHE-GENOMICA K (CI 95%)</th>
<th>ROCHE-LIPA K (CI 95%)</th>
<th>GENOMICA-LIPA K (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any &gt;150</td>
<td><strong>0.58</strong> (0.46-0.70)</td>
<td><strong>0.75</strong> (0.61-0.88)</td>
<td><strong>0.69</strong> (0.54-0.85)</td>
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<tr>
<td>Any &gt; 400</td>
<td><strong>0.59</strong> (0.48-0.71)</td>
<td><strong>0.78</strong> (0.65-0.91)</td>
<td><strong>0.68</strong> (0.51-0.85)</td>
</tr>
<tr>
<td>HSIL &gt;150</td>
<td><strong>0.70</strong> (0.57-0.83)</td>
<td><strong>0.72</strong> (0.56-0.88)</td>
<td><strong>0.71</strong> (0.55-0.88)</td>
</tr>
<tr>
<td>HSIL &gt;400</td>
<td><strong>0.70</strong> (0.57-0.83)</td>
<td><strong>0.76</strong> (0.60-0.92)</td>
<td><strong>0.72</strong> (0.54-0.90)</td>
</tr>
</tbody>
</table>
HPV pre: 53
LEEP: 31
post: 31, 53

Semi-quantitative evaluation of HPV-DNA (pg/ml)

7 years follow-up

>>500 CD4

HAART

CIN1

CIN3

2x10^5

4x10^4

10^1

>>500 CD4
Semiquantitative evaluation of HPV-DNA (pg/ml)

HPV pre: 18, 33, 34, 39, 44, 45, 52, 66
LEEP: 18, 33, 34, 56
post: 18, 33, 34, 39, 44, 52, 66

CIN3

CIN3

CIN1

< 200 CD4

HAART

4 years follow-up
HPV pre: 16, 52, 56, 58, 59
LEEP: 18, 45, 58, 59
post: 52, 59

Semiquantitative evaluation of HPV-DNA (pg/ml)

5 years follow-up

CIN3

7x10^6

CIN1

5x10^5

<< 200 CD4

HAART

5 years follow-up
HR-HPV positive HIV + patients w/o cervical lesions (mean qt:239)

3 years follow-up

7 years follow-up

4 years follow-up

RT

HAART

> 500

< 200

HAART

HR - HPV positive, HIV + patients w/o cervical lesions.
<table>
<thead>
<tr>
<th>N°=126</th>
<th>LESION</th>
<th>HR-HPV LOAD (%)</th>
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<tr>
<td></td>
<td>PAP</td>
<td>HIST.</td>
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<tr>
<td>22</td>
<td>NEG</td>
<td>NEG</td>
</tr>
<tr>
<td>12</td>
<td>LGSIL</td>
<td>CIN1</td>
</tr>
<tr>
<td>32</td>
<td>HGSIL</td>
<td>CIN2+</td>
</tr>
<tr>
<td>26</td>
<td>LGSIL</td>
<td>CIN2+</td>
</tr>
<tr>
<td>5</td>
<td>NEG</td>
<td>CIN2+</td>
</tr>
<tr>
<td>8</td>
<td>NEG</td>
<td>CIN1</td>
</tr>
<tr>
<td>3</td>
<td>HGSIL</td>
<td>NEG</td>
</tr>
<tr>
<td>4</td>
<td>HGSIL</td>
<td>CIN1</td>
</tr>
<tr>
<td>5</td>
<td>LGSIL</td>
<td>NEG</td>
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</table>
EFFECT OF ARV THERAPY
EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON HPV PERSISTANCE

Lillo et al. JID 2001;184:547-51
EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON THE EVOLUTION OF HPV RELATED LESIONS

EFFECT OF DIFFERENT ANTIRETROVIRAL THERAPIES ON THE EVOLUTION OF HPV RELATED LESIONS

Lillo et al JID 2001;184:547-51
HPV: prophylactic vaccine
Prophylactic vaccine: **Background**

- “HPV is the *necessary cause* of cervical carcinoma” (X.Bosch, 2002)

- “Identification of an infectious agent as a *necessary cause* of disease implies that interfering with the infection should prevent development of the disease” (D.Lowy, 2003)

Prophylactic vaccine: **Objectives**

The major objectives of HPV vaccination are:

1. **morbidity from cervical carcinoma**
2. **mortality**
3. **costs**

- 1. removing any risk of disease in women effectively immunised
- 2. reducing exposure to infection amongst the rest of population

Reduction of other genital/non-genital cancer sites

*(G.Garnett, 2000)*
Epidemiological evidence

HPV infection naturally induces low titre of neutralising antibodies. Nevertheless, prior infection is host protective for the same genotype. (Frazer, 2002)

Disease in the immunocompromised

Impaired cellular immune function (HIV or renal transplantation) increase the incidence of HPV lesions (Garzetti G, 1994; Halpert R, 1986; Ozsaran A, 1999)

Therapy with immunomodulators

IFN
Imiquimod
The presence of persisting HPV infection suggests the virus is capable of evading many forms of immune surveillance. (C. Rock, 2000)

- No viremic phase which stimulate immune response
- Viral proteins are produced in very small amounts
- HPV gene expression
- Release of assembled virus: virus antigens are not easily detected
- Superficial epithelial layer where
- Keratinocytes are not lysed: there is no a “danger signal”
- No inflammatory response: (lack of valid CTLs response)

- Viral early proteins inhibit expression of MHC class I
  - E7 (HPV16,18) inhibits the induction of IFN
  - E5 inactivate the antigen processing system
Viral structural proteins which constitute the viral capsid. They interact with the epithelial cells during early stages of the infection. Once HPV is integrated into the human cells, the capsid proteins are not always present. Necessary to establish and to assure the maintenance of the viral infection. Continue to be expressed during the later stage of disease.
Prophylactic vaccine: Rationale

Virus-like particles (VLPs)

Are neither infectious, nor potentially oncogenic.

- VLPs resemble the conformation of authentic virions and induce high levels (>10000) of neutralizing antibodies.
- The immune-response is type-specific.
Prophylactic vaccine: *Rationale*

**Parenteral route**

**Preteens - Teenagers**

Immune Response (humoral + (?) cellular)

**Host protection**

VLP
Prophylactic vaccine: **Rationale**

**Ideal HPV-vaccine**

1. **Multivalent**
   - 1. Interruption of the most common STD.
   - 2. More attractive also for men.

2. **Easily acceptable**

3. **Safe**

4. **Immunogenic**

   - oncogenic strains
   - non-oncogenic strains
   - Developed area vs Underdeveloped area

   - 1. Animal model
   - 2. Human clinical trials
Design of the human clinical trial

Methodology

- Geographic area (developed vs developing)
- Population selection (female vs male and men) (rural vs urban)
- Numerical dimension (size of the study)
- Impact on cervical disease:
  - Mathematical models of transmission
  - Biological/immunological end-point
- Length and type of follow-up (ability to follow-up women over a long period)
- Projected population benefit

Prophylactic vaccine: Rationale

- High-risk areas in developing countries (→ HBV vaccination in Gambia or Taiwan) (M. Plummer & S. Franceschi, 2002)
- Organized mass screening programs
- Population based cancer registries (J. Paavonen, 2000)
Prophylactic vaccine: \textit{Rationale}

- Coverage of the population
- Theoretical efficacy by HPV-type

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{prophylactic_vaccine_rationale}
\caption{Graph showing incidence of cervical cancer with and without vaccination.}
\end{figure}

\textit{S. Goldie, 2003}
Prophylactic vaccine: *Rationale*

HPV in cervical carcinoma

(X. Bosch, 1995)

- HPV16: 50
- HPV18: 14
- HPV45: 8
- HPV31: 5
- Others: 23

Legend:
- HPV16
- HPV18
- HPV45
- HPV31
- Others
"The use of papillomavirus vaccines will require better definition of the local prevalence" (I.Frazer, 2004)
The rate of detection of new HPV-types appears to have accelerated instead of slowed down (L.Villa, E.Franco, 2002)

Five types previously classified as low-risk (HPV 26,53,66,73,82) should now be added to the list of high-risk (N.Munoz, 2003)

Local HPV prevalence
1. Life-style
2. Sexual behaviour
Prophylactic vaccine: *open questions*

**Developing countries**

- Local production
- Easy and cheap distribution
- Vaccine could be stable, easy storing
- Long-lasting immunity with a single dose
- Oral/mucosal vaccine better than for injection

**EDIBLE VACCINES**

Does malnutrition impact on immune response to HPV-vaccine?
GRAZIE