Teratogenesis, Mutagenesis, and Carcinogenesis

Principles of Environmental Toxicology
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University of Idaho

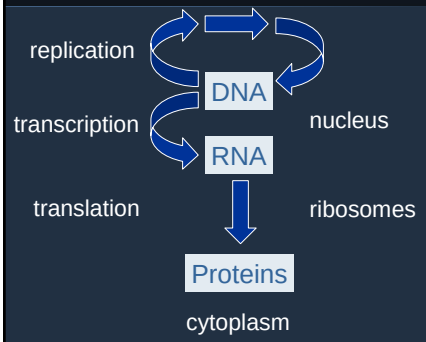
Learning Objectives

- Define teratogenesis, mutagenesis, and carcinogenesis.
- Describe the relevance of replication, transcription, and translation to teratogenesis, mutagenesis, and carcinogenesis.
- Summarize the mechanism of action for teratogenesis, mutagenesis, and carcinogenesis.
- Discuss examples of known teratogens, mutagens, and carcinogens.

Molecules of Life

- Toxicants can react with or modify DNA or RNA.
  - Can lead to heritable change in offspring or changes in cellular growth and development.
- Replication → perpetuate genetic information.
- Transcription and translation → express genetic information.

The Cell



Protein Functions

- Antibodies.
  - Recognize molecules of invading organisms.
- Receptors.
  - Part of the cell membrane; recognize other proteins, or chemicals, and inform the cell.
- Enzymes
  - Assemble or digest.
- Neurotransmitters, hormones
  - Trigger receptors.
- Channels and pores.

Endpoints

- Teratogenesis.
  - Origin or production of malformed fetuses or offspring.
- Mutagenesis.
  - Production of a mutation or change in the genetic code of an organism.
- Carcinogenesis.
  - Cancer formation including carcinoma and malignant neoplasms.

DNA Replication

- Structure implies replication
- Occurs via multiple enzyme action
- Helix unravels, strands part, DNA replicates
- Mitosis, meiosis
- Not always perfect
  - Repair enzymes

Replication

- Duplicates cell DNA.
- Mitosis - one somatic cell with 2n chromosomes divides to create two cells with 2n chromosomes (humans, n = 23).
- Number, quality and quantity of chromosomes per cell is conserved.
  - Triggers for mitosis (receptors + proteins).
    - External signals.
    - Hormones.
    - Internal factors.
    - Growth factors.

Replication, 2

- Meiosis - germ cells are cells that divide into gametes.
  - 2 cell divisions.
  - Four daughter cells.
    - Each with a different set of chromosomes.
    - Each with 1 set that will be joined by another in fertilization.

DNA Transcription

- DNA is copied via expendable mRNA
- mRNA codes for specific proteins
- Occurs in nucleus of cell

DNA Translation

- Occurs in the cytosol
- Interaction of mRNA, tRNA, amino acids and enzymes
- tRNA has three-base codons which correspond to different aa
- AA are added one at a time to form chain - polypeptide
- Polypeptide corresponds to protein with a specific aa sequence

Transcription and Translation

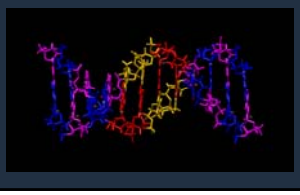
- DNA: double strand of nucleotides.
  - Nucleotide = **nucleic acid**, sugar and phosphate.
  - **C**ytosine, **T**hymine, **U**racil; **A**denine, **G**uanine.
  - Base pairing = A-T, G-C.
  - Gene: sequence of bases that code for a specific sequence of amino acids (protein).
  - Codon: sequence of 3 bases that code for a single amino acid, i.e.
    - AGC → Serine.
    - AAA → Lysine.

Transcription and Translation, 2

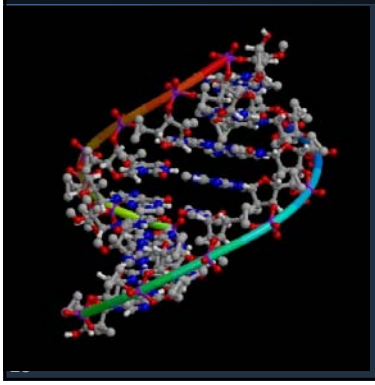
- Transcription = copying.
  - DNA unzips and enzymes make RNA "copy".
  - Differences:
    - T → U (UA not TA).
    - Deoxyribose → ribose.
  - mRNA formation; transport to cytoplasm.
- Translation = protein formation.
  - mRNA (blueprint).
  - rRNA (support).
  - TRNA (a.a. transport).

DNA Structure - Function

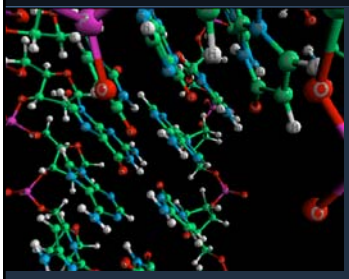
- Nucleotides form chains
- 3 nucleotides form a codon
- Multiple codons form genes
- Multiple genes form chromosomes
- Multiple chromosomes form DNA



DNA/RNA Complex



DNA/RNA Complex, 2



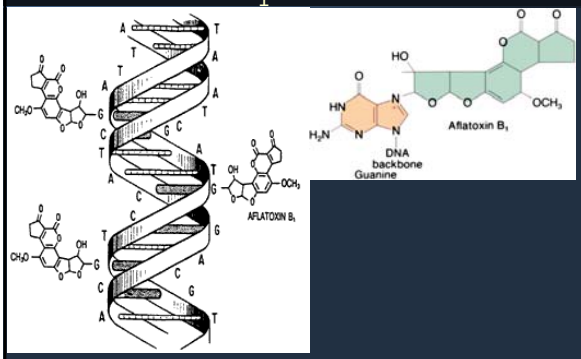
Errors in DNA Replication, Transcription and Translation

- Base pairing
- Repair enzymes and other enzymes
- Regulatory genes, operons, termination sequences
- Methylation patterns
- Post transcriptional/translational processing

DNA/Chemical Interactions

- Alkylation - covalent adduct between DNA and chemical
- Intercalation - noncovalent binding of chemical between two adjacent base pairs
- Cross-linkage - Inter or intrastrand covalent binding of chemical
- Breakage - scission of one or both strands of DNA

Afltoxin B_1 – DNA Adduct



Teratogenesis

- Teratology: the study of the frequency, causation, and development of congenital malformations.
- Complex mechanisms and timing of disruptive interaction during embryogenesis.
- Some natural "bad path" spontaneous abortion.
  - Humans: critical 1st 8 wks gestation.
    - Embryonic stage.
    - Morphological defects in specialized tissues and organs.
  - Fetal stage exposure.
    - Developmental or neoplastic endpoints.
- Known human teratogens.

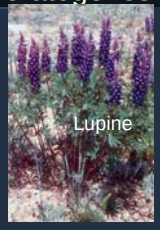
Example: Teratogenesis

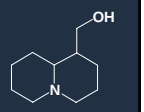
Five Legged Frog

Example: Teratogenesis, 2

Ovine Cyclopia
Veratrum Californicum
W. False Helebore

Example: Teratogenesis, 3





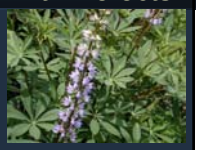
Quinolizidine alkaloids
Anagyrine

Case Study: Lupine Alkaloid Birth Defects

- In September 1980, a baby boy born in the mountainous back-country of northwestern California (Trinity County) was brought to the UC Medical Center in Sacramento with severe bone deformities in his arms and hands, including a partial absence of forearm bones (radial aplasia) and absent thumbs.
- Extensive medical histories and genetic analyses of his parents indicated that the probable cause was environmental rather than hereditary.
- His mother feared that somehow exposure to herbicide spraying was responsible. Association of forest spraying and a reportedly high incidence of birth defects in northwestern California and southern Oregon has been highly publicized in recent years and has become controversial. Indeed, it appears likely that this herbicide had been applied to a forested ridge several miles distant from the mother's home more than a year before the child's conception.

Case Study: Lupine Alkaloid Birth Defects

- The mother provided the evidence that her goats also gave birth to kids stillborn or with deformed legs during and after the period of her pregnancy, and that puppies born to a dog fed the goat's milk during pregnancy were likewise deformed.
- Local goat's milk has become a common food item in the area, and the child's mother drank it regularly herself throughout pregnancy.
- A thorough survey of nearby areas where the goats had regularly browsed at the time of the mother's early pregnancy showed that a perennial lupine, identified as the widely distributed Lupinus latifolius, often formed the principal low-growing forage as well as wild tobacco (Nicotiana), poison hemlock (Conium), and skunk cabbage (Veratrum).
- Circumstantial evidence.



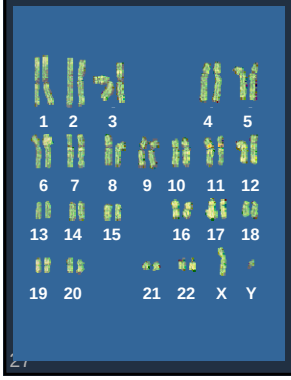
UC Davis Env Tox Newsletter 2:3 November 5, 1981

Mutagenesis

- Somatic cell mutations → metabolic dysfunction; carcinogenesis.
- Germ cell mutation → heritable change.
- Point mutation.
  - Base substitution (including analogues).
  - Frame shift.
- Chromosomal aberration.
  - Structural anomaly.
  - Numerical anomaly.

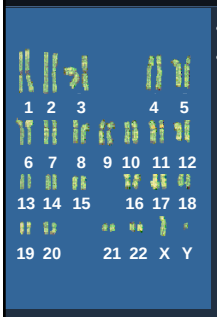
Hughes

Karotypes



Patterns photographed during metaphase help examine for chromosomal defects.

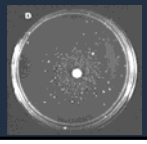
Abberations



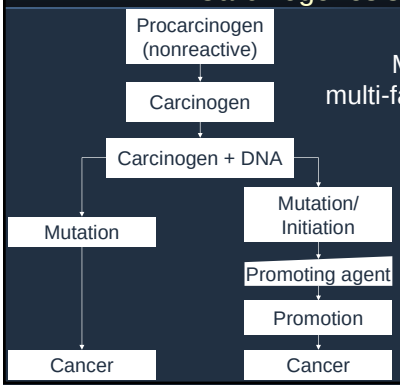
- Aneuploidy/Polyploidy
- Loss or gain of complete chromosomes
  - Microscopically visible
  - Down's Syndrome - (47,21+) trisomy
  - Klinefelder's Syndrome (47,XXY) trisomy
  - Turner's Syndrome (45,XO) monosomy

Ames Test for Chemical Mutagenicity

- Salmonella bacteria strain with histidine coding defect.
- Mutagenic chemicals can change the defect to allow cell division and growth.
- Add salmonella + test chemical + rat hepatocytes (for biotransformation).
  - Growth indicates mutagenic effect.



Carcinogenesis



Multi-step, multi-factorial disease

Cancer Definitions

- Cancer. NLM
  - A malignant tumor that has the ability to metastasize or invade into surrounding tissues.
- Tumor (Neoplasm).
  - A general term for the uncontrolled growth of cells that becomes progressively worse with time.
- Neoplasia.
  - The growth of new tissue with abnormal and unregulated cell proliferation.

Cancer Definitions, 2

- Benign tumor.
  - A tumor that does not metastasize.
- Metastasis.
  - Ability to establish secondary tumor growth at a new location.

NLM

Cancer Definitions, 3

- Carcinoma. NLM
  - Malignant tumor arising in the epithelium.
  - Most common form of cancer.
  - Usually spreads in the lymphatic system.
- Sarcoma.
  - Malignant tumor in muscle or connective tissue.
  - Usually spread in the blood stream.
  - Frequently metastasizes to the lung.

Multistage Carcinogenesis: Initiation

- Chemical-virus-spontaneous causes DNA lesion
- Cell division perpetuates DNA lesion
- No outcome if not promoted
  - Some chemicals can initiate and promote
  - May remain indefinitely if not promoted
- One hit
  - No threshold; irreversible

Properties of Initiated Cells

- No phenotypic differences
- Excess/deficiency of enzymes
  - e.g. δ-GT, G-6-P, Fe exclusion, ATPase
- Resistance to cytotoxic chemicals
  - Faster or slower metabolism
- Impaired cellular communication
- Enhanced response to growth factors
- Resistance to terminal differentiation

Multistage Carcinogenesis: Promotion

- Change in micro-environment of cells
- Chemical, viral, spontaneous-induced clonal proliferation of initiated cells
- Growth control factors; receptors; immune function; endocrine control; communication; metabolic; apoptosis
- Multi-hit, high dose
  - Reversible
  - Threshold

Multistage Carcinogenesis: Progression

- Complete loss of growth control
- Karyotype instability
- Loss/gain of chromosomal fragments
- DNA demethylation/deregulation
- Gene amplification
- Error prone DNA repair
- Irreversible
- Same mechanisms as promotion

Classification of Carcinogens

Hughes

- Genotoxic.
  - Act directly on DNA or expression of DNA during translation.
    - DNA replication errors.
    - Point mutations.
    - Chromosomal aberration.
- Epigenetic.
  - Non-DNA reactive.
  - Potentiators.
  - Cell, hormone, immune function modifiers.

Genotoxic Carcinogens

- Chemical capable of producing cancer by directly altering the genetic material of target cells.
- Direct carcinogens (no metabolic activation).
  - Alkylating agents.
- Indirect carcinogens (metabolic activation).
  - Polycyclic aromatic hydrocarbons.
  - Aromatic amines.
  - Nitrosamines.
  - Natural substances.
    - Mycotoxins.
  - Inorganic carcinogens.
    - Ni, Cr, Cd, As.

Marquardt

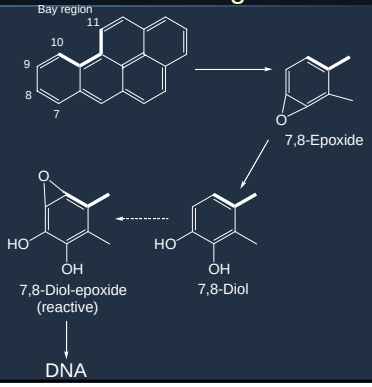
Epigenetic Carcinogens

Marquardt

- Cytotoxic carcinogens.
  - Nitrillotriacetate, BHA, BHT.
- Tumor promotors.
  - DDT, Dioxin
- Hormones.
  - Estradiol, DES
- Immunosuppressants.
  - Cyclosporin A
- Particulates.
  - Asbestos.

PAH Carcinogenic Activation

Marquardt



Proven Human Carcinogens

Marquardt

- Chemicals.
  - Aflatoxins, 4-aminobiphenyl, As, benzene, benzidine, Be, bis-chloroethylether, Cd, Cr(VI), soot, mineral oils, mustard gas, 2-naphthylamine, Ni, vinyl chloride.
- Substance abuse.
  - Alcohol, betel nuts, cigarettes.
- Dust and fiber.
  - Asbestos, silica, soots, talcum, wood dust.
- Chronic infection.
  - *H pylori*, Hepatitis B/C, HIV, liverfluke, papilloma virus, schistosomes.

Initiator Chemicals in Food

- Most genotoxic chemicals
- PAHs
- Aromatic amines
- Heterocyclic amines
- Mycotoxins
- Nitrosamines
- Nitrosamides

Promoting Agents in Food

- Butylated hydroxy toluene (BHT)
- Saccharin
- Cholic acid
- Tetrachloro-dibenzo-dioxin (TCDD)
- Alcohol

Chemical Cancer Assessment

Group A	Human carcinogen	Sufficient human evidence
Group B1	Probable human	Limited human evidence
Group B2	Probable human	Inadequate human evidence
Group C	Possible human	Limited animal evidence
Group D	Not classifiable	Inadequate animal evidence
Group F	No evidence	2 animal tests or epidem. and animal

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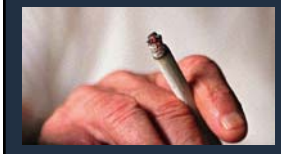
Causes of Cancer

- Diet, 35%
- Tobacco, 30%
- Sexual behavior, 7%
- Alcohol, 3%
- Infection, 10%
- Occupational exposure, 4%
- UV/radiation, 3%
- Pollution, 2%



 NLM

2006 Estimated US Cancer Cases*

Men 720,280		Women 679,510	
Prostate	33%	31%	Breast
Lung & bronchus	13%	12%	Lung & bronchus
Colon & rectum	10%	11%	Colon & rectum
Urinary bladder	6%	6%	Uterine corpus
Melanoma of skin	5%	4%	Non-Hodgkin lymphoma
Non-Hodgkin lymphoma	4%	4%	Melanoma of skin
Kidney	3%	3%	Thyroid
Oral cavity	3%	3%	Ovary
Leukemia	3%	2%	Urinary bladder
Pancreas	2%	2%	Pancreas
All Other Sites	18%	22%	All Other Sites

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
Source: American Cancer Society, 2006.

2006 Estimated US Cancer Deaths*

Men 291,270		Women 273,560	
Lung & bronchus	31%	26%	Lung & bronchus
Colon & rectum	10%	15%	Breast
Prostate	9%	10%	Colon & rectum
Pancreas	6%	6%	Pancreas
Leukemia	4%	6%	Ovary
Liver & intrahepatic bile duct	4%	4%	Leukemia
Esophagus	4%	3%	Non-Hodgkin lymphoma
Non-Hodgkin lymphoma	3%	3%	Uterine corpus
Urinary bladder	3%	2%	Multiple myeloma
Kidney	3%	2%	Brain/ONS
All other sites	23%	23%	All other sites

ONS=Other nervous system.
Source: American Cancer Society, 2006.

Lifetime Probability of Developing Cancer, by Site, Men, 2000-2002*

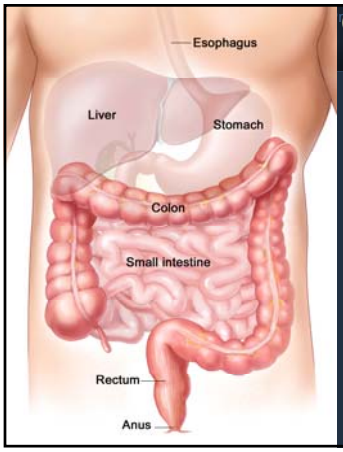
Site	Risk
All sites†	1 in 2
Prostate	1 in 6
Lung and bronchus	1 in 13
Colon and rectum	1 in 17
Urinary bladder‡	1 in 28
Non-Hodgkin lymphoma	1 in 46
Melanoma	1 in 52
Kidney	1 in 64
Leukemia	1 in 67
Oral Cavity	1 in 73
Stomach	1 in 82

* For those free of cancer at beginning of age interval. Based on cancer cases diagnosed during 2000 to 2002.
‡ Includes invasive and *in situ* cancer cases
† All Sites exclude basal and squamous cell skin cancers and in situ cancers except urinary bladder.
Source: DevCan: Probability of Developing or Dying of Cancer Software, Version 6.0 Statistical Research and Applications Branch, NCI, 2005. http://srab.cancer.gov/devcan

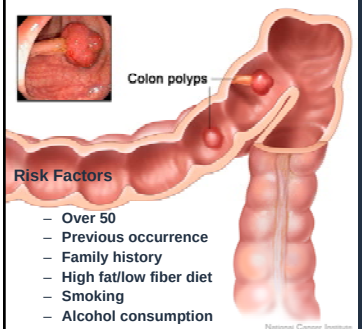
Lifetime Probability of Developing Cancer, by Site, Women, US, 2000-2002*

Site	Risk
All sites†	1 in 3
Breast	1 in 8
Lung & bronchus	1 in 17
Colon & rectum	1 in 18
Uterine corpus	1 in 38
Non-Hodgkin lymphoma	1 in 55
Ovary	1 in 68
Melanoma	1 in 77
Pancreas	1 in 79
Urinary bladder‡	1 in 88
Uterine cervix	1 in 135

Colorectal Cancer



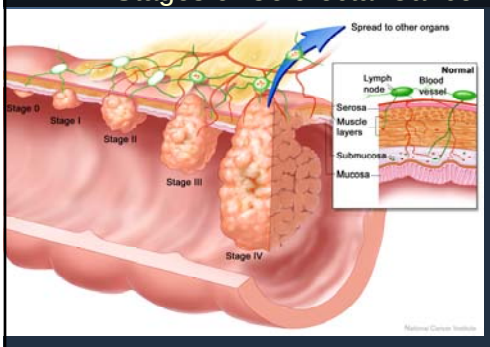
Colon Polyps



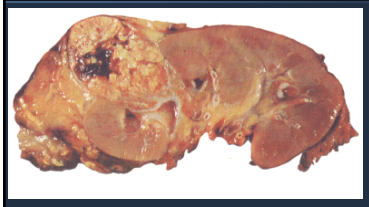
Risk Factors

- Over 50
- Previous occurrence
- Family history
- High fat/low fiber diet
- Smoking
- Alcohol consumption
- Sedentary
- Over weight

Stages of Colorectal Cancer



Kidney Cancer



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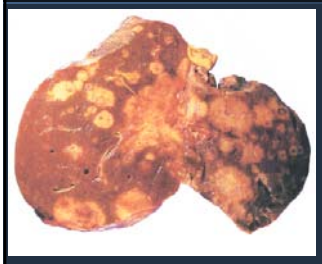
Liver Cancer



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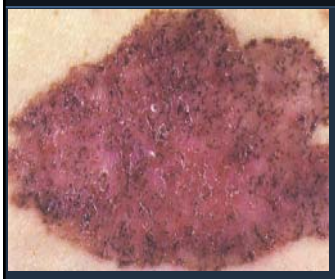
Basal Cell Carcinoma



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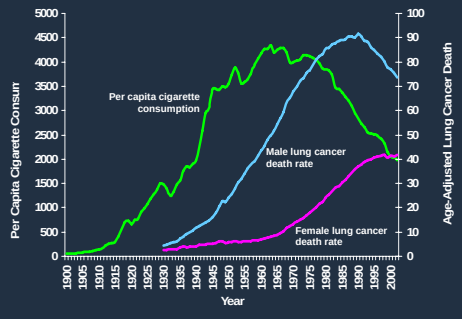
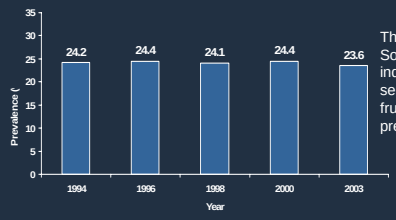
Tobacco Use in the US, 1900-2002



*Age-adjusted to 2000 US standard population.
Source: Death rates: US Mortality Public Use Tapes, 1960-2002, US Mortality Volumes, 1930-1959, National Center for Health Statistics, Centers for Disease Control and Prevention, 2005. Cigarette consumption: US Department of Agriculture, 1900-2002.

Trends in Consumption of Five or More Recommended Vegetable and Fruit Servings for Cancer Prevention, Adults 18 and Older, US, 1994-2003



The American Cancer Society recommends that individuals eat five or more servings of vegetables and fruits a day for cancer prevention.

Note: Data from participating states and the District of Columbia were aggregated to represent the United States.
Source: Behavioral Risk Factor Surveillance System CD-ROM (1984-1995, 1996, 1998) and Public Use Data Tape (2000, 2003), National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, 1997, 1999, 2000, 2001, 2004.