



Genes &
Environment
Laboratory

The Key Characteristics of Carcinogens

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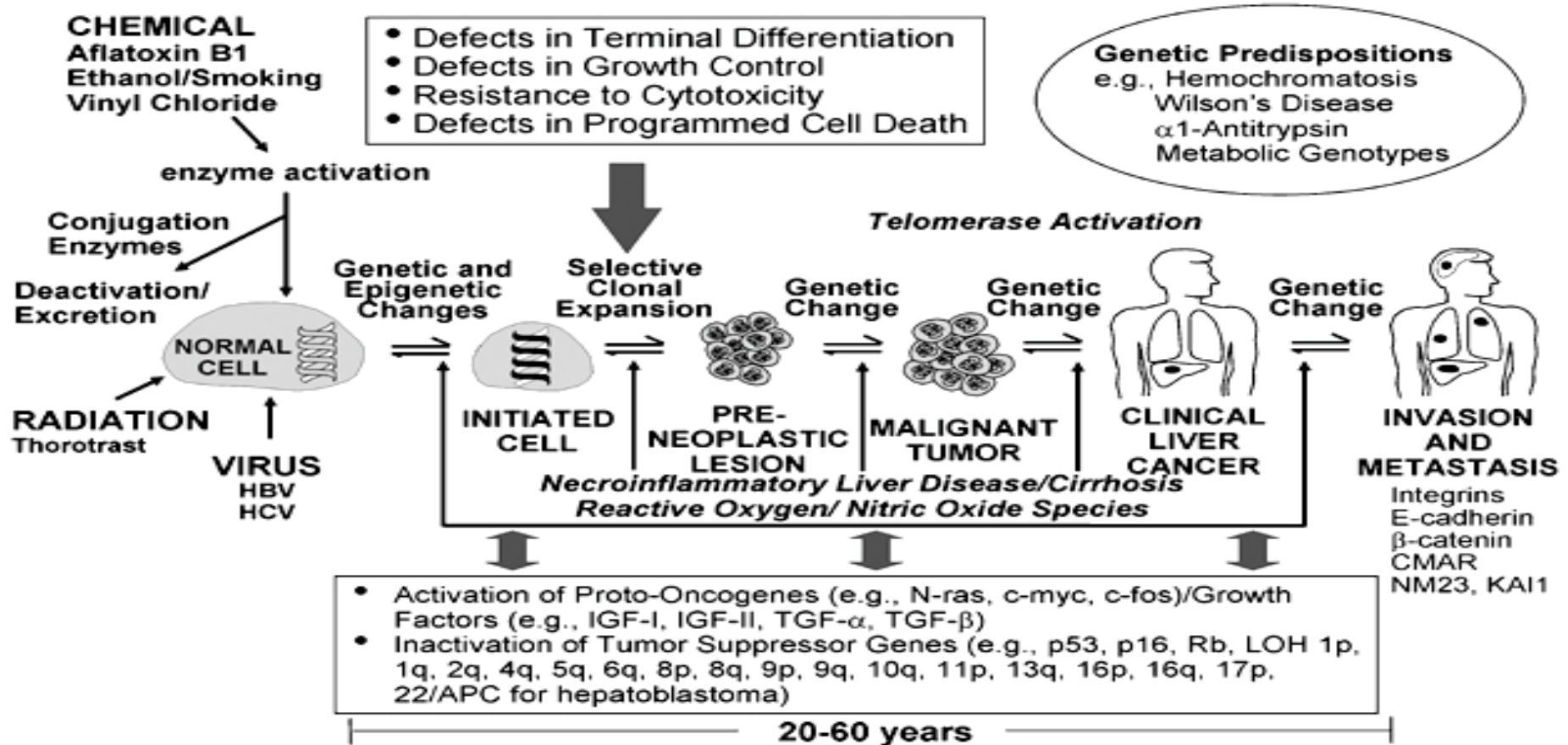
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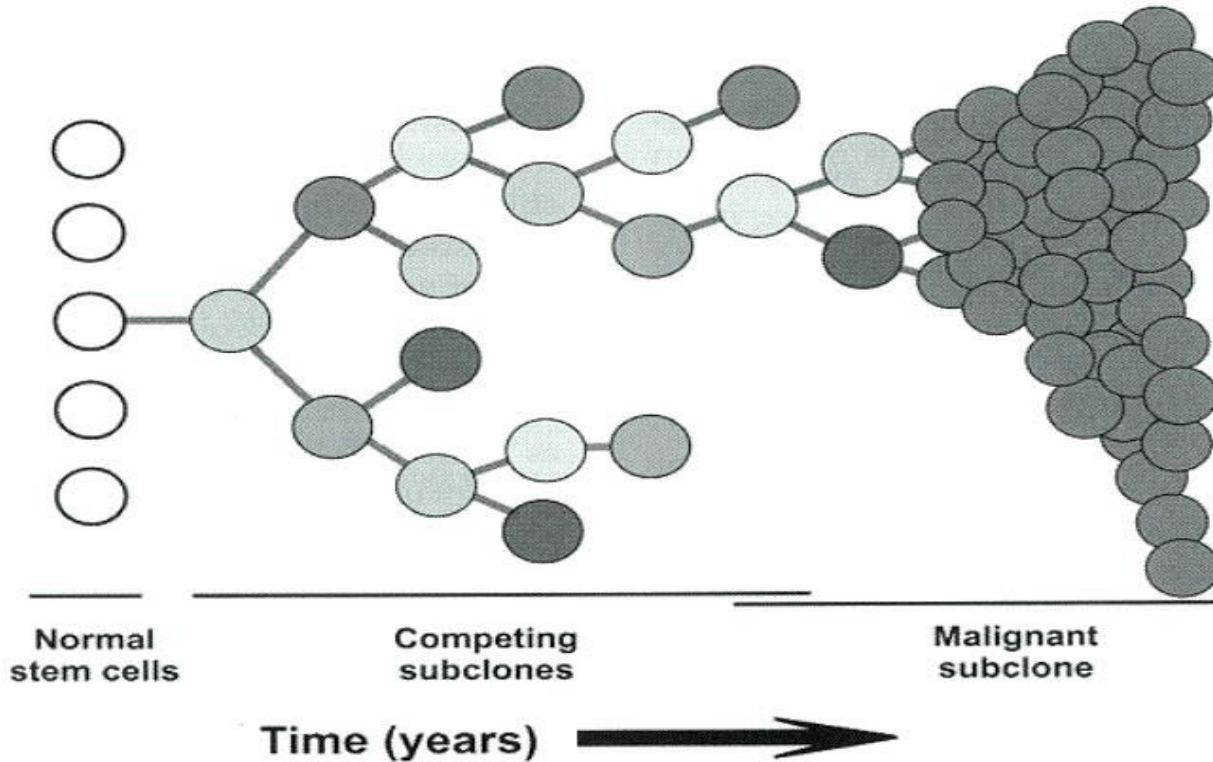
Mechanistic data - Problems to address

- 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?

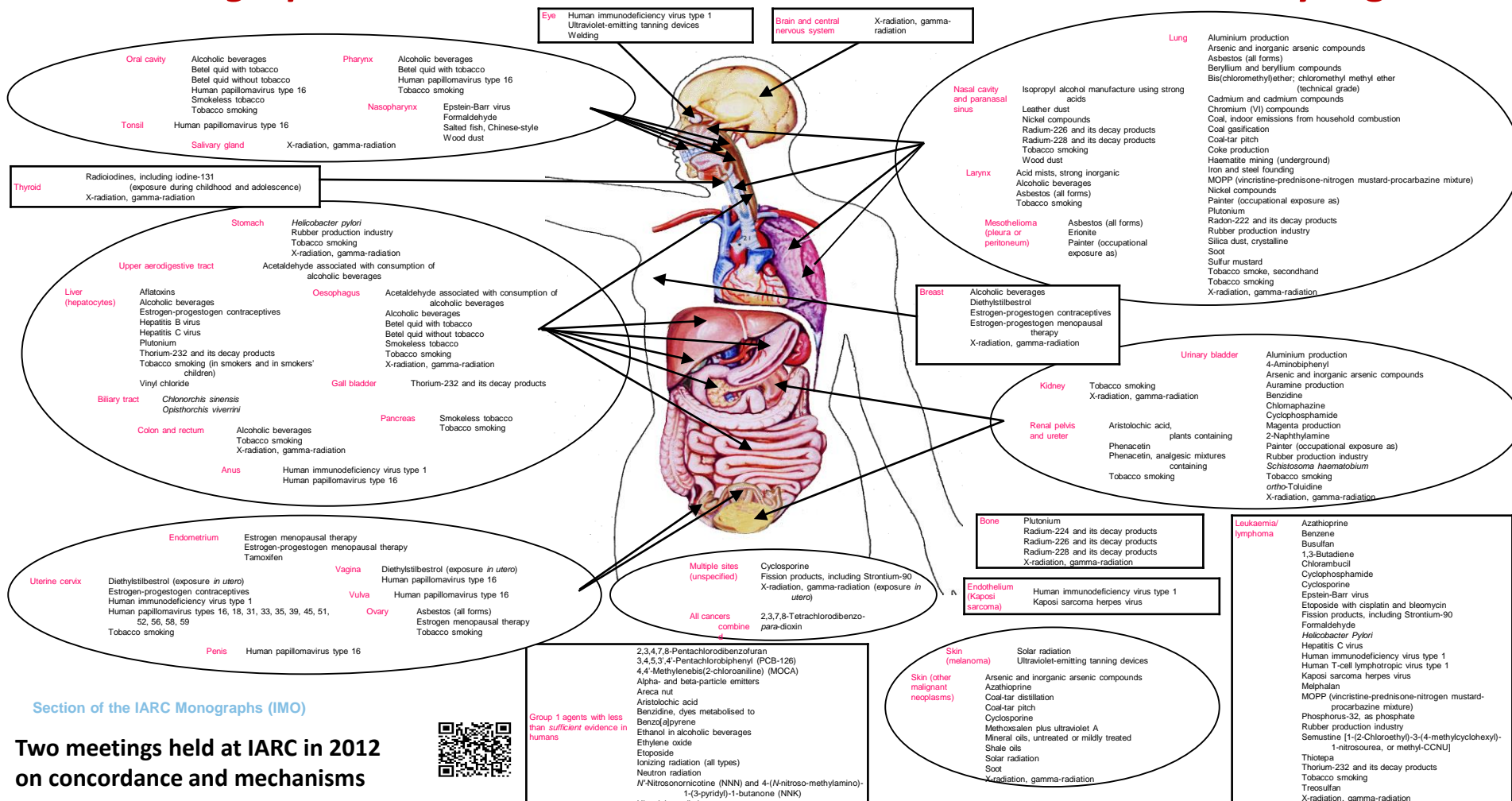
Human Tumors and Stages of Carcinogenesis



Clonal Selection Model of Neoplastic Progression



IARC Monographs Volume 100: The known causes of human cancer by organ site

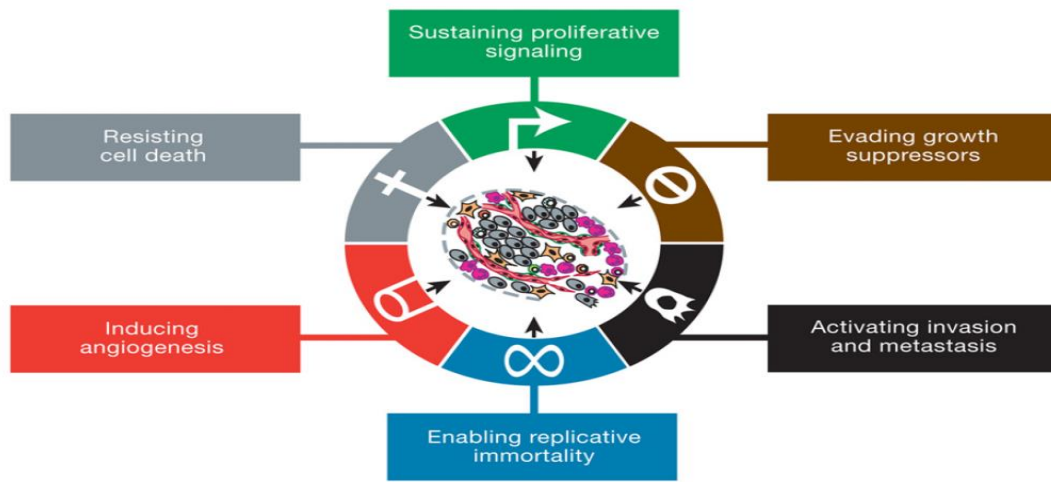


Section of the IARC Monographs (IIMO)



Group 1 agents with less than sufficient evidence in humans

Two meetings held at IARC in 2012 on concordance and mechanisms



HALLMARKS OF CANCER

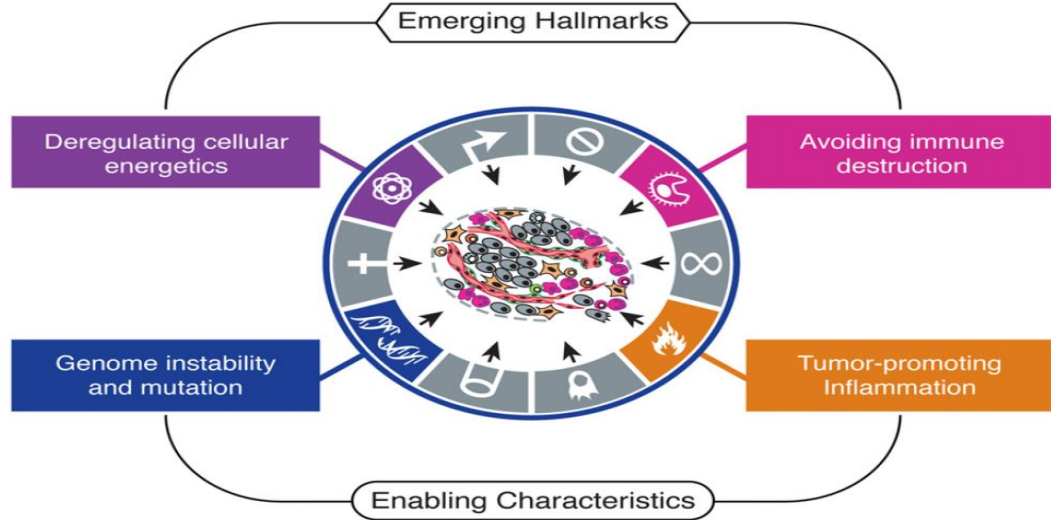
1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death
4. Enabling replicative immortality
5. Inducing aberrant angiogenesis
6. Activating invasion & metastasis

Emerging Hallmarks

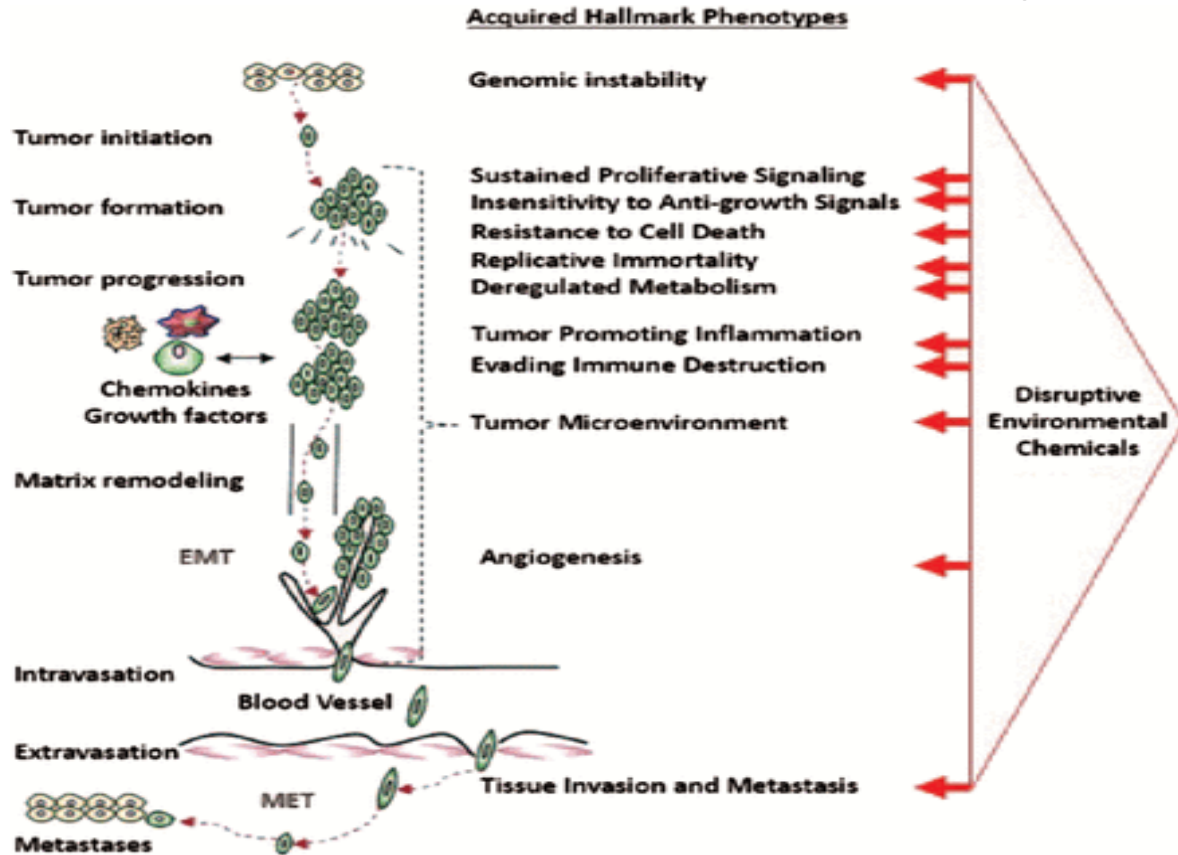
- Reprogramming energy metabolism
- Evading immune destruction

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...”

Goodson et al. Carcinogenesis. 2015 Jun; 36(Suppl 1): S254–S296.

Review team	Chemical name	Disruptive action on key mechanism/pathway	Low-dose effect (LDE, LLDE, NLDE, threshold, unknown)
Angiogenesis	Diniconazole	Vascular cell adhesion molecule and cytokine signaling	Threshold (H-PC) (36 = TOXCAST)
	Chlorothalonil	Thrombomodulin, vascular proliferation and cytokine signaling	Unknown (H-PC) (36), NLDE (A- <i>in vivo</i>) (38 in Amphibians)
Immune system evasion	Pyridaben	Chemokine signaling, TGF- β , FAK, HIF-1 α , IL-1 α pathways	Unknown (H-CL, H-PC, A-CL) (36,139,140), threshold (A-I) (141)
	Triclosan	Chemokine signaling, TGF- β , FAK, IL-1 α pathways	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

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

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]

Mechanisms	Carcinogen			
	AFB1	As+3	Asbestos	Benzene
DNA damage	+	+	-	+
Gene mutation	+	-	+	-
Chrom mutation	+	+	+	+
Aneuploidy	-	+	+	+
Epigenetic	+	+		+
Receptor signaling	-	+	+	
Other signaling	-	+		+
Immune effects	+	+	+	+
Inflammation	+	+	+	+
Cytotoxicity	+	+	+	+
Mitogenic	-	+		-
Gap junction	+	+		+

Key Characteristics of Human Carcinogens

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

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

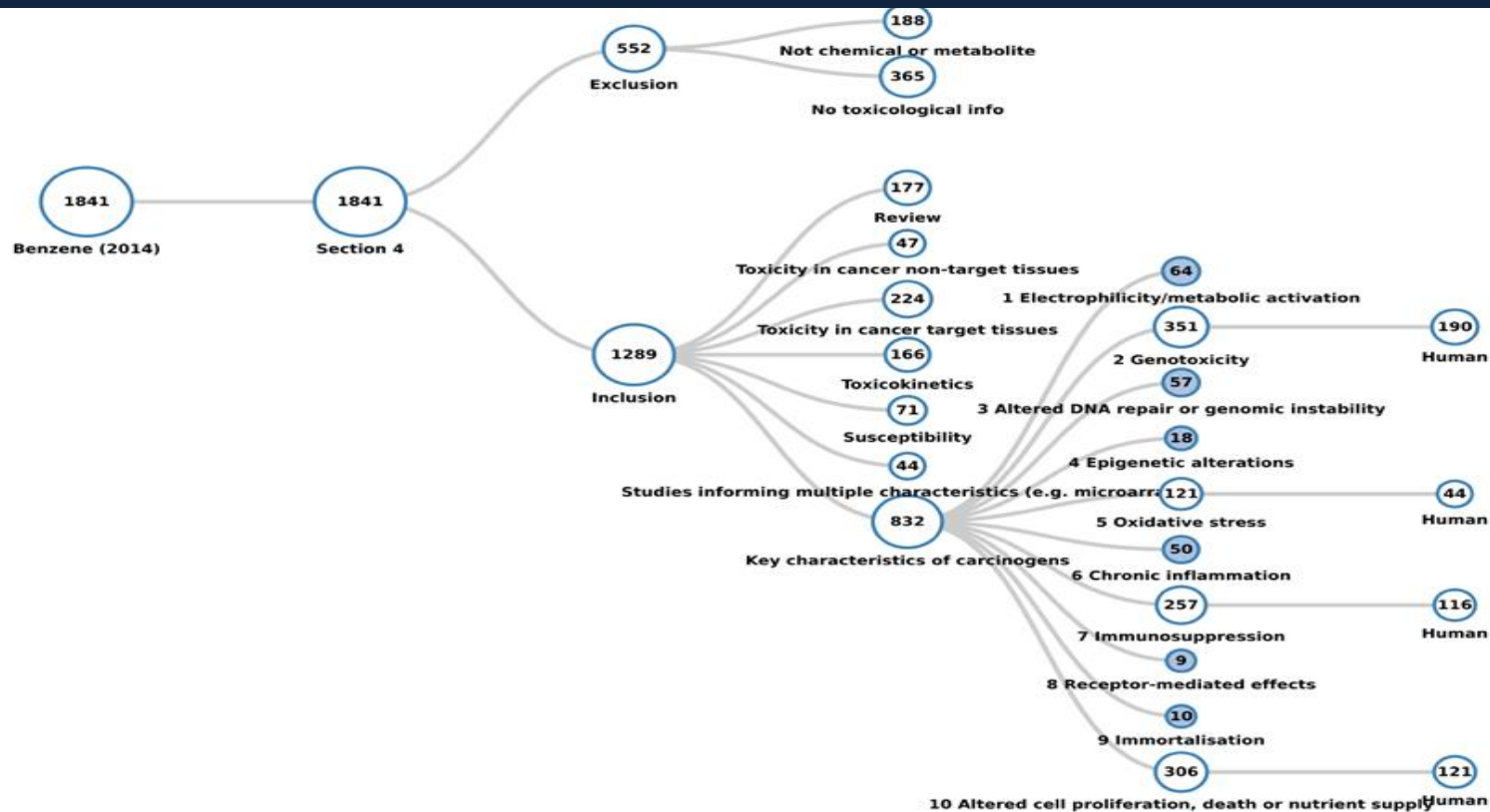
Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, DeMarini DM, Caldwell JC, Kavlock RJ, Lambert PF, Hecht SS, Bucher JR, Stewart BW, Baan RA, Cogliano VJ and K Straif. *Env Health Persp.*, 124(6), 713, 2016.

Characteristic	Examples of relevant evidence
1. Is Electrophilic or Can Be Metabolically Activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc), formation of DNA and protein adducts.
2. Is Genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei).
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces Epigenetic Alterations	DNA methylation, histone modification, microRNA expression
5. Induces Oxidative Stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)

Characteristic	Examples of relevant evidence
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is Immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes Immortalization	Inhibition of senescence, cell transformation, altered telomeres
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Benzene Mechanistic Data Search

conducted using the Health Assessment Workplace Collaborative (HAWC)
Literature Search tool (<https://hawcproject.org/>)



Benzene Example: An Adverse Outcome Pathway?

Benzene Exposure

Electrophilic
metabolites

Metabolic Activation

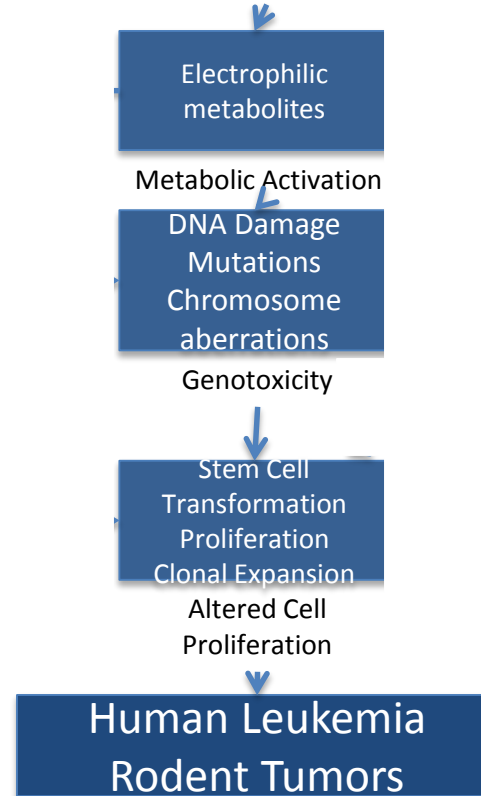
DNA Damage
Mutations
Chromosome
aberrations

Genotoxicity

Stem Cell
Transformation
Proliferation
Clonal Expansion

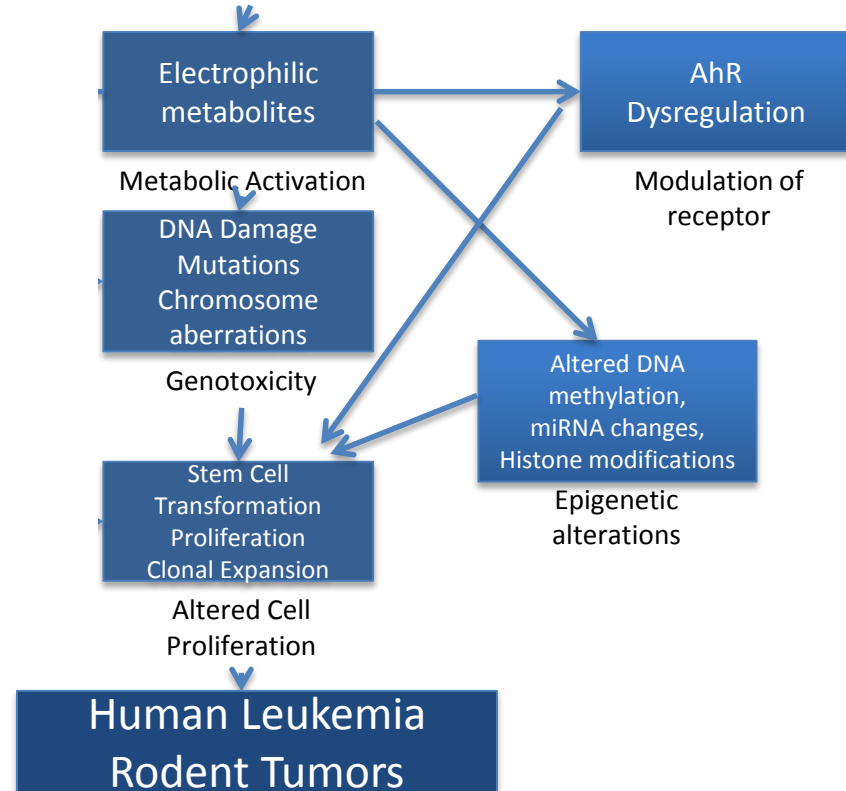
Altered Cell
Proliferation

Human Leukemia
Rodent Tumors

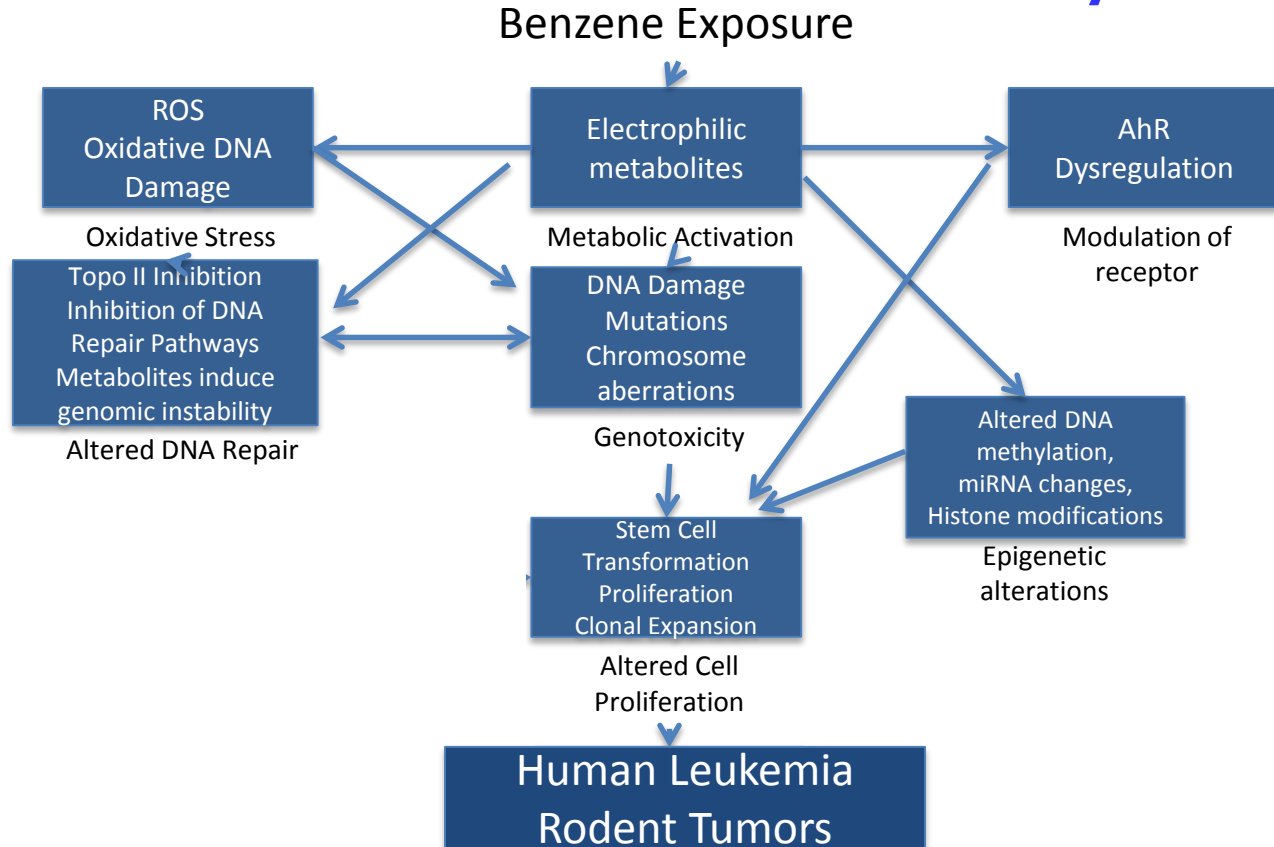


Benzene Example: An Adverse Outcome Pathway?

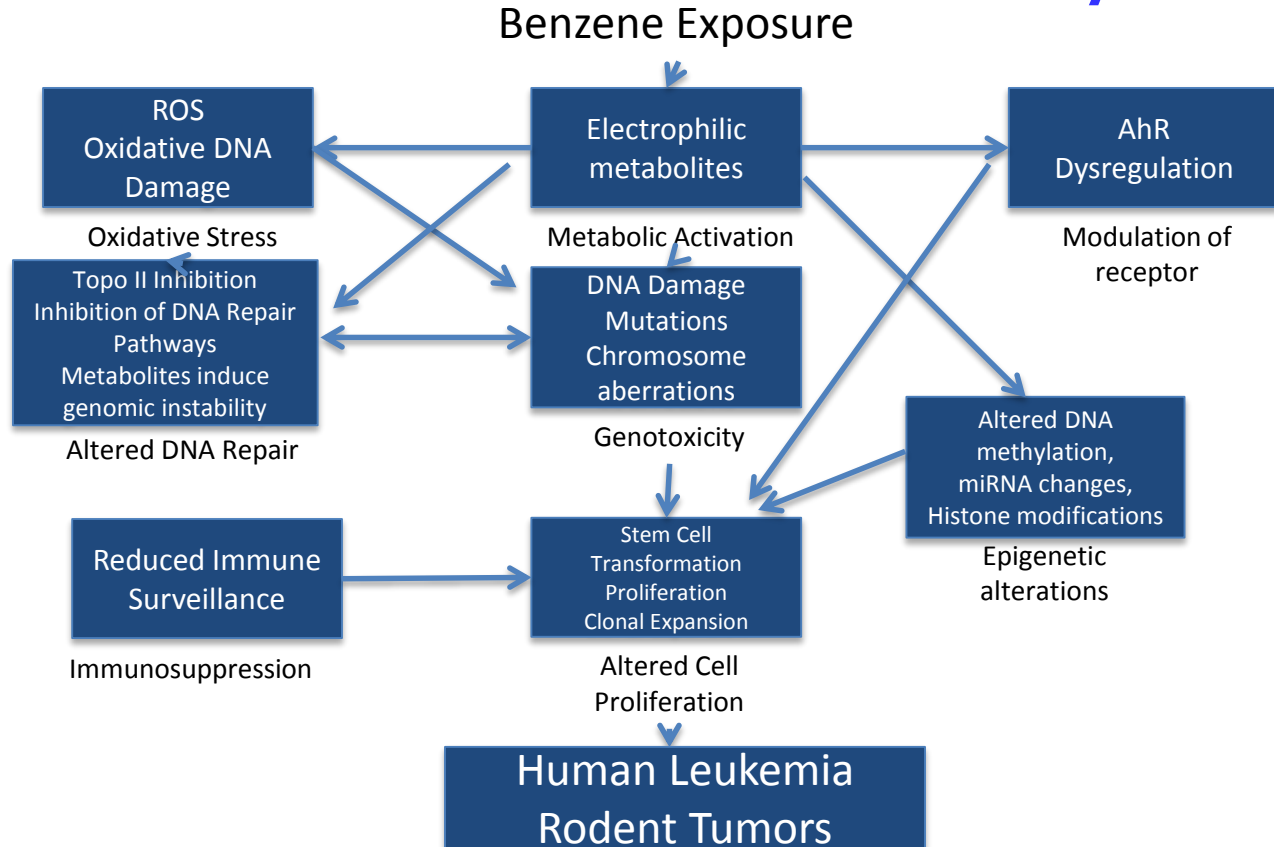
Benzene Exposure



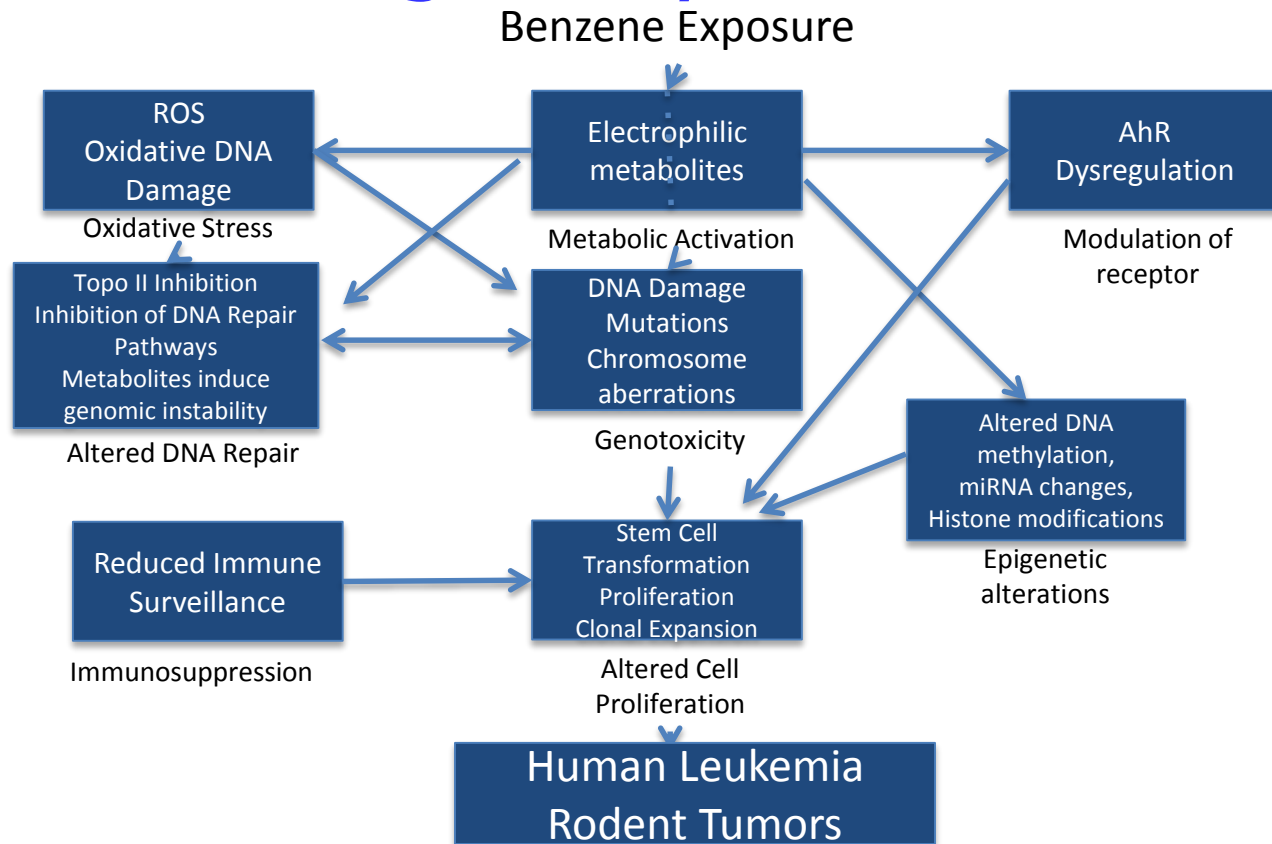
Benzene Example: An Adverse Outcome Pathway?



Benzene Example: An Adverse Outcome Pathway?



Benzene Example: An Adverse Outcome Network Involving 8 Key Characteristics



Mechanistic Conclusions in Recent IARC Monographs Evaluations

Agent	Cancer in humans	Cancer in animals	Mechanistic evidence (key characteristic)	Group
Tetrabromo-bisphenol A	Inadequate	Sufficient	Modulates receptor-mediated effects, is immunosuppressive, induces oxidative stress (5,7,8)	2A*
Diazinon	Limited (NHL, leukemia, lung)	Limited	Is genotoxic, induces oxidative stress (2,5)	2A
Glyphosate	Limited (NHL)	Sufficient	Is genotoxic, induces oxidative stress (2,5)	2A
Malathion	Limited (NHL, prostate)	Sufficient	Is genotoxic, induces oxidative stress, induces chronic inflammation, modulates receptor-mediated effects, and alters cell proliferation or death (2,5,6,8,10)	2A
Parathion	Inadequate	Sufficient		2B
DDT	Limited (NHL, liver, testis)	Sufficient	Is immunosuppressive, induces oxidative stress, modulates receptor-mediated effects (5,7,8)	2A
2,4-D	Inadequate	Limited	Induces oxidative stress (5)	2B

Summary

- Scientific findings providing insights into cancer mechanisms play an essential role in carcinogen hazard identification
- **The key characteristics of known human carcinogens provide the basis for an objective, systematic approach for identifying and evaluating mechanistic data**
- Shows carcinogens tend to act through multiple mechanisms in producing human and animal tumors – separation into genotoxic and non-genotoxic of little value
- Recent IARC Monographs evaluations have illustrated the applicability of this approach
- Is compatible with HT assays, but need to develop based on characteristics and hallmarks. Current ones flawed
- These developments lay groundwork for future evaluations where such data may fill important gaps in evidence of carcinogenicity

Question for the Future

If a chemical possesses multiple key characteristics can we classify it as a possible/probable human carcinogen without any animal bioassay or epidemiological data?

A Large Collaboration

- **IARC:** Kathryn Z. Guyton, Robert A. Baan and Kurt Straif
- **US EPA:** Catherine F. Gibbons, Jason M. Fritz, David M. DeMarini, Jane C. Caldwell, Robert Kavlock, Vincent Coglianor
- **NTP:** John R. Bucher
- **Academia:** Ivan Rusyn, Paul F. Lambert, Stephen S. Hecht, Bernard W. Stewart
- **EDF:** 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.
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