

Carcinogenesis

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Carcinogenesis Overview

- Brief History of Chemical Carcinogenesis
- Multi-stage Nature of Cancer
- Mutagenesis and Genomic Instability
- Tumor Microenvironment (Tumors are not Monoclonal)
- Tumor Epigenome
- Hallmarks of Cancer—Altered Pathways
- Therapeutics Based on Hallmarks of Cancer
- What Causes Cancer?
- Testing Chemicals for Carcinogenicity

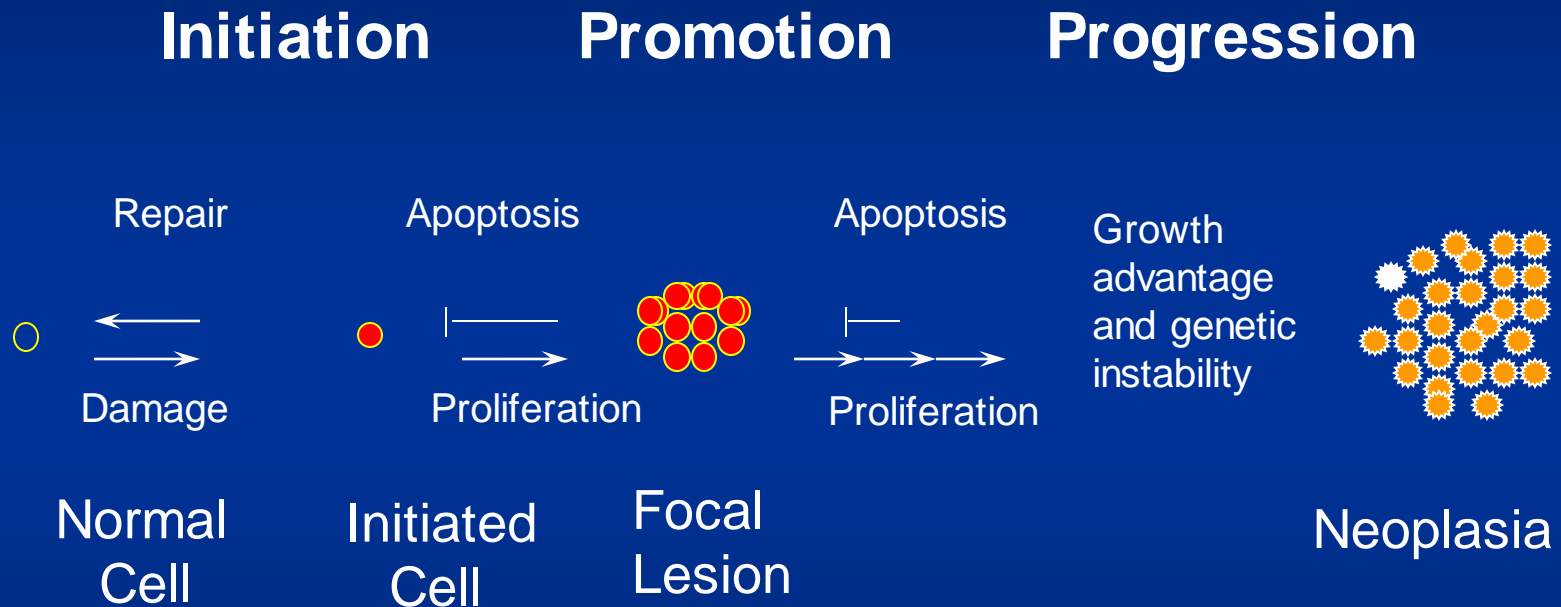
Brief History of Chemical Carcinogenesis

- Percival Pott
 - Described in 1775 scrotal cancer in chimney sweeps in London--caused by soot collecting under their scrotum.
- John Hill
 - One of the first to recognize the dangers of tobacco in his 1761 book entitled "Cautions Against the Immoderate Use of Snuff."

First Experimental Carcinogenesis

- K. Yamagawa and K. Ichikawa in 1915 in Japan showed that coal tar applied to the skin of rabbit ears was carcinogenic.
- R.D. Passey, J.A. Campbell did similar studies on mouse skin in the 1920s and 30s in Britain, and E. Kennaway isolated benzo[a]pyrene from coal tar in the 1930s.

Multi-stage Model of Cancer



The Cancer Process

- Cancer occurs by a series of mutations and epigenetic changes altering at least 6 functional pathways in the cell.
- The genomes of cancer cells contain hundreds or thousands of mutations; however, most of these are “passenger mutations” that result from genomic instability.

The Cancer Process (*cont.*)

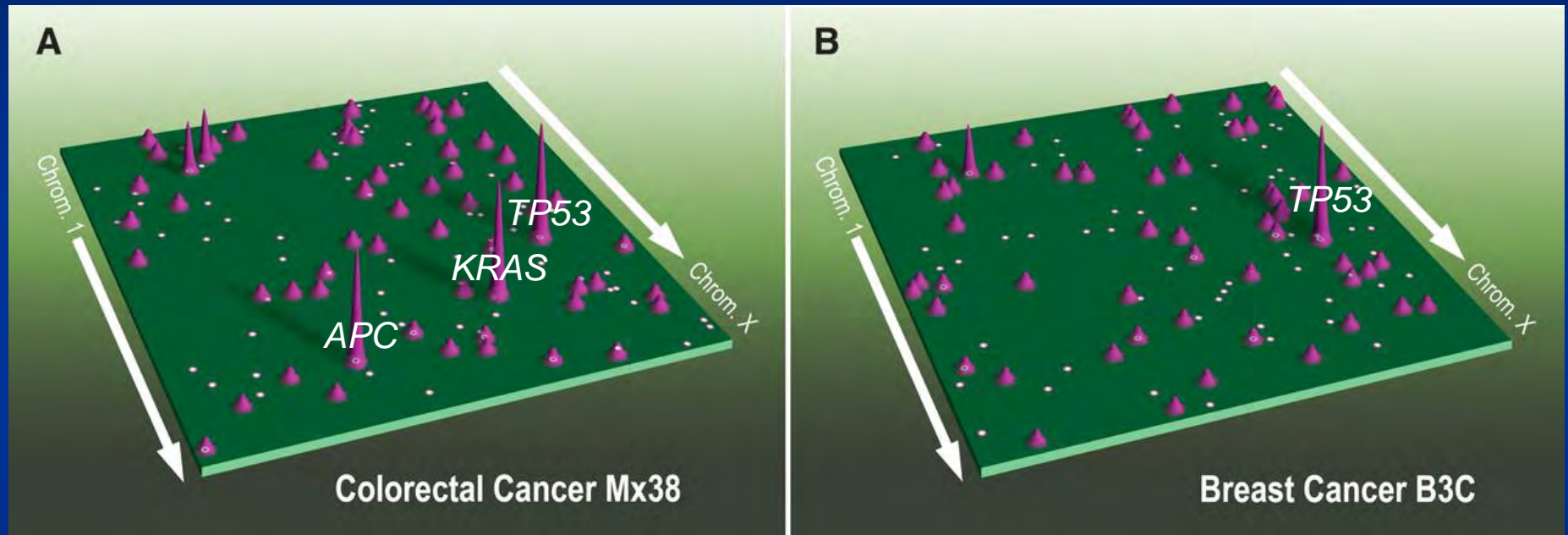
- Cancer proceeds like Darwinian evolution. There is a succession of mutations and epigenetic changes, each conferring a growth advantage, leading to the conversion of a normal cell to a cancerous cell.
- Mutagenesis, epigenesis, and selection the are the essential features of carcinogenesis.

Mutagenesis & Genomic Instability

1. Many familial (inherited) cancers are caused by mutations in DNA repair or DNA Damage Response (DDR) pathways.
2. Gene, chromosomal, and genomic mutations (aneuploidy) are found in nearly all tumors.
3. Aneuploidy can initiate or progress tumors.

Genomic Landscape of Mutations in Cancer Cells

[LD Wood et al., Science 318:1108, 2007]

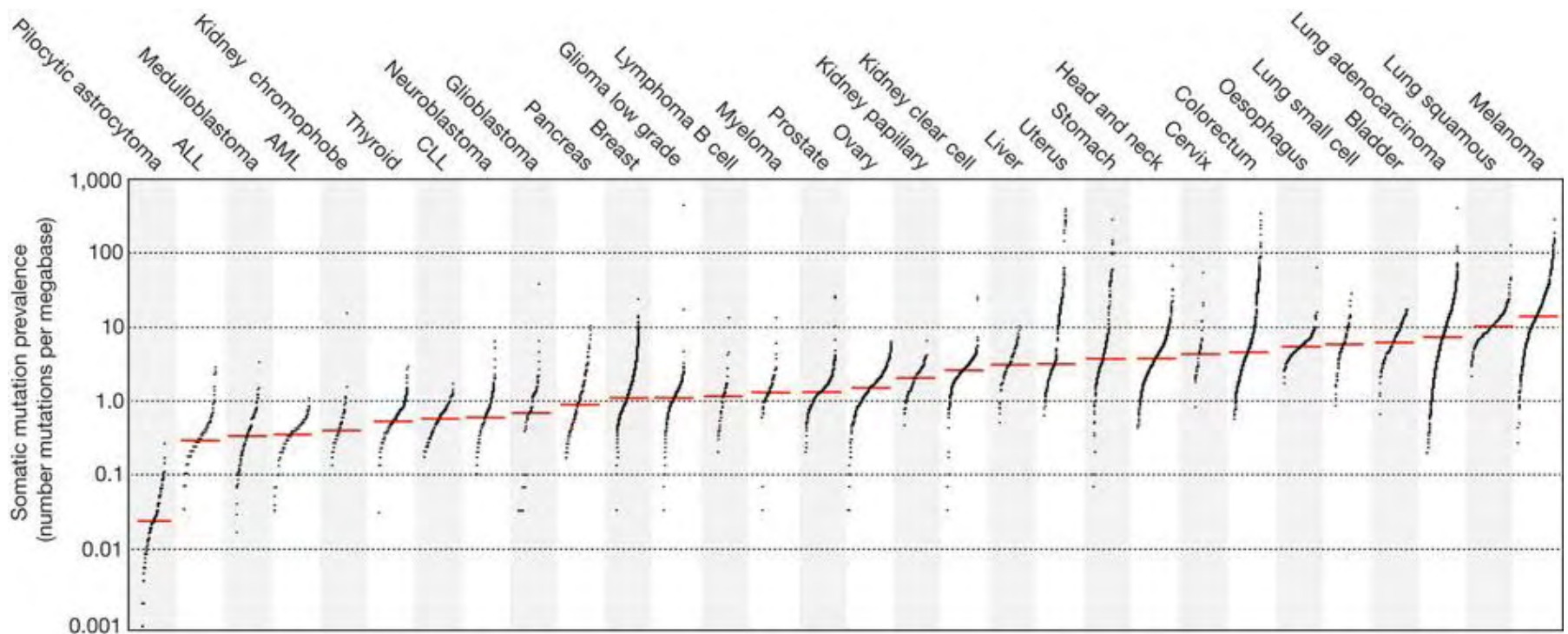


Tumors are composed of a small number of commonly mutated genes (mountains) and a much larger number of less-frequently mutated genes (hills). These “hills” may drive the cancer process through 6-8 pathways.

Frequency of Somatic Mutations in Various Types of Cancers

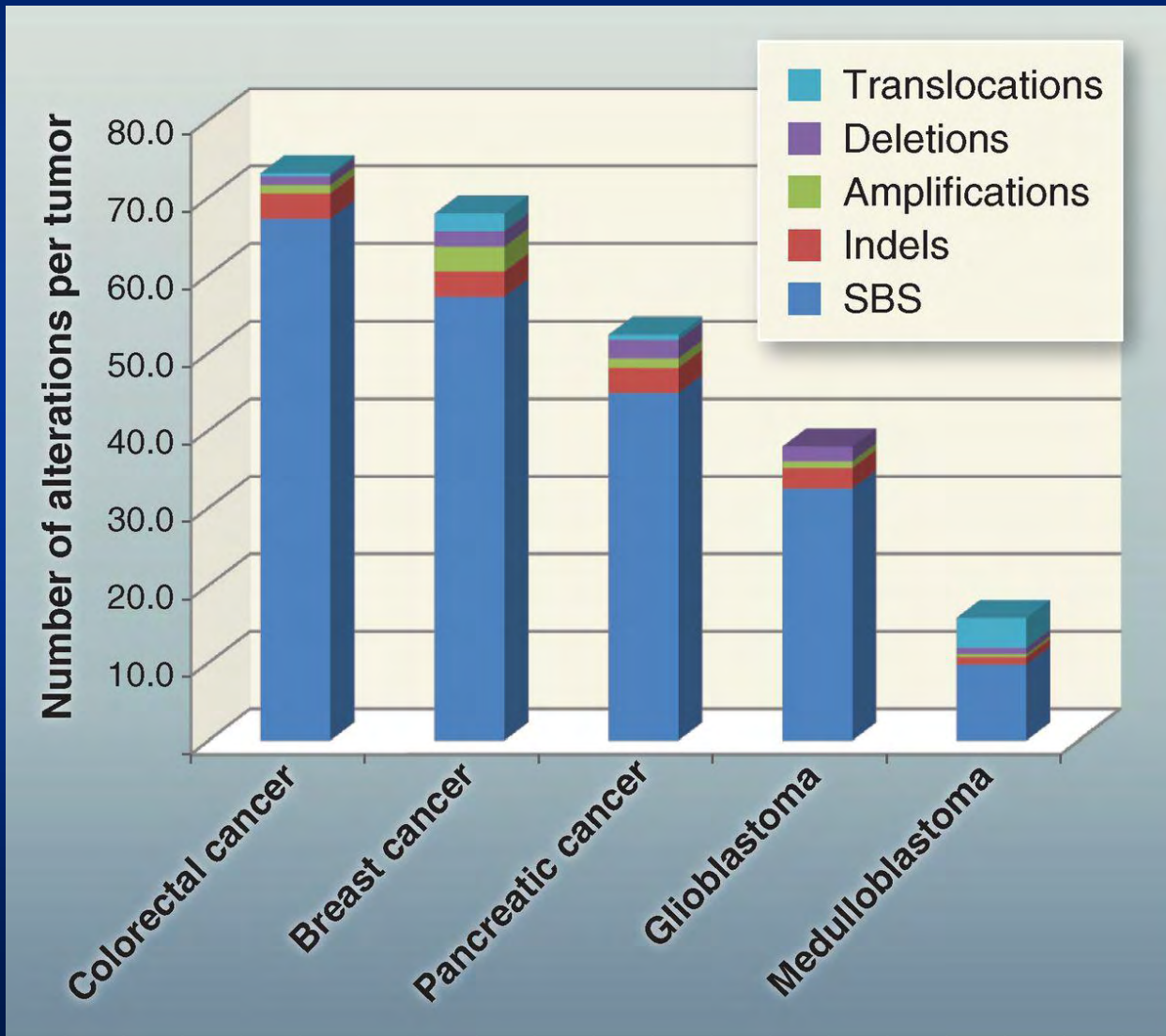
[Alexandrov et al., Nature 500:415, 2013]

20 distinct mutational signatures (combinations of mutations). Some are in many cancers; others in only one type. Some cancers have regions of hyper-mutation.



Proportion of Various Mutations in Coding Genes in Selected Human Tumors

(B Vogelstein et al., Science 339:146, 2013)



The vast majority of mutations in tumors are single base substitutions (SBS)

Cell-Signaling Pathways & Processes They Regulate

(B Vogelstein et al., Science 339:146, 2013)



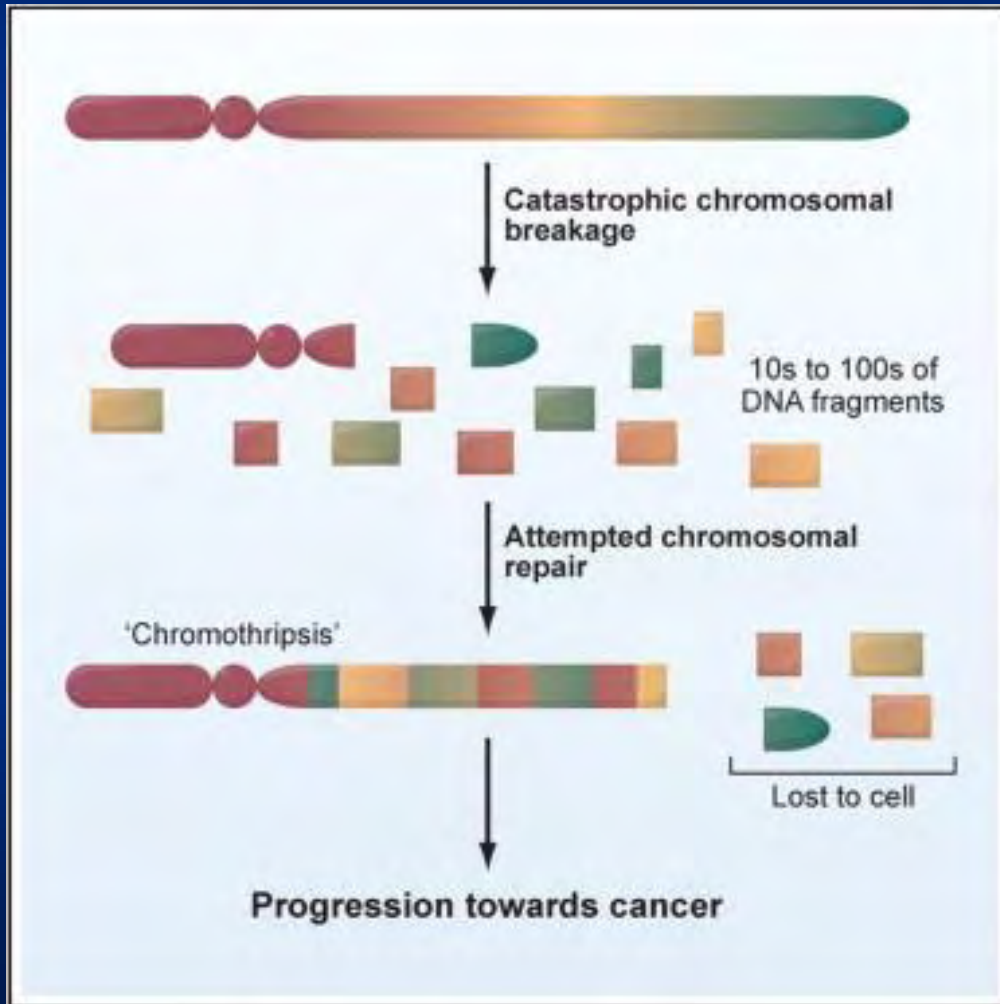
Inner circle lists the 12 common driver genes in cancer cells; the outer circle divides them into three cellular processes.

Cancer Genome Landscapes

(B Vogelstein et al., Science 339:1546, 2013)

- Most human cancers are caused by 2-8 alterations (mutations/epigenetic changes).
- Each alteration gives a selective growth advantage.
- 1000 to 100,000 genes may be mutated in a tumor, but only ~140 genes are directly involved in cancer.
- The driver genes function through 12 signaling pathways that regulate 3 cellular processes: cell fate, survival, and genome maintenance.
- Each tumor has distinct genetic alterations, but the pathways affected in different tumors are similar.
- Cells within a tumor are heterogeneous.

Chromothripsis: Massive Chromosomal Rearrangement in 1-3% of Tumors



PJ Stephens et al.,
Cell 144:27, 2011

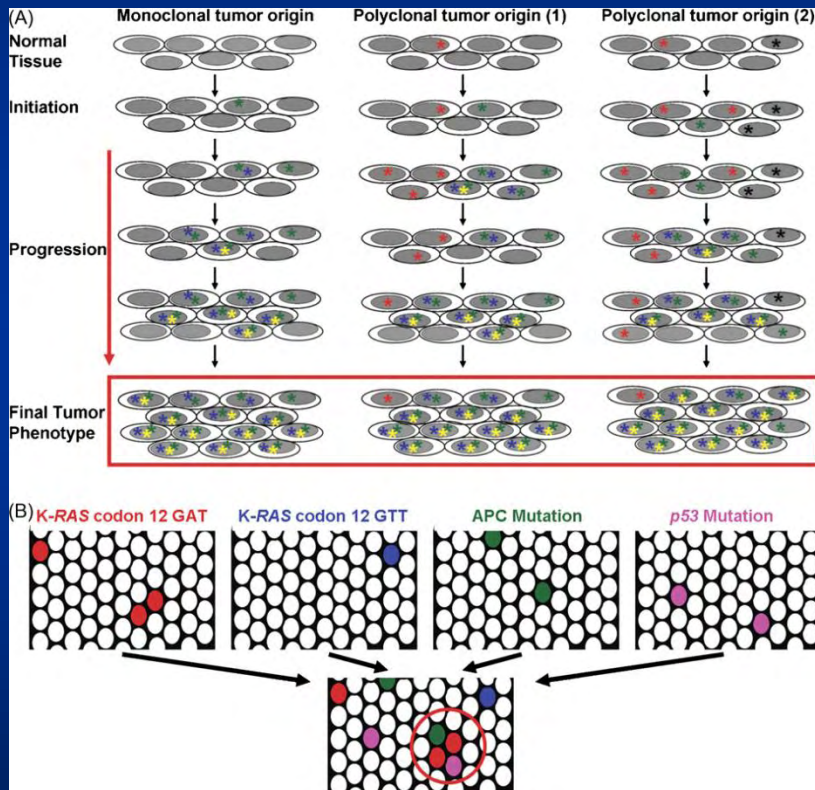
Chromothripsis Occurs
in Micronuclei

K Crasta et al., Nature
482:53, 2012

CA Maher & RK Wilson,
Cell 148:29, 2012

Tumors Are Typically Not Monoclonal

[BL Parsons, Mutat Res 659:232, 2008]



Intra-tumor Heterogeneity

M Gerlinger et al., NEJM 366: 883, 2012

63-69% of all somatic mutations were not present in all regions of kidney tumors analyzed by exon sequencing, chrom ab analysis, & mRNA expression analysis.

Epigenetics

An epigenetic alteration is a heritable change in gene expression that is NOT due to a change in DNA sequence—or is it?

Epigenetic Mechanisms

- **DNA methylation:** represses gene expression (Hsiao et al. 2009)
- **Histone modifications:** methylation, acetylation, phosphorylation, and ubiquitination alter gene expression, DNA repair, and chromosome condensation (Ellis et al. 2009)
- **Noncoding RNAs:** microRNAs, which are small RNAs ~22 nucleotides long that alter gene expression (Garzon et al. 2009)

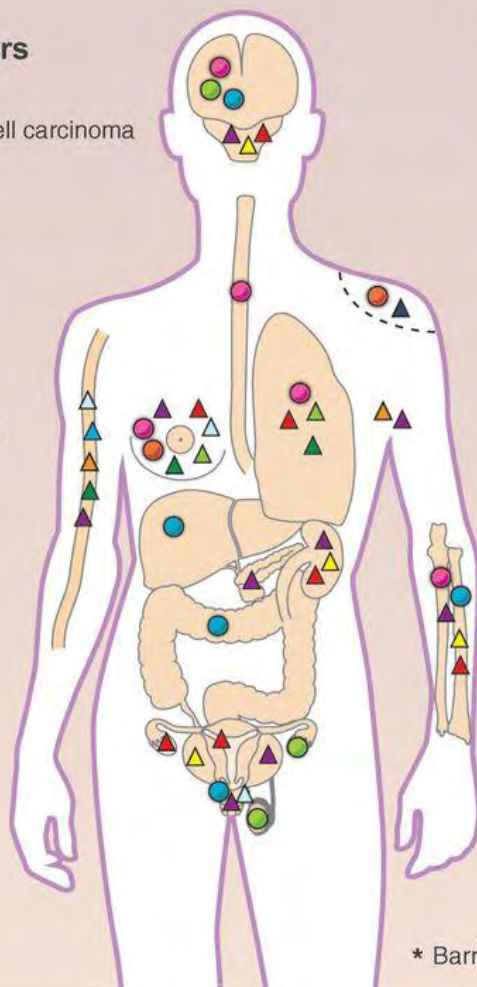
Epigenetic Reprogramming in Cancer

[Suva et al., Science 339:1567, 2013]

A

Transcription factors

- **SOX2**
Esophageal squamous cell carcinoma
Lung carcinoma
Glioblastoma
Breast carcinoma
Ewing sarcoma
- **KLF4**
Breast carcinoma
Skin malignancies
- **NANOG**
Hepatocellular carcinoma
Glioblastoma
Colon carcinoma
Prostate carcinoma
Ewing sarcoma
- **OCT4**
Germ cell tumors
- **C-MYC**
Multiple malignancies
- **LIN28**
Multiple malignancies



B

Chromatin regulators

- ▲ **SUV39H1***
Acute promyelocytic leukemia (APL)
- ▲ **SETDB1***
Melanoma
- ▲ **G9a***
Lung carcinoma
Breast carcinoma
- ▲ **UTX**
Multiple myeloma
Clear cell renal cell carcinoma
Transitional cell carcinoma of bladder
Medulloblastoma
- ▲ **PRC2**
Follicular and large B-cell lymphomas
Myelodysplastic syndromes
T-cell acute lymphoblastic leukemia
Overexpressed in multiple malignancies
- ▲ **ARID1A**
Ovarian clear cell carcinoma
Endometrioid carcinoma
Renal cell carcinoma
Neuroblastoma
Medulloblastoma
Lung carcinoma
Breast carcinoma
- ▲ **MLL1**
Acute myeloid leukemia (AML)
Acute lymphoblastic leukemia (ALL)
Transitional cell carcinoma of bladder
- MLL2**
Large B cell and follicular lymphoma
Medulloblastoma
Prostate carcinoma
Renal carcinoma
- MLL3**
Medulloblastoma
Transitional cell carcinoma of bladder
Breast carcinoma
Pancreatic adenocarcinoma
- ▲ **LSD1**
Acute myeloid leukemia (AML)
Breast carcinoma
Prostate carcinoma
- ▲ **DOT1L***
Mixed lineage leukemia (MLL)
- ▲ **KDM2B**
Acute myeloid leukemia (AML)
- ▲ **DNMT3A/B**
Acute myeloid leukemia (AML)
Breast carcinoma
Lung carcinoma

* Barrier to reprogramming.

Mutations in Genes Involved in Epigenetic Modifications May Explain Epigenetic Effects

[P Peltomäki, Exp Cell Res 318:299, 2012]

[RJH Ryan & BE Bernstein, Science 336:1513, 2012]

- Mutations in DNA methyltransferase genes (*DNMT1*, *DNMT3A*) in colorectal cancer or AML
- Mutations in histone lysine methyltransferases or demethylases (*HK4*, *H3K9*, *H3K27*) in kidney & colon cancer
- Mutations in histone acetyltransferases (*H3K18*, *H3K27*) in ALL
- Mutations in miRNAs in various cancers

Six Hallmarks of Cancer

[D Hanahan & RA Weinberg, Cell 144:646, 2011]

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

Hallmarks (cont.)

Emerging Hallmarks

- Reprogramming energy metabolism
- Evading immune destruction

Enabling Characteristics

- Genomic instability and mutation
- Inflammation

Alterations in 3 Classes of Genes are Needed for Carcinogenesis

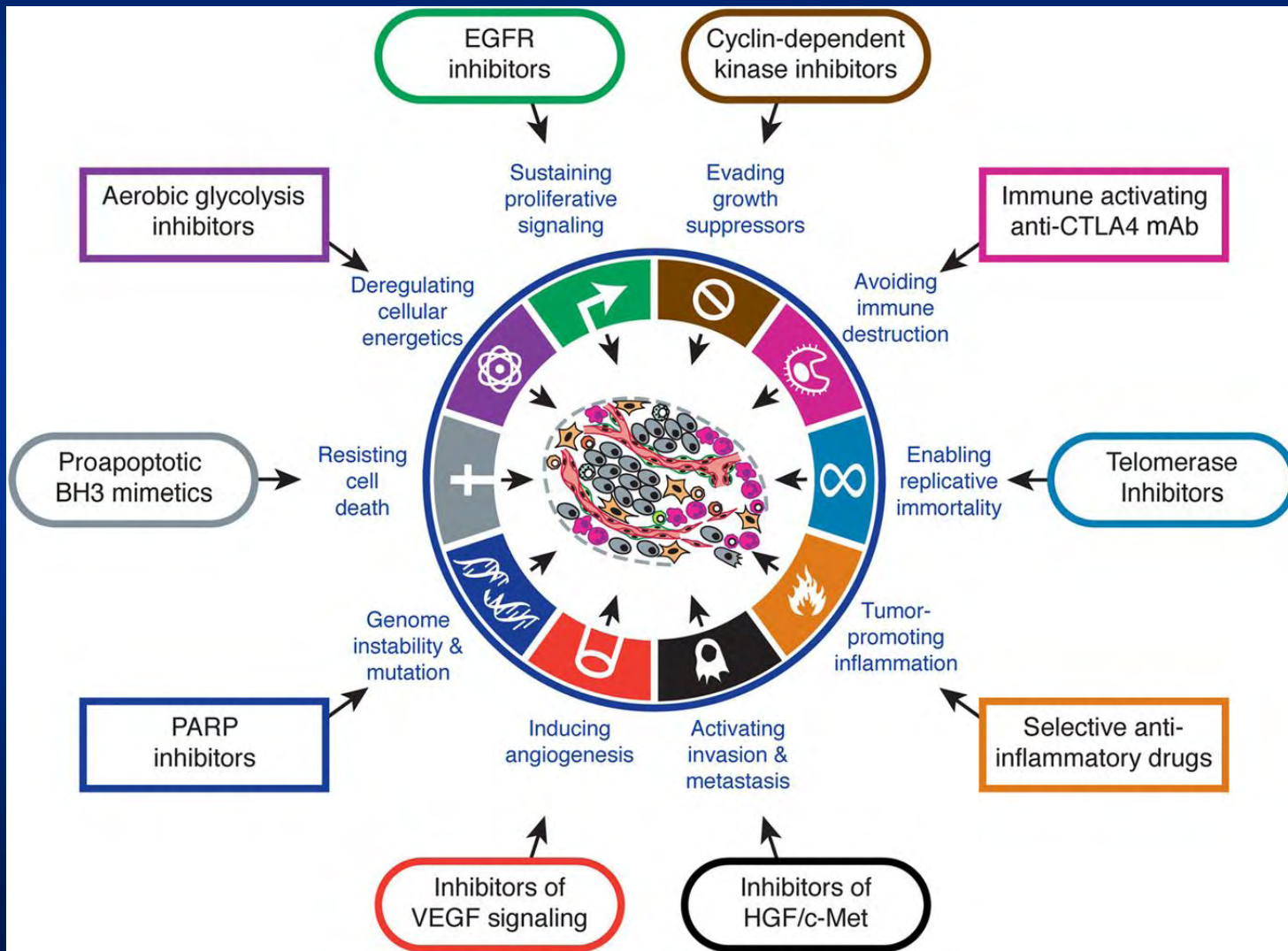
Tumor-Suppressor Genes (*P53, RB, APC*). Inhibit cell death, like dysfunctional breaks.

Oncogenes (*MYC, MET, KIT*). Stimulate cell division, like stuck accelerator.

Genomic-Stability Genes (*BER, NER, MMR*). More mutations, like an inept mechanic

Hallmarks of Cancer: Therapeutic Targets

[D Hanahan and RA Weinberg, Cell 144:646, 2011]



Drugs Targeted to Alter Epigenetics

- Azanucleosides are DNA methylation inhibitors that are now approved by the US FDA for treatment of myelodysplastic syndrome.
- SAHA is a histone deacetylase inhibitor approved by the US FDA for treatment of T-cell cutaneous lymphoma.

The Causes of Cancer

[R. Doll and R. Peto, Oxford University Press, 1981]

[P. Boffetta, Mutat. Res. 608:157, 2006]

- 35% Diet
- 30% Tobacco
- 10% Infection
- 7% Reproductive/Sexual Behavior
- 4% Occupational Exposures
- 3% Alcohol
- 2% Pollution
- 1% Medical Procedures
- 1% Industrial Products

Cancer Viruses

[AG Georgakilas et al., Mol. Biosyst. 6:1162, 2010]

- Account for much of cancer (most cervical cancer—HPV).
- Mechanisms involve inactivation of tumor-suppressor genes, stimulation of oncogenes, interference by viral proteins of transcription, signal transduction, DNA repair, apoptosis, induction of chronic oxidative stress, and DNA methylation (epigenetic gene regulation).

Types of Chemical Carcinogens

- DNA-Reactive (genotoxic or mutagenic) Carcinogens. Many of the 105 IARC human carcinogens are of this type.
- Non-DNA Reactive (not mutagenic or genotoxic) Carcinogens. Half of the rodent carcinogens are of this type.
- International Agency for Research on Cancer (IARC) in Lyon, France, declares agents as human carcinogens.

IARC Carcinogen Classification

- **Group 1.** Agent is carcinogenic to humans (sufficient evidence in humans).
- **Group 2A.** Agent is probably carcinogenic to humans (limited evidence in humans; sufficient evidence in experimental animals).
- **Group 2B.** Agent is possibly carcinogenic to humans (limited evidence in humans but less than sufficient evidence in experimental animals; or inadequate in humans but sufficient in animals.)
- **Group 3.** Agent is not classifiable (inadequate evidence in humans; inadequate or limited in animals).
- **Group 4.** Agent is probably not carcinogenic to humans (evidence suggesting lack of carcinogenicity in humans and experimental animals; caprolactam is the only one).

Many Human Carcinogens Are Mutagenic (IARC Vol 100)

Human Carcinogens	SAL	CA/MN
Aflatoxins, 4-Aminobiphenyl, Azathioprine, Benzidine, Chlorambucil, Chlornaphazine, Chromium IV, Cyclophosphamide, Mephalan, Methyl CCNU, Myleran, 2-Naphthylamine, Nickel, Paracetamol, Sulfur mustard, Thiotepa, Tobacco, Treosulphan, Vinyl Chloride	+	+
Benzene, DES, Arsenics	-	+
Bis(chloromethyl)ether, PUVA, Radon, (tars, soots, oils)	+	

Shelby (1998) Mutat. Res. 240:3-15

Shelby and Zeiger (1990) Mutat. Res. 234:257-261

Ashby and Paton (1993) Mutat. Res. 286:3-74

Mechanisms of Group 1 Carcinogens

[Guyton et al., Mutat 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	+	+		+

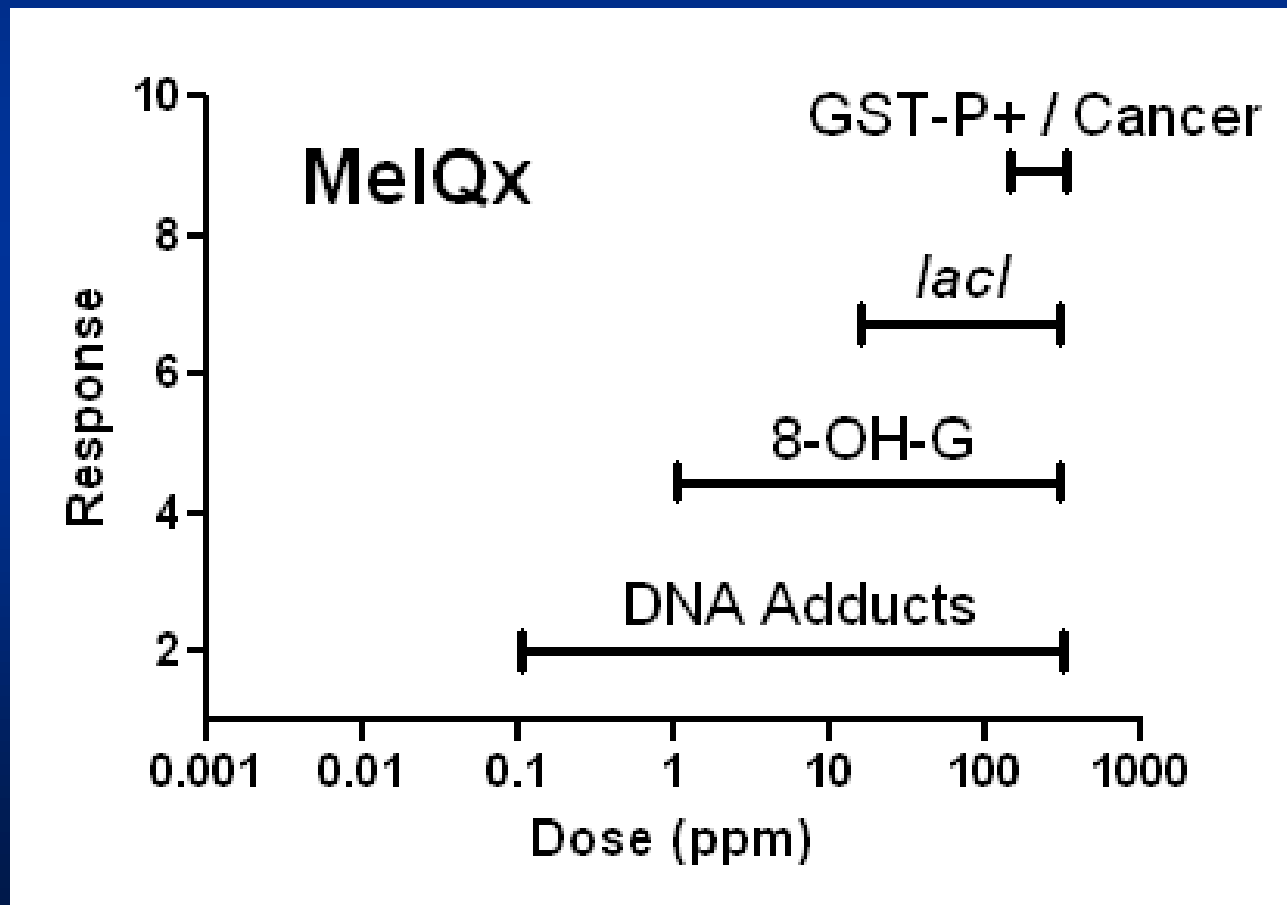
Two-Year Rodent Bioassay for Carcinogenesis

- F344 rats (50 males/50 females) and B6C3F1 mice (50 males/50 females)
- Exposed to maximum tolerated dose (MTD) determined from a 90-day exposure
- Takes 6 years and costs 6 million dollars.
- An agent that is a trans-species carcinogen (i.e., is carcinogenic in mice and rats, etc.) and is genotoxic in a variety of test systems has the characteristic of a human carcinogen.

Threshold for Genotoxic Carcinogens

[Fukushima et al., Genes Environ 31:33, 2009]

1,145 F344 male rats, 16-32 wks in feed



Mechanisms of Non-genotoxic Carcinogens

- Increase cell growth/proliferation
 - increased DNA synthesis or cytotoxicity
 - decreased cell death (decrease apoptosis)
- Modulation of intercellular communication
 - inhibition of gap junction intercellular communication
- Modulation of gene expression
 - Hypo- or hyper-methylation
 - Transcription-factor activation
 - Histone modification
- Binding and/or modification of receptors
 - Hormones, growth factors

Weight-of-Evidence Assessment

- Exposure to humans
- Evidence for carcinogenicity in humans
 - Epidemiology and biomarker studies
- Evidence for carcinogenicity in experimental systems
 - Studies in rodents or other animals
- Mechanistic evidence in humans and experimental systems
 - Mutagenicity, DNA damage, transformation, structure-activity assessment, altered gene expression, epigenetic effects

Summary

- Cancer is a genetic disease that proceeds in an evolutionary fashion with successive mutations or epigenetic changes being selected for a growth advantage.
- At least 6 functions must be altered to produce a cancer cell.
- Mutations and/or epigenetic changes among a subset of several hundred genes, which occur among 3 classes (tumor suppressors, oncogenes, genomic stability genes), must affect at least 1 gene in a set of critical pathways to produce a tumor.
- Germ-cell mutations that predispose to cancer may be inherited, and somatic-cell mutations may be acquired that contribute to the risk for cancer.

Summary (cont.)

- Carcinogens may modify DNA directly (genotoxic) or not (nongenotoxic).
- Short-term tests are used to identify mutagens, with the assumption that mutagens are likely to be carcinogens.
- Rodent assays provide definitive experimental demonstration of carcinogenicity.
- A variety of evidence (epidemiology, laboratory studies, structure-activity) must be used in a weight-of-the-evidence assessment to assess if an agent is a likely human carcinogen.

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