TRANSFORMATION
AND
ONCOGENESIS

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Lecture file:
http://www.howard.edu/rcmi_proteomics/events.html
Objectives:

• Terminology
• Types of cancers caused by viruses
• History of oncogenic viruses discovery
• Cell transformation, division, cell cycle
• Avian leukemia virus
• Viral oncogenes
• Types of transforming retroviruses
• Strategies and mechanisms of viral transformation
• HTLV oncogenesis
• Viral hepatitis oncogenesis
Cancer

**Benign:** cell growth without infiltration, not a malignant

**Cancer:** a malignant tumor that spreads out and infiltrates tissues, lymph nodes and different organs

- Genetic disease
- Leads to growth of cells with mutations
- Mutations may affect cells communication, growth and proliferation
- Mutations can be inherited or acquired
- Acquired mutations could be due to carcinogens or viral infections

**Carcinogenesis:** Complex multistage process by which cancer develops (5-7 mutations on average needed)

**Viral cancers:** ~20% of human cancers (EBV, HBV, HCV, HTLV-1, HPV)
Types of Cancer

**Adenocarcinoma:** cancer of epithelium in glandular tissue

**Carcinoma:** cancer of epithelial tissue

**Endothelioma:** overproduction of erythrocytes

**Fibropapilloma:** solid tumor of cells derived from connective tissue cells.

**Hepatocellular carcinoma:** cancer of liver epithelial cells

**Leukemia:** cancer of white blood cells

**Lymphoma:** cancer of lymphoid tissue

**Retinoblastoma:** cancer of retinal cells

**Sarcoma:** cancer of fibroblasts
<table>
<thead>
<tr>
<th>Common Cancer Types</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bladder Cancer</strong> (carcinomas)</td>
</tr>
<tr>
<td><strong>Breast Cancer</strong> (ductal or lobular carcinoma)</td>
</tr>
<tr>
<td><strong>Colon and Rectal Cancer</strong> (adenocarcinoma)</td>
</tr>
<tr>
<td><strong>Endometrial Cancer</strong> (adenocarcinoma in uterus)</td>
</tr>
<tr>
<td><strong>Kidney (Renal Cell) Cancer</strong> (renal cell carcinomas)</td>
</tr>
<tr>
<td><strong>Leukemia</strong> (overproliferation of leukocytes)</td>
</tr>
<tr>
<td><strong>Lung Cancer</strong> (in tissues of the lung, usually in the cells lining air passages)</td>
</tr>
<tr>
<td><strong>Melanoma</strong> (cancer of melanocytes)</td>
</tr>
<tr>
<td><strong>Non-Hodgkin Lymphoma</strong> (B and T cells lymphomas, including Burkitt lymphoma)</td>
</tr>
<tr>
<td><strong>Pancreatic Cancer</strong> (in the tissues of the pancreas)</td>
</tr>
<tr>
<td><strong>Prostate Cancer</strong> (in tissues of the prostate)</td>
</tr>
<tr>
<td><strong>Thyroid Cancer</strong> (papillary, follicular, medullary, and anaplastic thyroid cancer)</td>
</tr>
</tbody>
</table>
Cancers Caused by Viruses

- Major cause of liver and cervical cancer in humans
- Malignancy is not required for viral replication
- Cancer is a side effect of host response or host-viral interaction.

<table>
<thead>
<tr>
<th>Virus Family</th>
<th>Associated Cancer(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RNA viruses</strong></td>
<td></td>
</tr>
<tr>
<td><em>Flaviviridae</em></td>
<td></td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td><em>Retroviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hematopoietic cancers, sarcomas, carcinomas</td>
</tr>
<tr>
<td><strong>DNA viruses</strong></td>
<td></td>
</tr>
<tr>
<td><em>Adenoviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Various solid tumors</td>
</tr>
<tr>
<td><em>Hepadnaviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td><em>Herpesviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphomas, carcinomas and sarcomas,</td>
</tr>
<tr>
<td><em>Papillomaviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Papillomas and carcinomas</td>
</tr>
<tr>
<td><em>Polyomaviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Various solid tumors</td>
</tr>
<tr>
<td><em>Poxviridae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Myxomas (connective tissue, in heart) and fibromas (benign)</td>
</tr>
</tbody>
</table>
Common Features of Cancers Caused by Viruses

• Transformation is a single-hit process (infection with a single particle is sufficient to cause transformation)

• In most cases, transformation is accomplished by continuous expression of viral genes

• Infected cells do not produce viral particles. Viral transforming proteins alter cell proliferation by a limited number of molecular mechanisms
Discovery of Oncogenic Viruses

1908: Ellerman and Bang show that avain leukemia could be transmitted through filtered extracts or serum from infected birds. But leukemia was not recognized as a cancer at the time.

1911: Rous injected a cell free filtrate obtained from a solid tumor in chicken and showed formation of the same tumor (Nobel Prize in 1966)

1933: Shope isolates papillomavirus from warts

1962: Injection of polyoma virus into hamseter kidney BHK21 cells led to cells transformation

1964: Injection of SV40 into 3T3 cells led to cells transformation

1966: Discovery of Epstein-Barr virus in cells from Burkitt’s Lymphoma

1976: Bishop and Varmus showed that oncogenes are activated proto-oncogenes (Nobel Prize in 1989)
Rous Sarcoma Virus

A

B

Cell Transformation

Normal cells

- Growth and death are controlled
- Short-lived cells: intestinal and white blood ($t_{1/2} \sim \text{few days}$)
- Long lived, red blood cells, $t_{1/2} > 100$ days;
  - Neuronal cells, $t_{1/2} \sim \infty$

Transformation

- Immortality: can grow indefinitely
- Reduced requirement for serum growth factors
- Loss of capacity for growth arrest upon nutrient deprivation
- High saturation densities
- Loss of contact inhibition
- Anchorage independent (can grown in soft agar)
- Altered morphology (rounded and refractile)
- Tumorogenic (form tumors when transplanted into animals)
Primary cells in culture quickly reach a non-proliferative state known as senescence or mortality checkpoint (M1).

Cells can overcome M1 through inactivation of Rb or p53 and then reach the second mortality checkpoint 2 (M2) or crisis, which is associated with shortening telomere sequences.

Culturing primary cells also requires supplementation with serum and pro-angiogenic factors.

Cells immortalized with SV40 large T antigen and telomerase grow up to 60 passages but upon withdrawal of growth factors quickly die.
Mortality Checkpoint 1

Mortality Checkpoint 2

C

Control media                             293-E4 media

SV-40 large T antigen IHC

Control                            SV

-40 large T antigen IHC

Tumor in Mice Injected with Induced Pluripotent Stem Cells

(a) Transfection Passage onto MEF Clones formation Monitoring and picking of clones

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>2</th>
<th>7</th>
<th>24</th>
</tr>
</thead>
</table>

(b) i (293T derived iPSC) ii (293T-KD-derived iPSC)

Adopted from Jerebtsova et al., Biology, 2012
Mitogen-activated Signal Transduction

Fig 7.3 (Flint et al., Principles of Virology, Vol II).
Eukaryotic Cell Cycle

Fig 7.4 (Flint et al., Principles of Virology, Vol II).
Control of Cell Cycle by CDKs

Fig 7.5 (Flint et al., Principles of Virology, Vol II).
Transformation and Oncogenesis

• Cultured cells can be transformed
• Oncogenesis – development of tumor in animals
• Oncogenesis requires genetic changes
• Transformed cells may or may not be oncogenic
• Transformed cells may be oncogenic in some but not in all species
• Oncogenesis requires a concerted effort of viral infection and host cell mutation(s)
Transformation by Viral Infection

- Cytopathic effects should not be lethal (i.e. cells should survive the infection)
- Viral replication should be reduced or eliminated
- The cells should continue proliferation and division
Avian Leukosis Virus (ALV)

- Discovered in 1908
- Endemic in chicken
- Retrovirus with ssRNA genome
- Most chickens are infected in the first few months after hatching
- Leukosis (leukemia) occurs at ~ 14 weeks of age with 3% rate
- All birds have viremia, but in most it is controlled
- Does not result in solid tumor formation
- In aged animals, solid tumors appear and if virus is isolated it can cause Sarcoma
- ALV isolated from animals can often replicate only in the presence of original ALV
Retrovirus Replication Cycle

Infecting virus → Receptor binding and membrane fusion → Uncoating and reverse transcription → Viral RNA

Integration → Viral RNA encapsidation → Viral protein production → Virus release and maturation
### v-Oncogenes in Retroviruses

**Avian transducing retroviruses**

<table>
<thead>
<tr>
<th>gag</th>
<th>pol</th>
<th>env</th>
<th>Typical progenitor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Avian leukosis virus</td>
</tr>
</tbody>
</table>

- src: Rous sarcoma virus
- fps: PRC II avian sarcoma virus
- fps: Fujinami sarcoma virus
- myb: Avian myeloblastosis virus BA1
- myb ets: Avian myeloblastosis virus E26
- myc: Avian myelocytoma virus MC 29
- mil myc: Avian myelocytoma virus MH2
- yes: Avian sarcoma virus Y73
- jun: Avian sarcoma virus 17
- erbA erbB: Avian erythroblastosis virus ES4
- rel: Avian reticuloendotheliosis virus
- sea: S13 avian erythroblastosis virus
- ros: UR2 avian sarcoma virus

**Mammalian transducing retroviruses**

<table>
<thead>
<tr>
<th>gag</th>
<th>pol</th>
<th>env</th>
<th>Typical progenitor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Murine leukemia virus</td>
</tr>
</tbody>
</table>

- abl: Abelson murine leukemia virus
- mos: Moloney murine sarcoma virus
- raf: 3611 murine sarcoma virus
- fes: Gardner-Arnstein feline sarcoma virus
- fms: McDonough feline sarcoma virus
- sis: Simian sarcoma virus
- kit: HZ4 feline sarcoma virus
- ras: Harvey murine sarcoma virus

Fig 7.7 (Flint et al., Principles of Virology, Vol II).
v-Oncogenes:
- Growth factors (similar to normal growth factors)
- Growth factor receptors
- Intracellular signal transducers
- Transcription factors
- Tumor suppressor genes

Viral oncogene products:
- E1A, sT and LT, …. May contain pieces of cellular DNA
<table>
<thead>
<tr>
<th>Transducing oncogene</th>
<th>Function of cellular homolog</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth factors</strong></td>
<td></td>
</tr>
<tr>
<td><em>Sis</em></td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td><strong>Tyrosin kinase growth factor receptor</strong></td>
<td></td>
</tr>
<tr>
<td><em>erb2</em></td>
<td>Epithelial growth factor receptor</td>
</tr>
<tr>
<td><em>Kit</em></td>
<td>Hematopoietic receptor</td>
</tr>
<tr>
<td><strong>Hormone receptors</strong></td>
<td></td>
</tr>
<tr>
<td><em>erbA</em></td>
<td>Thyroid hormone receptor</td>
</tr>
<tr>
<td><strong>G-proteins</strong></td>
<td></td>
</tr>
<tr>
<td><em>H-ras, K-ras</em></td>
<td>GTPases</td>
</tr>
<tr>
<td><strong>Adapter proteins</strong></td>
<td></td>
</tr>
<tr>
<td><em>Crk</em></td>
<td></td>
</tr>
<tr>
<td><strong>Nonreceptor tyrosine kinase</strong></td>
<td></td>
</tr>
<tr>
<td><em>src, abl</em></td>
<td>Signal transduction</td>
</tr>
<tr>
<td><strong>Serine/threonine kinases</strong></td>
<td></td>
</tr>
<tr>
<td><em>mos</em></td>
<td>germ cell maturation</td>
</tr>
<tr>
<td><em>Akt</em></td>
<td>signal transduction</td>
</tr>
<tr>
<td><strong>Nuclear proteins</strong></td>
<td></td>
</tr>
<tr>
<td><em>jun, fos</em></td>
<td>transcriptional regulator (Ap-1 complex)</td>
</tr>
<tr>
<td><em>Myc</em></td>
<td>transcriptional regulator</td>
</tr>
</tbody>
</table>
Activation of Cellular Oncogenes

Mutation in the host gene
- Integration of retrovirus mutates cellular genes
- Proviral promoters can activate transcription of nearby genes
- Transformation can occur if the nearby gene is an oncogene
- Transformation can also occur if insertion disrupts tumor suppressor genes.

Change or loss of expression control
- Regulation of expression is altered
- Fusion to another promoter
- Stabilization of unstable proteins

Null mutation in tumor suppressor gene
Mechanisms of Oncogene Capture by Retroviruses

1. Integration within a proto-oncogene
   - LTR gag pol env LTR onc
   - Deletion of virus and cell sequence
   - Packaging of read-through transcript and wild-type genomes

2. Deletion of virus and cell sequence
   - Wild-type mRNA
   - Packaging of deleted and wild-type genomes

3. Nonhomologous recombination during reverse transcription in newly infected cell
   - Additional rearrangements and point mutations may occur
   - Point mutation
   - Rearrangement

Fig 7.8 (Flint et al., Principles of Virology, Vol II).
# Oncogenic Retroviruses

<table>
<thead>
<tr>
<th>Property</th>
<th>Transducing Viruses</th>
<th>Non-transducing Viruses</th>
<th>Long Latency Viruses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency of tumor formation</td>
<td>High (100%)</td>
<td>High to intermediate</td>
<td>Very low (&lt;5%)</td>
</tr>
<tr>
<td>Tumor latency</td>
<td>Short (days)</td>
<td>Intermediate (weeks, month)</td>
<td>Long (months, years)</td>
</tr>
<tr>
<td>Viral genome</td>
<td>Viral-cellular recombinant; replication defective</td>
<td>Intact; replication competent</td>
<td>Intact; replication competent</td>
</tr>
<tr>
<td>Oncogene</td>
<td>Cell oncogene in viral genome</td>
<td>Cellular oncogene activate by virus</td>
<td>Virus-encoded protein activating transcription</td>
</tr>
<tr>
<td>Mechanism</td>
<td>Oncogene transduction</td>
<td>cis-acting provirus</td>
<td>trans-acting protein</td>
</tr>
<tr>
<td>Transformation of cultured cells</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>
Three Types of Oncogenic Retroviruses
# Viral Transforming Gene Products

<table>
<thead>
<tr>
<th>Transducing oncogene</th>
<th>Gene Product</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adenoviridae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human adenovirus type2</td>
<td>E1A</td>
<td>Transforms rodent cells</td>
</tr>
<tr>
<td></td>
<td>E1B</td>
<td>Transforms E1A-expressing cells</td>
</tr>
<tr>
<td></td>
<td>E4</td>
<td>transforms; Ad9 protein causes mammary tumors</td>
</tr>
<tr>
<td><strong>Papillomaviridae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV 16 and 18</td>
<td>E6</td>
<td>Immortalization</td>
</tr>
<tr>
<td></td>
<td>E7</td>
<td>Transform rodent cells, cooperate with E6</td>
</tr>
<tr>
<td>BPV1</td>
<td>E5</td>
<td>transforms bovine and rodent fibroblasts</td>
</tr>
<tr>
<td></td>
<td>E6</td>
<td><em>support transformed phenotype of</em></td>
</tr>
<tr>
<td><strong>Polyomviridae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>LT</td>
<td>Immortalize primary cells</td>
</tr>
<tr>
<td></td>
<td>mT</td>
<td>Transforms established cell lines</td>
</tr>
<tr>
<td>Simian virus 40</td>
<td>LT</td>
<td>Immortalizes primary cells</td>
</tr>
<tr>
<td></td>
<td>sT</td>
<td>Requires for efficient LT transformation</td>
</tr>
</tbody>
</table>
Cellular Proteins Interacting with DNA Virus Transforming Proteins

A

- p27Kip1
- p21Cip1
- E1A
- Rb
- p300/Cbp

B

- Pdz proteins
- Paxillin
- E6
- p53
- p300/Cbp
- c-Myc
- Nfx1-91

Fig 7.9 (Flint et al., Principles of Virology, Vol II).
Genetic Paradigm for Cancer

Proto-oncogenes

G₀

G₂

S

M

G₁

Stop

Tumor suppressor genes
Strategies of Viral Transformation

Permanent activation of cellular signal transduction cascades

Disruption of cell cycle regulation
C-Src Tyrosin Kinase

Fig 7.10 (Flint et al., Principles of Virology, Vol II).
Regulation of Cell Proliferation and Adhesion by Src

Fig 7.11 (Flint et al., Principles of Virology, Vol II).
Expression of Human Homologous Genes by HHV-8

Fig 7.12 (Flint et al., Principles of Virology, Vol II).
Insertional Activation of c-Myc by AVL

Human X-SCID gene Therapy
- Defect in IL2 receptor
- Correction with retroviral gene delivery
- ~30 patients developed leukemia
- Insertion in LMO2 gene

Activation of signal Transduction by Epstein-Barr LMP-1
Activation of Src by Polyomavirus mT
SV40 small T controls Protein Phosphatase 2A

Fig 7.17 (Flint et al., Principles of Virology, Vol II).
Passage Through the Restriction Point in Mammalian Cells

Fig 7.18 (Flint et al., Principles of Virology, Vol II).
Rb Deregulation by SV40 Large T

Fig. 7.19 (Flint et al., Principles of Virology, Vol II).
Additional Mechanisms Of Cell Cycle Regulation

Alteration of cellular plasma membrane receptors
- E5 of BPV1 binds to tyrosine kinase receptors (such as Src) and activates them

Production of virus specific cyclins
- HHV-8 and Herpesvirus Saimiry produce v-cyclin
- Binds to CDK6 and phosphorylates Rb
- Promotes G1 → S transition

Inactivation of Cyclin-Dependent Kinase Inhibitors
- E7 protein of HPV16 binds to p21
- Inactivates p21 and increases p53
- Inactivates Rb
Signaling Pathways that Promote Cell Growth and Survival

Fig 7.21 (Flint et al., Principles of Virology, Vol II).
Function of p53

- p53 is a tumor suppressor gene
- Sensor for DNA damage and hypoxia
- p53 promotes cell cycle arrest
- p53 promotes apoptosis
- Virus infection activates p53
- Many viruses alter p53 activity
Increasing Stability of p53

1. **E2f-Dp1** → **E1A** → **p300/Cbp**
2. **Mdm-2** → **Arf**
3. **p53** → **Mdm-2**
4. **p53** → **Hausp**
5. **p53** → **Proteasome**
6. **p53** → **dsDNA break**
7. **p53** → **t_{1/2} \leq 30 \text{ min}**
8. **p53** → **t_{1/2} > 2 \text{ h}**
9. **p53** → **Acetylation**
10. **p53** → **Tetrameric p53**
11. **p53** → **p53-responsive promoters (e.g., p21^{Cip1}, Bax, Mdm-2)**

**Key Points**
- **p53** is a key protein involved in cellular stress response.
- **Mdm-2** is a negative regulator of p53.
- **E1A** and **p300/Cbp** are involved in transcriptional activation.
- **Hausp** is an ubiquitin-specific protease.
- **Proteasome** is responsible for protein degradation.
- **dsDNA break** indicates DNA damage response.
- **t_{1/2} > 2 \text{ h}** indicates a long-term stability of p53.

**Additional Notes**
- **Atm** and **c-Abl** are involved in phosphorylation and acetylation processes.
Inactivation of p53 Protein

Adenovirus, papillomavirus, or polyomavirus infection

HPV-16 or HPV-18 E6 protein

SV40 LT

E6-Ap

Ub

Proteasome

Degradation

p53 accumulation

Repression of transcription; Binding to apoptosis inducing proteins

Increased transcription of specific cellular genes

p21^{Cip1}

Bax, Fas

Apoptosis

Cell cycle arrest

Apoptosis

E6 ORF6 + E1B 55kDa

Sequestered p53

E1B 55kDa

E4 ORF6

p53-E4 ORF6

p53-E1B 55kDa

?
Human T-cell Leukemia Virus

- Infection leads to Adult T cell leukemia and lymphoma (~ 20-30 years)
- After ATLL detection, survival 6-24 months
- HAM/TSP (spinal cord inflammation), uveitis, arthropathy, ...
- Infection of T-cells and monocytes
- Autoimmune or cytokine damage
- Global prevalence (~20 M): Japan, Carribeans, South America, Australia, Africa
HTLV-1 Genome Organisation and Transcription

Fig 7.26 (Flint et al., Principles of Virology, Vol II).
Progression of HTLV-1-infected cells to ATLL
Effects of Tax on checkpoint factors at various points of the cell cycle are diagrammed.

Hepatitis Virus Oncogenesis

- Hepatitis B (Hepadnavirus), Hepatitis C (Flavivirus)
- Persistent infections
- Sustained low level liver damage due to immune system attack
- Lots of cell proliferation/regeneration
- Lots of cellular DNA replication + Lots of oxidative stress
- = Increased chance of mutation
Hepatitis Virus Oncogenesis

- Aflatoxin B1
  - p53 mutations
  - p53 inactivation
  - Proliferation and loss of growth control
  - Genetic alterations

- HBV or HCV viral and/or host factors
  - Oxidative stress
  - Inflammation
  - Expanded or altered stem cell compartment (?)
  - Possible mutagen: modulation of cancer-relevant signalling pathways
  - Necrosis
  - Regeneration
  - Genetic alterations

- HCV viral and/or host factors
  - Cirrhosis
  - Microenvironmental changes
  - Expanded or altered stem cell compartment (?)

- Alcohol
  - Inflammation
  - Expanded or altered stem cell compartment (?)

- Hepatocellular carcinoma
Retroviral Envelope Syncytin Genes Are Required for Fusion of Placental Syncytiotrophoblasts

Dupressoir A et al. PNAS 2011;108:18591-18592