Environmental factors

- CHEMICALS
- RADIATION
- NATURAL TOXINS
- VIRUSES

Chemical Exposure (air, water, food, etc.)
   - Internal Exposure
   - Metabolic Activation
   - Macromolecular Binding
   - DNA
   - RNA
   - Protein (Biomarker)
   - Detoxication

Biologically Effective Dose
   - Efficiency of Mispairing
   - Cell Proliferation
   - Initiation

What factors influence the development of cancer?

- Dose—amount and length of exposure. The lower the dose the least likely you are to develop cancer or related diseases.
- Environmental or “lifestyle” factors.
  - Cigarette smoking (co-carcinogen)
  - Alcohol consumption (co-carcinogen)
  - Diet—high fat consumption, natural antioxidants
  - Geographic location—industrial areas, UV light
  - Therapeutic drugs—some are known carcinogens
  - Inherited conditions

Human carcinogens - environmental

- Aflatoxins
- Asbestos
- Benzene
- Cadmium
- Coal tar
- Creosote
- DDT
- Polycyclic aromatic hydrocarbons
- Radon
- Solar radiation

Human carcinogens - drugs/therapeutic agents

- Adriamycin (doxorubicin)
- Androgenic steroids
- Chlorambucil
- Cisplatin
- Cyclophosphamide
- Cyclosporin A
- Diethylstilbestrol
- Ethylene oxide
- Melphalan
- Tamoxifen

How is chemical carcinogenicity determined?

- Epidemiological studies determine the relationship between a cancer suspect chemical and a human population over a long period of time.
- Animal studies directly induce cancer in test animals using a large sample of animals, usually of two or more species with varying dose and time parameters.
- Experiments with animals are based on the premise that chemicals that produce cancer in animals will have similar effects on human cells. Most known human carcinogens produce cancer in experimental animals.
Ames' test to detect the mutagenic property of a chemical.

**CHEMICAL CARCINOGENESIS**

Which classes of chemicals tend to be carcinogens?

- **Epoxides:**
  - Ethylene oxide
  - Propylene oxide

- **Organohalogen comp.:**
  - Vinyl chloride
  - Carbon tetrachloride
  - Chloroform
  - Hexachlorobenzene
  - Trichloroethylene

- **Hydrazines:**
  - Hydrazine (and salts)
  - 1,2-Dimethylhydrazine

- **N-Nitroso compounds:**
  - N-Nitrosodimethylamine

- **Aromatic Amines:**
  - Benzidine
  - Aniline
  - o-Aminodimethylamine
  - o-Toluidine

- **Aromatic hydrocarbons:**
  - Benzene
  - Benz[a]anthracene
  - Benz[a]pyrene

**Classes of carcinogens (cont.)**

- **Misc. organic compounds:**
  - Formadehtyde
  - Acetaldehyde
  - 1,4-Butanediol
  - Ethyl carbamate
  - 2-Nitropropane
  - Styrene
  - Thiourea
  - Thiuramates

- **Misc. inorganic comp.:**
  - Arsenic and compounds
  - Chromium and compounds
  - Thorium dioxide
  - Beryllium and compounds
  - Cadmium and compounds
  - Lead and compounds
  - Nickel and compounds
  - Selenium sulfide

In 1930, Sir Ernest Kennaway and collaborators obtained 7 gr of powder by distilling 2 tons of tar; the powder was proven to contain Polycyclic Aromatic Hydrocarbons (PAH), of which 3,4-benzopirene was the most prominent.

In 1930, Saraki e Yoshida showed that feeding rats with the dye o-aminooazotoluene could cause cancer in the LIVER, not in the intestine.

Thus, cancer developed in an organ different and distant from the organ of absorption! This observation indicates that some (pro-)carcinogen need to be metabolized in specific organs to become active.
In 1969 Elizabeth E. James Miller suggested that in order to cause a mutation, the CHEMICAL CARCINOGEN must form an adduct with the Nitrous Base, and to this end it must possess electrophilic properties.

Electrophilic molecules can easily bind to electron-rich macromolecules (containing numerous N) such as Nucleic Acids and Proteins.

Some carcinogens intrinsically possess the electrophilic property, others need to be transformed (activated) by the DMS to acquire an electrophilic group (such as the epoxide).

Thus CHEMICAL CARCINOGENS are classified as DIRECT or INDIRECT (or PRO-Carcinogen).

An adduct to the Nucleic Base can also be formed through covalent binding of an 'alkyl group' = alkylation.

**DIRECT ACTING CARCINOGENS**

- Direct-acting agents require no metabolic conversion to become carcinogenic, have highly reactive electrophile groups that directly damage DNA, leading to mutations and eventually cancer.
- Examples are: cancer chemotherapeutic drugs (e.g., alkylating agents) used as Rx of e.g., leukemia, lymphoma, Hodgkin lymphoma, and ovarian carcinoma, non-neoplastic disorders, such as rheumatoid arthritis or Wegener granulomatosis.
- may evoke later a second form of cancer, usually leukemia.

**INDIRECT-ACTING CARCINOGEN**

- The designation indirect-acting agent refers to chemicals that become activated, generating an electrophile, to an ultimate carcinogen before they become active.
- indirect-acting agents are not active until converted to an ultimate carcinogen by endogenous metabolic pathways e.g., endogenous enzymes like cytochrome P-450 oxygenase.
- Examples: Benzopyrene, Polycyclic hydrocarbons, aromatic amines and Azo dyes, Aflatoxin B1, insecticides, fungicides, nitrates used as food preservatives.

**Mechanisms of Action of Chemical Carcinogens**

- Initiation
- Promotion
- the application of an initiator may cause the mutational activation of an oncogene such as RAS
- subsequent application of promoters leads to clonal expansion of initiated (mutated) cells.
- Forced to proliferate, the initiated clone of cells accumulates additional mutations, developing eventually into a malignant tumor.

**Table 7-3. MAJOR CHEMICAL CARCINOGENS**

**DIRECT-ACTING CARCINOGENS**

*Alkylation Agents*
- Nitrosomorpholine
- Ethyl nitrite
- Ethylnitrosourea
- Methyl hypophosphoramide, chlorambucil, nitroso-urea, and others

**Active Agents**
- Acetyl derivatives
- Diethylnitrosamine chloride

**PROCARCINOGENS THAT REQUIRE METABOLIC ACTIVATION**

- Pyridine and heterocyclic aromatic hydrocarbons
- Benzo(a)pyrene
- 3,4-benzopyrene
- Acryl-Nitrosamines
- 2-Acetylaminofluorene
- Aromatic Amines, Amides, Azo Dyes
- 2-Alkylaminofluorene (butter chloroform)
- Barbiturates
- 2-Acetylaminofluorene (butter, yellow)
- Natural Plant and Microbial Products
- Alkaloids
- Cytosines
- Safranine
- Barbiturates
- Others

**Some modifications of pre-carcinogens leading to the formation of electrophilic groups**

- **Oxidation** at carbon
  - Products
  - Alkylation or activating agents
- **Oxidation** at nitrogen
  - Activating agent
- **Reduction** at nitrogen
  - Inactivating agent
- **Conjugation**
  - Activating agent
  - Deactivating agent
Induction of Metabolizing Enzymes

- May be a reason for tissue- and/or species-selectivity of carcinogens
- Metabolites may be ligands for receptors
- Production of reactive oxygen species

Metabolites may be ligands for receptors

Production of reactive oxygen species


Carcinogenic hydrocarbons

PAH

Chemical carcinogens: chemicals that cause tumor formation

1. have a very broad range of structures with no obvious unifying features
2. are genotoxic and can be classified into two broad categories based on their action mechanisms:
   a. Direct-acting carcinogens
      - react with nitrogen and/or oxygen atoms in DNA
      example: ethylmethane sulfonate (EMS)
   b. Indirect-acting carcinogens
      - become reactive after metabolic activation
      examples: aflatoxin, benz(a)pyrene

*genotoxic: an agent or process that interacts with cellular DNA, resulting in alteration of DNA structure
The direct-acting carcinogens interact with macromolecules through the covalent bond formation between an electrophilic form of the carcinogen and the nucleophilic sites in proteins (e.g., S, O, and N atoms in cysteine, tyrosine, and histidine, respectively) and nucleic acids (e.g., N and O atoms in purine or pyrimidine), such as N-methyl-N-nitrosourea, a chemically-reactive alkylating agent.

Some agents can intercalate (嵌入) into the DNA double helix by forming tight noncovalent bonds (e.g., daunorubicin).

Most of carcinogens are indirectly-acting; they do not interact in vitro with macromolecules until it has been incubated with liver homogenates or liver microsomal fractions. Thus, metabolic activation of certain carcinogenic agents is necessary to produce the “ultimate carcinogen” that actually reacts with crucial molecules in target cells.

1. Cytochrome P450 catalyses initial epoxidation.
2. With the exception of the 1-2 and 2-3 oxides that convert to phenols, epoxide hydrolase may catalyse the formation of aldehydes.
3. Benzo[a]pyrene-7,8-dihydrodiol is further metabolized at the olefinic double bond by cytochrome P450 to form 9,10-epoxy-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.
4. The highly unstable arene ring opens spontaneously to form a carbocation.
5. The electrophilic species forms a covalent bond between the 10 position of the hydrocarbon and the exocyclic amino group of deoxynucleosine.

Metabolic activation of benzo[a]pyrene

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Metabolic activation of aflatoxin

Aflatoxin B1, a toxin from a mold (Aspergillus flavus oryzae) that grows on grain and peanuts when they are stored under humid tropical conditions. It is thought to be a contributory cause of liver cancer in the tropics.

Indirect CARCINOGENS

Proposed mechanism of DNA damage, apoptosis, and carcinogenesis induced by benzene metabolites in the presence of NADH and Cu(II)

Metabolic activation of Benzo(a)pirene (PB)
The experiment proving the need for a temporal sequence of events in carcinogenesis

1. X = Application of Initiator
2. X = Application of Initiator
3. X = Application of Initiator
4. X = Application of Promoter
5. X = Application of Initiator

DNA adduct formation

Since most chemical carcinogens react with DNA and are mutagenic, interactions with DNA have been viewed as the most important reactions of these agents.

The principal reaction products of the nitrosamines and similar alkylating agents with DNA are N7 and O6 guanine derivatives. Reactions also occur with other DNA bases.

Purines and Pyrimidines

A. N-7 (benz[a]pyrene-6-yl)guanine
B. N-(deoxyguanosin-8-yl)-[acetyl]ammodiumphane
C. 8-alkyl-8-deoxycytidine-2'-O-sulfate
D. 4-Oxo-4-(3-pyridyl)butyl)guanine, a mutagenic lesion formed by the metabolism of the tobacco-specific nitrosamine, NNK
E. N-7-methyldeoxyguanosine

Examples of carcinogen-DNA adducts

Potential biological consequences of DNA-adduct formation

a. An insertion of the flat planar rings of a polycyclic hydrocarbon between the stacked bases of double-helical DNA may distort the helix, leading to a frame-shift mutation during DNA replication past the point of the intercalation.

b. Alkylated bases in DNA can mispair with the wrong base during DNA replication – for example, O6 methyguanine pairs with thymine instead of cytosine during DNA replication, leading to a base transition (i.e., GC→AT) type of mutation during the next round of DNA replication.
c. Many of the base adducts formed by carcinogens involve modifications of N-3 or N-7 positions on purines that induce an instability in the glycosidic bond between the purine base and deoxyribose. This destabilized structure can then undergo cleavage by DNA glycosylase, resulting in loss of the base.

d. Interaction with some carcinogens has been shown to favor a conformational transition of DNA from its usual double-helical B form to a Z-DNA form. This could alter the transcribability of certain genes, since B-Z conformational transitions are thought to be involved in regulating chromatin structure.

Mechanism of Carcinogenesis: Genotoxic Carcinogens

1. Carcinogen activation
2. DNA binding
3. Cell proliferation (fix mutation)
4. Gene mutation

Mechanism of Carcinogenesis: Non-Genotoxic Carcinogens

Classification of Carcinogens According to the Mode of Action

NON-GENOTOXIC:
- Do not directly cause DNA mutation
- Mechanism of action is not completely understood
- Difficult to detect - requires rodent carcinogen bioassay
Mechanisms of Non-Genotoxic Carcinogenesis (what’s in a “black box”?)

- Increased cell proliferation
- Decreased apoptosis
- Changes in gene expression
- Induction of metabolizing enzymes
- Activation of receptors (signaling)
- Oxidative stress
- ???

Cell Replication is Essential for Multistage Carcinogenesis

- Decreases time available for DNA repair
- Converts repairable DNA damage into non-repairable mutations
- Necessary for chromosomal aberrations, insertions, deletions and gene amplification
- Clonally expands existing cell populations

Cell Replication is Essential for Multistage Carcinogenesis

- Changes in cell behavior (- increased cell proliferation)
- Inhibition of apoptosis
- No direct structural alteration in DNA by agent or its metabolites

Table 1 Infection and inflammation associated with cancers

<table>
<thead>
<tr>
<th>Cause</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking tobacco/voil irritation</td>
<td>Oral cancers</td>
</tr>
<tr>
<td>Smoking/comed benignous</td>
<td>Lung</td>
</tr>
<tr>
<td>Asbestos</td>
<td>Mesothelioma</td>
</tr>
<tr>
<td>Radiation exposure</td>
<td>Breast adenocarcinoma of the breasts</td>
</tr>
<tr>
<td>Chronic immune suppression</td>
<td>Gastro esophageal and</td>
</tr>
<tr>
<td>Chronic lung inflammation</td>
<td>lymphoma</td>
</tr>
<tr>
<td>Chronic lung cancer</td>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td>Chronic lung cancer (for du rate)</td>
<td>Cholangiocarcinoma</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td>Hepatobrachial carcinomas</td>
</tr>
<tr>
<td>Human papilloma virus</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>Aneuploid carcinomas</td>
</tr>
<tr>
<td>Malignant melanoma (for du rate)</td>
<td>Nuclear cancer</td>
</tr>
<tr>
<td>Malignant melanoma (for du rate)</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>Chronic inflammatory disease</td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Chronic inflammatory disease</td>
<td>“Gray” mean arising from the lung, skin and other areas of existing</td>
</tr>
</tbody>
</table>

Asbestos

- Widely used in construction, insulation, and manufacturing
- Family of related fibrous silicates
- Chrysotile
- Crocidolite

NON GENOTOXIC CARCINOGENESIS

ASBESTOS

It is a silica-based natural fiber widely employed as termo-acoustic-insulator and fireproof.

Fibers with length >5 μm and diameter <3 μm are the most dangerous (as they reach the alveoli)

Serpentine: Chrysotile (a);
Anfiboli: Crocidolite (b);
Antofillite (c); Ricerite (d);
Tremolite (e); Amosite (f)
Malignant Mesothelioma

• Mainly occurs in pleural and peritoneal cavities
• Rare in general population
• Latent period of $\geq 20$ years