HPV & Cervical Cancer: Mechanisms

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Cervical Cancer Basics

- Cancer of the cervix
- 2\textsuperscript{nd} most common cancer in women worldwide
- Profiles like an STD (sexually transmitted disease) because of STD-dependent development
HPV is Necessary Cause of Cervical Cancer

- Human papillomavirus DNA required for development of cervical cancer
- HPV DNA detected in 90-100% of cervical cancer specimens compared to 5-20% in epidemiological control specimens

**Figure 3** Prevalence of human papillomavirus (HPV) DNA in cases and controls in the IARC multicentre case–control study.24-30

Bosch et al., 2002.
HPV is Epitheliotropic

- All characterized strains only infect epithelial cells, specifically
  - skin
  - anogenital mucosa
  - oropharyngeal mucosa

Human Papillomavirus model*

*Human Papillomavirus model is a graphic representation of the virus structure.
HPV Genome

• E1-E7 = “Early” genes (nonstructural)
• L1, L2 = Capsid genes
• URR = upstream regulatory region

• E6 & E7 proteins play major role in immortality & malignant transformation of infected cells
• E5 has role, but not required to maintain cancer phenotype

Munoz et al. 2006.
HPV Classification: Carcinogenic Risk

- Over 100 HPV strains identified
- Risk assessment based on transformative potential of a strain’s E proteins

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Strains</th>
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<tbody>
<tr>
<td>Low Risk</td>
<td>60, 11, 42, 43, 44</td>
</tr>
<tr>
<td>Intermediate</td>
<td>31, 33, 35, 51, 52, 58</td>
</tr>
<tr>
<td>High Risk</td>
<td>16, 18, 45, 56</td>
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</table>

- **Low** → found in benign lesions only
- **Intermediate** → found in benign lesions & invasive cancers
- **High** → usually found in carcinomas; occasionally seen in benign lesions

Furumoto et al., 2002.
Early Genes Hijack Cell Cycle Checkpoint

- HPV’s E6 & E7 proteins interact with key cell cycle proteins including pRB & p53, effectively over-riding the G₁/S-phase checkpoint

Mechanism

1. **E7** binds & phosphorylates pRB, activating E2F transcription factor
2. DNA replication proteins of host cell are then expressed; unchecked S-phase occurs
3. **E6** marks p53 for proteolytic degradation so it cannot activate apoptosis (*note*: absence of p53 is not necessary for E6 to cause immortalization)
E6 & E7 in Cervical Cancer Progression

Furumoto et al., 2002.
Consequences of the HPV Hijack

- Keratinocyte differentiation retarded

- Checkpoint dependence gone
  - Chromosomal instability; accumulation of oncogenic mutations
  - Increased loss of cell cycle/growth control

- Cancer
### High-Risk HPV Oncoproteins: E6

<table>
<thead>
<tr>
<th>E6 Identified Function</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Cell immortalization</td>
<td>(1) Band et al., 1990</td>
</tr>
<tr>
<td>(2) Binding of E6-associated protein results in degradation of specific host cell</td>
<td>(2) Werness et al., 1990 &amp; Sheffner et al., 1993</td>
</tr>
<tr>
<td>proteins [p53]</td>
<td></td>
</tr>
<tr>
<td>(3) Anti-apoptotic effect</td>
<td>(3) Werness et al., 1990 &amp; Thomas, 1998</td>
</tr>
<tr>
<td>(4) Chromosomal destabilization</td>
<td>(4) White et al., 1994</td>
</tr>
<tr>
<td>(5) Enhancement of foreign DNA integration &amp; mutagenicity</td>
<td>(5) Kessis et al., 1996 &amp; Havre et al., 1995</td>
</tr>
<tr>
<td>(6) Activation of telomerase</td>
<td>(6) Klingelhutz et al., 1996</td>
</tr>
<tr>
<td>(7) Blockade of interferon functions</td>
<td>(7) Ronco et al., 1998</td>
</tr>
<tr>
<td>(8) Degradation of Bak protein</td>
<td>(8) Banks et al., 1998 &amp; 1999</td>
</tr>
</tbody>
</table>

E6 is Pleiotropic

- Stimulates expression of transcription factor HIF-1α
- Prognostic Marker: Higher levels of HIF-1α expression in early-stage invasive cervical cancer correlated to shorter overall survival time

HPV-16 E6 protein from SWISS-Model Repository (P03126)
Significance of HIF-1α Expression in Cervical Cancer

- In cells with normal functioning p53, HIF-1α is expressed in instances of hypoxia (as its name, hypoxia-inducible factor, implies)
- HIF-1α binds & stabilizes p53 to induce apoptosis of hypoxic cells, however p53 is degraded by E6 in HPV-infected cells
- Instead, HIF-1α stimulates neoangiogenesis for tumor cells, providing the vascularization necessary for cancer progression
Evidence of HIF-1α Overexpression in Cervical Cancer

Invasive cervical cancer specimens exhibiting strong (A) & weak (B) HIF-1α expression

- No expression of HIF-1α in normal specimens
  - Antibody treatment less likely to disrupt normal cells
- HIF-1α expression identified by nuclear staining/immunohistochemistry

Birner et al., 2000
Candidate for Anti-angiogenesis Therapy?

- **Reduction of** **HIF-1α**-induced angiogenesis may slow progression rate by cutting off oxygen & nutrient supply to tumor cells

- **HIF-1α** mediates angiogenesis through activation of **VEGF** pathway
  - *Vascular endothelial growth factor* stimulates angiogenesis & release of similar factors
  - **AntiHIF-1α** or **antiVEGF** antibody treatment may control progression of cervical cancer
## High-Risk HPV Oncoproteins: E7

<table>
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<th>E7 Identified Function</th>
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</tr>
</thead>
<tbody>
<tr>
<td>(1) Cell immortality</td>
<td>(1) Munger &amp; Phelps, 1993</td>
</tr>
<tr>
<td>(2) Activation of cyclins E &amp; A</td>
<td>(2) Arroyo et al., 1993 &amp; Zerfass et al., 1995</td>
</tr>
<tr>
<td>(3) Induction of apoptosis</td>
<td>(3) Putthenveettil et al., 1996</td>
</tr>
<tr>
<td>(4) Inhibition of cyclin-dependent kinase inhibitors</td>
<td>(4) Jones et al., 1997 &amp; Funk et al., 1997</td>
</tr>
<tr>
<td>(5) Enhancement of foreign DNA integration &amp; mutagenicity</td>
<td>(5) Kessis et al., 1996 &amp; Reznikoff et al., 1996</td>
</tr>
<tr>
<td>(6) Degradation of Blk tyrosine kinase</td>
<td>(6) Oda et al., 1999</td>
</tr>
</tbody>
</table>

E7 is Pleiotropic

- Inactivation of $p^{21}_{CIP-1}$ & $p^{27}_{KIP-1}$ (cdk inhibitors) results in growth stimulation of infected cells
- Inactivation of tumor suppressor transcription factor interferon 1 (IRF-1) through direct interaction

HPV-1a E7 protein from SWISS-Model Repository (P06465)
May explain the immune-resistance mechanism of HPV-infected cervical cancer cells

1. IRF-1 activated during exposure to viral infection, IFNs, TNF\(\alpha\), etc.
2. Histone deacetylase (HDAC) mediates accessibility to chromatin of IRF-1 inducible genes, such as IFN-\(\beta\)
3. IFN-\(\beta\) expression stimulates anti-proliferative effect on cell

Normal role of IRF-1 in tumor suppressor mechanism
Mechanism of IRF-1 Inactivation

- E7 interacts with HDAC and IRF-1
- Blocks expression of IRF-1 inducible genes by inhibiting HDAC
- Result: Cell proliferation evades immune response

Park et al., 2000.
Notch1 Signaling Pathway in HPV-Cervical Cancer

- **Notch1** expression would inhibit expression of HPV regulatory region (URR) & subsequent E6/E7 expression.
- Novel protective role against HPV-induced transformation.

Talora et al., 2002.
Consequences of Notch1 Downregulation

- Downregulation of Notch1 expression inhibits cell growth & differentiation
- Required for maintenance of malignant phenotype in later stages of invasive cervical cancer (maintains E6/E7 expression)
- Mechanism poorly understood, but may eventually reveal drug target

A/B: Notch1 staining in normal cervical biopsy

Notch1 staining in CIN lesion (C) & invasive cervical cancer (D) biopsies

Talora et al., 2002.
E6 & E7 Integrate into Host Genome

• Progression of the tumor condition requires integration of viral genes into host genome
  • Chromatin remodeling or negative regulation of transcription (involving E2)
  • Benign or pre-malignant lesions show viral genes to be extrachromosomal

• Consequences
  • Stabilization of mRNA transcripts of viral genes
  • Results in constant E6 & E7 levels required to maintain phenotype of malignant cells
Carcinogenesis by HPV

Figure 10  Mechanisms of human papillomavirus (HPV) carcinogenesis. HSIL, high grade squamous intraepithelial lesion; LSIL, low grade squamous intraepithelial lesion; RB, retinoblastoma gene.

Bosch et al. 2002.
Preventative Intervention

- Vaccination against HPV infection
- HPV testing (PCR) is useful as an alternative primary screening tool for cervical cancer
  - Clinical trials have indicated HPV testing as a way to solve cases where cytology-based screening results are ambiguous
  - Determination of strain (e.g. HPV-16) characterizes associated carcinogenic risk
CIN: Pre-Cancerous Warning

- Cervical intraepithelial neoplasia (CIN) observed in disease progression
  - New, abnormal, disorganized growth of cervix epithelium
- Gynecological CIN Diagnosis
  1. Atypical Pap smear (not definitive!)
  2. Culposcopy: definitively determines if CIN present by examining specimen under culposcope
## Stages of CIN

1. **CIN I**
   - Number & depth of abnormal cells is low

2. **CIN II**
   - Abnormal cell growth penetrates about $\frac{1}{2}$ the thickness of cervical epithelium

3. **CIN III**
   - “carcinoma in-situ”
   - Abnormal cell growth penetrates entire thickness of cervical epithelium

4. **Invasive Cervical Cancer**
   - Abnormal cell growth penetrates beyond cervical epithelium
Stages of CIN: Histology

Furumoto et al., 2002.

NORMAL  CIN I  CIN II  CIN III
Cervical Cancer Cofactors

- HPV is NOT *sufficient* cause for cervical cancer
- Combination of HPV & 1 or more cofactors increase risk of cancer progression
  - Hormonal contraceptives
  - Smoking
  - Parity
HPV Vaccination

- Gardasil® is only FDA-approved viral vaccine for HPV 16*, 18*, 6 & 11
  - *high risk strains
- Contains purified virus-like particles (VLPs) of L1 gene product to activate humoral immune response in host
  - L1 = major capsid (structural) protein
  - VLPs = self-assembled capsid proteins in immuno-relevant organization
Treatment Options

- **Cryotherapy/laser surgery** – freezing off or cutting away of abnormal cervical epithelial cells
- **Partial/full hysterectomy** – removal of uterus & cervix, sometimes ovaries & fallopian tubes
- **Radiation therapy/chemotherapy**
### References