DNA: Mutation, Repair and the Environment

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The Genetic Basis of Cancer and Theodor Boveri 1862 - 1915

- Established that chromosomes carry the hereditary information by showing that aberrant segregation of chromosomes leads to certain phenotypes in sea urchin eggs.

- Suggested that aberrant segregation of human chromosomes could be responsible for a normal cell becoming a tumor cell.

- Suggested that some chromosomes promoted cell growth and others inhibit cell growth.

Marcella O'Grady Boveri (1865-1950) also contributed.
Marcella O'Grady Boveri (1863-1950) also contributed to Boveri's theory.

She was the first woman student to graduate from MIT with a Biology Major in 1885!

Chromosomes from a Normal cell

Chromosomes from a Tumor cell
Chromosomes from a Pancreatic Tumor Cell
**DNA the molecule of life**

**Trillions of cells**

Each cell:

- 46 human chromosomes
- 2 meters of DNA
- 3 billion DNA subunits (the bases: A, T, C, G)
- Approximately 20,000 genes code for proteins that perform most life functions

http://iusd.k12.ca.us/uhs/cs2/images/DNA.jpg
DNA is constantly being damaged by endogenous and exogenous agents.
Central Dogma of Molecular Biology

- **Replication**: DNA → RNA → Protein
- **Transcription**: mRNA
- **Translation**: Ribosome → Protein

- **Inheritance of DNA language**
- **Applications of DNA language**
- **Cell structures and functions**
Damage to DNA can create permanent changes in the genetic information. Inactive proteins or proteins with altered function are produced.
DNA damage → Cell Death → Mutation → DISEASE
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Mutation

DNA repair
DNA damage \rightarrow Cell Death \rightarrow DISEASE\\ \downarrow \\
Cell cycle arrest \rightarrow DNA repair \\
\downarrow \\
Mutation
Why do we care about DNA damaging agents in our environment??
Eat

Medicine

Environmental Exposures

Drink

Absorbed

Breathe

Infection

Food: www.boarhouse.ru
Drink: www.terlyn.com
Air: www.npl.co.uk
Pharmaceutical: www.butterworth-labs.co.uk
Sun: www.epa.gov
Helicobacter: microbewiki.kenyon.edu
One dramatic example - Xeroderma Pigmentosum
Two thymine residues

Before

Thymine-thymine dimer residue

After
Lack of DNA Repair speeds up the carcinogenic process, presumably because mutations accumulate more rapidly.
Another dramatic example – deficiencies in DNA damage induced cell cycle arrest.
The first signs of ataxia telangiectasia (A-T) usually appear in the second year of life as a lack of balance and slurred speech. It is a progressive, degenerative disease characterized by cerebellar degeneration, immunodeficiency, radiosensitivity (sensitivity to radiant energy, such as x-ray) and a predisposition to cancer.
Ataxia Telangiectasia - Cancer Prone

Defective DNA Damage Responses can affect both neurodegeneration and cancer susceptibility
How do genes get mutated?

How can we stop them from being mutated?
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Aspergillus flavus

Aflatoxin

Contaminated Corn

Contaminated Peanuts
Exposures that increase risk of Hepatocellular carcinoma (HCC)

- Aflatoxin in food
  - Relative Risk: 3 X

- HBV liver infection
  - Relative Risk: 7 X

- Combined Exposure
  - Relative Risk: 60 X
This Guanine has a large DNA adduct that is derived from a compound secreted by mold that grows on peanuts.

Normal DNA bases can become damaged when they react with chemicals that come from our food.
8,9-Dihydro-8-(N⁷-guanyl)-9-hydroxyaflatoxin B₁
Original Sequence: CTCGC

Mutant Sequence: CTCGA

The ‘C’ was replaced with ‘A’
How do genes get mutated?

How can we stop them from being mutated?
All these responses to DNA damage serve to prevent mutations accumulating, and thus prevent CANCER.
DNA Repair

- Direct Repair
- Base Excision Repair
- Nucleotide Excision Repair
- Transcription Coupled Repair
- Mismatch Repair

Sunlight
Xeroderma Pigmentosum ~ 1/250,000

Interindividual Variation in DNA Repair Capacity

Frequency

Defective → Deficient → Normal → Super

XP patients

Relative repair capacity (%)

Genome population

Wei et al., Clinical Chemistry, Vol. 41, No. 12, 1995
DNA Repair Strategies

• Direct Reversal
  Photolyase, Methyltransferase, Oxidative demethylase

• Excision Repair
  Base excision, nucleotide excision, mismatch repair

• Lesion Avoidance
  Translesion synthesis, DNA recombination

• Double strand break repair
Many genes can influence whether or not an environmental exposure leads to disease.
Sources of DNA Alkylating Agents

**Exogenous – outside us**
- Tobacco Smoke
- Fuel Combustion Products
- Food Constituents
- Food Preservatives
- Chemotherapeutic Agents

**Endogenous – inside us**
- S-Adenosylmethionine
- Nitrosation of Amines
- Lipid Peroxidation
Many genes can also influence whether or not cancer chemotherapy is effective.
DNA damage → Cell Death → Mutation → DISEASE

DNA repair → Cell cycle arrest
How can we identify ALL pathways that influence cell death and mutation?
Made a start with the budding yeast *Saccharomyces cerevisiae*

- Transcriptional profiling
- Genomic Phenotyping
Many genomes are now sequenced!!!

- **E. coli**
  - 4,200 genes
  - Sequenced in 1997

- **S. cerevisiae**
  - 5,800 genes
  - Sequenced in 1997

- **D. melanogaster**
  - 14,000 genes
  - Sequenced in 2000

- **mouse**
  - ~22,500 genes
  - Sequenced in 2005

- **chimpanzee**
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Made a start with the budding yeast *Saccharomyces cerevisiae*

- Transcriptional profiling
- Genomic Phenotyping

5,800 genes to analyze!!
The global or systems approach – studying all genes and all pathways together

Transcriptional profiling

Genomic phenotyping

Jelinsky and Samson 1999
Jelinsky et al 2000

>30% of genome is transcriptionally responsive to DNA Damaging agents

Begley et al 2002
Begley et al 2004

>30% of proteins are involved in recovery after exposure to DNA Damaging agents
Transcriptional profiling - Jelinsky and Samson 1999
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>30% of genome is transcriptionally responsive to DNA Damaging agents
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The global systems approach – studying all genes and all pathways together

PATHWAYS REPRESENTED

**DNA Repair**
**Cell Cycle**

**DNA Replication**
**Transcription**
**Signaling**
**Transport**
**Protein Degradation**
**Protein Synthesis**
**Amino Acid Metabolism**
**RNA Metabolism**
**Cell Membrane Biosynthesis**
**Chromatin structure**
Of 4,733 strains tested

<table>
<thead>
<tr>
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<th>Sensitive</th>
<th>Resistant</th>
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<tr>
<td>MMS</td>
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<tr>
<td>t-BuOOH</td>
<td>447</td>
<td>78</td>
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<tr>
<td>4NQO</td>
<td>819</td>
<td>2</td>
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<tr>
<td>UV</td>
<td>288</td>
<td>1</td>
</tr>
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Assembling the damage recovery system

Phenotype Data
- Sensitive
- No Phenotype
- Essential

Molecular Interaction Data
- Protein-Protein
- Protein-DNA
The Yeast Protein Interaction Network viewed in Trey Ideker's Cytoscape

4,684 proteins connected by 14,993 p-p interactions
Phenotypic Annotation of the Interactome

Tom Begley & Trey Ideker

- Essential, Toxicity Modulating (MMS, 4NQO, UV, t-BuOOH), No Phenotype
Filter out subnetworks with high clustering coefficients – indicative of protein complexes.
<table>
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- RNA Pol II and mediator complex includes genes like SFL1, S4B5, SIN4, ADR1, ROX3, GAL11, and MED1.
- Swi/snf complex includes genes like MUD13, TIF4632, STO1, PUB1, SWI3, HIR1, and SNF2.
- NER complex includes genes like RAD1, RAD4, RAD7, and RAD10.
- Vacuolar sorting includes genes like VPS8, VPS16, PEP3, and VPS33.

**Vacuolar membrane complex:**

- **Nuclear pore complex:**

- **RNA Pol II C-terminal kinase:**

- **Transcription regulation complex:**

*Maya Said and Tom Begley*
DNA Repair deficiency results in striking differential network activation

Repair proficient Treated

166 nodes/854 edges
11 GO categories

Repair deficient - Treated

590 nodes/3008 edges
21 GO categories
How can we identify ALL pathways that influence cell death and mutation?

DNA damage → Cell Death → DISEASE

DNA repair and cell cycle arrest plus MANY other unexpected pathways also play a role
Many genomes are now sequenced!!!

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The expression of each mouse or human gene can be dampened!!! This requires robotics and high throughput technology.
Determine what genes influence cancer susceptibility and whether cancer chemotherapy will be effective.
MAJOR POINTS

• The Genome is an extremely large and critical target for toxic agents – exposure to which is unavoidable.

• Damage to the Genome can result in cell death, small mutations and large chromosome rearrangements all of which contribute to aging and disease, including cancer.

• Sophisticated mechanisms exist to ensure that damage in the genome is repaired.

• Cells activate a plethora of pathways upon exposure to DNA damaging agents and not doing so can lead to devastating disease.