Transformation and Oncogenesis

Lecture 18
Biology W3310/4310
Virology
Spring 2014

Cause and effect, means and ends, seed and fruit, cannot be severed; for the effect already blooms in the cause, the end pre-exists in the means, the fruit in the seed.

RALPH WALDO EMERSON
Transformation
The puzzling properties of transformed cells in the laboratory

- Immortal: Grow indefinitely (HeLa)
- Loss of anchorage dependence
- Loss of contact inhibition
- Colony formation in semi-solid media
- Decreased requirements for growth factors (serum)
Oncogenesis

• The development of cancer
  - A malignant tumor; growth that is not encapsulated and infiltrates into surrounding tissues, replacing normal cells

• Cancer is a genetic disease

• Mutations affect steps in cell communication, growth, and proliferation

• Mutations may be inherited, caused by DNA damage, environmental carcinogens, or infectious agents including viruses
Transformation and oncogenesis are distinct

- Studying virus-transformed cells provides insight into molecular events that establish oncogenic potential
- No virus can do it all
<table>
<thead>
<tr>
<th>Family</th>
<th>Associated cancer(s)</th>
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</thead>
<tbody>
<tr>
<td>RNA viruses</td>
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<tr>
<td><em>Flaviviridae</em></td>
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<td>Hepatitis C virus</td>
<td>Hepatocellular carcinoma</td>
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<tr>
<td><em>Retroviridae</em></td>
<td>Hematopoietic cancers, sarcomas, and carcinomas</td>
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<td>DNA viruses</td>
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<td><em>Adenoviridae</em></td>
<td>Various solid tumors</td>
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<tr>
<td><em>Hepadnaviridae</em></td>
<td>Hepatocellular carcinoma</td>
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<td><em>Herpesviridae</em></td>
<td>Lymphomas, carcinomas, and sarcomas</td>
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<td><em>Papillomaviridae</em></td>
<td>Papillomas and carcinomas</td>
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<td><em>Polyomaviridae</em></td>
<td>Various solid tumors</td>
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<td><em>Poxviridae</em></td>
<td>Myxomas and fibromas</td>
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</tbody>
</table>

Contributing factor in ~20% of human cancers

- EBV, HBV, HCV, HTLV-1, HIV-1, HPV, HHV-8, MCPyV
Virus-induced cancer

Transformation and malignancy is not required for replication of any virus
On October 1, 1909, Dr. Peyton Rous removed a tumor from an English hen and injected a cell-free filtrate from the tumor into another healthy chicken, which later developed the same type of tumor.

Cancer could be caused by a viral infection!
It took the world almost 50 years to accept this idea

Dr. Rous lived long enough to be awarded the Nobel Prize for Physiology and Medicine in 1966 for his research

His Legacy: RSV; Rous Sarcoma Virus, a key player in two more Nobel Prizes
“By the 1950s, cancer researchers had split into three feuding camps.

The virologists, lead by Rous, claimed that viruses caused cancer, although no such virus had been found in human studies.

Epidemiologists...argued that exogenous chemicals caused cancer, although they could not offer a mechanistic explanation.

The third camp possessed weak, circumstantial evidence that genes internal to the cell might cause cancer...
In 1951, a young virologist named Howard Temin arrived at Cal Tech to study the genetics of fruit flies. Restless and imaginative, he soon grew bored with fruit flies. Switching fields, he chose to study Rous sarcoma virus in Renato Dulbecco’s laboratory.

Until the late fifties, Rous sarcoma virus had been shown to cause tumors only in live chickens. Temin imagined creating cancer in a petri dish. In 1958, in his seventh year in Dulbecco’s lab, Temin succeeded.
He added Rous sarcoma virus to a layer of normal cells in a petri dish. The infection of the cells incited them to grow uncontrollably, forcing them to form tiny distorted heaps containing hundreds of cells that Temin called foci. The foci, Temin reasoned, represented cancer distilled into its essential, elemental form: cells growing uncontrollably, unstoppably - pathological mitosis.

_Temin went on to discover RT in RSV_

**Avian cells transformed by RSV**

_A_ **Fusiform**  
_B_  
_C_ **Round, refractile**  
_D_
Transformation of cells by viruses

- **1962:** After infection with polyomavirus, *rare* BHK21 cells changed shape, kept growing

- **1964:** After infection of Swiss 3T3 cells with SV40, *rare* cells grew as colonies

Most of the infected cells died, but rare cells did not

*They were “transformed”*
How can a viral infection transform a cell?

• Cytopathic effects must be reduced or eliminated
  - *The infected cell does not die*

• Viral replication must be reduced or eliminated
  - *Transformed cells do not produce virions*

• The cell must continue to divide
  - *It becomes immortal*
What happens to the viral genome in transformed cells?

- Some transformed cells contain all or parts of viral genomes integrated into the host genome.
- Sometimes no viral nucleic acid remains in the transformed cell.
- *These were key, but mystifying early observations.*
Route to understanding viral transformation of cells in culture and relationship to cancer was convoluted

(1900s) Retroviruses

(1950s) in vitro studies with RSV

Convergence (1960s, 1970s)

(1943) in vitro Cancer Biology

(1920s) DNA Viruses

(1959) in vitro studies with polyomavirus

(present) UNIFIED THEORY OF CELL GROWTH CONTROL
How does Rous sarcoma virus cause tumors in chickens and transform cells in vitro?
Avian leucosis retroviruses (ALV) are ENDEMIC in virtually all chicken flocks around the world

- Ellerman & Bang 1908
- Most chickens infected with ALV within a few months of hatching
- Leucosis (leukemia) occurs sporadically in infected birds >14 wk old (3%)
- 97% of birds have transient viremia, become immune, don’t get leukemia
Infected birds develop other cancers as they age

- Connective tissue tumors or sarcomas (solid tumors)
- Virus isolated from these solid tumors *rapidly* cause sarcomas, *not* leucosis
  - *Rous isolated one of these viruses: Rous Sarcoma virus* - RSV
- Most of these sarcoma viruses are DEFECTIVE

*As you will see, Peyton Rous was very lucky with his first viral isolate!*
How does RSV, but not ALV, cause sarcomas?

• Key finding: the viral genomes from solid tumors were RECOMBINANTS!

• A piece of the ALV genome is replaced with a *segment of host DNA encoding an oncogene*

**J. Michael Bishop and H. Varmus identified the oncogene (v-SRC) carried by Rous sarcoma virus in 1976**

*Nobel Prize to both in 1989 for this discovery*
Major insight

• ALV infected birds came down with a variety of rare tumors
• These rare tumors all contained retroviruses derived from ALV, but most were defective and all were different
• Rous was lucky in that his RSV isolate was not defective

The retrovirus genomes isolated from each new solid tumor had different host DNA, NOT the v-SRC gene found in RSV

Each new DNA segment had a novel chicken oncogene

A gold mine for molecular oncology
Proviral DNA sequences

**Avian transducing retroviruses**

- **gag**
- **pol**
- **env**
- **src**
- **fps**
- **myb**
- **myb ets**
- **myc**
- **mil myc**
- **yes**
- **jun**
- **erbA erbB**
- **rel**
- **sea**
- **ros**

**Typical progenitor**

- Avian leukosis virus
- Rous sarcoma virus
- PRV II avian sarcoma virus
- Fujinami sarcoma virus
- Avian myeloblastosis virus BA1
- Avian myeloblastosis virus E26
- Avian myelocytoma virus MC 29
- Avian myelocytoma virus MH2
- Avian sarcoma virus Y73
- Avian sarcoma virus 17
- Avian erythroblastosis virus ES4
- Avian reticuloendotheliosis virus
- S13 avian erythroblastosis virus
- UR2 avian sarcoma virus

**Mammalian transducing retroviruses**

- **gag**
- **pol**
- **env**
- **abi**
- **mos**
- **raf**
- **fes**
- **fms**
- **sis**
- **kit**
- **ras**

**Murine leukemia virus**

- Abelson murine leukemia virus
- Moloney murine sarcoma virus
- 3611 murine sarcoma virus
- Gardner-Arnstein feline sarcoma virus
- McDonough feline sarcoma virus
- Simian sarcoma virus
- HZ4 feline sarcoma virus
- Harvey murine sarcoma virus
Defective vs non-defective retroviruses

- Defective viruses require helper virus to produce more virus
- Usually missing envelope proteins
- Envelope genes deleted during oncogene capture
Mechanism for oncogene capture

1. Integration within a proto-oncogene
2. Deletion of virus and cell sequence
   - Packaging of deleted and wild-type genomes
3. Nonhomologous recombination during reverse transcription in newly infected cell
Proto-oncogenes

- >60
- In all cells, control cell growth
- Highly regulated
- Normal cellular genes abbreviated as c-ONCS, e.g., c-SRC, c-MYC, c-MOS, C-RAS
- Certain retroviruses isolated from tumors carry altered copies of c-ONCS abbreviated as v-ONCS, e.g., v-SRC, v-MYC, v-MOS, v-RAS
Subcellular location of major classes of oncoproteins

**Growth Factors**
- sis

**Growth factor receptors**
- erbB, fms, kit, ros, sca

**Membrane-bound protein kinases**
- abl, src, yes
- ras

**G-proteins**

**Cytoplasmic protein kinases**
- fps, mos, raf

- erbA, ets, fos, jun, myb, myc, rel, ski

**Nuclear oncogenes, transcriptional regulators, cell cycle regulators**

**Original phenotype in cultured cells:**

**TRANSFORMATION**

**Uncontrolled cell growth**
The cell cycle

Proto-oncogenes

Revealed by studying *transforming* retroviruses

*Dominant oncogenes*
Retroviruses transform cells by three mechanisms

- **Rapid tumor formation**: eg RSV; 2 weeks
  - *RSV has activated dominant oncogene in genome (v-SRC)*
  - *Protein produced immediately when virus replicates*

- **Intermediate kinetics of tumor formation**: eg ALV; months
  - *ALV carries no dominant v-ONC gene*
  - *cis-activation: provirus turns on expression of endogenous oncogene*

- **Slow kinetics of tumor formation**: eg HTLV; years
  - *HTLV carries no dominant v-ONC gene*
  - *Does not cause cis-activation of local oncogenes*
  - *A viral regulatory protein activates oncogenes by trans-activation*
Proviruses with different transforming potential

- **TRANSDUCING**
  - Rapid
  - LTR \( \rightarrow \) v-onc \( \rightarrow \) LTR
  - env

- **cis-ACTIVATING**
  - Intermediate
  - LTR \( \rightarrow \) gag \( \rightarrow \) pol \( \rightarrow \) env \( \rightarrow \) LTR \( \rightarrow \) c-ONC

- **trans-ACTIVATING**
  - Slow
  - LTR \( \rightarrow \) gag \( \rightarrow \) pol \( \rightarrow \) env \( \rightarrow \) X \( \rightarrow \) LTR

  - \( x: \text{viral transcription activator} \)
  - cellular gene

  e.g., IL2 and the IL2 receptor (autocrine growth)
Mammalian transforming retroviruses

- Retroviruses transform cells as a mistake or byproduct of their life cycle - they must integrate their DNA
- No obvious viral requirement for transformation or for oncogenesis
How the study of DNA virus transformation revealed how the cell cycle was regulated
DNA tumor viruses

First oncogenic DNA virus discovered was papillomavirus that causes warts (papillomas) in cottontail rabbits - Richard Shope, 1933
DNA tumor viruses: *Polyomaviridae*

- Ludwig Gross discovered murine polyomaviruses in 1953
- Caused rare tumors under certain conditions
  - Natural host is the mouse
  - Ubiquitous in mice; no role in mouse cancer
  - Makes tumors of many tissues (poly-oma) in infant hamsters, rats, rabbits
DNA tumor viruses: *Polyomaviridae*

- Eddy and Hilleman showed that SV40, a contaminant of early poliovirus vaccines, induced rare tumors in newborn hamsters - 1962

- Several million Americans were infected with SV40 by poliovirus immunization (text box 7.4)
  - *Natural host is monkey*
  - *Causes no tumors in monkeys*
  - *Does not transform monkey cells in culture*
Response of different cells to infection

<table>
<thead>
<tr>
<th>Species</th>
<th>SV40</th>
<th>Mouse polyomavirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey</td>
<td><em>Permissive</em></td>
<td>Non-permissive</td>
</tr>
<tr>
<td>Mouse</td>
<td>Non-permissive</td>
<td><em>Permissive</em></td>
</tr>
<tr>
<td><em>Hamster</em></td>
<td><em>Semi-permissive</em></td>
<td>Semi-permissive</td>
</tr>
<tr>
<td>*Rat</td>
<td><em>Semi-permissive</em></td>
<td>Semi-permissive</td>
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</tbody>
</table>

* *tumors*
Polyomaviral transformation of cultured cells is rare

• 1 transformed cell per 100,000 infected cells
• Why is it so rare?
• How does this relate to rare tumor formation in animals?
**Adenoviridae**: Another family of transforming DNA viruses

- Many human serotypes, do NOT cause cancer in humans
  - Ad 12-18 tumors in hamsters
  - Ad 7-11 poorly tumorigenic in hamsters

- Tumors and transformation of cultured cells: like polyomaviruses and papillomaviruses, very **rare** events
Key finding: Viral T antigens found in tumors and transformed cells

- SV40: Large T, small T
- Polyomaviruses: Large T, middle T, small T
- Papillomaviruses: T encoded by E5, E6, E7 genes
- Adenoviruses: T antigens are E1A, E1B

All different proteins
T antigens are encoded by essential viral genes

- Required for replication
- Activate viral transcription
- Required for viral DNA synthesis
- Only viral genes that are always retained in tumor cells or transformed cells
- T antigen alone can transform cultured cells
Three seemingly unconnected discoveries in DNA virus biology were critical to understanding the link between virology, transformation, and the cell cycle.

1. A cellular protein of 53 kDa bound to SV40 T antigen.

2. Transcription of a set of adenovirus early genes (the E2 gene cluster) required a cellular protein called E2f (E2 factor).
   - Now a family of proteins called the E2f family.

3. E2f was found to bind a cellular protein called Retinoblastoma protein (Rb).

p53, Rb and E2F were subsequently discovered to be critical players in control of the normal cell cycle.
The cell cycle

**G** is for “gap” Resting cells are in “G zero”
When stimulated to divide, cells enter the G1 phase and then into S where they replicate their DNA and prepare for cell division
A "go/no go" decision to turn on the cell cycle is determined by nutrient concentration and growth factors in media.

-Is the outside world rich enough to replicate the cell?

Remember: The detectors and signaling proteins for growth were discovered primarily as activated oncogenes carried by transforming retroviruses.
If conditions are not right, the cell cycle pauses at the \textit{restriction point}.

\textbf{The cell does not make DNA and does not divide.}\n
\textbf{The protein that regulates the restriction point decision is Rb.}\n
\textbf{Retinoblastoma protein (Rb) — if both copies of the Rb gene are lost, develop retinal tumors of retinoblasts that form retina - these cells are gone by age 5.}\n
\textbf{It is a recessive oncogene (the wild type protein is a tumor suppressor).}
But DNA viruses need cells in S phase so they can replicate their DNA

*T antigens kick quiescent cells into S phase!!*
When viral T antigens bind to Rb, E2f proteins are released and initiate S phase transcription.
The entry into S decision is under MORE control

DNA damage or unscheduled DNA synthesis is monitored by p53

"Don't duplicate damaged genetic information!"
P53: the master gate-keeper of the cell cycle

- p53 recognizes DNA damage in cells and stops the cell cycle for repair
- p53 also recognizes viral DNA intermediates as "abnormal"
- If E2F is free (Rb has been bound by T antigens), then E2F cooperates with p53 to induce APOPTOSIS
- An intrinsic defense of cells against DNA virus infection

Viruses must counter p53
How do viruses counter p53?

- Adenovirus, papillomavirus, or polyomavirus infection
- HPV-16 or HPV-18 E6 protein
- SV40 LT
- E4 ORF6 + E1B 55 kDa
- E1B 55 kDa
- p53-E1B 55 kDa
- p53 degradation

Proteasome
Two more mysteries remain

1. Why are all the viral genes EXCEPT T-antigen encoding genes deleted or turned off in SV40, polyoma, and adenovirus-transformed cells?

2. Why is transformation by these viruses so rare?
Transformation is rare because two low probability events are required

• *Lethal late genes must not be expressed*

  - A rare spontaneous deletion of late genes must occur, or
  - Virus infects semi-permissive cells where late gene expression is blocked

• *T antigen must be on constitutively and transmitted to every cell*

  - Viral DNA encoding T antigen must be integrated into the host DNA
  - T antigen protein must be produced
Clearly, transformation and tumor formation are abnormal, epigenetic processes for these DNA “tumor” viruses.

These events are not required for the normal viral life cycle or transmission.
Transformation is an epiphenomena of a unique life style

• DNA tumor viruses must start the cell synthetic machinery to make DNA

• T antigens turn on the cell cycle to start the G1 to S phases through inactivation of normal inhibitors (p53 and Rb)

• Inactivation of p53 blocks apoptosis

If lytic events are blocked, cells making T antigens continue to divide – they are transformed
- They are on their way to becoming cancer cells
Revealed by studying transforming retroviruses

Dominant oncogenes

Revealed by studying DNA tumor viruses

Recessive oncogenes

Proto-oncogenes

Go

G₀

G₂

M

S

G₁

Stop

Tumor suppressor genes

Principles of Virology, ASM Press