HPV proteins and Dendritic Cells in the "metaplasia-dysplasia-cancer" Sequence of the Uterine Cervix

Philippe Delvenne
Laboratory of Experimental Pathology
University of Liege
Why cervical cancer?

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

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

3) 1 etiologic agent identified (Human papillomavirus; HPV)

4) 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
HPV: necessary agent for cervical cancers

Do HPV-negative cervical carcinomas exist?

Walboomers JM, Meijer CJ. J Pathol 1997; 181: 253-254
HPV: virology

* DNA sequences: > 100 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 particule 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)
HPV: necessary for cervical cancer

- HPV DNA in > 95% of cervical carcinoma and SILs
- 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)

Dyson N et al. Science 1989; 243: 934-937
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)
→...
Work hypothesis (1)

The oncogenic potential of viral E6/E7 proteins is based on their ability to inactivate cell proteins important for the immune responses

E6/E7 ↔ cytokines/chemokines?
↔ adhesion molecules?

→ No immune recognition of HPV-infected keratinocytes → cancer development?
Dendritic Cells: role in antitumor immune responses?

* Detection in a variety of cancers
* Infiltration correlated with a significant prolongation of survival
* Ability to present tumor antigens to the immune system?

Decreased density of LC in cervical SILs and SCC

Al-Saleh et al. J Pathol 1998; 184:283-290
Hubert et al. J Pathol 2005; 206; 346-355
The alterations of LC in SILs are associated with a lower expression of MIP3α/CCL20

| Table 1 - Differential Expression of MIP3α |
|-----------------|---|---|---|
| Biopsy          | +  | +/− | −  |
|                 | (%)| (%)| (%)|
| Exocervix       | 66 | 22 | 11 |
| TZ              | 11 | 33 | 55 |
| SIL             | 22 | 44 | 33 |

TZ, transformation zone; SIL, squamous intraepithelial lesion.

Biopsies were stained with mAbs or isotype matched controls and evaluated for MIP3α expression. The biopsies were grouped into 1 of 3 categories based on the relative expression of MIP3α in the epithelium: uniform (+), intermediate (+/−) and no expression (−). Nine biopsies were analysed for each group and all SILs were HPV +.

The low production of MIP3a/CCL20 in SILs is correlated to a decreased migration of Langerhans cells and to the expression of the viral E6 and E7 oncogenes

Cancer Immunol Immunother
DOI 10.1007/s00262-008-0522-5

Increased migration of Langerhans cells in response to HPV16 E6 and E7 oncogene silencing: role of CCL20

Jean-Hubert Caberg · Pascale Hubert · Ludvine Herman · Michael Herfs · Patrick Roncarati · Jacques Boniver · Philippe Delvenne

Received: 16 January 2008 / Accepted: 11 April 2008 © Springer-Verlag 2008
Work hypothesis (2)

Is it possible to induce the recruitment of DC/LC in a neoplastic squamous epithelium formed in vitro

Organotypic cultures of HPV transformed keratinocytes + LC/DC

Control

With GM-CSF

With MIP3a

**Work hypothesis (3)**

Is it possible to induce the recruitment of DC/LC in a neoplastic squamous epithelium formed in vivo

Animal model of HPV-associated neoplasia (in collaboration with LBTD)

HPV+ keratinocytes placed on a collagen gel, grafted in SCID mice and protected by a silicon chamber

GM-CSF stimulates the infiltration of DC in organotypic cultures of HPV+ keratinocytes transplanted in SCID mice

Anti-HPV vaccines

HPV is a necessary event in the cervical carcinogenesis

→ Anti-HPV VACCINES:

- **therapeutic:**
  → prevention of cervical cancer death by eliminating a neoplastic process already established

- **prophylactic:**
  → primary prevention of cervical cancer by eliminating the cause of cancer (HPV)
Prophylactic anti-HPV vaccines

Immunogens? Virus Like Particles (VLPs):

→ L1 capsid protein (Zhou et al. 1991)
- no DNA → no carcinogenic potential
Anti-HPV vaccines vs the local immune system

Systemic administration of vaccines?

Dendritic cell (DC)

HPV

Langerhans cell (LC)

Local immune system of the cervix?

-> The L1 major capsid protein of HPV16 differentially modulates the antigen-presenting cell functions according to the cervical or vaccinal context
HPV16 VLP activate DC

Increased expression of maturation markers

Increased expression of CXCR4

Increased migration of VLP DC in the presence of CXCL12

$\rightarrow$ DC activated by vaccine VLP could have a greater ability to migrate to the draining lymph nodes and to present viral antigens to T lymphocytes

HPV16 VLP do not activate LC

No expression of maturation markers

Increased production of PGE2 by normal cervical keratinocytes in the presence of VLP

Decreased migration of LC in the presence of conditioned media of VLP-treated keratinocytes

→ HPV16 VLP, in the presence of normal cervical keratinocytes, induce high secretion levels of PGE2 which affect the migration LC

Epithelial metaplasia

HPV infections in the TZ of the uterine cervix

Endocervix

Exocervix

Transformation zone

G = Glandular epithelium
M = Metaplastic squamous epithelium
E = Squamous epithelium
LC derived from TZ have a lower ability to induce the proliferation of allogeneic PBMC compared to LC derived from normal exocervix.

Some factors related to cervical epithelial metaplasia could also contribute to cancer development by increasing the permissivity to HPV infections.

Factors which could influence the migration and the function of DC

A. Cytokines/chemokines/Defensins

- GM-CSF, MIP3a, HD-5,
- + : GM-CSF, TNFa,...
- - : TGFb, IL10,...

B. Cell adhesion molecules

E-cadherin for DC/Kc interactions (Tang et al, 1993)
The expression of E-cadherin is correlated to the density of LC

Slug et Snail transcription factors inhibit E-cadherin expression and decrease the adhesion of LC to keratinocytes

Role of immune alterations induced by HPV in the development of cervical cancer: summary

- Infection of keratinocytes
- HPV
- E6/E7
- Cytokines/Chemokines
- Adhesion molecules
- Deficiency of LC
- Functional alterations of antigen presentation
- Absence of immune recognition of HPV antigens
- Cancer

- L1-VLP
- Squamous metaplasia
Research Unit: Cancer of the uterine cervix, HPV and the « metaplasia-dysplasia-cancer » sequence

PI: Pascale Hubert
    Anca Reschner
    Michael Herfs

Students: Ludivine Herman
          Stéphanie Demoulin
          Joan Somja

Technicians: Patrick Roncarati
             Estelle Dortu