Chemical Carcinogenesis:
Initiation, Promotion and Progression

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Promotion (reversible)

Initiation (irreversible)

More mutations
Progression (irreversible)

malignant metastases

Normal Cell

Initiated Cell

Preneoplastic Focal Lesion

Repair

DNA Damage

Apoptosis

Proliferation

Proliferation

Neoplasia
Different Steps of Carcinogenesis

**Initiation:** Mutation in one or more cellular genes controlling key regulatory pathways of the cell (irreversible)—must be a heritable DNA alteration.

**Promotion:** selective growth enhancement induced in the initiated cell and its progeny by the continuous exposure to a promoting agent.

**Progression:** results from continuing evolution of unstable chromosomes; further mutations from genetic instability during promotion—results in further degrees of independence, invasiveness, metastasis, etc.
Initiation
Initiation is the induction of a mutation in a critical gene involved in the control of cell proliferation.

As with mutational events, initiation requires one or more rounds of cell division for the “fixation” of the process.

The metabolism of initiating agents to non-reactive forms and the high efficiency of DNA repair of the tissue can alter the process of initiation.

Initiation is irreversible although the initiated cell may eventually die during the development of the neoplasm.
Types of mutations

Chemical carcinogens can cause:

1. **Point mutations**- the replacement of a single nucleotide base with another nucleotide.

2. **Frameshift mutations**- addition or deletion of a nucleotide such that the protein sequence from that point onward is altered.

3. **Chromosomal aberrations**- any change in the normal structure or number of chromosomes

4. **Aneuploidy**- chromosome number is not a multiple of the normal haploid (23)

5. **Polyploidy**- more than twice the haploid number of chromosomes
Mechanisms of DNA Repair

The persistence of chemically-induced DNA adducts is predominantly the result of failure of DNA repair, due to either:

- carcinogen-induced mutational inactivation of DNA repair enzymes.
- failure of the DNA repair mechanisms to recognize carcinogen-induced mutation.
Table 8-7
Types of DNA Repair

1. Direct reversal of DNA damage
   Alkyltransferases
2. Base excision repair
   Glycosylase and AP endonuclease
3. Nucleotide excision repair
   T-T, C-C, C-T repair
   “Bulky” adduct repair
4. Double-strand-break repair
   Homologous recombination (HR)
   Nonhomologous DNA end joining (NHEJ)
5. Mismatch repair
   Repair of deamination of 5-Me cytosine
   Repair of mismatches in DNA due to defective repair, etc.

SOURCE: Modified from Myles and Sancar (1989) and from Lieber (1998), with permission.
Targets of Initiation

Chemical carcinogens initiate cells via:

1. Mutational activation of oncogenic (proliferative) pathways (e.g. growth factor receptors and downstream signaling proteins, proteins involved in cell cycle checkpoints).

2. Mutational inactivation of apoptotic (cell death) pathways (e.g. growth inhibitory receptors, proteins involved in apoptosis, tumor suppressors).

3. Mutational inactivation of DNA repair mechanisms (e.g. BER, NER, etc).

4. Mutational inactivation of antioxidant response (e.g. SOD).
Tumor suppressor p53 signaling

- p53 is an important tumor suppressor (transcriptional factor) that controls cell cycle, apoptosis, DNA repair mechanisms.
- Mdm2 is a negative regulator of p53 that functions both as an E3 ubiquitin ligase and an inhibitor of p53 transcriptional activation.
p53—tumor suppressor:

Mutated in most cancers.

Carcinogens often mutationally inactivate p53 as well as proteins that control p53 function (e.g. Mdm2, p14)

DNA damage, cell damage
- ATM kinase activated
  - phosphorylates p53 so it can't bind Mdm2
  - phosphorylates Mdm2 prevents ubiquitination of p53
  - increased p53 (tetrameric TF)
    - binds tandem sequence of PuPuPuC A/t T/a G PyPyPy
      - increased Fas receptor
      - increased Bax
        - Bax dimer depolarizes mitochondrial membrane
          - cyt c released into cytosol
            - cyt c, Apaf-1, caspase 9 form apoptosome
              - activates executioner caspases 3,6,7

apoptosis
Ras oncogene: involved in control of cell cycle progression and apoptosis

- norepinephrine, serotonin, etc
  - binds G-protein coupled receptor
    - PLC
    - IP3, DAG
    - Ca2+
    - PKC
      - CaMK
      - Raf
        - MEK
          - ERK
            - p90
              - CREB
                - cyclin D, E2F1-3
                  - CDK4 decreases p21, p15
                    - Fos
                      - AP1
                        - increased cyclin D
                          - cell cycle progression

- growth factor (PDGF, IGF, EGF, NGF)
  - binds receptor tyrosine kinase and dimerizes to autophosphorylate cytosolic Tyr on receptor
    - recruits Grb2/Sos to phospho-Tyr
    - Ras-GDP
      - Ras-GTP
        - PI3-Kinase
          - PI4,5-P2
            - PIP3
              - Akt/PKB and is activated by phosphorylation by PDK
                - P-bad (inactive)
                  - P-p21, P-p27 (inactive)
                    - P-Mdm2 (sequesters p53)
                      - inhibits TSC1/2 (mTor active)
                        - activates protein synthesis

- apoptosis suppressed
  - MAP Kinase pathway
benzo[a]pyrene

(+)-benzo[a]pyrene 7,8-oxide

(-)-benzo[a]pyrene 7,8-dihydrodiol

(+) benzo[a]pyrene 7,8-dihydrodiol-9,10-epoxide

ULTIMATE CARCINOGEN

BaP-N\(^2\)-dG DNA adduct
Benzopyrene Leads to Mutations in K-Ras and p53 in the Genomic Loci Found to be Mutated in Smoking-Induced Lung Cancers

- **K-Ras and p53** are the two **oncogenes** most frequently mutated in smoking-related lung cancers.
- If not corrected by the cell’s DNA repair mechanism, this guanine “adduct” is misread as a thymine by the DNA polymerase that copies chromosomes during replication.
- Ultimately, the original G—C base pair may be replaced by a T—A base pair, a mutation called a **traversion**.
- Cells treated with Benzopyrene show the same spectrum of G—T transversions as found in the K-RAS and p53 of smokers.
- These mutational “hot spots” map well to the guanine binding sites of BaP epoxide.
Promotion
Promotion

- Epigenetic event—change in gene expression without change in DNA.
- **Mitogenic** (Not mutagenic) Stimulates proliferation. Causes both mutated and normal cells to proliferate.
- Enhances the effect of the genotoxic initiating agent by establishing clones of initiated cells.
- Long delay possible between administration of initiating agent and promoting agent.
- Promotion is reversible.
Promoters

1. Reactive Oxygen Species (ROS) and redox active xenobiotics and metals
2. Phorbol esters (e.g. TPA)
3. Polycyclic aromatic compounds (e.g. Dioxin)
4. Peroxisome Proliferators (oxidized fats)
5. Endocrine Disruptors (estradiol, DES)
Structures of Representative Promoters

TPA and other phorbol esters activate protein kinase C, which leads to signal transduction pathways that increase DNA replication, cell division.

TCDD (dioxin) activates aryl hydrocarbon receptor (AhR) and induces the expression of cytochrome P450 \( \rightarrow \) increases oxidative stress \( \rightarrow \) can oxidatively activate oncogenic pathways (e.g. RAS).
Endocrine Receptors and Carcinogenesis

Endocrine disruptors are involved in breast, ovarian, colon, prostate cancers.

1. ERβ/ERα (estrogen receptors) ratio is decreased in cancers (ligands include estradiol); ERs are transcription factors.

2. ERβ inhibits ERα
   a. ERα-ERα dimerization (homodimer) leads to mitogenic activation.
   b. ERβ-ERα dimerization (heterodimer) leads to an inactivation.

3. Androgen Receptor (prostate) (AR) can also homodimerize with AR leading to mitogenic activation; AR can heterodimerize with ERβ to cause growth arrest (prostate also dependent on estrogenic signals).
Estrogen Receptor Interactions

ERbeta

ERalpha

estrogen

mitogenic  no proliferation

cytosol

nucleus
Examples of Endocrine Disruptors

B. Synthetic

Other examples include dioxin, polychlorinated biphenyls (PCBs), DDT, bisphenol A (BPA) and atrazine.
Progression
Mechanisms of Progression

Progression is an irreversible process and leads to metastasis.

Progression requires:

1. Further mutations from genetic instability (chromosomal instability) during promotion.

2. Recruitment of inflammatory immune cells to the tumor.

3. The tumor cell acquiring “wound-healing” characteristics (secretion of chemo-attractants to attract inflammatory immune cells, angiogenesis factors, proteases, etc).

Examples of progressor agents: inflammation, asbestos fibers, benzene, benzoyl peroxide, other peroxides, oxidative stress, inflammation
A Changing View of Cancers

The 'old' view

Cancer cells

The 'new' view

Cancer cells
Fibroblasts
Immune cells
Endothelial cells

Cancers are composed of different types of cells that interact with each other (heterotypic interactions) to facilitate the growth and spread of the tumor mass.
Chronic Toxicant Exposure
- Decreased ATP, increased Ca\(^{2+}\), increased oxidative stress

Cellular Necrosis
- Intracellular contents (e.g. ATP, dsDNA)

Activation of Resident Macrophages
- Cytokines, chemokines, Eicosanoids (TNF\(\alpha\), IL1\(\beta\), PGE2)

Recruitment and Activation of More Macrophages
- TGF\(\beta\), IGF1, PDGF, TNF\(\alpha\)

Intracellular contents (e.g. ATP, dsDNA)

Growth factors (e.g. TGF\(\beta\), IGF1, PDGF, ROS)

Cell proliferation

Genetic instability
- Mutations
- Cell proliferation
- Cellular transformation

Malignant progression of cancer cells
- TGF\(\beta\)

Epithelial-to-mesenchymal transition (EMT)

Cytokines, chemokines, Eicosanoids (TNF\(\alpha\), IL1\(\beta\), PGE2)

Recruitment and Activation of More Macrophages
- Proteases, TGF\(\beta\)

EMT and breakdown of ECM

Fibroblast proliferation, differentiation
- VEGF

Excessive formation of hardened extracellular matrix (ECM)

TGF\(\beta\), IGF1, PDGF, TNF\(\alpha\)

Infiltration of more immune cells into damaged tissues

Leakier basement membrane

Tissue Cells And Macrophage Cellular Necrosis

TGF\(\beta\)

Epithelial-to-mesenchymal transition (EMT)

Angiogenesis

Cell proliferation

Angiogenesis

Genetic instability
- Mutations
- Cell proliferation
- Cellular transformation

Malignant progression of cancer cells
- TGF\(\beta\)

Epithelial-to-mesenchymal transition (EMT)

Cytokines, chemokines, Eicosanoids (TNF\(\alpha\), IL1\(\beta\), PGE2)

Recruitment and Activation of More Macrophages
- Proteases, TGF\(\beta\)

EMT and breakdown of ECM

Cancer cells extravagate with macrophages and blood supply into circulation

Tissue dysfunction, tissue damage, degeneration, organ failure

Metastasis
Inflammation and Cancer

- Inflammation acts at all stages of tumorigenesis
- It may contribute to tumor initiation through mutations, genomic instability
- Inflammation activates tissue repair responses, induces proliferation of premalignant cells, and enhances their survival
- Inflammation also stimulates angiogenesis, causes localized immunosuppression, and promotes the formation hospitable microenvironment in which premalignant cells can survive, expand, and accumulate additional mutations
- Inflammation also promotes metastatic spread.