Summer Academy 2009

Molecular Mechanisms of Human Disease

Solid Tumors: Transcripts, Tyrosine Kinases, and Therapeutics

Molecular Carcinogenesis and Cancer Genes

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Lecture Goals
What causes cancer?
Define the process of carcinogenesis?
Define cancer genes
Introduce concepts related to cancer genes
Describe general mechanisms of mutation
Introduce mechanisms of cancer gene activation/inactivation
Molecular Carcinogenesis and Cancer Genes

Lecture Outline

What causes cancer?

What is carcinogenesis?

Cancer as a genetic disease

What are cancer genes?

Oncogenes and tumor suppressor genes

General mechanisms of mutation

Examples of mutations affecting cancer genes
What Causes Cancer?

Through the history of man, diseases (including cancers) have been attributed to numerous causes, including…

Wrath of God?
Bad humors?
Defective immunity?
Viruses?
Other infectious agents?
What Causes Cancer?

Hippocrates and Galen
300 BC – 200 AD

Both regarded cancer to be a side effect of melancholia (extreme depression characterized by tearful sadness and irrational fears).

*Cancer results from an imbalance between the black humor (from the spleen) and the other three bodily humors (blood, plegm, and bile).*

These were the first to attribute cancer to natural causes.
What Causes Cancer?

In the Dark Ages
“Cancer families,” “cancer houses,” and “cancer villages” were described.

Environmental (infectious) causes for cancer?
Genetic predisposition to cancer development?
What Causes Cancer?

1775
Sir Percival Pott

Suggested a link between the high incidence of scrotal cancer in chimney sweeps and their exposure to “soot.”

P. Pott (1775)
Chirurgical Observations Relative to the Cataract, the Polypus of the Nose, the Cancer of the Scrotum, the Different Kinds of Ruptures, and the Mortification of the Toes and Feet.
Hawes, Clark, and Collins, London
What Causes Cancer?

“Natural Causes”

Environmental Agents
(Chemicals, etc.)

Environmental Exposures
(Radiation, occupational exposures, etc.)

Infectious Agents
(Viruses and other infectious agents)

Endogenous Agents
(Hormones, etc.)

Genetics
What Causes Cancer?

**Mechanisms of Carcinogenesis**
- Chemical Carcinogenesis
- Hormonal Carcinogenesis
- Viral Carcinogenesis
- Physical Carcinogenesis
- Spontaneous Carcinogenesis
Lecture Outline

What causes cancer?
What is carcinogenesis?
Cancer as a genetic disease
What are cancer genes?
Oncogenes and tumor suppressor genes
General mechanisms of mutation

Examples of mutations affecting cancer genes
Carcinogenesis

What is carcinogenesis?
What are carcinogens?
Examples of carcinogens
Carcinogenesis as a multi-step process
What is *Carcinogenesis*?

The pathogenic process or processes that constitute “carcinogenesis” represent the mechanism or mechanisms of cancer induction.
What is a Carcinogen?

“Carcinogens” are agents that drive the process of carcinogenesis.

...carcinogens cause cancer
Examples of *Carcinogens*?

- Chemical Agents
- Physical Agents
- Hormones
- Infectious Agents

*Mechanisms of Carcinogenesis*

- Chemical Carcinogenesis
- Physical Carcinogenesis
- Hormonal Carcinogenesis
- Viral Carcinogenesis
- Spontaneous Carcinogenesis
Exposure to Carcinogenic Agents

Occupational Exposure
Vinyl Chloride Exposure in Factory Workers

Pharmacologic Exposure
Contraceptive Steroids

Environmental Exposure
Aflatoxin B₁ Exposure in China and Africa

Habitual Exposure
Cigarette Smoking
Carcinogenesis is a Multistage Process

Multistage models of carcinogenesis were proposed to explain the observation that the age-specific incidence curves of many common cancers increase roughly with a power of age.


Carcinogenesis is a Multistage Process

Adapted from C.C. Harris (1991)
Chemical and physical carcinogenesis: Advances and perspectives for the 1990s.
*Cancer Res.* **51**:5023s-5044s.
Carcinogenesis is a Multistage Process

What are the various stages of carcinogenesis?
- Initiation
- Promotion
- Conversion
- Progression

What is the biological significance of each stage?
How were these stages defined?
Carcinogenesis is a Multistage Process

Initiation

Promotion

Conversion

Progression

Insult

Genetic Change

Selective Clonal Expansion

Genetic Change

Genetic Change

Genetic Change

Normal Cell

Initiated Cell

Preneoplastic Lesion

Malignant Tumor

Clinical Cancer

Advanced Clinical Cancer
Carcinogenesis is a Multistage Process

Initiation

*Definition and Biological Significance*

A rapid and typically irreversible process whereby a carcinogen (chemical or other) produces permanent changes in the DNA of target cells.

*Initiating agents directly interact with cellular DNA.*
Carcinogenesis is a Multistage Process

Promotion

*Definition and Biological Significance*

The process through which tumor formation is stimulated in tissues that have been exposed to an initiating carcinogen (chemical or other).

Promoting agents do not directly interact with the cellular DNA.

The process occurs over a long period of time (latency period) through a series of usually reversible tissue and cellular changes before the appearance of the first cancer cell.
Carcinogenesis is a Multistage Process

Progression

Definition and Biological Significance

The process through which the cells of a tumor evolve in a stepwise fashion into more aggressive forms with increasingly malignant behavior.
Initiating and Promoting Agents in Chemical Carcinogenesis

Initiation alone is not sufficient for tumor formation. Initiating agents are not complete carcinogens.

Promoting agents can induce tumors from initiated cells, but are not carcinogenic by themselves. Promoting agents are not complete carcinogens.

Complete carcinogens act as both initiating agent and promoting agent.
Carcinogenesis is a Multistage Process

How were these stages defined?


Images obtained from the National Institute for Environmental Health Sciences
Defining Initiation and Promotion in the Mouse Skin Carcinogenesis Model

- ◯ Application of Initiating Agent
- ▲ Application of Promoting Agent

- X — No Tumors
- X ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ — Tumors
- ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ — Tumors
- ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ X — No Tumors
- ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ ▲ — No Tumors
Defining Initiation and Promotion in the Mouse Skin Carcinogenesis Model

Application of Initiating Agent

Application of Promoting Agent

Application of an initiating agent alone is not sufficient to cause carcinogenesis (tumor formation). Multiple applications of a promoting agent in the absence of initiation does not result in carcinogenesis (tumor formation).

Carcinogenesis requires initiation and promotion.
### Defining Initiation and Promotion in the Mouse Skin Carcinogenesis Model

<table>
<thead>
<tr>
<th>Application of Initiating Agent</th>
<th>Application of Promoting Agent</th>
</tr>
</thead>
</table>

- **X** Application of Initiating Agent
- ▼ Application of Promoting Agent

Application of an initiating agent after multiple applications of a promoting agent does not result in carcinogenesis (tumor formation).

*Carcinogenesis requires initiation prior to promotion.*
Defining Initiation and Promotion in the Mouse Skin Carcinogenesis Model

Application of an initiating agent followed by multiple applications of a promoting agent results in carcinogenesis (tumor formation). The time interval between initiation and promotion can be long. *Initiation of carcinogenesis is long-lasting and may be permanent.*
Lecture Outline

What causes cancer?

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Cancer as a genetic disease

What are cancer genes?

Oncogenes and tumor suppressor genes

General mechanisms of mutation

Examples of mutations affecting cancer genes
“…a particular, incorrect chromosome combination which is the cause of abnormal growth characteristics passed on to daughter cells…”

T. Boveri (1914)
Zur Frage der Entstehung Maligner Tumoren
(“Concerning the Origin of Malignant Tumours”)
Gustave Fischer Verlag, Jena

Images taken from http://www.gk-639.uni-wuerzburg.de
Cancer as a Disease State

“Cancer is, in essence, a genetic disease.”

Simple Genetic Diseases

Simple genetic diseases are caused by inherited mutations in a single gene that are necessary and sufficient to determine the phenotype.

For example:
Duchenne Muscular Dystrophy

Dystrophin Mutation → Muscular Dystrophy
Complex Genetic Diseases

Complex genetic diseases involve single gene mutations that can predispose patients to pathological conditions, but the defective gene itself is not sufficient to guarantee development of disease.

*For example:*  
**Atherosclerosis**

- **LDL Receptor Mutation** → **Lipid Accumulation** → **Atherosclerosis**
Cancer as a Genetic Disease

Cancers become manifest following the accumulation of a critical number of genetic (and epigenetic) alterations, in response to imperfections in the DNA replication machinery or through DNA damage caused by environmental mutagens.

Ultimately…

*Cancer is a disease of abnormal gene expression.*
Cancer is a Genetic Disease
DNA is the Biological Target of Carcinogenesis

Are all carcinogens are mutagens?
Are all mutagens carcinogens?

DNA damaged by 2-aminofluorene.  
Left, 2-aminofluorene in the DNA major groove.  
Right, 2-aminofluorene inserted in the DNA helix with displacement of the damaged guanine.

Image taken from http://commons.wikimedia.org
DNA is the Biological Target of Carcinogenesis

Are all carcinogens are mutagens?
Are all mutagens carcinogens?

Mutagens cause changes in the base composition of the DNA. Carcinogens cause cancer.

Is mutation necessary for cancer induction?
Lecture Outline

What causes cancer?
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Cancer Pathogenesis

“The development of cancer in humans involves a complex succession of events that usually occur over many decades. During this multistep process, the genomes of incipient cancer cells acquire mutant alleles of proto-oncogenes, tumor suppressor genes, and other genes that control, directly or indirectly, cell proliferation.”

What Are Cancer Genes?

“...proto-oncogenes, tumor suppressor genes, and other genes that control, directly or indirectly, cell proliferation.”

W.C. Hahn and R.A. Weinberg (2002)
Rules for making human tumor cells.
What Are Cancer Genes?

…Genes that contribute to cancer…

There are a number of genes and classes of genes that contribute to neoplastic transformation that do not conform to the definition of classic oncogenes or classic tumor suppressor genes.
Cancer Genes
Caretakers and Gatekeepers

Gatekeepers
...Genes that cause cancer...

Caretakers
...Genes that contribute to cancer...

Familial cancer syndromes: The role of caretakers and gatekeepers.
In: The Genetic Basis of Human Cancer, Second Edition
Cancer Genes

Caretakers and Gatekeepers

*Gatekeepers* are genes that directly regulate the growth of tumors by inhibiting their growth or by promoting their death.

*Caretakers* are genes that encode proteins that do not directly regulate tumor growth. The products of *caretaker* genes promote genetic stability.
Cancer Gene Classification

Proto-oncogenes
Tumor Suppressor Genes
Caretakers
Gatekeepers

*Positive Mediators*

*Negative Mediators*
What is the contemporary definition of cancer gene?

Cancer genes encode for positive mediators and negative mediators of neoplastic development.
Molecular Carcinogenesis and Cancer Genes

Lecture Outline

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Oncogenes, Proto-oncogenes, and Positive Mediators of Neoplastic Development

The term *oncogene* traditionally refers to a cancer-causing gene of viral origin.

The term *proto-oncogene* historically referred to a cellular homologue of a viral oncogene.

Today, *oncogene* can be used to refer to any cancer-causing gene, and *proto-oncogene* refers to the normal counterpart of an activated oncogene.

*Positive mediators of neoplastic development* include oncogenes, as well as other cancer-promoting genes.
Positive Mediators of Neoplastic Development

Positive mediators of neoplastic development include oncogenes, as well as other cancer-promoting genes.

Positive Growth Factors
*EGF, TGF\(\alpha\), HGF, and others*

Growth Factor Receptors
*EGFR, IGFII-R, and others*

Positive Regulators of Cell Cycle Progression
*Cyclins and Cyclin-dependent Kinases*

Neoplastic disease is characterized by uncontrolled cell proliferation. Positive mediators of neoplastic development function to promote cell cycle progression and cell proliferation.
Functional Subcellular Localization of Proto-oncogene Products

Growth Factors

Growth Factors Receptors

Cytoplasmic Serine-Threonine Kinases

Nuclear Transcription Factors

GTPase Proteins

Guanine Nucleotide Exchange Proteins

Cytoplasmic Membrane-associated Tyrosine Kinases
Identification of Cancer Promoting Genes

Transforming genes in human tumors.
S. Pulciani, E. Santos, A.V. Lauver, L.K. Long,
and M. Barbacid (1982)

Oncogenes in human tumor cell lines: Molecular cloning of a transforming gene from human bladder carcinoma cells.
S. Pulciani, E. Santos, A.V. Lauver, L.K. Long,
Identification of Cancer Promoting Genes

High Molecular Weight DNA From Tumor Cells

DNA Calcium Phosphate

Transfect

Mouse 3T3 Fibroblasts

2-3 Weeks

Focus of Transformed Cells (Neoplastic?)

Tumorigenicity Assay

Focus of Transformed Cells (Neoplastic?)
Detection of the First Human Oncogene (EJ-ras)

Human Bladder Carcinoma Cells → High Molecular Weight DNA From Tumor Cells → Transfect → Mouse 3T3 Fibroblasts → Focus of Transformed Cells (Neoplastic?)
Detection of Cellular Proto-oncogenes by Gene Transfer

Normal DNA

Transfect

Transformed Focus

Transfect DNA from Transformed Focus
Detection of Cellular Proto-oncogenes by Gene Transfer

DNA from Transformed Cells

Transfect

Transfect DNA from Transformed Focus
What is a Tumor Suppressor Gene?

Genes that encode for proteins that function to suppress neoplastic transformation and/or tumor formation?

What is the normal function of these genes?
What is a Tumor Suppressor Gene?

“…a distinct class of genes that function as negative regulators of neoplastic disease… [a class of genes whose] loss of function leads to tumor development…”

What is a Tumor Suppressor Gene?

A tumor suppressor gene is any gene that is lost or inactivated during the development of human cancer.

What are the Normal Functions of Tumor Suppressor Genes?

- Regulation cell cycle progression
- Cellular differentiation
- Cell adhesion and cell structure
- Cell signaling
- Regulation of gene expression
- DNA replication and repair
- And others?
Evidence for Tumor Suppressor Genes

Lessons Learned From Cell Fusion Studies

It was commonly believed that neoplastic transformation (and tumorigenic potential) were dominantly inherited traits.

Cell fusion studies enabled this idea to be investigated.

Suppression of malignancy by cell fusion.
H. Harris, O.J. Miller, G. Klein, P. Worst, and T. Tachibana (1969)
Nature 223:363-368.
Evidence for Tumor Suppressor Genes

Lessons Learned From Somatic Cell Hybrids

Normal Cell
(Fibroblast)

Cell Fusion

Tumor Cell

Somatic Cell Hybrid
Evidence for Tumor Suppressor Genes

*Lessons Learned From Somatic Cell Hybrids*

When malignant cells are fused with nonmalignant cells, the resulting somatic cell hybrid is usually nontumorigenic. *This observation contradicts the idea that neoplastic transformation is the result of dominant genetic change.*
Evidence for Tumor Suppresser Genes

Lessons Learned From Somatic Cell Hybrids

Somatic Cell Hybrid Cultures (Over Time)

Over time in cell culture, somatic cell hybrid cell lines would change and re-express tumorigenic potential. Reexpression of tumorigenic potential by somatic cell hybrid cells was associated with chromosomal alterations, specifically loss of “normal” chromosomes.

This suggest that tumor suppression is associated with specific chromosomal loci.
Knudson examined the frequency and age of development of inherited versus sporadic forms of retinoblastoma, and proposed that the development of this tumor required at least two mutational events.


The Two-Hit Hypothesis for Development of Retinoblastoma

The two-mutation model accounts for the dominant inheritance of susceptibility to retinoblastoma. However, it was recognized that the susceptibility gene did not function as a single dominant determinant of neoplastic transformation at the cellular level.

A.G. Knudson, Jr. (1971)
Mutation and cancer: Statistical study of retinoblastoma.
Hereditary Retinoblastoma

\[ \text{Rb}^{\text{mut}} \]

- Egg

\[ \text{Rb} \]

- Sperm

\[ \text{Rb}^{\text{mut}} \text{ Rb}^{\text{mut}} \]

- Homozygous Mutant Tissue

Somatic Mutation

\[ \text{Rb}^{\text{mut}} \text{ Rb}^{\text{mut}} \]

- Development of Retinoblastoma (usually with multiple tumors)

\[ \text{Rb}^{\text{mut}} \text{ Rb}^{\text{mut}} \]

- Heterozygous
Sporadic Retinoblastoma

Rb Rb
Homozygous Normal Individual

First Somatic Mutation

Rb<sup>mut</sup> Rb
Heterozygous Tissue

Rb<sup>mut</sup> Rb<sup>mut</sup>
Homozygous Mutant Tissue

Second Somatic Mutation

Development of Retinoblastoma (usually with a solitary tumor)
Evidence for Tumor Suppressor Genes

Lessons Learned From Cancer Cytogenetics

Deletion of genetic material in cancer cells suggests that *loss of function* contributes to abnormal cell proliferation or loss of cellular differentiation.

Thus, loss of some inhibitory or regulatory gene (or genes) may cause cancer in conjunction with alterations in positive mediators of neoplastic transformation (proto-oncogenes).

With the exception of leukemias and lymphomas (where chromosomal rearrangements dominate), the most commonly observed chromosomal abnormality in solid tumors is *chromosomal deletion*.

Chromosomal deletion may be manifest as partial loss of a chromosomal region, or as loss of an entire chromosome resulting in numerical abnormalities.
Evidence for Tumor Suppressor Genes

Lessons Learned From Chromosome Transfer

Methods for monochromosome transfer...

Microcell-mediated transfer of murine chromosomes into mouse, Chinese hamster, and human somatic cells.
R.E.K. Fournier and F.H. Ruddle (1977)


Selective transfer of individual human chromosomes to recipient cells.

Identification of TumorSuppressor Loci Using Monochromosomal Transfer

Colcemid 48-72 Hours

Micronucleate
Cytochalasin B

Select
No Colonies

Select
Microcell Hybrid

46 X

Microcells
Evidence for Tumor Suppressor Genes

Lessons Learned From Chromosome Transfer

Evidence for Tumor Suppressor Genes

Lessons Learned From Chromosome Transfer

Evidence for Tumor Suppressor Genes

Lessons Learned From Chromosome Transfer

Suppression of tumorigenicity in Wilms tumor by the p15.5-p14 region of chromosome 11.
Science 254:293-295.

- Transfer of Human Chromosome 11 → Suppression
- Transfer of Chromosome 11del(p13) → Suppression
- Transfer of Chromosome 11del(p15.5-p14.1) → Tumorigenic

G401 Wilms Tumor Cells
Evidence for Tumor Suppressor Genes

Lessons Learned From Chromosome Transfer

Suppression of tumorigenicity in Wilms tumor by the p15.5-p14 region of chromosome 11.
Science 254:293-295.

Normal Human Chromosome 11

Suppressive

Nonsuppressivve

Wilms’ tumor suppressor
11p15.5 (WT2)
Evidence for Tumor Suppressor Genes

Somatic cell hybrids generated from tumorigenic and nontumorigenic cell lines are typically not tumorigenic, suggesting that specific chromosomes confer tumor suppression.

The statistical analysis of hereditary retinoblastoma suggested the recessive nature of the inherited defect that predisposes to cancer.

Cancer cytogenetic studies demonstrate that chromosomal deletion is the most common chromosomal abnormality in solid tumors, suggesting that loss of function contributes to neoplasia.

Chromosome transfer studies demonstrate that specific chromosomes, or chromosomal regions can confer suppression in specific tumor types.
Molecular Carcinogenesis and Cancer Genes

Lecture Outline

What causes cancer?
What is carcinogenesis?
Cancer as a genetic disease
What are cancer genes?
Oncogenes and tumor suppressor genes

General mechanisms of mutation
Examples of mutations affecting cancer genes
Common Mutational Mechanisms and Human Cancer Genes

*Chromosomal Abnormalities*
- Large-Scale Deletions
- Amplifications
- Numerical Abnormalities
- Rearrangements

*Sequence Alterations*
- Point Mutations
- Insertions
- Deletions
Chromosomal Abnormalities
Large-Scale Deletions, Amplifications, Rearrangements

Chromosomal alterations that represent nonlethal genetic damage can lead to carcinogenesis.

**Most chromosomal alterations represent structural aberrations that produce various functional consequences.**

Some chromosomal alterations are without biological consequence.
Chromosomal Abnormalities

Chromosomal Deletions

Terminal Deletion

Normal Human Chromosome 11

Interstitial Deletion
**Chromosomal Abnormalities**

Chromosomal Amplification

- Amplification *in situ*
- Normal Human Chromosome
- Homogeneously Staining Region (HSR)
- Double Minute Chromosomes
Mechanisms of Proto-oncogene Activation

Chromosomal Amplification

c-myc was the first cellular proto-oncogene found to be amplified in human neoplasms…

S. Collins and M. Groudine (1982)

Mechanisms of Proto-oncogene Activation

Consequences of Chromosomal Amplification

Gene amplification results in a dramatic increase in the copy number of genes in the affected chromosomal region.

Oncogenic activity results from overexpression of the proto-oncogene product as a consequence of gene expression from multiple alleles.

Increased gene copy number and associated elevation of gene expression.
Consequences of Chromosomal Amplification

*N-*myc Amplification in Neuroblastoma

Amplification of the N-*myc* gene occurs in approximately 40% of advanced pediatric neuroblastomas. This gene amplification may be associated with double minutes (DMs) or homogenously staining regions (HSRs). For unknown reasons, amplification of the N-*myc* gene leads to a bimodal distribution of gene copy number, with some tumors having 10-30 copies, and other tumors having 100-150 copies.

Adapted from R.A. Weinberg. *The Biology of Cancer*. Garland Science, 2007, Figure 4.11
Consequences of Chromosomal Amplification

HER2/Neu Amplification is Associated with Poor Long-term Survival Among Breast Cancer Patients

The HER2/neu gene encodes a member of the epidermal growth factor receptor family (erbB2). Overexpression of HER2/neu is oncogenic by virtue of hyperstimulation of growth factor receptor signaling pathways. Breast cancers with amplified HER2 demonstrate are prone to relapse in the first 18 months after diagnosis and have poor overall prognosis, indicative of more aggressive disease.

Adapted from R.A. Weinberg. The Biology of Cancer. Garland Science, 2007, Figure 4.6.
Chromosomal Abnormalities

Chromosomal Rearrangements

- Translocation
- Normal Human Chromosome 11
- Inversion
Gene Activation by DNA Rearrangement

Promoter

1 2 3

Recombination

Fusion Protein
The Philadelphia Chromosome occurs in 95% of chronic myelogenous leukemias (CML) and was the first chromosomal aberration to be identified as a causative factor in the development of human cancer.
The Philadelphia Chromosome in CML

\( t(9,22) \)

\( t(9,22)(q34;q11.1) \)

P.C. Nowell and D.A. Hungerford (1960)
Chromosome studies on normal and leukemic human leukocytes.

P.C. Nowell and D.A. Hungerford (1960)
A minute chromosome in human granulocytic leukemia.
*Science* 132:1497.
The *bcr/abl* Fusion Gene Arising From t(9;22) Translocation
*Acute Lymphocytic Leukemia*
The *bcr/abl* Fusion Gene Arising From *t(9;22)* Translocation

*Chronic Myelogenous Leukemia*

8.5 kb mRNA
Mechanisms of Proto-oncogene Activation

Consequences of Chromosomal Translocation

The Philadelphia chromosome translocation [t(9,22)], results in the activation of the c-abl proto-oncogene through production of the bcr/abl fusion gene.

Expression of the bcr/abl gene is driven by the bcr gene promoter, and the transcript produced represents a chimera consisting of the 5’ portion of the bcr gene, and the 3’ portion of c-abl.

The bcr/abl protein expresses enhanced tyrosine kinase activity compared to the normal ABL protein.

Oncogenic activity results from inappropriate expression of the bcr/abl gene, and from enhanced tyrosine kinase signaling activity (gain of function).
Mechanisms of Proto-oncogene Activation

Consequences of Chromosome Rearrangement

Chromosome rearrangement can result in the generation of chimeric genes that encode a fusion protein.

Oncogenic activity results from inappropriate expression or overexpression of proto-oncogene function as a consequence of changes in protein structure.
Gene Activation by DNA Rearrangement

Promoter

Protein Overexpression
Mechanisms of Proto-oncogene Activation

Chromosomal Rearrangements

Burkitt’s lymphoma is characterized by t(8;14) which places the c-myc proto-oncogene in proximity to the immunoglobulin heavy chain (IgH) locus on chromosome 14.

Cellular myc oncogene is altered by chromosome translocation to the immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas.

Mechanisms of Proto-oncogene Activation
Chromosomal Rearrangements

Normal c-myc Proto-oncogene

Rearranged c-myc Oncogene

Translocation to IgH Locus

Transcription and RNA Splicing

Normal MYC Protein
Mechanisms of Proto-oncogene Activation

Consequences of Chromosomal Translocation

The t(8;14) in Burkitt’s lymphoma links the c-myc proto-oncogene to the IgH locus, resulting in overexpression of the c-myc gene, and excess production of MYC protein.

Overexpression may be related to the influence of the proximal Ig enhancer sequences, or to the loss of the c-myc control regions that are located in the untranslated exon 1.

Oncogenic activity results from inappropriate and excess expression of the c-myc gene. This translocation is associated with Burkitt’s lymphoma by virtue of the normal high-level expression of immunoglobulin genes in cells of the lymphatic tissue.
Mechanisms of Proto-oncogene Activation

Consequences of Chromosome Rearrangement

Chromosome rearrangement can result in dissociation of the coding portion of a proto-oncogene from its normal regulatory sequences (promoter or other), producing a chimeric gene that is abnormally expressed.

Oncogenic activity results from inappropriate expression or overexpression of the proto-oncogene product as a consequence of normal regulation of the chimeric promoter.
Common Mutational Mechanisms and Human Cancer Genes

**Sequence Alterations**

**Point Mutations**
Can produce silent mutations or non-conservative amino acid changes.

**Insertions**
Small sequence insertions (nucleotide additions) can result in frame-shift mutations and/or in-frame amino acid additions.

**Deletions**
Small sequence deletions (nucleotide loss) can result in frame-shift mutations and/or in-frame amino acid deletions.
**Sequence Alterations**

*Point Mutations*

Non-conservative Amino Acid Substitutions

ATG-ATA-CCA-GGA-TTT

\[\xrightarrow{\text{Met-Ile-Pro-Gly-Phe}}\]

ATG-ATA-\textcolor{red}{CAA}-GGA-TTT

\[\xrightarrow{\text{Met-Ile-\textcolor{red}{Gln}-Gly-Phe}}\]

ATG-ATA-CCA-\textcolor{orange}{GAA}-TTT

\[\xrightarrow{\text{Met-Ile-Pro-\textcolor{orange}{Glu}-Phe}}\]
Sequence Alterations

Point Mutations
Nonsense and Silent Mutations

ATG-ATA-CCA-CAG-TTT

↓

Met-Ile-Pro-Gln-Phe

ATG-ATA-CCG-GGA-TTT

↓

Met-Ile-Pro-Gly-Phe

Silent Mutation

ATG-ATA-CCA-TAG-TTT

↓

Met-Ile-Pro-Stop

Nonsense Mutation
Frameshift mutations very often result in the generation of premature stop codons.
Sequence Alterations

Insertion and Deletion Mutations
In-frame Amino Acid Addition

ATG-ATA-CCA-CAG-TTT-ATG-AAG-GCG…
↓
Met-Ile-Pro-Gln-Phe-Met-Lys-Ala…

ATG-ATA-CCA-CAG-GGG-AGC-TTT-ATG-AAG-GCG…
↓
Met-Ile-Pro-Gln-Gly-Ser-Phe-Met-Lys-Ala…

The functional consequences of amino acid insertions will depend upon the nature of the inserted amino acids and their location within the protein.
Sequence Alterations

Insertion and Deletion Mutations

Deletion-related Frameshift Mutation

ATG-ATA-CCA-CAG-TTT-ATG-AAG-GCG…

↓

Met-Ile-Pro-Gln-Phe-Met-Lys-Ala…

ATG-ATA-CCC-AGT-TTA-TGA-AGG-CG…

↓

Met-Ile-Pro-Ser-Leu-Stop…

Frameshift mutations very often result in the generation of premature stop codons.
Sequence Alterations

Insertion and Deletion Mutations

In-frame Amino Acid Deletion

ATG-ATA-CCA-CAG-TTT-ATG-AAG-GCG…

↓

Met-Ile-Pro-Gln-Phe-Met-Lys-Ala…

ATG-ATA-CCA-ATG-AAG-GCG…

(delete CAGTTT)

↓

Met-Ile-Pro-Met-Lys-Ala…

(delete Gln-Phe)

The functional consequences of amino acid deletion will depend upon the nature of the lost amino acids and their location within the protein.
Sequence Alterations

*Biological Consequences*

**Gene Activation/Protein Activation**
- Gain of gene/protein function
- Loss of gene/protein regulation

**Gene Inactivation/Loss of Protein Function**
- Loss of gene expression
- Loss of normal protein function
The crystal structure of RAS demonstrates close proximity between the frequently mutated Gly12 residue and the γ-phosphate of bound GTP.

Adapted from R.A. Weinberg. *The Biology of Cancer*. Garland Science, 2007, Figure 5.31, Courtesy of A. Wittinghofer
Regulation of Ras Signaling

Upstream Signals
- Guanine Nucleotide Releasing Factors (Sos1)

Inactive Ras
- GDP
- GTPase-activating Proteins (GAP, NF1)

Active Ras
- GTP
- Downstream Signals (Raf1, MAPK, MAPKK, RSK, c-jun)
Activation of the c-H-ras Proto-oncogene by Point Mutation

**Upstream Signals**
- Activation of the c-H-ras Proto-oncogene by Point Mutation

**Guanine Nucleotide Releasing Factors (Sos1)**
- GDP

**Inactive Ras**
- GDP

**GTPase-activating Proteins (GAP, NF1)**
- GTP

**Active Ras**
- GTP

**Downstream Signals (Raf1, MAPK, MAPKK, RSK, c-jun)**
- GTP
Identification of the Transforming Region of the EJ-*ras* Gene

Cancer Cells

Normal Cells

Transfection

2-3 Weeks

2-3 Weeks
Identification of the Transforming Region of the EJ-\textit{ras} Gene

Human Placental H-\textit{ras}

\rightarrow

No Transformation Activity in NIH 3T3 Cells

EJ-\textit{ras}

\rightarrow

Efficient Transformation of NIH 3T3 Cells
Identification of the Transforming Region of the EJ-ras Gene

Normal

EJ-ras

Recombinants

DNA region responsible for transforming activity
Activation of the c-H-ras Proto-oncogene by Point Mutation

Normal c-H-ras

1 2 3 4 5 6 7 8 9 10 11 12 13 188 189
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Gly Gly Leu Ser
ATG ACG GAA TAT AAG CTG GTG GTG GTG GGC GCC GGC GGT ... CTC TCC
ATG ACG GAA TAT AAG CTG GTG GTG GTG GGC GCC GTC GGT ... CTC TCC
Met Thr Glu Tyr Lys Leu Val Val Val Gly Ala Val Gly Leu Ser

Mutant EJ-ras

Mechanisms of Proto-oncogene Activation

Consequences of Point Mutation

Point mutational alteration of a proto-oncogene sequence results in a change of amino acid in the protein structure, typically reflecting a nonconservative change. These amino acid changes can alter protein function.

Oncogenic activity results from gain of function. In the case of c-ras, point mutation results in loss of GTPase activity, resulting in constitutive activation of the signaling function of the protein.
Inactivation of Tumor Suppressor Proteins in Neoplastic Transformation

*Loss of Protein Function*

**Small Sequence Mutations**
- Missense mutants result in defective protein
- Insertion mutations result in defective protein

**Chromosomal Alterations**
- Gene deletion results in loss of expression
- Gene rearrangement results in defective protein

**Epigenetic Regulation**
- Overexpression of negative regulator inhibits protein function
- Epigenetic silencing through DNA methylation
Mutations of the $p53$ Tumor Suppressor Gene in Human Tumors

Codon Distribution of Single Base Substitutions

Somatic mutations in $p53$ from 20,819 tumors.


Tumor Suppressor Gene Mutational Spectra

Somatic Mutations

p53

18,216 Missense (73%)
2,237 Frameshift (9%)
1,902 Nonsense (8%)
463 Splice Site (2%)
1,057 Silent (4%)
192 Intrinsic (<1%)
569 Other (2%)

IARC TP53 Mutation Database
Release 13 (November 2008)
Tumor Suppressor Gene Mutational Spectra

Prevalence of p53 Somatic Mutations by Tumor Site

2,040/4,264  Ovary  (47.8%)
5,475/12,667  Colorectum  (43.2%)
1,772/4,110  Esophagus  (43.1%)
2,116/5,206  Head and Neck  (40.6%)
  310/768  Larynx  (40.4%)
2,604/6,751  Lung  (38.6%)
  756/2,157  Skin  (35%)
299/918  Pancreas  (32.6%)
1,097/3,432  Stomach  (32%)
1,010/3,171  Liver  (31.9%)
  1,554/5,756  Brain  (27%)
1,031/3,911  Bladder  (26.4%)
3,405/13,608  Breast  (25%)
  231/1,128  Uterus  (20.5%)
214/1,089  Soft Tissues  (19.7%)
301/1,575  Lymphoma  (19.1%)
  193/1,106  Prostate  (17.5%)

p53 Mutational Hotspots in Human Cancer

Codon 157
- Lung: 61%

Codon 175
- Colon: 66%

Codon 179
- Lung: 52%

Codon 248
- Head & Neck: 27%

Codon 249
- Liver: 69%

Codon 278
- Skin: 59%
p53 Tumor Suppressor Function

Interaction between the p53 protein and the DNA molecule is required for tumor suppressor function. *p53 is a transcription factor.*
p53 and Cellular Homeostasis

DNA Damage → p53 → p53 Protein → GADD45

WAF1/CIP1

p21 Protein

Cdk2-cyclin E

Prevents phosphorylation of pRB

Cell Cycle Progression
p53 Functions as a Tetramer

DNA Binding Requires Higher Order Structure
Regulation of Cell Cycle Progression by p53

- **p21<sup>WAF1/CIP1</sup>**
- p53 Tetramer
- Activate Transcription
- Inhibition of cdks
p53 Tumor Suppressor Function

Amino acid residues in the p53 protein that contact the DNA molecule are frequently mutated.

Image adapted from http://www.bioinf.org.uk/p53/
Mutations of the $p53$ Tumor Suppressor Gene in Human Tumors

~50% of all mutations

Nearly all mutations
Regulation of Cell Cycle Progression by p53

Activation of transcription by p53 tetramer leads to the expression of p21WAF1/CIP1, which inhibits cdk activity.
Effects of Mutant p53 on the Regulation of Cell Cycle Progression

- p53
- No p21<sup>WAF1/CIP1</sup> Synthesized
- Unregulated cdk Activity

No Transcription
p53 Functions as a Tetramer

*Mutant p53 Inhibits Tetramer Function*

Wild-type p53

Mutant p53

Functional Tetramer

Nonfunctional Tetramers
Effects of Mutant p53 on the Regulation of Cell Cycle Progression

No p21\textsuperscript{WAF1/CIP1} Synthesized

Unregulated cdk Activity
Loss of p53 Due to Chromosome Deletion

- p53
- p21\textsuperscript{WAF1/CIP1}
- Inhibition of cdks

X

Activate Transcription

X

p21\textsuperscript{WAF1/CIP1}
Effects of mdm2 Overexpression on p53 Regulation of Cell Cycle Progression

- **mdm2**
- **p53**
- No transcription
- **p21^{WAF1/CIP1}**
- No transcription
- No synthesized p21^{WAF1/CIP1}
- Unregulated cdk Activity
Cancer Genes

Positive and Negative Mediators of Neoplastic Progression

Gain of Function
Inappropriate Expression
Loss of Function
Loss of Expression
Carcinogenesis is a Multistage Process

The Pathogenesis of Human Colorectal Cancer

Images used with permission from The Internet Pathology Laboratory for Medical Education
http://library.med.utah.edu.edu/WebPath/webpath.html
Carcinogenesis is a Multistage Process

The phenotypic changes occurring in cells undergoing neoplastic transformation are the direct reflection of changes in gene expression patterns.

Inappropriate Expression or Loss of Expression
Gain of Function or Loss of Function
Carcinogenesis is a Multistage Process

Initiation  Promotion  Conversion  Progression

Chemical Radiation Viral

Genetic Change Selective Clonal Expansion Genetic Change Genetic Change Genetic Change Genetic Change

Normal Cell Initiated Cell Preneoplastic Lesion Malignant Tumor Clinical Cancer Advanced Clinical Cancer

Activation of Proto-oncogenes  Inactivation of Tumor Suppressor Genes

Defects in Cellular Differentiation  Defects in Growth Control  Resistance to Cytotoxicity
The Molecular Pathogenesis of Human Colorectal Cancer

- Normal Colonic Mucosa
- Hyperplasia
  - APC and MCC Mutation or Loss
  - Altered DNA Methylation
    - c-K-ras Mutation
  - Other Genetic Alterations?
- Early Adenoma
- Late Adenoma
  - DCC Loss
    - p53 Mutation or Loss
- Carcinoma
- Metastasis
In this lecture, essential concepts related to cancer pathobiology were introduced, including (i) a description of the general process of carcinogenesis, (ii) definition of cancer as a genetic disease, (iii) definition of cancer genes, (iv) an introduction to oncogenes and tumor suppressor genes, and (v) a description of general mechanisms of mutation.

Throughout the lecture, examples of mutations affecting cancer genes were used to illustrate these concepts.