Mechanisms of Viral Oncogenesis

Denise A. Galloway  
FHCRC

<table>
<thead>
<tr>
<th>Human Cancers Associated with a Viral Etiology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carcinomas</strong></td>
</tr>
<tr>
<td>Anogenital HPV</td>
</tr>
<tr>
<td>Hepatocellular HBV, HCV</td>
</tr>
<tr>
<td>Nasopharynx EBV</td>
</tr>
<tr>
<td>Oropharynx HPV</td>
</tr>
<tr>
<td>Stomach EBV</td>
</tr>
<tr>
<td>Merkel Cell MCPyV</td>
</tr>
<tr>
<td><strong>Lymphomas</strong></td>
</tr>
<tr>
<td>Post-transplant EBV</td>
</tr>
<tr>
<td>Burkitt EBV</td>
</tr>
<tr>
<td>Hodgkin EBV</td>
</tr>
<tr>
<td>Adult T-cell HTLV-1</td>
</tr>
<tr>
<td><strong>Sarcoma</strong></td>
</tr>
<tr>
<td>Kaposi HHV8</td>
</tr>
</tbody>
</table>
Donald Maxwell Parkin*
Clinical Trials Service Unit and Epidemiological Studies Unit,
University of Oxford, Headington,
Oxford OX3 7LF, United Kingdom

The global health burden of infection-associated cancers in the year 2002

2006 Wiley-Liss, Inc.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cancer</th>
<th># cases</th>
<th>% all Ca.</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. Pylori</td>
<td>Stomach</td>
<td>592,000</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
<td>11,500</td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>Cervix</td>
<td>492,800</td>
<td>5.2</td>
</tr>
<tr>
<td></td>
<td>Anogenital</td>
<td>53,880</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Oropharynx</td>
<td>14,500</td>
<td></td>
</tr>
<tr>
<td>HBV, HCV</td>
<td>Liver</td>
<td>535,000</td>
<td>4.9</td>
</tr>
<tr>
<td>EBV</td>
<td>Nasopharynx</td>
<td>78,100</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Hodgkin L.</td>
<td>28,600</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Burkitt L.</td>
<td>6,700</td>
<td></td>
</tr>
<tr>
<td>HIV/HHV8</td>
<td>Kaposi sarcoma</td>
<td>66,200</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Non-Hod. L.</td>
<td>36,100</td>
<td></td>
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<tr>
<td>Schistosomes</td>
<td>Bladder</td>
<td>10,600</td>
<td>0.1</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>ATL</td>
<td>3,300</td>
<td>0.03</td>
</tr>
<tr>
<td>Liver flukes</td>
<td>Bile duct</td>
<td>2,500</td>
<td>0.02</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1,932,800</td>
<td>17.8</td>
</tr>
</tbody>
</table>
# I-A Ca in Developing and Developed Countries

<table>
<thead>
<tr>
<th>Site</th>
<th>Agent</th>
<th>Developed Countries</th>
<th>Developing Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># cases</td>
<td>% all Ca</td>
<td># cases</td>
</tr>
<tr>
<td>Liver</td>
<td>HBV, HCV</td>
<td>48,000</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Flukes</td>
<td>0</td>
<td>2,500</td>
</tr>
<tr>
<td>Cervix</td>
<td>HPV</td>
<td>83,400</td>
<td>1.7</td>
</tr>
<tr>
<td>Stomach</td>
<td>H. pylori</td>
<td>192,000</td>
<td>3.8</td>
</tr>
<tr>
<td>Kaposi</td>
<td>HHV8</td>
<td>3,700</td>
<td>0.1</td>
</tr>
<tr>
<td>NHL</td>
<td>HIV/EBV</td>
<td>9,300</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>EBV (BL)</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H. pylori</td>
<td>5,600</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HTLV-1</td>
<td>550</td>
<td></td>
</tr>
<tr>
<td>Anogenital</td>
<td>HPV</td>
<td>22,450</td>
<td>0.4</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>EBV</td>
<td>6,500</td>
<td>0.1</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>HPV</td>
<td>5,600</td>
<td>0.1</td>
</tr>
<tr>
<td>Hodgkin L.</td>
<td>EBV</td>
<td>11,500</td>
<td>0.2</td>
</tr>
<tr>
<td>Bladder</td>
<td>Schistos</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>389,000</strong></td>
<td>7.7</td>
</tr>
</tbody>
</table>

## Why Study Infection Associated Cancers?

- Prevention - vaccines, eliminate agent
- Diagnosis - identify precursors expressing agent
- Treatment - anti-virals, antibiotics
- Provides insights into mechanism
- Important problem- large world wide burden of cancer
Principles of Viral Oncology

- Viruses have evolved to replicate and be transmitted to new hosts, and to establish latency/persistence, NOT TO CAUSE CANCER.

- Most viral infections are mildly pathogenic and are controlled by the host immune response.

- Viruses contribute to the etiology of human cancers in diverse ways; by direct and indirect means.

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Principles of Oncology

- The induction of proliferation involves exit from $G_0$ or $G_1$ into $S$ phase.
• Uncontrolled proliferation induces apoptosis

**p53**

- Noxa, Puma
- Bak, Bax
- BclxL, Mcl1
- Mule

Cyt C → caspases

Bak/Bax

**Principles of Oncology**

• Uncontrolled proliferation induces senescence through telomere shortening (or S phase DNA damage).

Telomerase (hTERT) is activated in cancer cells to maintain telomere length.

**telomere length**

<table>
<thead>
<tr>
<th>cell divisions/age</th>
</tr>
</thead>
<tbody>
<tr>
<td>undetectable telomeres</td>
</tr>
<tr>
<td>End-to-end fusions</td>
</tr>
</tbody>
</table>
How do HPVs cause cancer?

Why do only some HPVs cause cancer?

Papillomaviruses are a Large Family

de Villiers et al 2004
Natural History of Cervical HPV Infections

Atypia/ASCUS/CIN1-2/LSIL

CIN2-3/CIS/HSIL

Adapted from Wright and Schiffman

Natural History of Cervical Neoplasia: Median Age of Events

Sexual debut
HPV infection
CIN/SIL

Prevalent CIS
Micro invasive CxCA
Invasive CxCA

Koutsky, Kiviat, Winer and others
Human papillomavirus (HPV)

- NCR
- E6
- E7
- L1
- L2
- E5
- E4
- E2
- E1

~8000 bp

Viral oncogenes

Non-coding regulatory sequences

Viral capsid proteins

Transcriptional control and viral DNA replication

The HPV Life Cycle

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Doorbar, J.Clin.Vir. 2005
Targets of the HR E6 and E7 oncoproteins

- E2F
- S phase genes
- HDAC
- E7
- Rb/p107/p130

- E7
- HDAC
- E2F
- S phase genes
- E6-AP
- E6
- E6-AP
- E6
- p53
- BAK
- telomerase activity
- AAUCCCAA
- PDZ
- E6
- induces
- HPV E6/E7 persist; Rb + p53 are wild type

HPV Expression in Cervical Cancers

E6/E7 expand the proliferative compartment inactivate cell cycle checkpoints, block apoptosis, induce telomerase activity
Strategies for HPV Vaccination

HPV L1 Assembles into Virus-Like Particles
GARDASIL® [Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine]

**GARDASIL: A Quadrivalent HPV Vaccine**

- Quadrivalent HPV 6/11/16/18 L1 virus-like particle (VLP) vaccine
- VLPs are produced in *Saccharomyces cerevisiae*.
  - The L1 proteins self-assemble into VLPs.
  - Purified VLPs are adsorbed on aluminum-containing adjuvant.
  - The adjuvant is amorphous aluminum hydroxyphosphate sulfate (225 μg per dose).
- Each 0.5-mL dose contains HPV Types 6/11/16/18 (20/40/40/20 μg L1 protein, respectively).

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**GARDASIL Is Efficacious Against HPV 16– and 18–Related CIN 2/3 or AIS**

- 16- to 26-year-old females naïve to the relevant vaccine HPV type at enrollment and through 30 days Postdose 3
- Over a period of 2 to 4 years
- Analysis included Protocol 005.

CIN = cervical intraepithelial neoplasia; AIS = adenocarcinoma in situ.
HPV in other Anogenital and Oropharyngeal Cancers

- Same HPV types; predominantly HPV 16
- Viral genome persists and E6/E7 expressed
- Wild type Rb and p53

Why don’t low risk HPVs that infect the genital tract cause cancer?
Targets of the LR E6 and E7 oncoproteins

Papillomaviruses are a Large Family

de Villiers et al 2004
Genus Beta HPVs and Squamous cell Skin Cancer

- HPVs first found with Epidermodysplasia verruciformis then in other SCSCs.
- Beta HPVs are prevalent on normal skin.
- Viral genome is not present in most cells of the tumor.
- UV exposure is the major risk factor for SCSC, also Immunosuppression.
- p53 mutation and p16/ARF loss are very frequent

Targets of the β E6 and E7 oncoproteins

Some E6s induces telomerase activity

- HDAC
- E2F
- E7
- Rb/p107/p130
Beta HPVs and Skin Cancer

- E6/E7 may expand the proliferative compartment
- E6 blocks the apoptotic response to UV
- Carcinogenesis is driven by mutation
- Persistent HPV is not required and may be Selected against

HBV and HCV in the etiology of Hepatocellular Carcinoma

- Prevalence of HCC is correlated with endemic chronic infection with HBV and HCV
- Chronic HBV and HCV infections result in cirrhosis in ~15% of subjects in 25-30 years, with HCC arising in ~1% of subjects with cirrhosis.
- Non-viral cirrhosis is also a risk factor for HCC.

HCC only arises in the context of cirrhosis.
Hepatitis viruses are very diverse

- HBV is a hepadna virus - a DNA, gapped double stranded genome

  Infection induces inflammation, cytokine signalling

  HBV DNA is integrated into some, but not all HCCs. Integration to activate hTERT has been documented.

  HBV X is frequently expressed
  
  - transactivates promoters indirectly
  - regulates proteosome function
  - regulates mitochondrial function
  - modulates calcium function

Hepatitis viruses are very diverse

- Hepatitis C virus (HCV) is a positive strand RNA Flavivirus.

  HCV core induces signalling pathways

  E2 interferes with interferon signalling
Do HBV and HCV induce Cancer?

- HBV and HCV induce unique gene expression profiles indicating that the virus has an effect on the phenotype of the cancer.

- The HBV vaccine has reduced the incidence of HCC.

EBV is associated with multiple cancers

- Burkitt’s lymphoma
- Post transplant lymphomas
- Hodgkin’s lymphoma
- Nasopharyngeal carcinoma
- Gastric cancer
EBV is associated with multiple cancers

- EBV infection occurs in > 90% of the population; cancer is very rare.

- Most EBV associated cancers are associated with immunosuppression - transplantation (drugs); HIV; malaria;

- EBV causes cancer in both B cells, where the virus is latent and productive, and in epithelial cells that do not support viral latency.

EBV associated cancers

- Naïve B cell
- Germinal Center
- Memory B cell
- Other B cells
- PTLD: EBNA1-3, LMPs1-2b
- Hodgkin’s: EBNA1, LMP1,2A
- Burkitt’s: EBNA1 only
EBV associated cancers

- Nasopharyngeal Carcinoma
  - EBNA1, LMP1, 2a

HHV-8 (KSHV) and Cancer

- Kaposi’s sarcoma - spindle cell (endothelial origin)
- Pulmonary Effusion Lymphoma
- Multicentric Castleman’s Disease - B cell (non-cancer)

Viral genome encodes homologues for cellular genes that block innate and adaptive immunity, block apoptosis, induce proliferation.
Are there other Cancers with a Viral Etiology?

Cancers that increase disproportionately with Immunosuppression

- non EBV+ lymphomas
- skin cancers
- Merkel cell cancer
- lung cancer

Before I came here I was confused about this subject. Having listened to your lecture, I am still confused – but at a higher level.

Enrico Fermi