Biomarkers in Human Carcinogenesis

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University of Georgia
### US Mortality, 2019 (Age-adjusted rates)*

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cause of death</th>
<th>No. of deaths</th>
<th>% of total deaths</th>
<th>Rate of mortality (per 10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Heart Diseases</td>
<td>659,041</td>
<td>23.1</td>
<td>161.5</td>
</tr>
<tr>
<td>2</td>
<td>Cancer</td>
<td>599,601</td>
<td>21.0</td>
<td>146.2</td>
</tr>
<tr>
<td>3</td>
<td>Accidents (unintentional injuries)</td>
<td>173,040</td>
<td>6.1</td>
<td>49.3</td>
</tr>
<tr>
<td>4</td>
<td>Chronic lower respiratory diseases</td>
<td>156,979</td>
<td>5.5</td>
<td>38.2</td>
</tr>
<tr>
<td>5</td>
<td>Cerebrovascular diseases</td>
<td>150,005</td>
<td>5.3</td>
<td>37.0</td>
</tr>
<tr>
<td>6</td>
<td>Alzheimer disease</td>
<td>121,499</td>
<td>4.3</td>
<td>29.8</td>
</tr>
<tr>
<td>7</td>
<td>Diabetes mellitus</td>
<td>87,647</td>
<td>3.1</td>
<td>21.6</td>
</tr>
<tr>
<td>8</td>
<td>Nephritis (nephrotic syndrome)</td>
<td>51,565</td>
<td>1.8</td>
<td>12.7</td>
</tr>
<tr>
<td>9</td>
<td>Influenza and pneumonia</td>
<td>49,783</td>
<td>1.7</td>
<td>12.3</td>
</tr>
<tr>
<td>10</td>
<td>Intentional self-harm (Suicide)</td>
<td>47,511</td>
<td>1.7</td>
<td>13.9</td>
</tr>
<tr>
<td></td>
<td>All other causes</td>
<td>758,167</td>
<td>26.6</td>
<td></td>
</tr>
</tbody>
</table>

* Source, USCDC, 2019.
US Cancer Facts

• More than 16.9 million Americans were alive with a history of cancer on January 1, 2019;

• About 1,898,160 new cancer cases are expected to be diagnosed in 2021;

• About 608,570 Americans are expected to die of cancer in 2021, about 1,670 deaths per day;

• At least 42%, about 750,000 new cases, are potentially avoidable (19% due to smoking and 18% due to a combination of excess body weight, physical inactivity, excess alcohol consumptions, and poor nutrition).
Causes of Cancer

A

Environment 90-95%

Genes 5-10%

B

Genes

Breast 2.1
Melanoma 1.9
Prostate 2.0
Kidney 2.9
Colorectal 2.5
Lung 2.6
Multiple myeloma 4.3
Laryngeal 5.0
Thyroid 8.5
Testicular 8.6

C

Environment

Others 10-19%
Alcohol 4-6%
Obesity 10-20%
Infections 15-20%
Diet 30-35%
Tobacco 25-30%

www.thelancet.com/oncology Published online May 9, 2012 DOI:10.1016/S1470-2045(12)70137-7
Worldwide Cancer Causes

Developed Countries

- Diet or nutrition: 30%
- Infections: 8%
- Tobacco: 16%
- Other: 39%
- Occupational exposures: 5%
- Environmental pollution: 2%
Worldwide Cancer Causes

Developing Countries

- Infections 26%
- Diet or nutrition 20%
- Tobacco 10%
- Other 44%
<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Estimated % of total cancer deaths attributable this factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco</td>
<td>30</td>
</tr>
<tr>
<td>Adult diet/obesity</td>
<td>30</td>
</tr>
<tr>
<td>Sedentary lifestyle</td>
<td>5</td>
</tr>
<tr>
<td>Occupational factors</td>
<td>5</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td>5</td>
</tr>
<tr>
<td>Virus/other biologic agents</td>
<td>5</td>
</tr>
<tr>
<td>Perinatal factors/growth</td>
<td>5</td>
</tr>
<tr>
<td>Reproductive factors</td>
<td>3</td>
</tr>
<tr>
<td>Alcohol</td>
<td>3</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>3</td>
</tr>
<tr>
<td>Environmental pollution</td>
<td>2</td>
</tr>
<tr>
<td>Ionizing/ultraviolet radiation</td>
<td>2</td>
</tr>
<tr>
<td>Prescription drugs/medical procedures</td>
<td>1</td>
</tr>
<tr>
<td>Salt/other food additives and contaminants</td>
<td>1</td>
</tr>
</tbody>
</table>
The Biomarker Paradigm

EXPOSURE → INTERNAL DOSE → BIOLOGICALLY EFFECTIVE DOSE → EARLY BIOLOGICAL EFFECT → ALTED STRUCTURE/FUNCTION → DISEASE

SUSCEPTIBILITY FACTORS

NRC, 1987
Concepts

• A continuum of biological events, multiple stages, multiple factors;

• Indicators signaling these events or stages;

• Relationship between exposure and impairment;

• Modified by host-susceptibility factors at every stage.
Biomarkers

• Molecular, biochemical or cellular alterations that is measurable in biological media, such as tissues, cells, or fluids.

• Three types of biomarkers were proposed in 1987: biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility.
Biomarkers of Exposure

• Indicate the presence and magnitude of the previous exposure to the chemical compounds;

• Measure chemical and its metabolite or its interaction with a biological molecule;

• Vary consistently and quantitatively with the extent of exposure;

• Specific for the environmental exposure of concern;

• Markers of internal dose usually are good biomarkers of exposure.
Biomarkers of Effect

• Indicate the presence and magnitude of biological response to exposure to the chemical compounds;

• A measurable biochemical, physiological, behavioral or other alteration within an organism that is associated with an established or possible health impairment or disease;

• Can be at levels of whole organism, organ function, tissue, individual cells and subcellular components.
Biomarkers of Susceptibility

• An indicator or a measure of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical compound.

• Concerned with factors in kinetics and dynamics of uptake and metabolism of exogenous chemicals.

• The concept encompasses enzymes of activation and detoxification, repair enzymes, and changes in target molecules for toxic chemicals.
TOXICOLOGICAL RESEARCH FIELDS

INTERNAL
BIOLOGICALLY EFFECTIVE DOSE
EARLY BIOLOGICAL EFFECT
ALTERED STRUCTURE/FUNCTION
DISEASE

TOXICOLOGICAL RESEARCH FIELDS

SUSCEPTIBILITY FACTORS

EXPOSURE
INTERNAL DOSE
BIOLOGICALLY EFFECTIVE DOSE
EARLY BIOLOGICAL EFFECT
ALTERED STRUCTURE/FUNCTION
DISEASE

Interventions/Prevention Strategies

Exposure biomarkers
Effect biomarkers

Toxicology/Biomarkers/Prevention

Exposure biomarkers
Effect biomarkers
Process of Carcinogenesis

Harris, 1995
Application of Biomarkers in Carcinogenesis

• To assess human carcinogen exposure for cancer etiology studies, via measurement of biomarkers of exposure, e.g., the internal dose of specific carcinogen;

• To assess carcinogenic effects at the early stage (initiation & promotion) with biomarkers of early effects;

• To assess carcinogenic effects at the middle and late stage (progression & metastasis) with biomarkers of later effects;

• To assess susceptibility in carcinogenic process with biomarkers of susceptibility;

• To assess efficacies of cancer prevention strategies with all three types of biomarkers.
Biomarkers of Early Effect

• Genotoxic Markers
  • DNA damage
  • Adducts: DNA adducts and Protein adducts
  • Oxidative damage

• Gene Mutation Markers
  • Somatic gene mutation in surrogate tissues
    • HPRT mutation
    • Glycophorin A
  • Gene mutation in target tissue
    • Oncogenes (Ras, Myc, etc.)
    • Tumor suppressor genes (Rb, APC, p53, etc.)
Biomarkers of Oxidative Damage

- 8-oxo-7,8-dihydroguanine (8-oxoGua)
- 8-oxo-7,8-dihydro-2’-deoxguanosine (8-oxodG) or 8-hydroxy-2’-deoxyguanosine (8-OHdG)
- Malondialdehyde (MDA)
- Nitrotyrosine
- F2-isoprostanes
Biomarkers of Mid-Effect

• Gene Regulation and Expression Markers
  • mRNA expression
  • micro RNAs and non-coding RNAs (Epigenetic markers)
  • Protein expression

• Cytogenetic Markers
  • Chromosomal aberrations
  • Sister chromatid exchanges
  • Micronuclei

• Enzymatic or Protein Markers
  • Organ-specific enzymes
  • Tumor markers (PSA)
Enzymatic Biomarkers

- Liver functions:
  - Alanine aminotransferase (ALT or SGPT)
    - Adults < 40 IU/L
  - Gamma Glutamyltransferase (GGT)
    - < 30 IU/L
  - Aspartate aminotransferase (AST, SGOT)
    - < 20 IU/L

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AST</th>
<th>ALT</th>
<th>Creatine Kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>7800</td>
<td>450</td>
<td>473</td>
</tr>
<tr>
<td>Liver</td>
<td>7100</td>
<td>2850</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5000</td>
<td>300</td>
<td>2500</td>
</tr>
<tr>
<td>Kidney</td>
<td>4500</td>
<td>1200</td>
<td>32</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1400</td>
<td>130</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Spleen</td>
<td>700</td>
<td>80</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Lungs</td>
<td>500</td>
<td>45</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Biomarkers of Late-effect

• Structural and functional changes
  • Symptoms or Signs

• Early disease status
  • Polyps, dysplasia, etc.

• Tumor markers

• Advanced disease status
  • Metastasis markers
Tumor Markers

• A tumor marker is a substance found in an increased amount in the blood, other body fluids, or tissues that may suggest the presence of a type of cancer.

• Tumor markers are the biochemical or immunological counterparts of the differentiation state of the tumor, which represent the re-expression of substances produced normally by embryogenically related tissue.

• Tumor markers in dynamics may represent the tumor progression and can serve for the clinical diagnosis.
Tumor markers (examples)

• Alpha-fetoprotein (AFP, found in 1963)
  • A 70 kD glycoprotein, 4% carbohydrates
  • < 15 ng/mL

• Carcinoembryonic antigen (CEA, found in 1965)
  • A family of glycoproteins, 50% carbohydrates
  • < 5.0 ng/mL

• Prostate specific antigen (PSA, found in 1980)
  • A 28.43 kD glycoprotein, 7% carbohydrates
  • < 4.0 ng/mL
Tumor markers (examples)

- Carbohydrate-related tumor markers
  - Antigens in tumor surface
  - CA 15-3 for Breast/ovarian cancers
  - CA 125 for Ovarian/endometrial cancers
  - CA 549 for breast/ovarian cancers
  - CA 19-5 for GI and pancreatic cancers
  - CA 19-9 for pancreatic and GI cancers
Biomarkers of Susceptibility (Examples)

• Phenotypic expression markers
  • Enzyme activities
  • Phase 1 and 2 metabolites
  • Protein (enzymes) expressions

• Genetic polymorphisms markers
  • Single nucleotide changes
  • Homozygous vs. heterozygous
  • Deletions
  • Insertions
Genes and Genetic Variations

- Human genome
  - 3 billion base pairs
  - 30,000 genes
  - 100,000 proteins
  - Variations in expression of proteins

- Single nucleotide polymorphisms (SNPs)
  - 10 million SNPs in the genome or 1/300 base pairs
  - Minimal effect in creating susceptibility to disease
  - Many genetic variants to have relatively weak contributions to chronic diseases, such as cancer, diabetes
  - Interactions with environment and non-genetic factors play important role in susceptibility.
Polymorphisms of CYP450s

- **CYP1A1:**
  - Mspl and Ile (AUU/AUC/AUA)-Val (GUU/GUC/GUA) variants (A-G),
  - Mspl/Mspl or Val/Val genotype have a high risk of possessing a mutant p53,
  - associated with lung and breast cancers.

- **CYP1B1:**
  - Leu (CUU/CUC/CUA)-Val in codon 432,
  - associated with breast and prostate cancers.

- **CYP1A2:**
  - No genotypic polymorphism has been found;
  - Phenotypic variations;
  - Associated with colorectal and bladder cancers.
  - Urinary caffeine metabolites are good biomarkers for evaluation of polymorphic phenotypes of CYP1A2 and NAT2.
Polymorphisms of CYP450s

- **CYP2D6:**
  - Also called debrisoquine hydrolase;
  - Poor metabolizers (PMs), lower risks for lung and bladder cancer;
  - Extensive metabolizers (EMs), high risks?

- **CYP2E1:**
  - Three types of genetic polymorphism;
  - DraI, Rsall, and PstI;
  - C1/C1, C1/C2, and C2/C2.
Polymorphisms of CYP450s

• CYP2C9:
  • Polymorphic was found in metabolizing tolbutamide, an antidiabetic drug;
  • Three polymorphic types were identified, called *1, *2, and *3.

• CYP2C19
  • Polymorphic was found in metabolizing mephenytoin, an anticonvulsant drug;
  • Three polymorphic types were also identified, called m1, m2, and m3.

• PMs and EMs were found for both CYP450s;
• Only 2-5% Caucasians for PMs, however, 13-23% Orientals for PMs.
## Ethnic variations of genetic polymorphisms of metabolic enzymes

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Caucasian (%)</th>
<th>Asian (%)</th>
<th>African-American (%)</th>
</tr>
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<tbody>
<tr>
<td>CYP1A1-MspI</td>
<td>7-14</td>
<td>33</td>
<td>23</td>
</tr>
<tr>
<td>CYP1A1-Ile-Val</td>
<td>3</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>CYP1A1-MspI (intron 7)</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>9</td>
</tr>
<tr>
<td>CYP2D6*</td>
<td>7-10</td>
<td>1</td>
<td>NA</td>
</tr>
<tr>
<td>CYP2E1-Dral</td>
<td>9</td>
<td>31</td>
<td>9</td>
</tr>
<tr>
<td>CYP2E1-Pstl</td>
<td>2</td>
<td>24</td>
<td>5</td>
</tr>
<tr>
<td>CYP2E1-Rsal</td>
<td>2</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>mEH-113 H/H</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>mEH-139R/R</td>
<td>5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>GSTM1**</td>
<td>43-52</td>
<td>48-60</td>
<td>22-35</td>
</tr>
<tr>
<td>GSTT1**</td>
<td>15-20</td>
<td>60</td>
<td>22</td>
</tr>
<tr>
<td>NAT2***</td>
<td>40-70</td>
<td>32-60</td>
<td>68</td>
</tr>
<tr>
<td>Paraoxonase</td>
<td>50</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>

* Poor metabolizer alleles; **null (deleted) alleles; ***slow acetylator alleles.
Variations in DNA Repair

- DNA repair systems are responsible for maintaining the integrity of the genome by minimizing replication errors, removing DNA damage, and minimizing deleterious rearrangements arising via aberrant recombination;

- There are many studies focused on identification of single nucleotide polymorphisms (SNPs) in DNA repair genes;

- A great deal of substitution variants have been found in genes in DNA repair pathways, which lead to variations in critical DNA repair enzymes.
Measurement of Global Repair Capacity

• Endogenous cellular repair capacities are more than adequate to compensate for background DNA damage (global repair capacity);

• Massive or frequent exposure to exogenous genotoxic agents may saturate the DNA repair capacity leading to human genetic disease;

• Global repair capacity assay can test this balance.
Major Polymorphic DNA Repair Genes

- ERCC
- hOGG
- RAD51
- XPC
- XPD
- XRCC
- BRAC1 and BRAC2
# Genetic variations in DNA repair pathways

<table>
<thead>
<tr>
<th>Pathway</th>
<th>No. of genes in pathway*</th>
<th>No. of genes Screened</th>
<th>No. of variants identified</th>
<th>No. of alleles &gt;2% frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base excision repair</td>
<td>25</td>
<td>19</td>
<td>68</td>
<td>15</td>
</tr>
<tr>
<td>Nucleotide excision repair</td>
<td>33</td>
<td>16</td>
<td>51</td>
<td>17</td>
</tr>
<tr>
<td>Double strand break repair</td>
<td>~30</td>
<td>11</td>
<td>31</td>
<td>8</td>
</tr>
<tr>
<td>Mismatch repair</td>
<td>7</td>
<td>6</td>
<td>41</td>
<td>13</td>
</tr>
<tr>
<td>Damage recognition &amp; cell cycle checkpoint</td>
<td>~30</td>
<td>6</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Total**</td>
<td>&gt;110</td>
<td>51</td>
<td>177</td>
<td>50</td>
</tr>
</tbody>
</table>

*Genes with roles in more than pathways are counted in all relevant pathways. **The total is unique genes and variants, not the sum of the column.
Summary

- Biomarker concept, once proposed, has been widely accepted by toxicological and many biomedical research fields;
- Biomarkers can be divided into biomarkers of exposure, effects, and susceptibility, and can be classified as genotypic and phenotypic biomarkers based on the current measurement tools;
- Biomarkers in carcinogenesis can be classified as early-, middle- and late-effect markers based on the specific events or stages in carcinogenic process;
- Biomarkers have been applied to assess human carcinogen exposure for cancer etiology studies; to assess carcinogenic effects at the early stages (initiation & promotion) and the middle and late stages (progression & metastasis);
- Biomarkers have been widely used to assess efficacies of cancer prevention strategies, and biomarkers of susceptibility were extensively used to study variations in cancer rates and individual/ethnic cancer risks.