MOLECULAR BASIS OF CANCER

Assoc.Prof. Işık G. Yuluğ
Bilkent University
Department of Molecular Biology and Genetics

yulug@fen.bilkent.edu.tr
Cellular Basis of Cancer

- Cancer is a collection of diseases characterized by abnormal and uncontrolled growth
- Cancer arises from a loss of normal growth control
- In normal tissues, the rates of new cell growth and old cell death are kept in balance
- In cancer, this balance is disrupted
- This disruption can result from 1) uncontrolled cell growth or 2) loss of a cell's ability to undergo apoptosis
Cancer Cell Do Not Grow Faster Than Normal Cells

Rather, Their Growth is Just Uncontrolled
1 fertilized egg

$10^{16}$ cell divisions/lifetime

Proliferation  Differentiation  Death

50 x $10^{12}$
Cancer: disruption of cellular equilibrium

Proliferation Differentiation\textsubscript{6} Death
Stem cells as the target of carcinogens

Stem cell

Post mitotic

Differentiated

Normal senescent differentiated cell

Benign tumor

Grade 2 malignancy

Grade 3 or 4 malignancy

7
Invasion and Metastasis

- Abnormal cells proliferate and spread (metastasize) to other parts of the body

- Invasion - direct migration and penetration into neighboring tissues

- Metastasis - cancer cells penetrate into lymphatic system and blood vessels
Malignant versus Benign Tumors

- Benign tumors generally do not spread by invasion or metastasis
- Malignant tumors are capable of spreading by invasion and metastasis
What causes Cancer?

• Cancer is caused by alterations or mutations in the genetic code

• Can be induced in somatic cells by:
  – Carcinogenic chemicals
  – Radiation
  – Some viruses

• Heredity - 5%
What is the molecular basis of cancer?

Cancer is a genetic disease.

- Mutations in genes result in altered proteins
  - During cell division
  - External agents
  - Random event

- Most cancers result from mutations in somatic cells
- Some cancers are caused by mutations in germline cells
Theories of cancer genesis

Standard Dogma

• Proto-oncogenes (Ras – melanoma)
• Tumor suppressor genes (p53 – various cancers)

Modified Dogma

• Mutation in a DNA repair gene leads to the accumulation of unrepaired mutations (xeroderma pigmentosum)

Early-Instability Theory

• Master genes required for adequate cell reproduction are disabled, resulting in aneuploidy (Philadelphia chromosome)
CANCER AND GENETICS

- Cancer: genome disease
- Causes of genomic changes
- Effects of genomic changes
- Revolution in cancer treatment: ‘Smart Bullets Period’
CANCER: GENOME DISEASE

- Loss of DNA
- Gain of DNA
- Changes in nucleotides
- Epigenetic effects
Signs for Genomic Changes in Cancer

- Changes in chromosome numbers
  - Aneuploidy
- Chromosomal changes
  - Increase in DNA copy number - 15 different region
  - Loss in chromosomal - 200,000 regions
- Micro changes
  - Microsatellite changes
    - Mikrosatellite - 100,000
  - Nucleotide changes
Chromosomal changes in the genome of cancer cells: tip of the iceberg

Terminal Deletion  Insertion  Inversion  Reciprocal translocation  Robertsonian Translocation  Isochromosomes

http://www.tokyo-med.ac.jp/genet/cai-e.htm
Nucleotide changes in the genome of cancer cells: unseen site of the iceberg

Nucleotide Deletions

Nucleotide Insertions

Nucleotide Substitutions

http://www.tokyo-med.ac.jp/genet/cai-e.htm
DNA Loss in cancer cells

**Figure 1** Representative results of allelotype analysis. Allelic patterns of five polymorphic loci on chromosomal arm 14q examined in a fibrillary astrocytoma (case 21) are shown. Allelic loss is indicated by arrow.

British Journal of Cancer (2002) **87**(2), 218–224
DNA Loss in cancer cells: beyond coincidence...

Early Brain Tumor (Astrocytoma Stage II)

Advance Brain Tumor (Glioblastoma Multiforme Stage IV)
Chromosomal loss:

Mostly, it is a sign for the loss of a tumor suppressor gene

- CDKN2 locus
- PTEN locus
- RB1 locus
- ??? locus
- p53 locus
Cancer: Genome Disease

Epigenetic effects
Genetic and **Epigenetic** Silencing of Tumor Suppressor Genes
THE CAUSES OF GENOMIC CHANGES IN CANCER

- Carcinogenic chemicals
- UV
- Replication Errors
- Radiation
- Viruses
- Normal cell
- Damaged DNA
- Point mutations
- Rearrangements (translocation, deletions, amplifications)
- Alters DNA of genes controlling cell proliferation. (Proliferation becomes abnormal)
- Cancer cell
THE CAUSES OF GENOMIC CHANGES IN CANCER:
Somatic Changes

<table>
<thead>
<tr>
<th>Hasar Etken Türü</th>
<th>Hasar Etkeni</th>
<th>Kanser Riski</th>
<th>İşareti</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morötesi İşimlar</td>
<td>Deri Ka., Melanoma</td>
<td>P53 (CC-TT)</td>
<td></td>
</tr>
<tr>
<td>Radyasyon</td>
<td>Tiroid Ka., Lösemi</td>
<td>Translokasyon</td>
<td></td>
</tr>
<tr>
<td>Benzopren</td>
<td>Akciğer Ka.</td>
<td>p53 (G-T)</td>
<td></td>
</tr>
<tr>
<td>Aflatoksin</td>
<td>Karaciğer Ka.</td>
<td>p53 (249 G-T)</td>
<td></td>
</tr>
<tr>
<td>Kimyasal</td>
<td>Oksidatif Stres</td>
<td>Yaşlılık Kanserleri</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBV</td>
<td>Karaciğer Ka.</td>
<td>P53 (C-T)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Virus DNA İntegrasyonu</td>
</tr>
</tbody>
</table>
### The Causes of Genomic Changes in Cancer: Hereditary Predisposition

<table>
<thead>
<tr>
<th>Genes</th>
<th>Disease (Gene)</th>
<th>Function</th>
<th>Inheritance</th>
<th>Cancer Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FA Genes</strong></td>
<td>F-A</td>
<td>DNA Damage response?</td>
<td>OR</td>
<td>Lösemi</td>
</tr>
<tr>
<td><strong>XP Genes</strong></td>
<td>X-P</td>
<td>NER Type DNA Repair</td>
<td>OR</td>
<td>Skin Ca.</td>
</tr>
<tr>
<td>BLM</td>
<td>Bloom</td>
<td>DNA Helicase?</td>
<td>OR</td>
<td>Various cancers</td>
</tr>
<tr>
<td>WRN</td>
<td>Werner</td>
<td>DNA Helicase?</td>
<td>OR</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>RECQ4</td>
<td>Rothmund-Thomson</td>
<td>DNA Helicase</td>
<td>OR</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>MLH1, MSH2, PMS1, PMS2</td>
<td></td>
<td>MMR DNA Repair</td>
<td>OD</td>
<td>Colon, Endometrium Ca.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td>Lösemi, NF1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>Breast, Ovary, Prostate, Pancreas Ca</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR</td>
<td>Lymphoma, Leukemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>Breast Ca. ?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>Various cancers</td>
</tr>
</tbody>
</table>
• Approximately 90-95% of all cancers are sporadic.

• 5-10% are inherited.
GENES PLAYING ROLE IN CANCER DEVELOPMENT

• Oncogenes
• Tumor suppressor genes
  • DNA repair genes
What are the genes responsible for tumorigenic cell growth?

**Normal**

- Proto-oncogenes +
- Tumor suppressor genes -

- Cell growth and proliferation

**Cancer**

- Mutated or “activated” oncogenes ++
- Loss or mutation of Tumor suppressor genes

- Malignant transformation
ONCOGENES

• Oncogenes are mutated forms of cellular proto-oncogenes.

• Proto-oncogenes code for cellular proteins which regulate normal cell growth and differentiation.
Five types of proteins encoded by proto-oncogenes participate in control of cell growth:

Class I: Growth Factors

Class II: Receptors for Growth Factors and Hormones

Class III: Intracellular Signal Transducers

Class IV: Nuclear Transcription Factors

Class V: Cell-Cycle Control Proteins
Functions of Cellular Proto-Oncogenes

1. Secreted Growth Factors
2. Growth Factor Receptors
3. Cytoplasmic Signal Transduction Proteins
4. Nuclear Proteins: Transcription Factors
5. Cell Growth Genes
A generic signalling pathway
Oncogenes

proto-oncogene = ras

Oncogene = mutated ras

Always activated
Always stimulating proliferation
<table>
<thead>
<tr>
<th>Ras gene</th>
<th>12</th>
<th>59</th>
<th>61</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-ras (H, K, N)</td>
<td>Gly</td>
<td>Ala</td>
<td>Gln</td>
<td>normal cells</td>
</tr>
<tr>
<td>H-ras</td>
<td>Gly</td>
<td>Ala</td>
<td>Leu</td>
<td>lung carcinoma</td>
</tr>
<tr>
<td></td>
<td>Val</td>
<td>Ala</td>
<td>Gln</td>
<td>bladder carcinoma</td>
</tr>
<tr>
<td>K-ras</td>
<td>Cys</td>
<td>Ala</td>
<td>Gln</td>
<td>lung carcinoma</td>
</tr>
<tr>
<td></td>
<td>Arg</td>
<td>Ala</td>
<td>Gln</td>
<td>lung carcinoma</td>
</tr>
<tr>
<td></td>
<td>Val</td>
<td>Ala</td>
<td>Gln</td>
<td>colon carcinoma</td>
</tr>
<tr>
<td>N-ras</td>
<td>Gly</td>
<td>Ala</td>
<td>Lys</td>
<td>neuroblastoma</td>
</tr>
<tr>
<td></td>
<td>Gly</td>
<td>Ala</td>
<td>Arg</td>
<td>lung carcinoma</td>
</tr>
<tr>
<td>H-ras</td>
<td>Arg</td>
<td>Thr</td>
<td>Gln</td>
<td>Harvey strain</td>
</tr>
<tr>
<td>K-ras</td>
<td>Ser</td>
<td>Thr</td>
<td>Gln</td>
<td>Kirsten strain</td>
</tr>
</tbody>
</table>
Activation mechanisms of proto-oncogenes

proto-oncogene --> oncogene
<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Translocation</th>
<th>Proto-oncogene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt lymphoma</td>
<td>t(8;14)</td>
<td>c-myc&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>t(8;22)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>t(2;8)</td>
<td></td>
</tr>
<tr>
<td>Chronic myelogenous leukemia</td>
<td>t(9;22)</td>
<td>bcr-abl&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acute lymphocytic Leukemia</td>
<td>t(9;22)</td>
<td>bcr-abl&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>c-myc is translocated to the IgG locus, which results in its activated expression

<sup>2</sup>bcr-abl fusion protein is produced, which results in a constitutively active abl kinase
## GENE AMPLIFICATION

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Amplification</th>
<th>Source of tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-myc</td>
<td>~20-fold</td>
<td>leukemia and lung carcinoma</td>
</tr>
<tr>
<td>N-myc</td>
<td>5-1,000-fold</td>
<td>neuroblastoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>retinoblastoma</td>
</tr>
<tr>
<td>L-myc</td>
<td>10-20-fold</td>
<td>small-cell lung cancer</td>
</tr>
<tr>
<td>c-abl</td>
<td>~5-fold</td>
<td>chronic myeloid leukemia</td>
</tr>
<tr>
<td>c-myb</td>
<td>5-10-fold</td>
<td>acute myeloid leukemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>colon carcinoma</td>
</tr>
<tr>
<td>c-erbB</td>
<td>~30-fold</td>
<td>epidermoid carcinoma</td>
</tr>
<tr>
<td>K-ras</td>
<td>4-20-fold</td>
<td>colon carcinoma</td>
</tr>
<tr>
<td></td>
<td>30-60-fold</td>
<td>adrenocortical carcinoma</td>
</tr>
</tbody>
</table>
Oncogenes are usually dominant (gain of function)

• cellular proto-oncogenes that have been mutated (and “activated”)

• cellular proto-oncogenes that have been captured by retroviruses and have been mutated in the process (and “activated”)

• virus-specific genes that behave like cellular proto-oncogenes that have been mutated to oncogenes (i.e., “activated”)

The result:

- Overproduction of growth factors
- Flooding of the cell with replication signals
- Uncontrolled stimulation in the intermediary pathways
- Cell growth by elevated levels of transcription factors
**Tumor suppressor genes**

- Normal function - inhibit cell proliferation
- Absence/inactivation of inhibitor --> cancer
- Both gene copies must be defective
Inactivation of a tumor suppressor gene requires two mutations, inherited mutation and somatic mutation.
**KNUDSON TWO HIT HYPOTHESIS IN SPORADIC CASES**

Inactivation of a tumor suppressor gene requires two somatic mutations.
## TUMOR SUPPRESSOR GENES

<table>
<thead>
<tr>
<th>Gene (locus)</th>
<th>Function</th>
<th>Disorders in which gene is affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCC (18q)</td>
<td>cell surface interactions</td>
<td>unknown, colorectal cancer</td>
</tr>
<tr>
<td>WT1 (11p)</td>
<td>transcription</td>
<td>Wilm's tumor, lung cancer</td>
</tr>
<tr>
<td>Rb1 (13q)</td>
<td>transcription</td>
<td>retinoblastoma, small-cell lung carcinoma</td>
</tr>
<tr>
<td>p53 (17p)</td>
<td>transcription</td>
<td>Li-Fraumeni syndrome, breast, colon, &amp; lung cancer</td>
</tr>
<tr>
<td>BRCA1(17q)</td>
<td>transcriptional</td>
<td>breast cancer, breast/ovarian tumors</td>
</tr>
<tr>
<td>BRCA2 (13q)</td>
<td>regulator/DNA repair</td>
<td></td>
</tr>
</tbody>
</table>
CELL CYCLE

- Daughter cell
- Mitosis
- Gateway
- Growth Factors
- DNA replication
- Control Point
- Cell cycle inhibitors
**Rb gene**

- Rb protein controls cell cycle moving past G1 checkpoint
- Rb protein binds regulatory transcription factor E2F
- E2F required for synthesis of replication enzymes
- E2F - Rb bound = no transcription/replication
- Growth factor --> Ras pathway
  --> G1Cdk-cyclin synthesized
- Active G1 Cdk-cyclin kinase phosphorylates Rb
- Phosphorylated Rb cannot bind E2F --> S phase
  - Disruption/deletion of *Rb* gene
  - Inactivation of Rb protein
- --> uncontrolled cell proliferation --> cancer
p53

- Phosphorylated p53 activates transcription of p21 gene
- p21 Cdk inhibitor (binds Cdk-cyclin complex --> inhibits kinase activity)
- Cell cycle arrested to allow DNA to be repaired
- If damage cannot be repaired --> cell death (apoptosis)

- Disruption/deletion of p53 gene
- Inactivation of p53 protein
  --> uncorrected DNA damage
  --> uncontrolled cell proliferation --> cancer
These are genes that ensure each strand of genetic information is accurately copied during cell division of the cell cycle.

Mutations in DNA repair genes lead to an increase in the frequency of mutations in other genes, such as proto-oncogenes and tumor suppressor genes.

i.e. Breast cancer susceptibility genes (BRCA1 and BRCA2) Hereditary non-polyposis colon cancer susceptibility genes (MSH2, MLH1, PMS1, PMS2) have DNA repair functions. Their mutation will cause tumorigenesis.
Molecular mechanisms of DNA double strand break repair
IMPORTANCE OF DNA REPAIR

- DNA damage
- DNA repair
  - Genetic stability
- Defective/incomplete DNA repair
  - Genetic instability
  - Cancer, hereditary disease
  - Genetic divergence

Nature Reviews | Cancer
Tumor Progression

Multiple mutations lead to colon cancer

Genetic changes --> tumor changes
Revolution in cancer treatment: ‘Smart Bullets Period’
Summary of 30 years of research (1971-2001)
Translocation and Bcr-Abl fusion in CML
STI-571 against Bcr-Abl
Smart bullet STI-571 locks itself to the target molecule
Thousands of Targets
~3.000.000.000 bp DNA

~30.000 genes
~300.000 protein
~3.000.000 interaction
1 human cell