Chapter 3 and 4
Tumor Viruses and Cellular Oncogenes

Viral sources of Cancer
Viruses were first found in chickens in 1908 by Ellerman and Bang (erythroid leukemia) and in 1911 (soft cell carcinoma) by Peyton Rous.

Initial “war on cancer” was thought to be primarily viral

- Initial study in 1909 Rous transmitted tumor from breast muscle to similar birds, later found cell extract (DNA) could transmit information creating foci and tumors
- Showed use the information of cancers was very small (filterable through sand) was a virus.
- At the time (1911) all/most diseases were thought to be infectious in nature – cancer was considered another such disease
Viral sources of Cancer

- The mechanism involves viral DNA being integrated into the host cell DNA, and that the protein products of viral genes maintain transformation to the neoplastic state.
- Most viruses are non-transforming - however, they may may play a role in reducing the host cell’s ability to inhibit apoptosis.
- Cells that are resistant to apoptosis by virtue of the viral genes that they express are more likely to survive genomic damage that will predispose to later neoplastic damage.
- KSHV (Kaposi sarcoma-related herpes virus has been implicated in AIDS-associated KS, the most common malignant tumor seen in patients with the acquired immunodeficiency syndrome.

Viral sources of Cancer

Viral oncogenes (v-onc, i.e. v-Ras) were used to find a large number of transforming cellular oncogenes. Viral participation in carcinogenesis has turned out to be rare however there are a few well known cases.

- KSHV (Kaposi sarcoma-related herpes virus has been implicated in AIDS-associated KS, the most common malignant tumor seen in patients with the acquired immunodeficiency syndrome.
- Human papilloma viruses are implicated in cervical cancer. There are over 65 variants of the virus and only 10 or so are high risk strains. 85% of the cervical tumors contain the high risk virus. The viral protein seems to interact with pRb or p53.
- Hepatitis B can lead to liver cancer and causes 0.5 million fatalities per year.
Life cycle of Virus

Viron particles bind to proteins on cell surface, shed the coating and replicate using the cells, machinery

- DNA is replicated for new particles
- RNA – Protein for new capsids (protein coating)
- Single cell will produce 100-1000 new virons

Retrovirus

HHMI Link (click here)
Transforming retroviruses and acquired oncogenes

<table>
<thead>
<tr>
<th>Name of virus</th>
<th>Viral oncogene</th>
<th>Species</th>
<th>Major disease</th>
<th>Nature of oncoprotein</th>
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</thead>
<tbody>
<tr>
<td>Rous sarcoma</td>
<td>src</td>
<td>chicken</td>
<td>sarcoma</td>
<td>non-receptor TK</td>
</tr>
<tr>
<td>Y73/Esh sarcoma</td>
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<td>chicken</td>
<td>sarcoma</td>
<td>non-receptor TK</td>
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<tr>
<td>Fujinami sarcoma</td>
<td>fyn</td>
<td>chicken</td>
<td>sarcoma</td>
<td>non-receptor TK</td>
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<tr>
<td>U92</td>
<td>ras</td>
<td>chicken</td>
<td>sarcoma</td>
<td>RTK; unknown ligand</td>
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<tr>
<td>Myelocytomatosis 29</td>
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<td>chicken</td>
<td>myeloid leukemia</td>
<td>transcription factor</td>
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<tr>
<td>Mill Hill virus 2</td>
<td>mif</td>
<td>chicken</td>
<td>myeloid leukemia</td>
<td>ser/thr kinase</td>
</tr>
<tr>
<td>Avian myeloblastosis E26</td>
<td>myb</td>
<td>chicken</td>
<td>myeloid leukemia</td>
<td>transcription factor</td>
</tr>
<tr>
<td>Avian myeloblastosis E26</td>
<td>cmy</td>
<td>chicken</td>
<td>myeloid leukemia</td>
<td>transcription factor</td>
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<tr>
<td>Avian erythroblastosis E54</td>
<td>erbA</td>
<td>chicken</td>
<td>erythroleukemia</td>
<td>thyroid hormone receptor</td>
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<td>Avian erythroblastosis E54</td>
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<td>erythroleukemia</td>
<td>EGF RTK</td>
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<tr>
<td>3611 murine sarcoma</td>
<td>ras*</td>
<td>mouse</td>
<td>sarcoma</td>
<td>ser/thr kinase</td>
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<tr>
<td>SKV770</td>
<td>ski</td>
<td>chicken</td>
<td>endometrioma (7)</td>
<td>transcription factor</td>
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<tr>
<td>Reticuloendotheliosis</td>
<td>r1l</td>
<td>turkey</td>
<td>immature B-cell lymphoma</td>
<td>transcription factor</td>
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<tr>
<td>Abelson murine leukemia</td>
<td>abl</td>
<td>mouse</td>
<td>pre-B-cell lymphoma</td>
<td>non-receptor TK</td>
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<tr>
<td>Moloney murine sarcoma</td>
<td>mos</td>
<td>mouse</td>
<td>sarcoma, erythroleukemia</td>
<td>ser/thr kinase</td>
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<tr>
<td>Harvey murine sarcoma</td>
<td>hras</td>
<td>rat, mouse</td>
<td>sarcoma</td>
<td>small G protein</td>
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<tr>
<td>Kirsten murine sarcoma</td>
<td>k-ras</td>
<td>mouse</td>
<td>sarcoma</td>
<td>small G protein</td>
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<td>FBJ murine sarcoma</td>
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<td>mouse</td>
<td>osteosarcoma</td>
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<td>Snyder-Heithel feline sarcoma</td>
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<td>cat</td>
<td>sarcoma</td>
<td>non-receptor TK</td>
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<td>McDonough feline sarcoma</td>
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<td>cat</td>
<td>sarcoma</td>
<td>CSF-1 RTK</td>
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<td>Gardner-Rasheed feline sarcoma</td>
<td>fgr</td>
<td>cat</td>
<td>sarcoma</td>
<td>non-receptor TK</td>
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</tbody>
</table>
Example of retrovirus-associated oncogenes discovered in altered form in human cancers

<table>
<thead>
<tr>
<th>Name of virus</th>
<th>Species</th>
<th>Oncogene</th>
<th>Type of oncoprotein</th>
<th>Homologous oncogene found in human tumors</th>
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<tbody>
<tr>
<td>Rous sarcoma</td>
<td>chicken</td>
<td>src</td>
<td>non-receptor TK</td>
<td>colon carcinoma(^a)</td>
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<tr>
<td>Abelson leukemia</td>
<td>mouse</td>
<td>abl</td>
<td>non-receptor TK</td>
<td>CML</td>
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<td>Avian erythroblastosis</td>
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<td>erb(^b)</td>
<td>receptor TK</td>
<td>gastric, lung, breast(^b)</td>
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<tr>
<td>McDonough feline sarcoma</td>
<td>cat</td>
<td>fms</td>
<td>receptor TK</td>
<td>AML(^c)</td>
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<tr>
<td>H-Z feline</td>
<td>cat</td>
<td>kit</td>
<td>receptor TK(^d)</td>
<td>gastrointestinal stromal</td>
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<tr>
<td>Murine sarcoma 3611</td>
<td>mouse</td>
<td>raf</td>
<td>ser/thr kinase(^e)</td>
<td>bladder carcinoma</td>
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<tr>
<td>Simian sarcoma</td>
<td>monkey</td>
<td>sis</td>
<td>platelet-derived growth factor (PDGF)</td>
<td>many types(^f)</td>
</tr>
<tr>
<td>Harvey sarcoma</td>
<td>mouse/rat</td>
<td>H-ras(^g)</td>
<td>small G protein</td>
<td>bladder carcinoma</td>
</tr>
<tr>
<td>Kirsten sarcoma</td>
<td>mouse/rat</td>
<td>K-ras(^h)</td>
<td>small G protein</td>
<td>many types</td>
</tr>
<tr>
<td>Avian erythroblastosis</td>
<td>chicken</td>
<td>erbA</td>
<td>nuclear receptor(^i)</td>
<td>liver, kidney, pituitary</td>
</tr>
<tr>
<td>Avian myeloblastosis E26</td>
<td>chicken</td>
<td>ets</td>
<td>transcription factor</td>
<td>leukemia(^l)</td>
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<tr>
<td>Avian myelocytoma</td>
<td>chicken</td>
<td>myc</td>
<td>transcription factor</td>
<td>many types</td>
</tr>
<tr>
<td>Reticuloendotheliosis</td>
<td>turkey</td>
<td>rau(^k)</td>
<td>transcription factor</td>
<td>lymphoma</td>
</tr>
</tbody>
</table>

Viruses Implicated in Human Cancer Causation

<table>
<thead>
<tr>
<th>Virus(^b)</th>
<th>Virus family</th>
<th>Cells infected</th>
<th>Human malignancy</th>
<th>Transmission route</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV(^b)</td>
<td>Herpesviridae</td>
<td>B cells</td>
<td>Burkitt's lymphoma</td>
<td>saliva</td>
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<tr>
<td></td>
<td></td>
<td>oropharyngeal epithelial cells</td>
<td>nasopharyngeal carcinoma</td>
<td>saliva</td>
</tr>
<tr>
<td></td>
<td></td>
<td>lymphoid</td>
<td>Hodgkin's disease(^e)</td>
<td></td>
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<tr>
<td>HTLV-I</td>
<td>Retroviridae</td>
<td>T cells</td>
<td>non-Hodgkin's lymphoma</td>
<td>parenteral, venereal(^f)</td>
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<tr>
<td>HHV-8(^b)</td>
<td>Herpesviridae</td>
<td>endothelial cells</td>
<td>Kaposi's sarcoma, body cavity lymphoma</td>
<td>venereal, vertical(^f)</td>
</tr>
<tr>
<td>HBV</td>
<td>Hepadnaviridae</td>
<td>hepatocytes</td>
<td>hepatocellular carcinoma</td>
<td>parenteral, venereal</td>
</tr>
<tr>
<td>HCV</td>
<td>Flaviviridae</td>
<td>hepatocytes</td>
<td>hepatocellular carcinoma</td>
<td>parenteral</td>
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<tr>
<td>HPV</td>
<td>Papillomaviridae</td>
<td>cervical epithelial</td>
<td>cervical carcinoma</td>
<td>venereal</td>
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<tr>
<td>JCV(^b)</td>
<td>Polyomaviridae</td>
<td>central nervous system</td>
<td>astrocytoma, glioblastoma</td>
<td>?</td>
</tr>
</tbody>
</table>
Behaviors of transformed cells

Monolayer cells stop growing upon touching each other (contact inhibition)
- transformed cells lose this phenomena
- they also lose density inhibition and can form clusters of cells (foci)
displaying a loss of topoinhibition.

Transformed cells excessively proliferate for long (indefinite?) passages –
imortalization

Non-transformed monolayer cells (epithelial…) grow poorly without solid
substrate – anchorage dependence - can grow suspended in agar

Increased Tumorigenicity and transport of glucose

Protoncogenes / Oncogenes

Virus/Retrovirus use proto-oncogenes as a strategy for survival and propagation, but most
cancers are not viral in nature

• Proto-oncogene: a normal cellular gene that
  upon alteration of DNA can acquire the ability
to function as an oncogene

• Oncogene: a protein capable of inducing
cancer (can transform cells).
  • c-ZYZ indicates a cellular, yet to be oncogenic
    (mutated) proto oncogene of cellular origin vs
  • v-XYZ is a viral oncogene origin (not often used)
  • Non-human oncogenes are in italics Xyz
  • Oncoproteins (not genes) start with capitals
    followed by lower case Xyz vs XYZ
Protoncogenes / Oncogenes

Protein (molecular) basis of cancer is found at the genetic level

• Malignant transformation occurs by chromosomal damage, proto oncogene mutation or increase in DNA activity
• DNA is the critical macromolecule in cancer. Daughter cells will retain the mutations and the transformation phenotypes and continue to recruit normal cells into transformation

Genetic Basis of Cancer

- Viral oncogenes - insert mutated DNA into cell and create oncogenes
- Translocation of chromosomes - movement of one segment of a chromosome to another
  — not normally a cause of cancer but used to find cellular proto oncogenes and study their effects
- Point Mutations - Alterations in specific sequences of critical genes (proto oncogene activation)
  - usually needs several mutations with one or more critical requirements for cancer to develop
- Alteration in promoter/enhancers - can occur due to chromosomal translocation (expression)
- Gene amplification (expression)
Retroviral – Gene Amplification

Increased copy number of a gene can lead to transformation, proliferation and cell migration or enhanced survival.

- Lead to increased protein production and as a result higher signaling

Myc – first found via avian MC29 myelocytomatosis transforming virus is 10-20 extra copies in leukemia and many other cancers

Myc – cellular (c-Myc) homolog and isoforms N, L and other Myc forms expressed and activated in nearly 70% of cancers.

- Is a transcription factor (helix-loop-helix leucine zipper) found in most organisms.
- Leads to activation via binding partners to activate proliferation and several signaling cascades – promising target for therapy as small inhibition seems to reduce tumor formation
Gene Amplification

erbB – first discovered in avian erythroblastosis virus – increased copy number in stomach, breast and brain tumors and many others.

• aka – Neu/Her/erbB: EGFR over expression – separate from mutation/truncation. Several subforms generate protein tyrosine kinase receptors.
  • Gene is amplified in 30% of breast cancers and are the target of several antibody drugs (biologics) Herceptin.

Direct correlation of amplification with poor breast cancer prognosis

• > 5 copies increases tumor formation and decreases 5 year survival rate by nearly half.

• Gene amplification alone can’t explain breast cancer
  • DNA amplification increases protein but mRNA can be increased independently.
  • Some amplifications stretch beyond Myc to enhance other oncogenes providing a synergistic effect
Gene mutations – may cause protein structural changes in oncogene activation

Point mutation – single DNA base pair can lead to altered amino acid and protein function. Ras is an example of a somatic mutation leading to cancer.

**Extracellular signal**

```plaintext
Val Gly Ala Val Gly Val
GTG GCC CCC GTC GGT GTG
Val Gly Ala Val Gly Val
```

**Signal transduction**

- Ras GTP
- Ras off
- Ras on
- Ras GTP
- Ras off
- Ras on
- Ras GTP

**Figure 4.3**: The Role of Complexes in Cell Signaling (C) Garland Science (1994).
Point Mutation - Ras

Rat Sarcoma discovered by Jennifer Harvey and Werner Kirsten

Three human Ras isoforms (3 different genes)
- NRas – initially found in neuroblastomas
- Kras – Kirsten – sarcomas
- Hras – Harvey rat sarcomas.

P-loop
- Binds second phosphate of GTP
- Gly-Val mutation decreases GTPase activity leaving Ras active

Enhanced gene – protein activity via translocation

Placement of oncogene in front of active gene/chromosomal arm will increase protein expression without gene copy
Chromosomal Translocation:

Translocation between chromosomes 8 and 14 found in Burkitt’s lymphoma (lymph system cancer / leukemia)

Burkitt's lymphoma is a B cell neoplasm characterized by small noncleaved cells that are uniform in appearance. This neoplasm is one of the fastest growing malignancies in humans.

Burkitt's lymphoma is characterized by a specific cytogenetic defect, a balanced, reciprocal translocation of genetic material from the long arm of chromosome 8 to the long arm of chromosome 14.

Chromosomal Translocation:

lymphoma are characterized by a specific cytogenetic defect, a balanced, reciprocal translocation of genetic material from the long arm of chromosome 8 to the long arm of chromosome 14. Two variants of Burkitt's lymphoma are recognized: African and non-African; although very similar in histologic and cytologic features, they have very different epidemiologic patterns and clinical presentations. African Burkitt's lymphoma presents most often as a jaw or orbital tumor and occurs endemically in central Africa. In contrast non-African Burkitt's lymphoma presents primarily as an abdominal mass.
Translocation – micro RNA

Nuclear Protein HMGA – translocates in a non-protein coding region to another gene producing a hybrid protein. 

Micro-RNA silencing (shRNA/RNAi) DNA is left behind.

Loss of degradation of mRNA for HMGA leads to enhanced and extended protein levels.

Proto-oncogene activation: Point mutations or translocations – lead to truncation or fusion proteins

Mutations (point mutation or translocation) can lead to a loss of regulation.

• “false” stop codons can be added or pauses in mRNA reading frames lead to truncated proteins.

• Examples include EGFR
Translocation within genes produce new oncogene

Two proto-oncogenes are “mixed and matched” to generate unregulated kinase expressed in the wrong tissue

- Abl and Bcr

Imatinib/GLEEVEC – Bcr-Abl specific inhibitor to treat myoleogenous leukemia

Figure 4.1: The Biology of Cancer (2nd edition) Science 2009

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Abl – Abelson Murine Leukemia Virus
Tyrosine kinase (non-receptor) involved in cell division, adhesion and stress response

Bcr– Break Point Cluster Region Protein
GTPase activating protein for small G proteins with mostly unknown normal function

Brc-Abl Fusion Protein
Unregulated PTK activating white blood cells to grow without cytokine control

Chronic myelogenous leukemia (CML)
White blood (leukemia) cancer due to myeloid cells with extensive proliferation in bone marrow. CML leads to ~15-20% of all leukemias
Summary

Virus – first discovered but not primary cause of cancer
DNA and Retroviral infection can lead to some cancer types
Over expression of protein by amplification of gene or over expression of gene can lead to transformation
Several mechanisms by which proto-oncogenes are activated.