Molecular mechanisms of carcinogenesis

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Aims

What is cancer at the cellular level?

How do chemical and physical agents cause cancer?

How do we test for carcinogens?

How can understanding mechanistic inform risk assessment?
Cellular basis of cancer

- Uncontrolled and inappropriate division of cells
- Genetic damage to critical genes that regulate cell growth
- Exposure to chemical carcinogens considered important
“normal” cells
Cancer cells
What kind of genes are damaged in cancer cells?

1) Oncogenes

2) Tumour suppressor genes

e.g. p53 “the guardian of the genome”
## Some Transgenic and Knock-out models

<table>
<thead>
<tr>
<th>TG Model</th>
<th>Effect on cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>myc</td>
<td>↑ (20% incidence)</td>
</tr>
<tr>
<td>mutated ras</td>
<td>↑ (40% incidence)</td>
</tr>
<tr>
<td>myc + mutated ras</td>
<td>↑↑ (100% incidence)</td>
</tr>
<tr>
<td>myc + bcl-2</td>
<td>↑↑</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KO Model</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 hemizygote</td>
<td>↑</td>
</tr>
<tr>
<td>p53 homozygote</td>
<td>↑↑</td>
</tr>
<tr>
<td>Cx32</td>
<td>↑</td>
</tr>
<tr>
<td>Ah receptor</td>
<td>↓ (in response to dioxin)</td>
</tr>
</tbody>
</table>
Hallmarks of cancer cells

- EGFR inhibitors
- Cyclin-dependent kinase inhibitors
- Immune activating anti-CTLA4 mAb
- Telomerase Inhibitors
- Selective anti-inflammatory drugs
- Inhibitors of VEGF signaling
- Inhibitors of HGF/c-Met
- Parp inhibitors
- Proapoptotic BH3 mimetics
- Resisting cell death
- Deregulating cellular energetics
- Sustaining proliferative signaling
- Evading growth suppressors
- Avoiding immune destruction
- Enabling replicative immortality
- Tumor-promoting inflammation
- Inducing angiogenesis
- Activating invasion & metastasis
- Genome instability & mutation

Hanahan and Weinberg 2011
Where are we exposed to carcinogens?

All of the time.....

Diagram showing the factors contributing to cancer:
- Diet
- Viruses
- Smoking
- Environment
- Radiation

With 10-70% (35%) from Diet.
Schematic diagram of Multistage Carcinogenesis

**Initiation**
- DNA damage
  - Genotoxic chemicals
  - Radiation, UV, viruses
- Initiated cell

**Promotion**
- Clonal expansion
  - Growth factors
  - Non-genotoxic chemicals
- Pre-neoplastic lesion

**Progression**
- Primary malignant tumour
- Secondary tumours
- Genetic instability
  - Suppressor gene loss
  - Gene amplification
Chemical carcinogens

**Genotoxic**

- Possess the ability to damage DNA
- Can lead to mutations in critical genes resulting in development of cancer
- Majority of known human carcinogens are genotoxic

**Non Genotoxic**

- Don’t damage DNA
- Alter the balance between cellular growth and death
- Lots of non genotoxic chemicals positive in animal studies
Genotoxic carcinogens often require metabolic activation:

Some directly acting e.g. alkylating agents but often need metabolic activation:

**Typically by the cytochrome P450 enzyme system:**

- e.g. PAHs, aflatoxin, vinyl chloride, PhIPs, NNK many more

**Other enzyme systems can also be important:**

- e.g. metabolism of benzene in bone marrow

**Products of metabolism are electrophilic and DNA reactive**
Case study: Benzopyrene

- Important occupational and environmental carcinogen:
  
  - DIET: charred food
  - OCCUPATION: Coke oven workers, mechanics
  - TOBACCO SMOKE
  - AIR POLLUTION: Diesel fumes
Benzo[a]pyrene: A Paradigm of metabolic activation

Benzopyrene

Detoxification

GST<sub>m</sub>

DNA

Cancer
Genetic variation in benzo[a]pyrene metabolism

- Known CYP1A1 polymorphic forms - CYP1A1, CYP1A*2A, CYP1A*2B

- Known GSTm polymorphic forms - GSTm*A, GSTm*B, GSTm*0
## Genetic variability and individual risk of exposure to BP

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BP-DNA adducts/10^8 in WBC DNA from workers</th>
<th>Odds ratio for susceptibility to lung cancers in Japanese smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1 *1/*1-GST_m active</td>
<td>&lt;0.2</td>
<td>Low dose: 1.0, High dose: 7.0</td>
</tr>
<tr>
<td>CYP1A1 *m1/*m1- GST_m active</td>
<td>&lt;0.2</td>
<td>~1.0, 9.3</td>
</tr>
<tr>
<td>CYP1A1 *1/*1- GST_m null</td>
<td>0.7</td>
<td>~2.0, 10.8</td>
</tr>
<tr>
<td>CYP1A1 *m2/*m2- GST_m null</td>
<td>5.8</td>
<td>~3.0, 16.3</td>
</tr>
<tr>
<td>CYP1A1 *m1/*m1- GST_m null</td>
<td>6.4</td>
<td>16.0, 20.0</td>
</tr>
</tbody>
</table>

Data taken from Nakachi et al (1996)
Vinyl Chloride
Situation is complex !!!
Reactive oxygen species (ROS)

- Highly reactive intermediates of molecular oxygen
- Produced as side products of endogenous metabolism
- Metabolism of many foreign substances also produce ROS
- Potentially genotoxic
Species variation also needs to be considered...

<table>
<thead>
<tr>
<th>Species</th>
<th>Route A</th>
<th>Route B</th>
<th>Liver Cancer?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>-/+</td>
<td>+++</td>
<td>no</td>
</tr>
<tr>
<td>Mice</td>
<td>+++</td>
<td>+</td>
<td>yes</td>
</tr>
<tr>
<td>Humans</td>
<td>-/+</td>
<td>++</td>
<td>no?</td>
</tr>
</tbody>
</table>
Testing for genotoxicity/carcinogenicity

Tiered approach

_in vitro_ – e.g. AMES test, mouse lymphoma assay

short term _in vivo_ tests – e.g. mouse micronucleus assay, SCE

long term (2 – 3 year) _in vivo_ carcinogenicity assays
THE AMES TEST

TEST COMPOUND

Metabolic activation (S9)

Ultimate mutagen

His -ve

Salmonella typhimurium

E.g. TA98-ACC-GCC-GGC-AGG

-ACC-GCC-CGG-CAG-G

Histidine deficient medium

Increased number of revertant colonies
“Green screen” - Gentronix
The Comet Assay

1) CELL POPULATION
2) SLIDE PREPARATION
3) FPG TREATMENT
4) ELECTROPHORESIS
5) CELL ANALYSIS

• Extremely sensitive and robust

• Can be modified to detect specific DNA lesions (e.g. 8-oxo dG, bulky DNA adducts) - further enhances sensitivity

• Non specific re origin of DNA damage
Non genotoxic carcinogens:

Are not DNA reactive and act by other mechanisms:

Potential to alter the balance of cell growth and proliferation…

mitogenic (mimic growth factors)
inhibit apoptosis (phenobarbitol – rodent liver cancers
cause cytotoxicity and “regenerative” hyperplasia – e.g. saccharin

“Receptor” based mechanisms

e.g. peroxisome proliferators – rodent liver cancer
d-limonene - male rat kidney
dioxins – possible human carcinogen

very often species specific and not relevant to humans

Epigenetic mechanisms – DES, arsenic, many others?
Dioxins

**Acute**

- Chloracne

**Long-term?**

- Cancer - soft tissue sarcomas, non hodgkins lymphoma ?
- Suppresion of immune system ?
Model of toxicity of dioxins

Dioxin

Ahr

Ahr

Ahr

DNA

CYP 1A1 mRNA

CYP1A1 protein
Nongenotoxic carcinogens are “tumour promoters”
SOME FOODS WHICH CONTAIN CHEMICALS WHICH CAUSE CANCER IN RATS

Parsley  Mushrooms  Cauliflower
Parsnip   Cabbage    Orange juice
Celery    Brussels sprouts   Mango
Nutmeg    Black pepper    Pineapple
Cocoa    Basil    Jasmine tea

BUT........!!
Need to consider dose-response models

Deterministic model

Stochastic model

Non genotoxic carcinogens

Genotoxic carcinogens “single hit hypothesis”
“Single-hit hypothesis” - Can a single asbestos fibre really cause mesothelioma?

This is what a stochastic model of genotoxic carcinogenesis predicts.

Is it true? Impossible to test...
Asbestos production in south Africa (circa 1950)

Limpet asbestos spraying (UK, circa 1960)
Asbestos exposure and relationship with incidence of mesothelioma in the UK population: