Cancer Biomarkers and Susceptibility Factors

Thomas Kensler, PhD
Bloomberg School of Public Health
Section A

Biomarkers of Exposure
Traditional Epidemiology

Exposure ← Exposure ← Disease

Traditional Toxicology

Exposure → Exposure → Disease
1952 London Fog Episode: Total Deaths

![Graph showing deaths and pollution during the 1952 London Fog episode. The diagram indicates a significant rise in deaths and pollution during the fog period, with a peak around December 9, 1952.](image)
The Problem

- To identify the role of specific risk factors, some of which may interact with host susceptibility factors, in the causation of environmentally-induced disease
  - Multiple exposures, multiple agents
  - Low levels of exposure
  - Wide range of susceptibility mechanisms
  - Long latency periods
Definitions

- **Biomarkers**: Molecular, biochemical or cellular alterations that are measurable in biological media, such as human tissues, cells, or fluids
- **Molecular epidemiology**: Incorporation of biomarkers into analytic, epidemiologic research
Fundamental Principles of Toxicology

Principle I

- The toxic action of a substance is a consequence of the physical/chemical interaction of the active form of that substance with a molecular target within the living organism
Fundamental Principles of Toxicology

**Principle II**

- The magnitude of the toxic effect will be a function of the concentration of altered molecular targets, which in turn is related to the concentration of the active form of the toxicant at the site where the molecular targets are located.
The Toxicological Process

EXPOSURE
- Absorption
- Distribution and Metabolism of Toxicant
- Storage
- Excretion

TOXICOKINETICS

INITIATION

Molecular Level → Cellular Response → Organ Response → Organism Response

T + M ⇌ M* → E1

Repair

E2 → E3 → E4 → E5 → ...

E6 → E7 → ...

Health → Pathology

Repair

TOXICODYNAMICS
The Toxicological Paradigm

Toxicokinetics  Toxicodynamics

Exposure

Internal Dose  Biologically Effective Dose  Early Biological Effects  Altered Structure/Function

Susceptibility Factors

Disease

Exposure Assessment  Risk Assessment
Biomarkers of Exposure
Biomarkers of Dose to Humans

Internal Dose

- Direct measure of toxic chemicals or their metabolites in cells, tissues, or body fluids (e.g., blood, urine, feces, milk, amniotic, sweat, hair, nails, saliva)
  - Integrates multiple portals of entry
  - Integrates fluctuating exposures
  - Relates exposure to dose
Patterns of Exposure

- **Cyclic**
- **Random**
- **Intermittent**
- **Concentrated**
- **Continuous**
Examples of Biomarkers of Internal Dose

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exhaled breath</td>
<td>Volatile organic chemicals (ethanol)</td>
</tr>
<tr>
<td>Blood levels</td>
<td>Styrene, lead, cadmium, arsenic</td>
</tr>
<tr>
<td>Fat concentrations</td>
<td>PCBs and PBBs, DDT &amp; TCDD</td>
</tr>
<tr>
<td>Metabolites in urine</td>
<td>Aflatoxin, benzene, arsenic</td>
</tr>
<tr>
<td>Mutagens in urine</td>
<td>Chemotherapeutic drugs, carcinogens</td>
</tr>
<tr>
<td>Hair sample</td>
<td>Arsenic</td>
</tr>
<tr>
<td>Blood carboxyhemoglobin</td>
<td>Carbon monoxide</td>
</tr>
<tr>
<td>Blood methemoglobinemia</td>
<td>Organic nitrates</td>
</tr>
</tbody>
</table>
Markers of Biologically-Effective Dose

Assessment of the interactions of toxicants with their molecular targets

- **DNA adducts**
  - Cellular DNA: e.g., benzo[a]pyrene-DNA adducts in peripheral lymphocytes of coke oven workers; cisplatinum-DNA adducts in WBC of chemotherapy patients; $0^6$-methyl deoxyguanosine in GI mucosa from nitrosamine ingestion
Markers of Biologically-Effective Dose

- Urine: E.g., aflatoxin-N\textsuperscript{7} – guanine adducts in urine of individuals consuming AFB\textsubscript{1}; oxidized DNA bases in urine following radiation and other forms of oxidative stress
Markers of Biologically-Effective Dose

- **Protein adducts**
  - Hemoglobin: Ethylene oxide, aromatic amines, tobacco-specific nitrosamines, cisplatinum
  - Albumin: Aflatoxin B$_1$
Examples of Assays
For Carcinogen-Macromolecular Adducts

- Cellular DNA adducts
  - Nonhydrolyzed: Immunoassays, fluorometry
  - Hydrolyzed concentration, e.g., high-pressure liquid chromatography or immunocolumn followed by: 32P-postlabeling, immunoassay, electrochemical conductance
Examples of Assays

*For Carcinogen-Macromolecular Adducts*

- Urinary DNA base adducts
- Protein adducts
  - Hemoglobin
  - Albumin
DNA Adducts Associated with Tobacco Smoking

- Current analytical capabilities
  - Complex mixtures of unidentified adducts ($^{32}$P-postlabeling)
  - Polycyclic aromatic hydrocarbons (BPDE)
  - Aromatic amines (4-ABP)
  - Tobacco-specific nitrosamines (NNN, NNK)
DNA Adducts Associated with Tobacco Smoking

- Tissues and cells containing smoking-related DNA adducts
  - Lung, bronchial epithelium, alveolar macrophages, oral mucosa
  - Urinary bladder, exfoliated urothelial cells
  - Placenta, fetal tissues
  - White blood cells
4-ABP-Hemoglobin Adduct Levels

In Smokers (Solid Bars) & Nonsmokers (Shaded Bars)

Nonsmokers: $\overline{x} = 28$

Smokers: $\overline{x} = 154$
Effect of Smoking Cessation on 4-ABP-Hemoglobin Adduct Levels (Five Different Individuals)
Section B

Biomarkers of Risk
Markers of Early Biological Effect

Assessment of molecular sequelae of toxicant-cell interactions

- **Genetic alterations in target and reporter genes**
  - Mutated oncogenes, hprt, thymidine kinase, glycophorin A
  - Loss of tumor suppressor genes
  - Gene rearrangements

- **Nuclear aberrations**
  - Single-strand breaks
  - Unscheduled DNA synthesis
  - DNA hyperploidy
  - Micronuclei
  - Sister chromatid exchanges
  - Chromosome gaps and break

- **Altered enzymatic activities**
  - Elevated protoporphyrin (Pb)
  - Decreased acetylcholinesterase (organophosphates)
  - Elevated xenobiotic metabolizing enzymes (TCDD)
Effects of Lead on Heme Synthesis in the Mammalian Erythrocyte
Early Detection Biomarkers

Somatic Mutations in Target Genes

- **Reporter genes**
  - HPRT, Glycophorin A

- **Oncogenes and suppressor genes**
  - Gatekeepers: Control net cellular proliferation, e.g., APC, K-ras, p53
  - Caretakers: Maintain genomic integrity, e.g., hMSH2, hMLH1
p53 Tumor Suppressor Protein

- First described in 1970s as a cellular protein that co-precipitated with the large T antigen of simian virus 40 (SV40) and whose synthesis was enhanced in chemically-transformed tumors
- p53 is the gene most frequently found to be mutated in human tumors
- Gene is located on chromosome 17p13; nuclear 53kD phosphoprotein
- p53 regulates expression of cell cycle-related genes: Wild type protein blocks cell cycle progression while mutant promotes cell proliferation
Mutational “Hot-Spots” in p53

- Colon: 175, 248, 273, 282
- Brain: 273
- Liver: 249
Markers of Altered Structure/ Function

Assessment of morphological and/or functional changes following toxicant-cell interactions

- **Serum markers of disease**: Elevated serum GSTs, ALA, SDH (liver toxicity), SGOT, creatinine kinase (myocardial infarction)
- **Proliferation markers**: Mitotic frequency, thymidine labeling index, nuclear antigens, ornithine decarboxylase activity, polyamine levels
- **Differentiation markers**: Cytokeratins, involucrin, transglutaminase
- **Differentially expressed genes**: EGF, TGF-β, serum α-fetoprotein, carcinoembryonic antigen
- **Cellular/ tissue changes**: Metaplyastic/dysplastic lesions, sperm counts/mobility, macrophage activity, lymphocyte ratios, RBC counts
Early Detection/ Prognostic Biomarkers: Altered Cell Structure

**Intraepithelial neoplasia:** precancerous lesions directly on causal pathway
- Genomic instability
- Independent, multicentric lesions within epithelium

<table>
<thead>
<tr>
<th><strong>Breast:</strong> DCIS, LCIS</th>
<th><strong>Esophagus:</strong> Barrett’s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prostate:</strong> PIN</td>
<td><strong>Oral cavity:</strong> Oral leukoplakia</td>
</tr>
<tr>
<td><strong>Cervix:</strong> CIN</td>
<td><strong>Stomach:</strong> Dysplastic lesions</td>
</tr>
<tr>
<td><strong>Lung:</strong> Bronchial dysplasia</td>
<td><strong>Colon:</strong> Adenomatous polyps</td>
</tr>
</tbody>
</table>
Early Detection/ Prognostic Biomarkers: Altered Cell Structure

- Most common intermediate endpoint currently in use in chemoprevention trials
- Detection: histopathological, quantitative computer-assisted image – nuclear morphology
Criteria for Selecting Biomarkers

- **Practical**
  - Simple, inexpensive, high throughput

- **Analytical**
  - Reliability: repeatability
  - Precision: sharply measured
  - Accuracy: measures “true” level
  - Validity: measures “true” change

- **Biological**
  - Dynamism: modulated by eliminating exposure or by chemopreventive agents
  - Surrogacy: amount of effect of chemopreventive agent explained by modulation of biomarker by agent
Suspect Human Carcinogen/Disease Linkage

Identify & develop methodologies for measuring specific biomarkers

Determine relation of biomarker to exposure and disease in experimental animals

Cross-sectional study of biomarker levels in exposed humans

Longitudinal study of biomarkers in humans

Validated exposure marker

Modulation of biomarker and disease in animal chemoprevention studies

Case-control studies
Cohort studies
Clinical trials

Validated risk marker
Section C

Susceptibility Factors: Overview
Can individuals at high risk be identified?
The Toxicological Paradigm

**Toxicokinetics**
- Exposure
- Internal Dose
- Biologically Effective Dose

**Toxicodynamics**
- Early Biological Effects
- Altered Structure/Function
- Disease

**Susceptibility Factors**

**Exposure Assessment**  **Risk Assessment**
Predisposal to Chemical Toxicity

- Heterozygous recessive traits that may predispose individuals to chemical toxicity
  - Sickle cell trait
    - Anemia producers: Benzene, lead, cadmium
    - Methemoglobin formers: Aromatic amines and nitro compounds
    - Blood O$_2$ tension reducers: CO, CN
  - Serum $\alpha$-1 antitrypsin deficiency
    - Predisposition to pulmonary disease following exposure to irritants
  - Glucose 6-phosphate dehydrogenase deficiency
    - Predisposition to hemolytic anemia
Inverse Correlation

- Examples of the Inverse Correlation Between Allelic Frequency & Cancer Risk Associated with Inherited Cancer Susceptibility Genes

Adapted from Hussain and Harris, Cancer Research 58: 4023-4037, 1998

**Germline Mutations**

- High (10³- to 10⁴-fold)
  - Rb (Retinoblastoma)
  - p53 (Li-Fraumeni)
  - APC (Familial Polyposis Coli)
  - NF1 (Neurofibromatosis)
  - XPA-G (Xeroderma Pigmentosum)
  - ATM (Ataxia Telangiectasia)
  - BRCA1 (Breast-Ovarian Ca)
  - HMLH1 (Hereditary Nonpolyposis Colorectal Ca)
- Moderate
  - CYP1A1
  - CYP2E1
  - NAT
  - GSTM1

- Low (1-5 per 10⁵ live births)

- Frequency of At Risk Allele
  - (2-50 per 10² live births)

Adapted from Hussain and Harris, Cancer Research 58: 4023-4037, 1998
Susceptibility Factors

Assessing inter-individual variability in—

- **Biotransformation**: Phase I and Phase II enzymes
  - **Phenotypic**
    - Direct *in vitro* determination of enzyme content and activity in biopsy specimens
    - Assay substrate/metabolite levels and/or kinetics (t 1/2s) in blood, urine, feces, saliva, milk, breath
  - **Genotypic**
    - Genetic polymorphisms, RFLPs
- **DNA repair**: repair proficiency by plasmid-host cell reactivating assay
DNA Repair Defects Increase Sensitivity to Carcinogens

- **Xeroderma pigmentosum**
  - Failure to repair UV damage to DNA

- **Ataxia telangiectasiasis**
  - Failure in repair or replication of double-strand breaks in DNA
Assay for DNA Repair Capacity

[pCMVcat 6.45 kb]

Genotoxic damage → cat → DEAE dextran → T-Lymphocytes → Damage Removal

Excised fragment & Chloramphenicol Acetyl transferase (CAT)

3 H-Acetyl-CoA + Chloramphenicol → 3 H-Mono- and Di-Acetylated Chloramphenicol
DNA Repair Capacity in Peripheral Blood Lymphocytes

Adapted from: Grossman & Wei (1994)
Correlation Between DNA Repair Capacity & Mutagen Sensitivity

Relationship Between Age at First Basal Cell Carcinoma & DNA Repair Capacity

Risk of Arsenical Cancer and DNA Repair Capacity Stratified by Length of Time of Arsenic Exposure

Arsenic Exposure

- < 12.6 ppm x years
- > 12.6 ppm x years

Odds Ratio

HIGH REPAIR

LOW REPAIR

15.8
(5.3-46.9)

3.3
(1.1-9.9)
Susceptibility Factors

Assessing inter-individual variability in—

- **Biotransformation**: Phase I and Phase II enzymes
  - **Phenotypic**
    - Direct *in vitro* determination of enzyme content and activity in biopsy specimens
    - Assay substrate/metabolite levels and/or kinetics (t 1/2s) in blood, urine, feces, saliva, milk, breath
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    - Genetic polymorphisms, RFLPs
- **DNA repair**: repair proficiency by plasmid-host cell reactivating assay
### Examples of Procarcinogens Activated by Human Cytochrome P-450 Enzymes

<table>
<thead>
<tr>
<th>I A2</th>
<th>III A4</th>
<th>II E1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-AA</td>
<td>6-AC</td>
<td>$\text{N,N-DiethylNitrosamine}$</td>
</tr>
<tr>
<td>2-AAF</td>
<td>$\text{AFB}_1$</td>
<td>$\text{N,N-DimethylNitrosamine}$</td>
</tr>
<tr>
<td>4-ABP</td>
<td>$\text{AFG}_1$</td>
<td>$\text{N-Butyl- N-methylNitrosamine}$</td>
</tr>
<tr>
<td>2-AF</td>
<td>BP-7, 8-idiol</td>
<td>$\text{N-Benzyl- N-methylNitrosamine}$</td>
</tr>
<tr>
<td>Glu-P-1</td>
<td>BFA-9, 10-idiol</td>
<td></td>
</tr>
<tr>
<td>Glu-P-2</td>
<td>Sterigmatocystin</td>
<td></td>
</tr>
<tr>
<td>IQ</td>
<td>Tris-(2,3-dibromopropyl) phosphate</td>
<td>$\text{Benzene}$</td>
</tr>
<tr>
<td>MelQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MelQx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp-P-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Heterocyclic Amines

- Produced by cooking meats
- Target breast, colon and prostate

![Chemical structure](image)

DNA adducts: \( \text{N}^2-(2'-\text{deoxyguanosin}-8-\text{yl})-\text{PhIP} \)
Acetylation & CYP1A2 Phenotypes in Controls & Cases with Colorectal Cancer or Polyps

% Rapid Phenotype

- Controls
- Cases

Acetylation

CYP1A2
Acetylation and CYP1A2 Phenotypes in Controls and Cases with Colorectal Cancer or Polyps

% of Population

- Colon & Polyp
  - Rapid/Rapid
  - Slow/Rapid
- Colon
  - Slow/Rapid
  - Slow/Slow
- Polyp
  - Rapid/Slow
  - Slow/Slow
- Controls
  - Rapid/Rapid
  - Slow/Rapid
  - Slow/Slow
Usual Preparation of Red Meat Among 50 Colorectal Patients and 96 Surgical Controls in the Washington, D.C. Area

<table>
<thead>
<tr>
<th>Degree of Doneness</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95%) CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rare to medium rare</td>
<td>12 (24%)</td>
<td>33 (34%)</td>
<td>1.0 (ref.)</td>
</tr>
<tr>
<td>Medium to medium well</td>
<td>15 (30%)</td>
<td>44 (46%)</td>
<td>0.9 (0.4–2.5)</td>
</tr>
<tr>
<td>Well done</td>
<td>23 (46%)</td>
<td>18 (20%)</td>
<td>3.5 (1.3–9.6)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>50 (100%)</strong></td>
<td><strong>95 (100%)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Interactions Between Phenotype and Dietary Exposure

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Phenotypes (NAT-2 and CYP1A2)</th>
<th>Meat Cooking Preference</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow-slow</td>
<td>Rare/medium</td>
<td>Slow-slow</td>
<td>1.00</td>
</tr>
<tr>
<td>Rapid-slow</td>
<td>Rare/medium</td>
<td>Slow-rapid</td>
<td>1.39</td>
</tr>
<tr>
<td>Slow-rapid</td>
<td>Rare/medium</td>
<td>Rapid-rapid</td>
<td>3.13</td>
</tr>
<tr>
<td>Rapid-rapid</td>
<td>Rare/medium</td>
<td>Well done</td>
<td>2.06</td>
</tr>
<tr>
<td>Slow-slow</td>
<td>Rare/medium</td>
<td>Rapid-slow</td>
<td>1.87</td>
</tr>
<tr>
<td>Slow-rapid</td>
<td>Rare/medium</td>
<td>Slow-rapid</td>
<td>2.86</td>
</tr>
<tr>
<td>Rapid-rapid</td>
<td>Rare/medium</td>
<td>Rapid-rapid</td>
<td>6.45</td>
</tr>
</tbody>
</table>

Determinants of Carcinogen-DNA Adduct Levels

<table>
<thead>
<tr>
<th>Carcinogen Exposure</th>
<th>Activation Deactivation</th>
<th>DNA Repair</th>
<th>Adduct Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Low</td>
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<td>Low</td>
<td>Low</td>
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<tr>
<td></td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
</tbody>
</table>

High

Low
Key Points

Uses Of Biomarkers in Public Health Research

- Improved accuracy of exposure measurement
- Identification of subclinical disease
- More homogeneous classification of disease
- Identification of susceptible individuals in presence of adverse exposures
Key Points
Uses Of Biomarkers in Public Health Research

- Improvement in methodology for preventive and therapeutic trials
- Increased knowledge of disease pathogenesis