CONCEPTS of CARCINOGENESIS II

JAMES E. TROSKO, Ph.D.
Dept. Pediatrics and Human Development
College of Human Medicine
246 Natl. Food Safety Toxicology Center
Phone: 517-353-6346
Fax: 517-432-6340
E-mail: james.trosko@ht.msu.edu
Home Page: http://www.phd.msu.edu/Trosko
Hygeia, the Greek Goddess for healthy people
I. OBJECTIVES OF LECTURE

A. REVIEW CONCEPTS OF CARCINOGENESIS

2. Stem Cell Theory.
4. Mutation versus Epigenetic Theory.
5. Oncogene/ Tumor Suppressor Gene Theory.
6. Integrative Theory Based on Cell-Cell Communication”
I. OBJECTIVES of LECTURE (Cont.)

B. OVERVIEW of CANCER as a MAJOR HUMAN DISEASE.

1. Infectious versus chronic diseases, such as birth defects, cancer, atherosclerosis, diabetes, Parkinson’s, etc.

2. Cancer as a disease of evolution, genes, culture, diet, life-style old age.
I. OBJECTIVES of LECTURE (CONT.)

C. DISCUSSION of the MECHANISMS of CARCINOGENESIS.

1. Role of Mutations (gene and chromosomal).
2. Role of Cell Death (Necrosis and Apoptosis).
3. Role of Altered Gene Expression or “epigenetic” Alterations.
II. NATURE and NURTURE THEORY

A. GENES and ENVIRONMENTAL INTERACTIONS.
II. NATURE and NURTURE THEORY (Cont.)

B. EXAMPLES of INHERITED CANCER-PREDOSPOSING SYNDROMES.
Nature and Nurture Theory - Genetic and Environmental Interactions

- Phenotype = Genotype x Environment

- Example: Xeroderma pigmentosum (XP)
GENETICALLY PREDISPOSED ABNORMAL DNA (i.e., as in xeroderma pigmentosum syndrome)

ENVIRONMENTAL FACTORS (i.e., Kinds and amounts of physical, chemical and biological initiators; physical and chemical promoters, repair inhibitors, gene de-repressors, cell division stimulators and immunological suppressors.)

DEVELOPMENT AND DIFFERENTIATION

GENETIC FACTORS (i.e., Drug metabolizing enzymes, repair enzymes, immunological factors)

DNA1 DNA2

ZYGOTE

“Normal” DNA

ENVIRONMENTALLY PREDISPOSED ABNORMAL DNA OR DNA EXPRESSION (i.e., as in cells treated with mutagens, carcinogens and tumor promoters)

DNA1

SPERM

DNA2

EGG
Xeroderma Pigmentosum
Xeroderma Pigmentosum
Xeroderma Pigmentosum
Fanconi’s Anemia
An advanced Retinoblastoma in a young child
Ataxia Telangiectasia
II. NATURE and NURTURE THEORY (CONT.)

C. EXAMPLES of ENVIRONMENTALLY-INDUCED CANCERS or BIRTH DEFECTS.
Psoriasis
Psoriasis plus treatment
III. STEM CELL THEORY

A. Stem Cells as “Target Cells” for the Start of the Carcinogenic Process. [“Oncogeny as partially-blocked ontogeny”].

B. What are stem cells?

C. The De-Differentiation Theory of Carcinogenesis. Differentiated cells as “target cells” for the carcinogenic Process.

D. Cancers formed from the Two Processes Require Different Treatment Strategies.
DEFINITION OF “STEM CELLS”

A cell which divides “asymmetrically” to give rise to one daughter cell which maintains “self-renewal” and to another daughter cell which goes down the pathway to terminal differentiation.
IV. MULTI-STAGE, MULTI-MECHANISM THEORY of CARCINOGENESIS.

A. “Initiation, Promotion, Progression” Theory of Carcinogenesis.

B. “initiation” is the Irreversible Alteration of a Cancer-Related Gene.
C. Promotion is the Clonal Expansion of the Initiated Cell.

1. Stimulation of Growth of Initiated cells by Mitogenic growth factors, hormones or compensatory hyperplasia caused by necrosis or cell removal (Surgery).


3. Promotion is an Interruptible or Reversible Phase
IV. MULTI-STAGE, MULTI-MECHANISM THEORY OF CARCINOGENESIS (CONT.).

D. PROGRESSION PHASE OF CARCINOGENESIS.

1. Stable Alteration of Genes in an Initiated Cell.

2. Either Mutations or Epigenetic Events may Confer the Malignant Phenotypes of Invasiveness and Metastasis.
THE INITIATION AND PROMOTION OF TUMORS

1) No Tumors
2) Many Tumors
3) No Tumors
4) Many Tumors
5) No Tumors
6) No Tumors

Symbols: Time
Initiator  Promoter
V. MUTATION/EPIGENETIC THEORIES of CARCINOGENESIS

A. Definitions of Mutagenesis: The Qualitative (Gene) and Quantitative (Chromosomal) Alteration of Genetic Information in the GENOME of a Cell.

B. Mechanism of Mutagenesis.
   1. Errors of Replication (i.e., Bloom’s Syndrome).
   2. Errors of DNA Repair (i.e., Xeroderma pigmentosum.)
V. MUTATION/EPIGENETIC THEORIES of CARCINOGENESIS (CONT.)

C. Mutagens

1. Physical Agents (X Rays; UV Light).

2. Electrophilic Chemicals (Nitrosamines, Benzo (a)pyrenes)
D. Definition of Epigenesis: Alteration of Gene Expression at the Transcriptional, Translational, or Posttranslational Levels.

1. Translational Level- Altered Methylation of DNA or Acetylation of Nuclear Proteins.

2. Translational Level- Alternative Splicing of mRNA.

3. Posttranslational Level: Modification of Proteins by Phosphorylation or Nitrosylation.

4. Down’s and Phenytoin Syndromes as Examples.
Down’s Syndrome
Fetal Alcohol Syndrome
V. MUTATION/EPIGENETIC THEORIES of CARCINOGENESIS (CONT.)

F. Epigenetic Carcinogens or Tumor Promoters.

1. Non-Mutagenic chemicals (e.g., DDT, Phenobarbital).

2. They Act as Either Mitogens and / or Inhibitors of Apoptosis.
Chemicals Which Down-Regulate GJIC In Normal Cells: Potential Epigenetic Toxicants

- NATURAL CHEMICALS: Phorbol Esters
- TOXINS: Vomatoxin, T-2 Toxic, LPS,
- HORMONES: Estrogens
- GROWTH FACTORS: EGF, PDGF, TGF-\(\alpha\), TNF-\(\alpha\)
- PESTICIDES: DDT, dieldrin
- HERBICIDES: 2,4,D,2,4,5-T
- CYTOKINES: interleukin-1\(\alpha\), ceramides, prosteglandins
Chemicals Which Down-Regulate GJIC In Normal Cells: Potential Epigenetic Toxicants (Cont.)

- POLLUTANTS: PCBs, PBB, TCCD,
- HEAVY METALS: methylmercury; cadmium
- SOLID PARTICLES: Airborne particulates; [60] fullerene
- NUTRIENTS: Unsaturated fatty acids,
- DRUGS: Phenobarbital
- FOOD ADDITIVES: Saccharin, carrageenan
VI. ONCOGENE/ TUMOR SUPPRESSOR GENE THEORY

A. Definition of Oncogenes.

B. Biological Functions of Oncogenes.

C. Definition of Tumor Suppressor Genes.
ONCOGENES

- Derived from normal “proto-oncogenes” in all cells

- Tumor-specificity (i.e., neu or ERB2 expressed in breast cancer)

- Can be “activated” by amplification of the normal proto-oncogene; mutated to become activated; abnormally expressed.
BIOLOGICAL FUNCTIONS OF ONCOGENES

1. Growth Factors/Hormones (e.g., SIS; PDGF)
2. Growth Factor/Hormone-Receptors (e.g., Neu; EGF-R)
3. Signal Transduction Enzymes (e.g., Ras; G-protein)
4. Transcription Factors (e.g., MYC)
TUMOR SUPPRESSOR GENE

• Must be “de-activated”
• Can be tumor specific (BRCA-1/BRCA-2 in breast tumors; rb in retinoblastoma, osteosarcoma)
• Can be ubiquitous (p53 in at least 50% of all tumors)
BIOLOGICAL FUNCTIONS OF TUMOR SUPPRESSOR GENES

1. Growth Inhibitors (e.g., TGF-β; glucocorticoids)
2. Growth Inhibitor Receptors
3. Signal Transduction Protein Inhibitors
4. Transcription Factors of Growth Inhibitors
VII. AN INTEGRATIVE THEORY OF CARCINOGENESIS

A. Cancer as a “Disease of Differentiation”, a “Stem Cell” Disease, or a “Disease of Homeostasis”.

B. Characteristics of Cancer Cells.
   1. “Immortal”.
   2. Loss of Growth control or “contact inhibition”.
   3. Unable to Terminally Differentiate or Apoptose.
   4. Have Invasive and Metatastic properties.
   5. Have Angiogenic Support System.
VII. AN INTEGRATIVE THEORY OF CARCINOGENESIS

C. Normal Cells are Homeostatically Regulated.


VII. AN INTEGRATIVE THEORY OF CARCINOGENESIS (CONT.)

3. Cancer Cells are Characterized by **not having** Functional Gap Junctional Intercellular Communication.

4. **What are Gap junctions?** – Protein Channels between cells allowing Ions and Small Molecules to Synchronize Electrotonic and Metabolic Functions needed for Homeostasis.

5. **Normal Cells have functional Gap Junctions, Tumor Cells Do Not.**
VII. AN INTEGRATIVE THEORY OF CARCINOGENESIS (CONT.)

6. Evidence for the Role of Gap Junctional Communication in Normal Growth Control and when Inhibited, in the Tumor Promotion/Progression Phase, by Epigenetic Chemicals or Oncogenes, Cells Proliferate, do not Differentiate or Apoptose.
An Integrated View of Carcinogenesis

- Target cells: Stem cells (and progenitor cells)
- Target genes: Specific oncogenes and tumor suppressor genes
- Individual susceptibility: Hereditary and endogenous/exogenous exposure to carcinogens
- Relevant alterations: Genetic (mutation) and epigenetic alteration of target genes in target cells.
- Mechanism of tumor development: Clonal expansion (tumor promotion) and genomic instability (tumor progression) result in accumulation of relevant alterations.
Gap Junctions in Cellular Homeostasis

Extracellular Communication

Translational Regulators
Transcriptional Regulators
Post translational Regulators

Intracellular Communication

Intercellular Communication

is = intercellular signal

Toxic Chemicals

receptor

Growth factor

Biological End Points

Cell Proliferation
Cell Differentiation
Apoptosis
Senescence
Adaptive responses of differentiated cells
A Gap Junction Plaque
GAP JUNCTION ROLES IN ORGAN HOMEOSTASIS
Scrape Loading/Dye Transfer Technique

**PRINCIPLE:** If cells have gap junctional communication, Lucifer yellow will diffuse only through gap junctions. Rhodamine Red Dextran will not.
Demonstration of cell-to-cell communication in normal cells.
HUMAN SKIN FIBROBLASTS (PY CULTURE)
A. CONTROL CELLS, NO TREATMENT
B. PRETREATMENT WITH TPA (10 ng/ml) 90 min
Most, if not all, tumor cells have dysfunctional homologous or heterologous GJIC.
Lack of cell-to-cell communication in human colon carcinoma cells as evidenced by the lack of dye transfer.
NEGATIVE REGULATORS OF CELL GROWTH

- Transforming growth factor beta (extracellular communication)
- Signal transduction with cell (intracellular communication)
- Gap junctional intercellular communication (intercellular communication)
- Apoptosis (induced programmed cell death)
VIII. ROLE OF CHRONIC BACTERIAL, VIRAL, PARASITIC INFECTIONS in CARCINOGENESIS.

A. Immortalized Cells-Properties of “Initiators”
   (SV40 virus; papilloma virus).

B. Induce Chronic Inflammation/Hyperplasia- Properties of Tumor Promoters. (Hepatitis Virus).
Apoptosis

A morphological description of a programmed sequence of events leading to single cell death within a population.
### Apoptosis vs. Necrosis

<table>
<thead>
<tr>
<th>Apoptosis</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Shrinkage</td>
<td>Cell swelling</td>
</tr>
<tr>
<td>Membrane integrity remains intact</td>
<td>Loss of membrane integrity</td>
</tr>
<tr>
<td>Energy requiring</td>
<td>No energy required</td>
</tr>
<tr>
<td>Single cells</td>
<td>Groups of cells</td>
</tr>
</tbody>
</table>
Apoptosis

The function of apoptosis is to clear tissues of unwanted cells:

• during normal development
• in response to environmental insult
• a mechanism to eliminate pre-cancer (initiated) cells.
Cell in green is an apoptotic cell.
IX. STRATEGIES for CANCER CHEMOTHERAPY

A. Kill Cells by Necrosis-Current Strategy.
B. Kill Cells by Apoptosis ("By-stander Effect")
C. Induction of Terminal Differentiation.
D. Targeted Immunotherapy.
E. Gene Therapy.
F. Oncogene Inhibitors.
G. Inhibitors of Cell cycle Enzymes.
H. Telomerase Inhibitors.
I. Angiogenesis Inhibitors.
J. Restoration of Gap Junctional Communication
K. Limitation/ Potentials of each.
X. STRATEGIES for PREVENTION of CANCER

A. Prevention of “initiation” or Mutagenesis Phase Carcinogenesis.
B. Prevention of Promotion Phase of Carcinogenesis.
C. Prevention of Progression- Similar to Prevention of Initiation Phase.
D. Role of Diet( Tofu, Tomatoes, Broccoli, Olive Oil, Green Tea, Red Wine).
E. Role of Cultural Behavior- Breast Cancer Example.
F. Role of Caloric Restriction- The Okinawa Example
XI. FROM THEORETIC ABSTRACTIONS TO SPECIFIC EXAMPLE OF HUMAN COLON CARCINOGENESIS.

A. Role of Genetics/Environmental/Diet and Culture on Human Colon Carcinogenesis.

B. Prevention of Tumor Promotion of Colon Cancer with Nutraceuticals, Diet, Drugs via Prevention of the Inhibition of Gap Junctional Communication.

C. DISCUSSION WITH PATIENT WHO HAS HAD COLON CANCER*** CONTINGENT OF PATIENT AVAILABILITY.
Stem cell differentiation in the crypt of the small intestine.
Somatic Mutation Model of Tumor Progression

Adapted from: Robert A. Weinberg
Scientific American - September, 1996

© Peter W. Laird
A Genetic Model for Colorectal Tumorigenesis

MUTATION OF MSH2, MLH1, PMS1 OR PMS2

MUTATION OR LOSS OF APC GENE

ALTERNED DNA METHYLATION

MUTATION OF K-RAS GENE

LOSS OF DCC GENE

MUTATION AND LOSS OF P53 GENE

OTHER ALTERATIONS

NORMAL EPITHELIUM

HYPERPLASIA

EARLY ADENOMA

INTERMEDIATE ADENOMA

LATE ADENOMA

CARCINOMA

METASTASIS

Adapted from Fearon and Vogelstein, 1
Adenomatous Polyposis Coli
Endoscopic views of colon polyps.
Familial adenomatous polyposis