Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis

Martyn Smith
Director, Superfund Research Program
martynts@berkeley.edu

School of Public Health
University of California, Berkeley
http://superfund.berkeley.edu
Mechanistic data - Problems to address

• There is no broadly accepted, systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision-making in cancer hazard identification

• Many human carcinogens act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis – How to capture these diverse effects that lead to cancer and other adverse outcomes for all types of agents?
Human Tumors and Stages of Carcinogenesis

- CHEMICAL: Aflatoxin B1, Ethanol/Smoking, Vinyl Chloride
- Enzyme activation
- Conjugation Enzymes
- Deactivation/Excretion
- RADIATION: Thorotrast
- VIRUS: HBV, HCV

Genetic and Epigenetic Changes → Selective Clonal Expansion

Defects in Terminal Differentiation
Defects in Growth Control
Resistance to Cytotoxicity
Defects in Programmed Cell Death

Telomerase Activation

Genetic Predispositions: e.g., Hemochromatosis, Wilson's Disease, α1-Antitrypsin, Metabolic Genotypes

NORMAL CELL → INITIATED CELL → PRE-NEOPLASTIC LESION → MALIGNANT TUMOR → CLINICAL LIVER CANCER

Necroinflammatory Liver Disease/Cirrhosis, Reactive Oxygen/Nitric Oxide Species

- Activation of Proto-Oncogenes (e.g., N-ras, c-myc, c-fos)/Growth Factors (e.g., IGF-I, IGF-II, TGF-α, TGF-β)
- Inactivation of Tumor Suppressor Genes (e.g., p53, p16, Rb, LOH 1p, 1q, 2q, 4q, 5q, 6q, 8p, 8q, 9p, 9q, 10q, 11p, 13q, 16p, 16q, 17p, 22/APC for hepatoblastoma)

20-60 years

Hussain et al., Oncogene, 2007
IARC Monographs Volume 100: The known causes of human cancer by organ site

Two meetings held at IARC in 2012 on concordance and mechanisms

Section of the IARC Monographs (IMO)
HALLMARKS OF CANCER

1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death
4. Enabling replicative immortality
5. Inducing aberrant angiogenesis
6. Activating invasion & metastasis

Emerging Hallmarks
- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics
- Genomic instability and mutation
- Inflammation
Chemicals and other stressors act at different points on the disease continuum.

“Considering the multistep nature of cancer and the acquired capabilities implied by each of these hallmarks, it is therefore a very small step to envision how a series of complementary exposures acting in concert might prove to be far more carcinogenic than predictions related to any single exposure might suggest. Interacting contributors need not act simultaneously or continuously, they might act sequentially…”


MT Smith, UCB Dec2015
<table>
<thead>
<tr>
<th>Review team</th>
<th>Chemical name</th>
<th>Disruptive action on key mechanism/pathway</th>
<th>Low-dose effect (LDE, LLDE, NLDE, threshold, unknown)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiogenesis</td>
<td>Diniconazole</td>
<td>Vascular cell adhesion molecule and cytokine signaling</td>
<td>Threshold (H-PC) (36 = TOXCAST)</td>
</tr>
<tr>
<td>Chlorothalonil</td>
<td></td>
<td>Thrombomodulin, vascular proliferation and cytokine signaling</td>
<td>Unknown (H-PC) (36), NLDE (A-in vivo) (38 in Amphibians)</td>
</tr>
<tr>
<td>Immune system evasion</td>
<td>Pyridaben</td>
<td>Chemokine signaling, TGF-β, FAK, HIF-1a, IL-1a pathways</td>
<td>Unknown (H-CL, H-PC, A-CL) (36,139,140), threshold (A-I) (141)</td>
</tr>
<tr>
<td>Triclosan</td>
<td></td>
<td>Chemokine signaling, TGF-β, FAK, IL-1a pathways</td>
<td>Threshold (H-CL, H-PC, A-I) (36,142–144), LDE (A-I, H-CL) (145,146) None of these papers (142-146) show immune evasion</td>
</tr>
</tbody>
</table>

Examples of endpoints used to support conclusions of Goodson et al. --
Problem is that assay endpoints don’t match hallmarks

MT Smith, UCB Dec 2015
Dilemma: Cancer or Carcinogens

• Hallmarks are the biological characteristics of cancer cells and tumors in general, NOT the characteristic properties of human carcinogens
• Need to identify the key characteristics of human carcinogens
• IARC Working Group did this in 2012 and subsequently scientists at EPA, IARC and elsewhere determined how these characteristics could be searched for systematically
## Multiple Mechanisms of IARC Group 1 Carcinogens
[KZ Guyton….MT Smith, Mut Res 681; 230, 2009]

<table>
<thead>
<tr>
<th>Mechanisms</th>
<th>AFB1</th>
<th>As+3</th>
<th>Asbestos</th>
<th>Benzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA damage</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Gene mutation</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chrom mutation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aneuploididy</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Epigenetic</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Receptor signaling</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Other signaling</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Immune effects</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inflammation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cytotoxicity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mitogenic</td>
<td>-</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Gap junction</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>
Key Characteristics of Human Carcinogens

<table>
<thead>
<tr>
<th>Key characteristic:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is Electrophilic or can be metabolically activated</td>
</tr>
<tr>
<td>2. Is Genotoxic</td>
</tr>
<tr>
<td>3. Alters DNA repair or causes genomic instability</td>
</tr>
<tr>
<td>4. Induces Epigenetic Alterations</td>
</tr>
<tr>
<td>5. Induces Oxidative Stress</td>
</tr>
<tr>
<td>6. Induces chronic inflammation</td>
</tr>
<tr>
<td>7. Is Immunosuppressive</td>
</tr>
<tr>
<td>8. Modulates receptor-mediated effects</td>
</tr>
<tr>
<td>9. Causes Immortalization</td>
</tr>
<tr>
<td>10. Alters cell proliferation, cell death, or nutrient supply</td>
</tr>
</tbody>
</table>

Evidence that these characteristics are observed, especially in humans or as intermediate biomarkers in human specimens can provide biological plausibility for epidemiological findings and/or early warning if no epidemiology exists.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Examples of relevant evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Is Electrophilic or Can Be Metabolically Activated</td>
<td>Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.</td>
</tr>
<tr>
<td>2. Is Genotoxic</td>
<td>DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).</td>
</tr>
<tr>
<td>3. Alters DNA repair or causes genomic instability</td>
<td>Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)</td>
</tr>
<tr>
<td>4. Induces Epigenetic Alterations</td>
<td>DNA methylation, histone modification, microRNA expression</td>
</tr>
<tr>
<td>5. Induces Oxidative Stress</td>
<td>Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Examples of relevant evidence</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6. Induces chronic inflammation</td>
<td>Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production</td>
</tr>
<tr>
<td>7. Is Immunosuppressive</td>
<td>Decreased immunosurveillance, immune system dysfunction</td>
</tr>
<tr>
<td>8. Modulates receptor-mediated effects</td>
<td>Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of exogenous ligands (including hormones)</td>
</tr>
<tr>
<td>9. Causes Immortalization</td>
<td>Inhibition of senescence, cell transformation, altered telomeres</td>
</tr>
<tr>
<td>10. Alters cell proliferation, cell death or nutrient supply</td>
<td>Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis</td>
</tr>
</tbody>
</table>
Benzene Mechanistic Data Search
conducted using the Health Assessment Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/)
Benzene Example: An Adverse Outcome Network Involving 8 Key Characteristics

Benzene Exposure

- ROS Oxidative DNA Damage
  - Oxidative Stress
  - Topo II Inhibition
    - Inhibition of DNA Repair Pathways
    - Metabolites induce genomic instability
  - Altered DNA Repair

- Electrophilic epoxides, aldehydes and quinones
  - Metabolic Activation
  - DNA Damage Mutations
    - Chromosome aberrations
  - Genotoxicity

- AhR Dysregulation
  - Modulation of receptor
  - Altered DNA methylation, miRNA changes, Histone modifications
    - Epigenetic alterations

- Reduced Immune Surveillance
  - Immunosuppression
  - Altered Cell Proliferation

- Stem Cell Transformation
  - Proliferation
  - Clonal Expansion

- Leukemia

- Altered Cell Proliferation

- Stem Cell Transformation
  - Proliferation
  - Clonal Expansion

- Leukemia
Number of IARC Group-1 Agents Demonstrating Multiple Key Characteristics

Implications of ‘key characteristics’

• Lays the groundwork for a structured evaluation of the strength of the mechanistic evidence base, and therefore its utility in supporting hazard classifications.
• Shows carcinogens tend to act through multiple mechanisms – separation into genotoxic and non-genotoxic actions of little value
• Allows development of credible Adverse Outcome Networks based on systematic review
• Could be developed for specific cancers and other adverse outcomes
• HT assays need to be developed based on characteristics and hallmarks. Current ones flawed.
An Agency-Academia Collaboration

- **IARC**: Kathryn Z. Guyton, Robert Baan and Kurt Straif
- **US EPA**: Catherine F. Gibbons, Jason M. Fritz, David M. DeMarini, Jane C. Caldwell, Robert Kavlock, Vincent Cogliano
- **NTP**: John R. Bucher
- **Academia**: Ivan Rusyn, Paul Lambert, Stephen S. Hecht, Bernard W. Stewart
- **Thun**: Christopher Portier
- **Other members** of the IARC WG: Lawrence Banks; Frederick A. Beland, James A. Bond; Maarten C. Bosland; Bice Fubini; Bernard D. Goldstein; Kari Hemminki; Mark A. Hill; Charles Jameson; Agnes B. Kane; Daniel Krewski; Ronald Melnick; Jerry M. Rice; Leslie Stayner; Robert L. Ullrich; Harri Vainio; Paolo Vineis; Michael P. Waalkes; and, Lauren Zeise.
- **MTS** was supported by NIEHS SRP grant P42ES004705.