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Epigenetics in disease control and prevention: when will it be ready for prime time?

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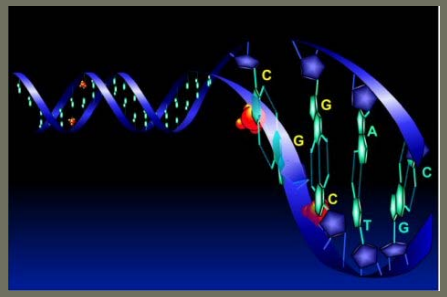
Division of Cancer Control and Population sciences (DCCPS)
National Cancer Institute (NCI)
National Institutes of Health (NIH)

PUBLIC HEALTH GENOMICS Seminar Series (August 26, 2009)



U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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Outline

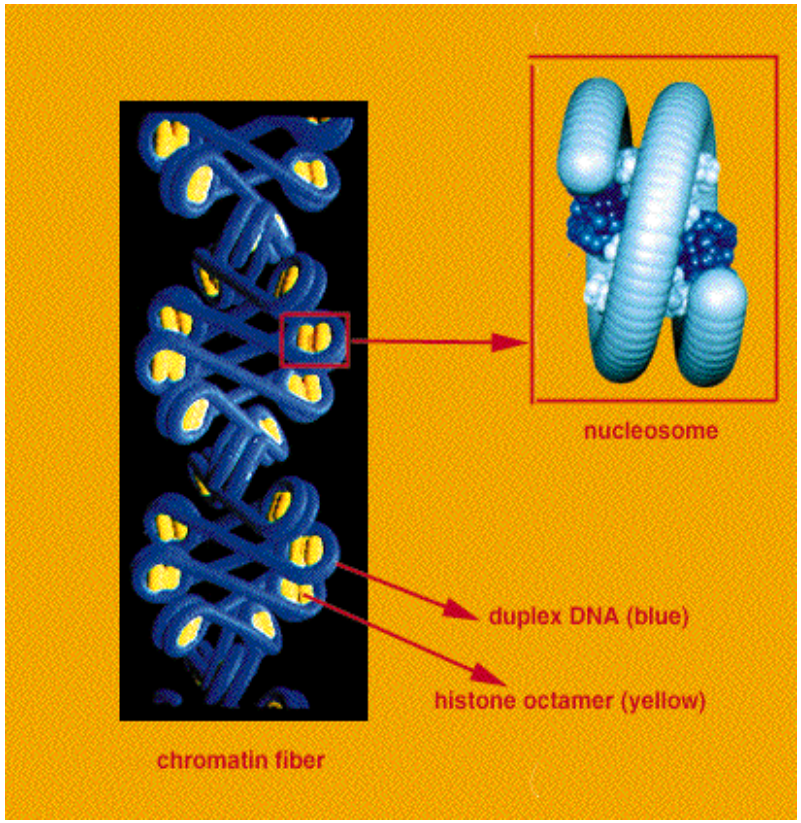
- Molecular Basis of Epigenetics
- Epigenetic Profiling: Technology
- Examples from Clinical Studies (Cancer)
- Implication in Personal Health
- NIH Roadmap: Epigenomics

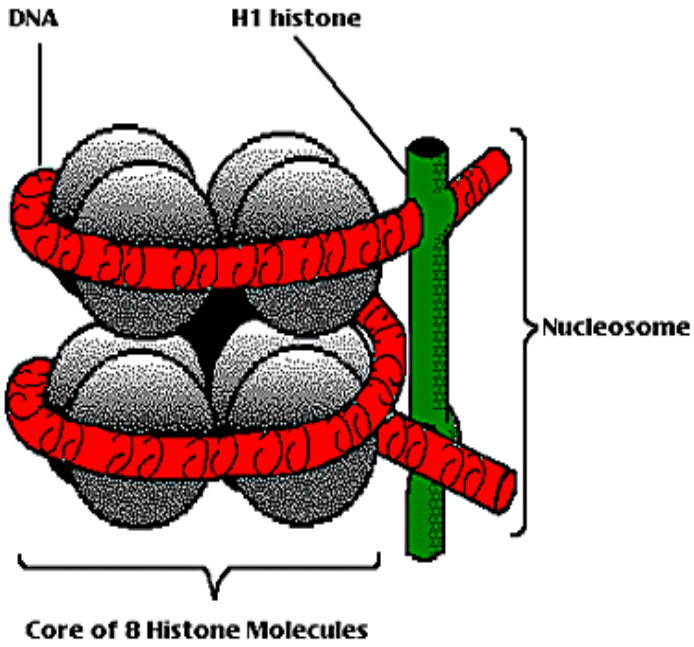
Nucleosomes (Units of Chromatin)

DNA
Histones H2a, H2b, H3, H4

} To neutralize charge and provide stability

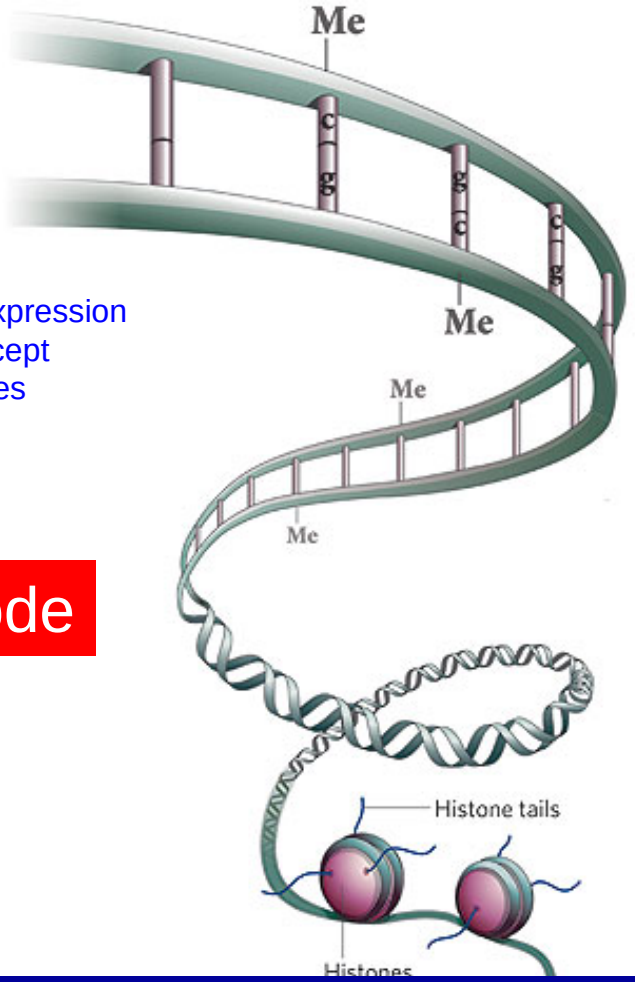
Nucleosome: two turns of DNA (146 base pairs) wrapped around an octomeric complex of two of each of histone types





Nucleosome

Epigenetics:
Stable alterations in gene expression by several mechanisms, except nucleotide sequence changes



The two main components of the epigenetic code

DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

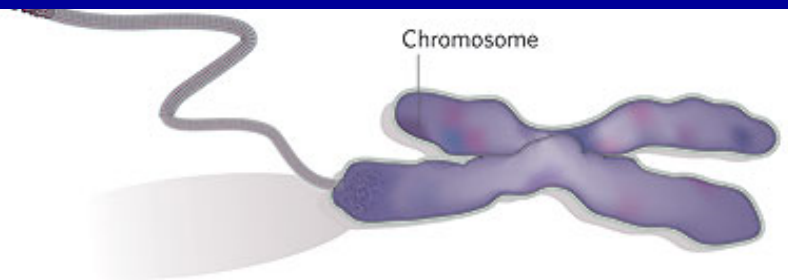
Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These

Methylation Code

Genetic Code

Histone Code

The genetic information provides the blue print for the manufacture of all the proteins necessary to create a living organism, whereas the epigenetic information provides the instructions on how, where and when the genetic information will be used.

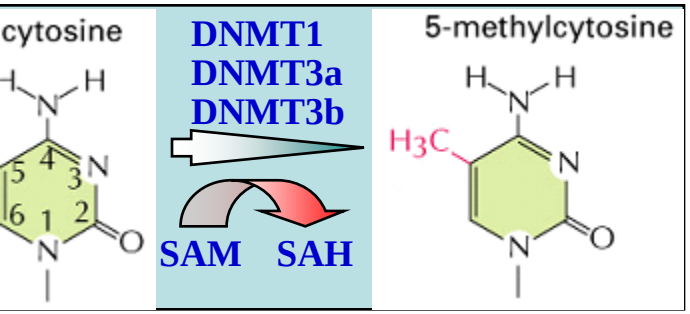


Qiu NATURE 441: 143

EPIGENETICS

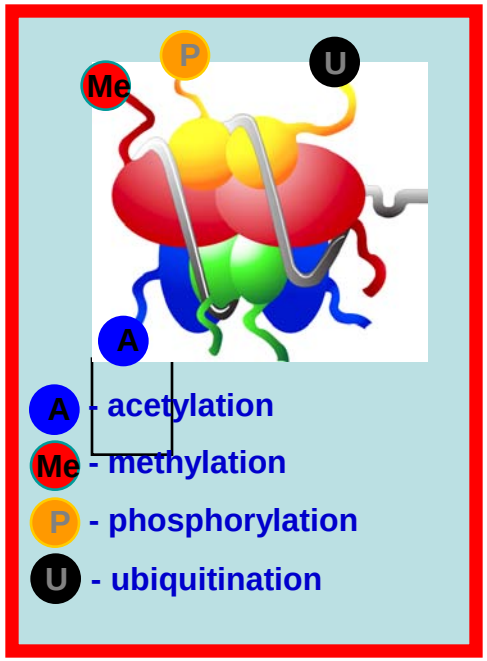
Epigenetic alterations – changes induced in cells that alter expression of the information on transcriptional, translational, or post-translational levels without change in DNA sequence

Methylation of DNA



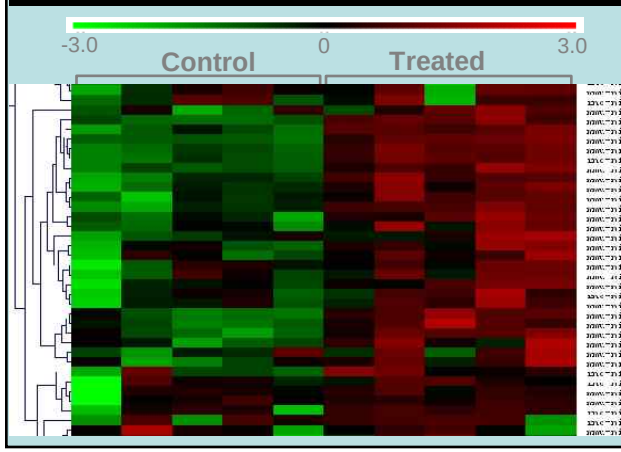
Genomic Imprinting

Modifications of histones



RNA-mediated modifications

- RNA-directed DNA methylation
- RNA-interference mediated chromatin remodeling
- RNAi, siRNA, miRNA …



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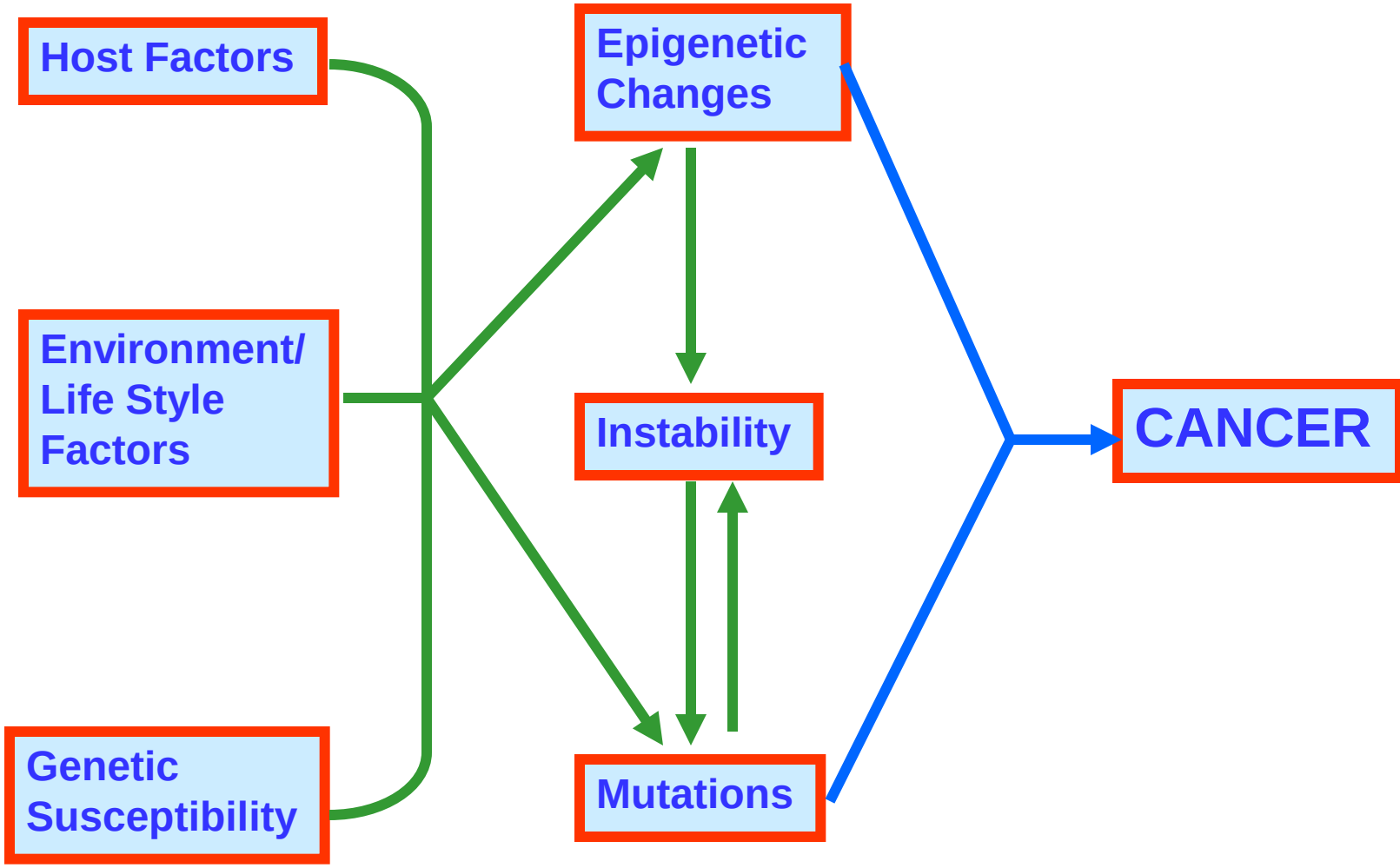
Background



Verma (2009) Hum Gen. Epidemiology. 2nd Edn.

A multistage model of carcinogenesis in skin

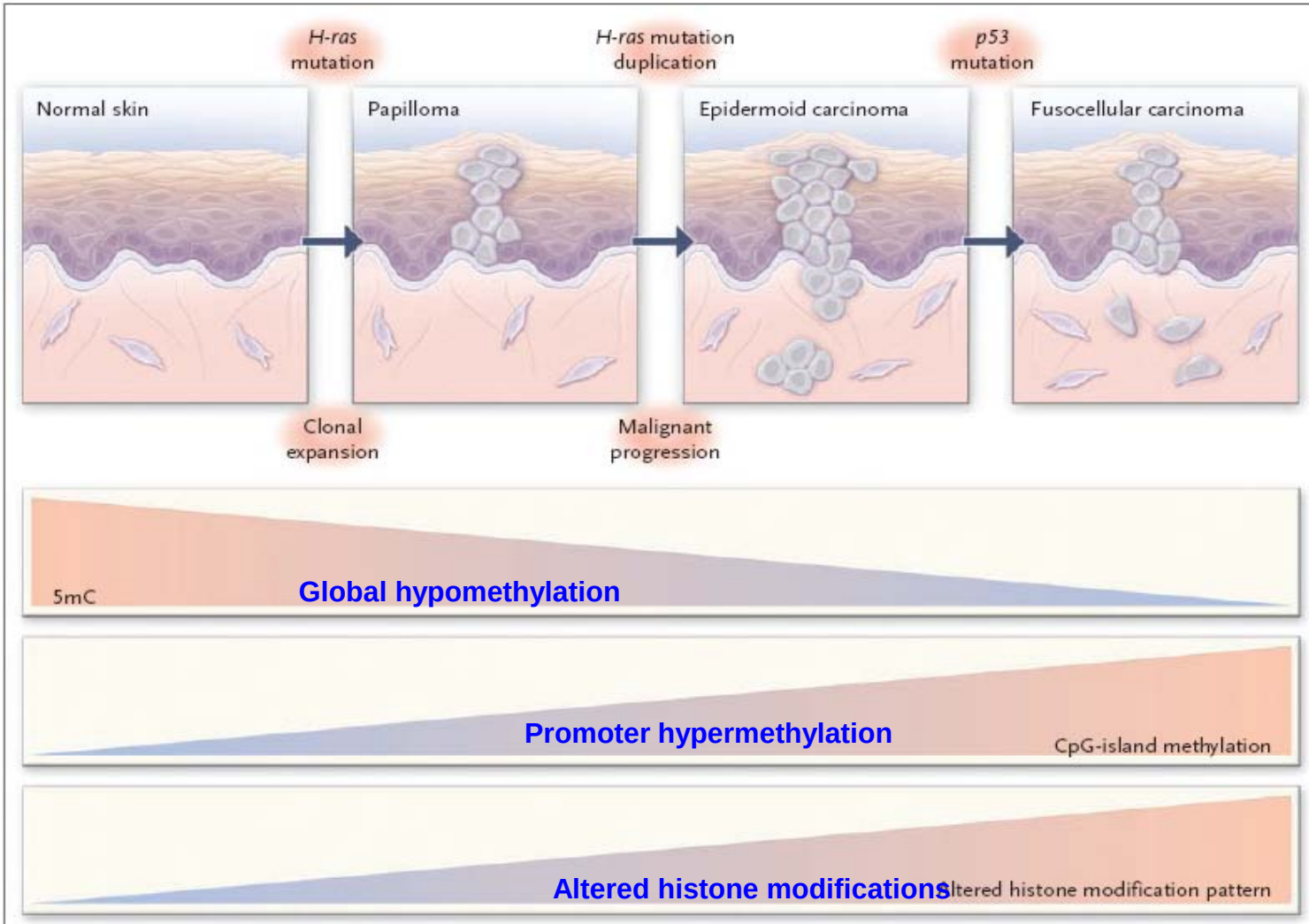


Figure 1. Epigenetic Alterations in Tumor Progression.
A multistage model of carcinogenesis in skin is shown. In conjunction with phenotypic cellular changes and the accumulation of genetic defects, there is a progressive loss of total DNA methylation content, an increased frequency of hypermethylated CpG islands, and an increased histone-modification imbalance in the development of the disease. *H-ras* denotes Harvey–ras oncogene, and 5mC 5-methylcytosine.

Cancer is a genetic and epigenetic disease

Esteller, NEJM, 358:1148-59, 2008

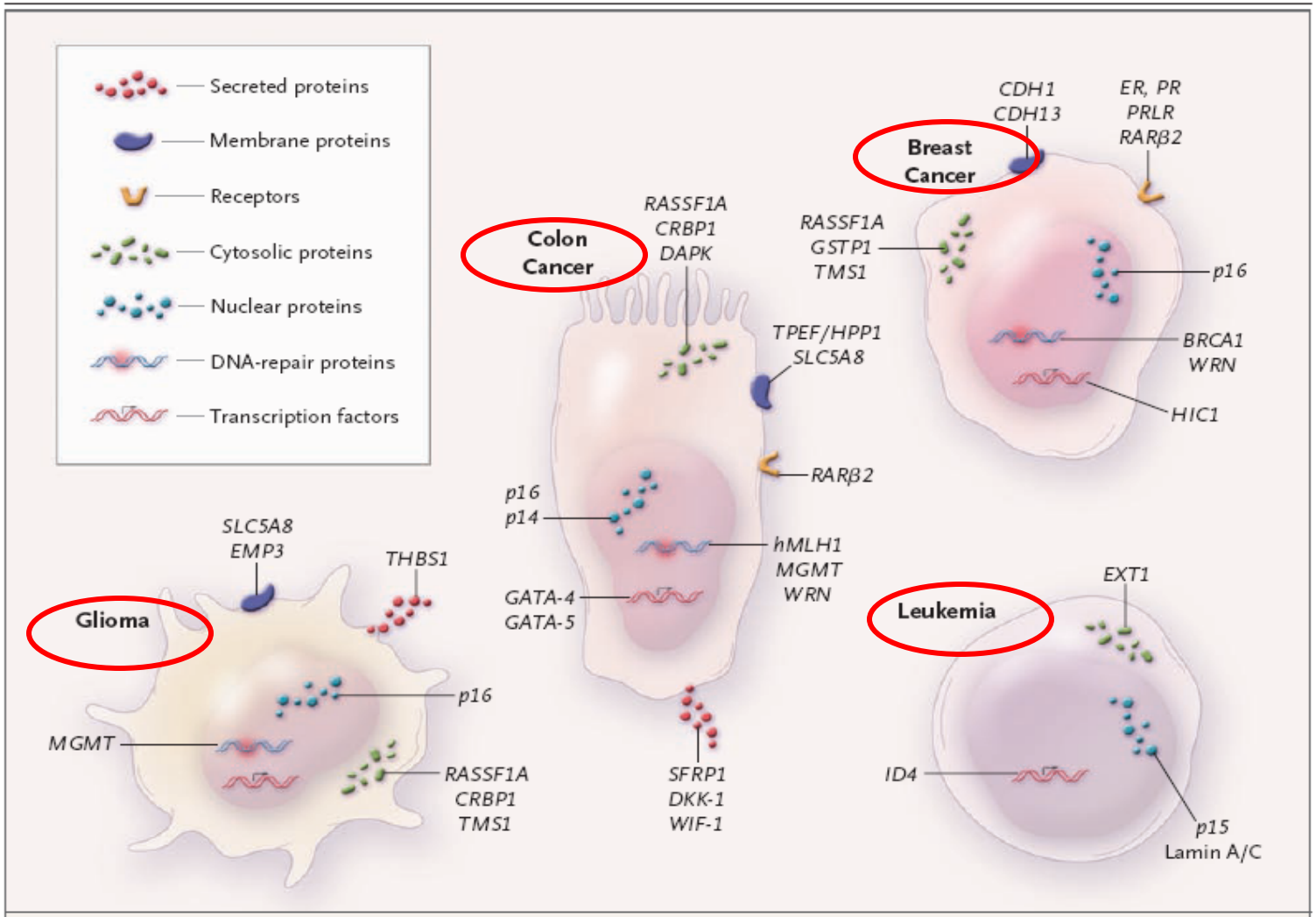


Figure 2. Profile of Hypermethylation of the CpG Island in the Promoter Region of Tumor-Suppressor Genes in Human Cancer.

Four tumor cells are shown undergoing transcriptional silencing by DNA hypermethylation of the regulatory regions of tumor-suppressor genes. In colon cancer, entrance into the cell cycle occurs by means of *p16*^{INK4a} methylation. In leukemia cells, *p15*^{INK4b} methylation initiates proliferation. In breast-cancer cells, defects in DNA repair are related to methylation of *BRCA1*, and in glioma cells, methylation of O^6-methylguanine–DNA methyltransferase (*MGMT*) initiates defects in DNA repair. Other depicted hypermethylated tumor-suppressor genes are *CDH1* (cadherin 1), *CDH13* (cadherin 11), *CRBP1* (cellular retinol binding protein 1), *DKK-1* (dickkopf homologue 1), *ER* (estrogen receptor), *GATA-4* (GATA-binding protein 4), *GATA-5* (GATA-binding protein 5), *HIC1* (hypermethylated in cancer 1), *PR* (progesterone receptor), *PRLR* (prolactin receptor), *RARβ2* (retinoic acid receptor β 2), *SLC5A8* (solute carrier family 5 iodide transporter member 8), *WIF-1* (WNT inhibitory factor 1), and lamin A/C.

Esteller, NEJM, 358:1148-59, 2008

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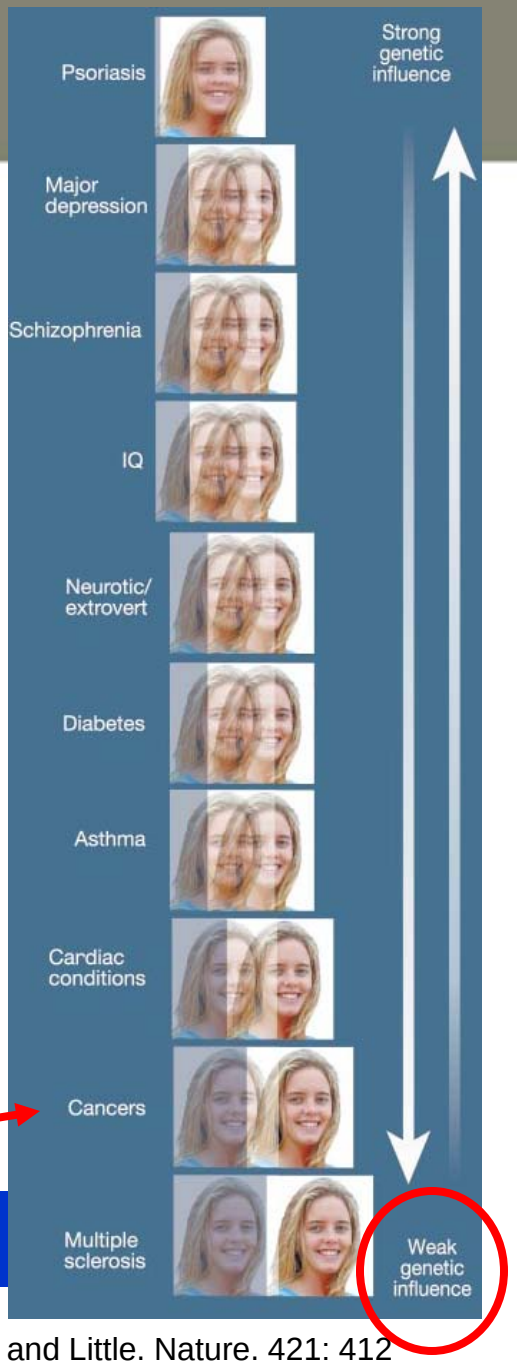
Epigenetics

Epigenetic events are alterations in gene expression without changes in the DNA coding sequence that are heritable through cell division

Epigenetic events occur early in the cancer development

Epigenetic changes occur during normal development but dysregulation of epigenetic events may result in cancer



Genetics of Epigenetics: Twin Studies

Chakravarti and Little. Nature. 421: 412

Epigenetic differences arise during the lifetime of monozygotic twins

Monozygous twins share a common genotype. However, most monozygotic twin pairs are not identical; several types of <u>phenotypic discordance</u> may be observed, such as differences in susceptibilities to disease and a wide range of <u>anthropomorphic features</u>.

Manel Estellar examined the global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins.

These findings indicate how an appreciation of epigeneticsis missing from our understanding of how different phenotypes can be originated from the same genotype.

EU Funded Twin Consortium http://www.genomeutwin.org

PNAS 102: 10604

Why Environmental Factors and Epigenetics?

- **More than 40% genes are regulated epigenetically**
- **Environmental carcinogens, such as arsenic and nickel, affect methylation of DNA and acetylation of histones, two key processes involved in epigenetic regulation of genes leading to cancer**
- **Arsenic gets methylated and thus depletes methyl group from the nutrients in the body**
- **Inhibition and reversal of nickel-induced transformation by the histone deacetylase inhibitor was observed**

Zhang et al.. Tox Appl Pharm 192: 201-211.

Loss (or Gain) of gene function in cancer

Most permanent → Most dynamic

Deletion
Point mutations
Amplification
Chromosomal Translocation (Ig rearrangement)

Chromatin Changes
Promoter Methylation Silencing

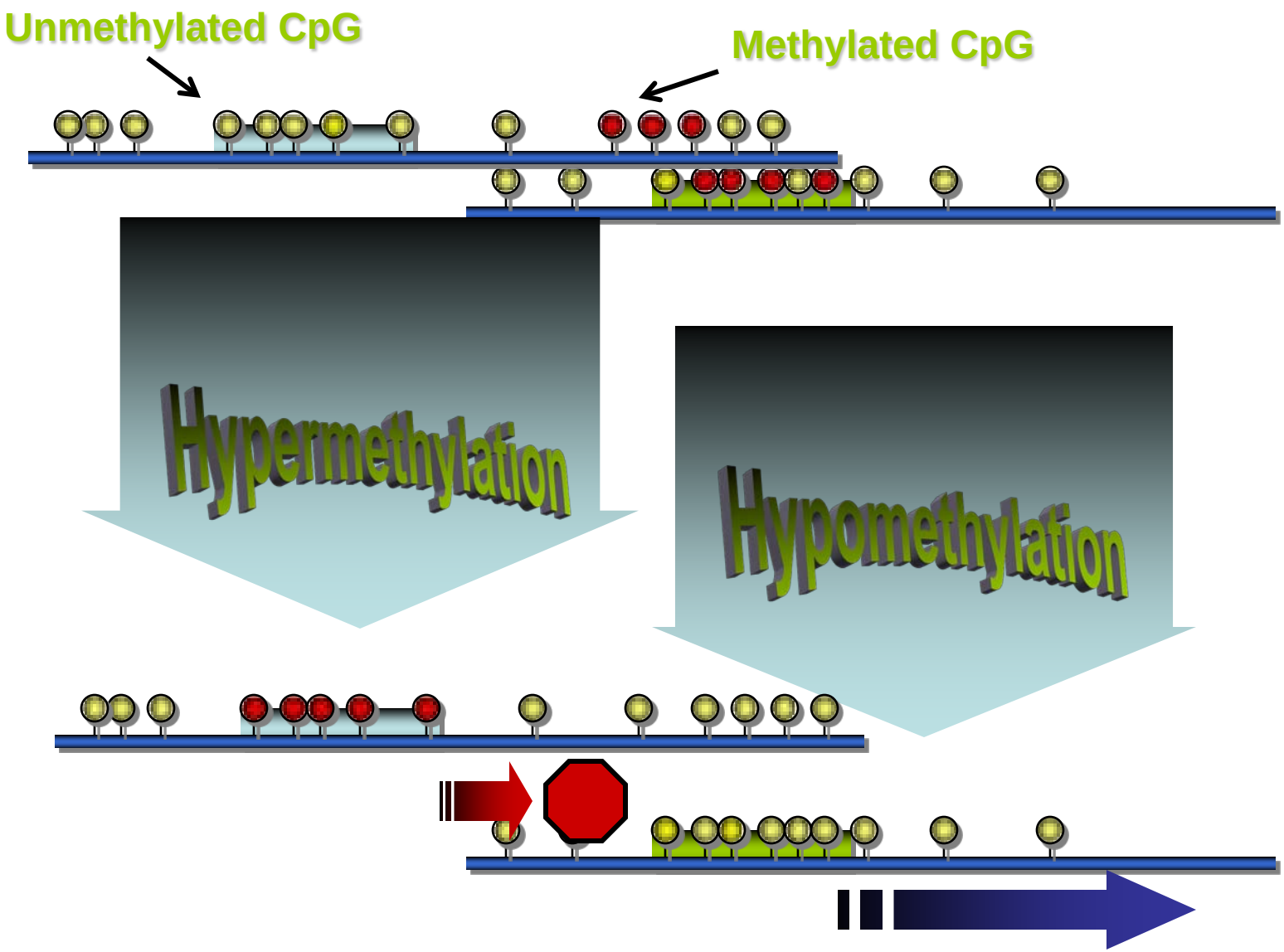
Transcription Factor Changes

Cell-cycle Regulated Changes

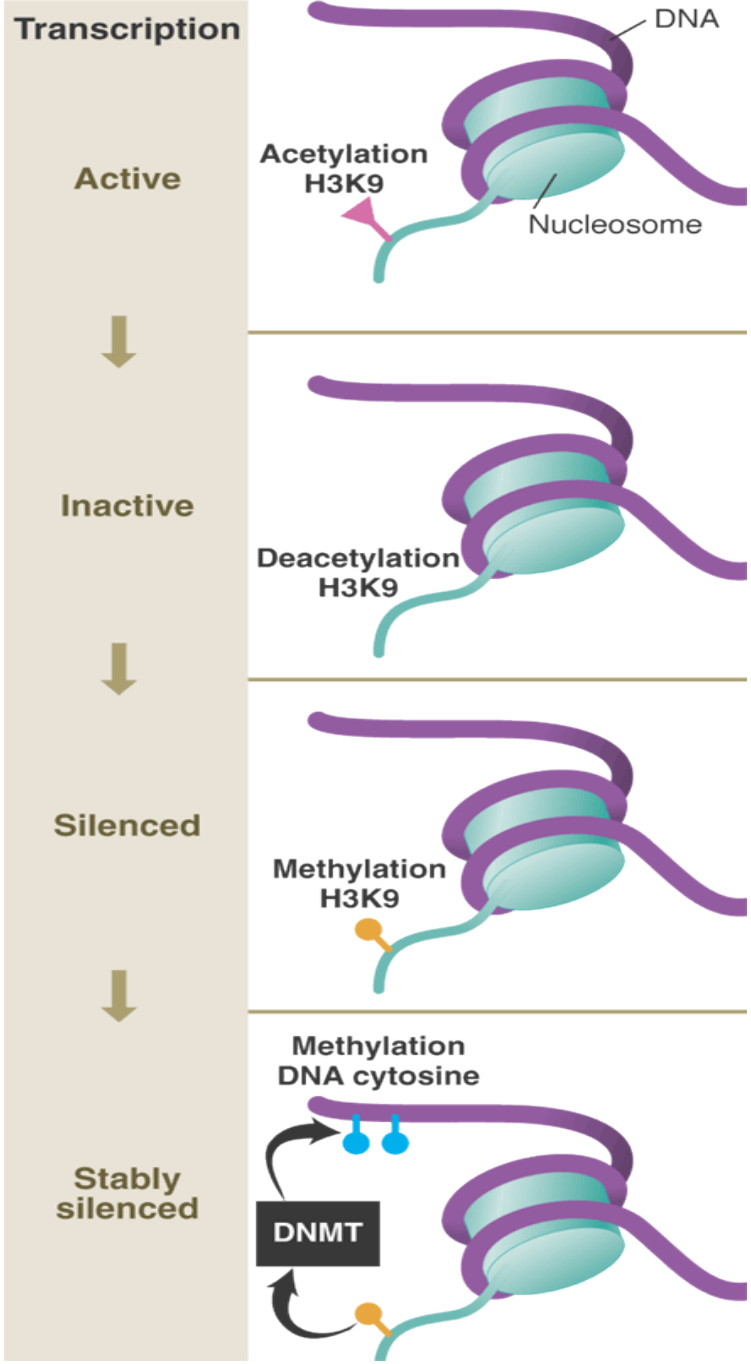
Genetic

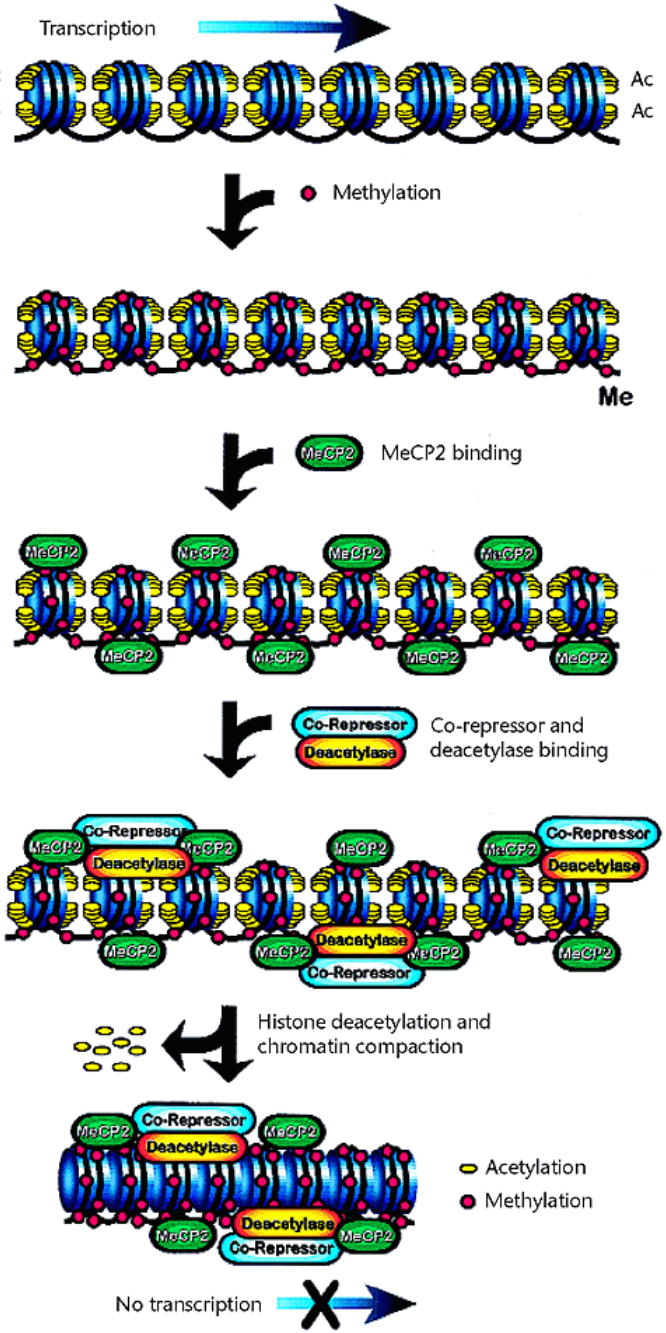
Epigenetic

Herman *Chest.* 125:119S-122S



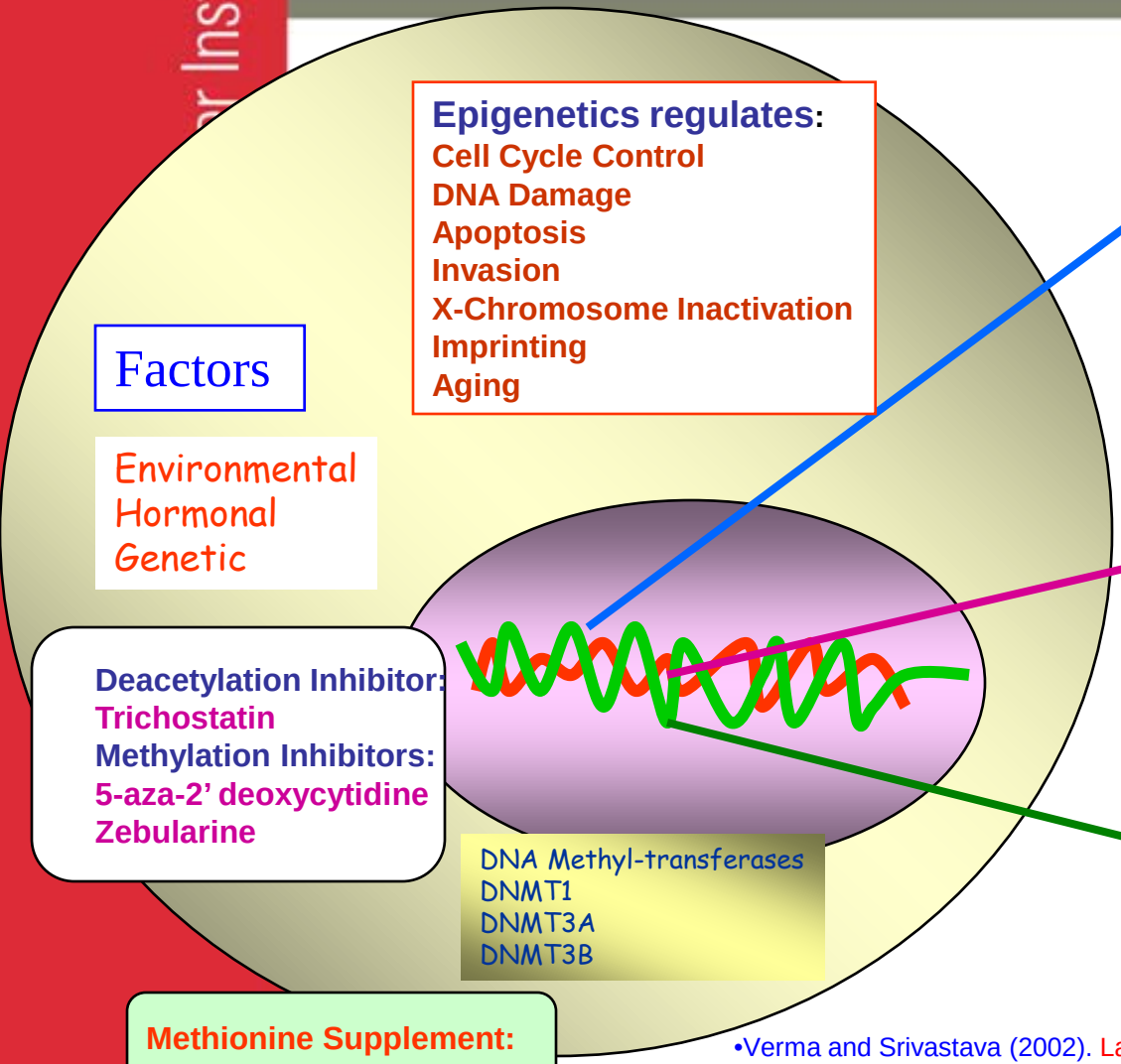
Asad Umar, NCI





Jones and Laird. *Nat Rev Gen* 21: 163

Epigenetic Markers and Targets



Hypermethylation

APC
BRCA1
ER
hMLH1
GSTP1
TIMP3
DAPK1

Hypomethylation

Raf
C-myc
C-fos
C-Ha-ras
C-K-ras

Acetylation/Deacetylation

Histone Acetyltransferase
Histone Decetylase
(Dynamic Equilibrium)

•Verma and Srivastava (2002). Lancet Oncol. 3: 755-363;
•Verma et al (2004) Crit. Rev. Clin. Sc. 41: 585-607;
•Verma and Manne (2006). Crit. Rev. Hematol. Oncol. 60: 9-18;
•Verma et al (2006). Mol. Diag.Therapy. 10: 1-15.

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May 3, 2005 • Volum

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Localized Prostate C
Deaths Remain Low
Years

Director's Update
Cancer Centers: Pro
Leadership and New
Opportunities

Spotlight
Cancer Epigenetics:

Patients

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Epigenetics and Cancer Prevention

Some cancers involve the inappropri-ate silencing or activation of genes through epigenetic changes—chemical modifications to DNA and proteins that control gene activity without causing a change in DNA sequence.

Though the epi
certain gene
life, epigen
stable than
may chang
epigenetic
by environ
drugs.

Epigenetic
essential f
lead to can
But the fla
potentially
even preve

For examp
mally prot
environme
prostate tu
by the addit
methyl groups
at Johns Hopkins Univ
oped an experimental
to detect this DNA
most common e
etic change.

"Cancer epigenetics is important for prevention because we may be able to use methylation markers to identify people at higher risk of cancer and perhaps detect cancer earlier," said Dr. Mukesh Verma in NCI's Division of Cancer Control and Population Sciences (DCCPS), one of several divisions conducting epigenetics research.

Investigators in DCP's Early Detection Research Network are engaged in analytical validation of *GSTP1* meth-ylation for prostate cancer, as well as

sites. A summary by Drs. Jacob Kagan and Sudhir Srivastava of DCP will be published soon in *Cancer Research*.

"We cannot reverse genetic mutations, but by using drugs we may be able to reverse changes in methylation," said Dr. Verma. "This approach has prom-ise for prevention."

Diet also may affect methylation. Giving pregnant mice diets rich in
including folic acid,
and genistein,
can modify the
f a certain gene
can cause a
at may be asso-
cancer risk.

n presumably
vironmental fac-
d that's intrigu-
ss of DCP.
understand-
lify epigenetic
h disease." ◆

betaine, choline, folate, B_{12}

Yellow Mouse
High risk of cancer, diabetes, obesity; reduced lifespan

Agouti Mouse
Lower risk of cancer, diabetes, obesity; prolonged life

Mouse models are helping researchers understand the genetic and epigenetic changes associated with cancer, as well as factors that can influence this risk.

"Cancer epigenetics is important for prevention because we may be able to use methylation markers to identify people at higher risk of cancer and perhaps detect cancer earlier"

- Mukesh Verma, DCCPS

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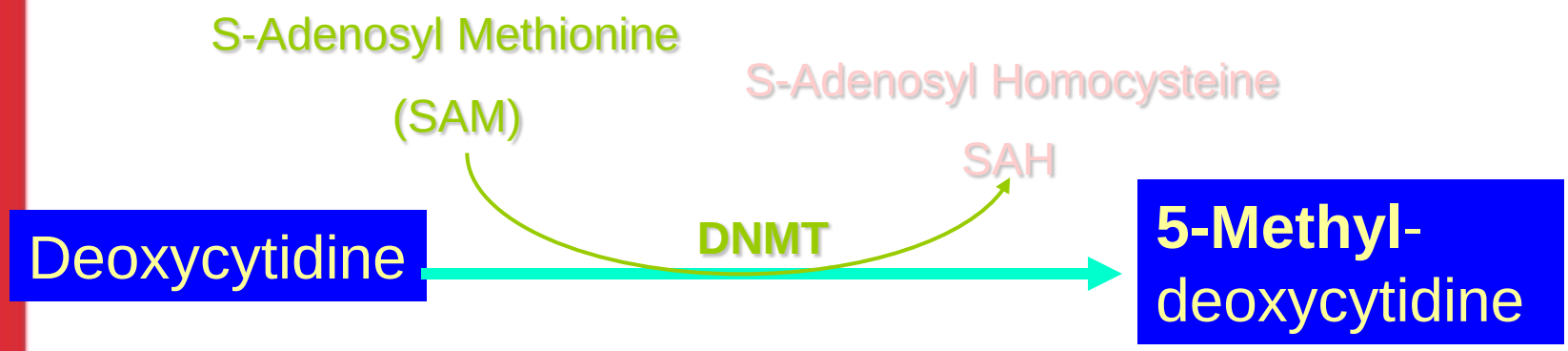
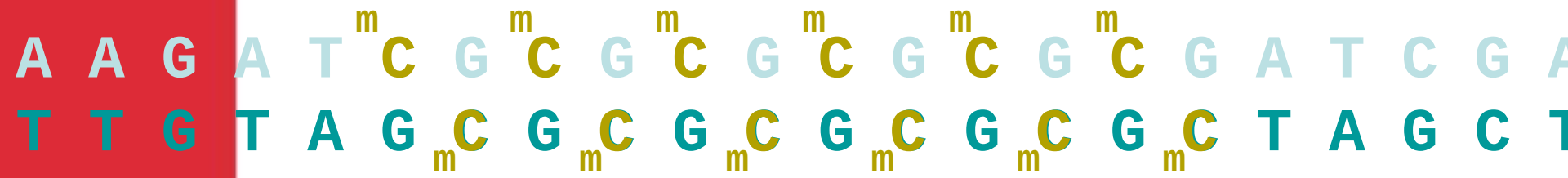
March 13, 2007

Mechanism of DNA methylation





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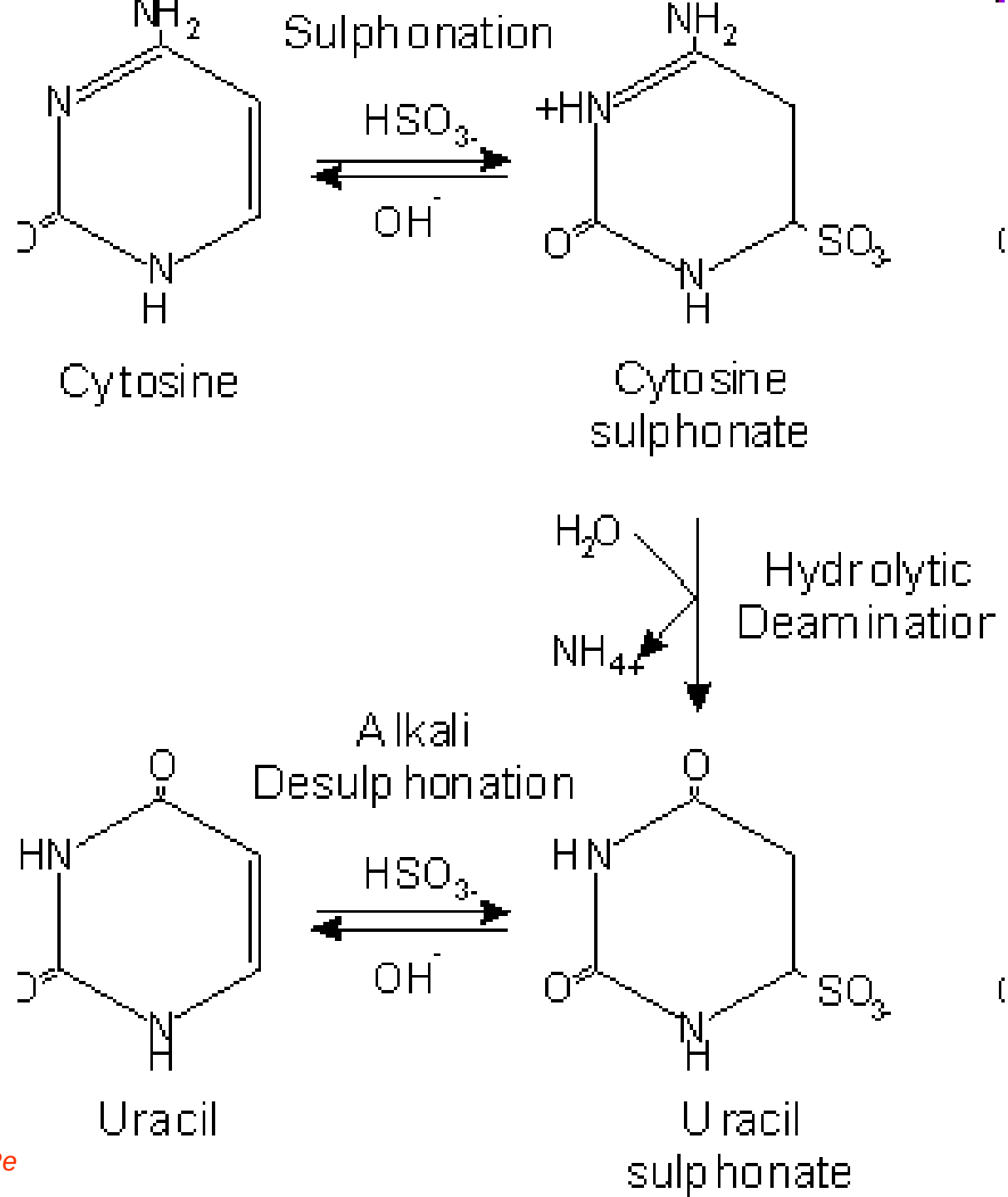
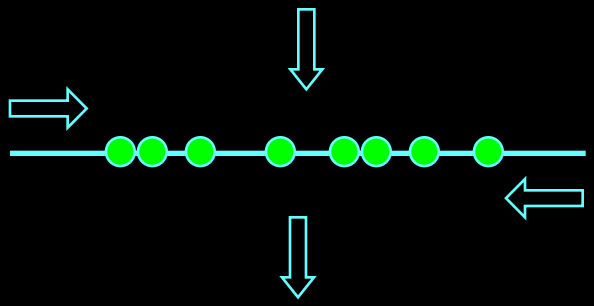
Asad Umar


Sulphonation
HSO_3^-
OH^-
Cytosine
Cytosine sulphonate
H_2O
NH_4^+
Hydrolytic Deamination
Alkali Desulphonation
HSO_3^-
OH^-
Uracil
Uracil sulphonate

Laird. *Nat Re*

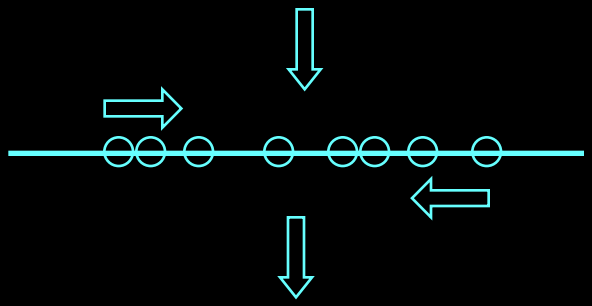
Bisulfite-Dependent DNA Methylation Analysis

Bisulfite PCR

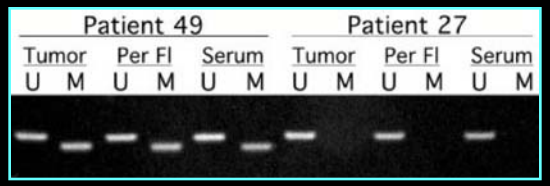


Analysis of PCR Product

Methylation-Specific PCR

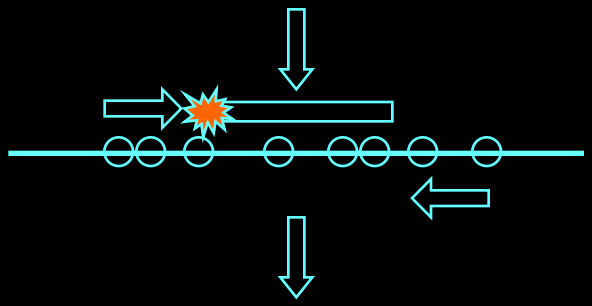


PCR Product Yes or No?

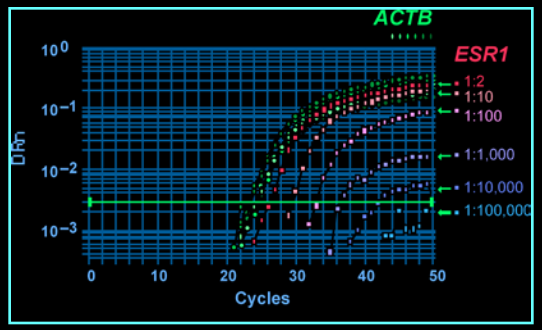


Herman (1996) PNAS 93, 9821

MethyLight



Real-Time PCR Analysis



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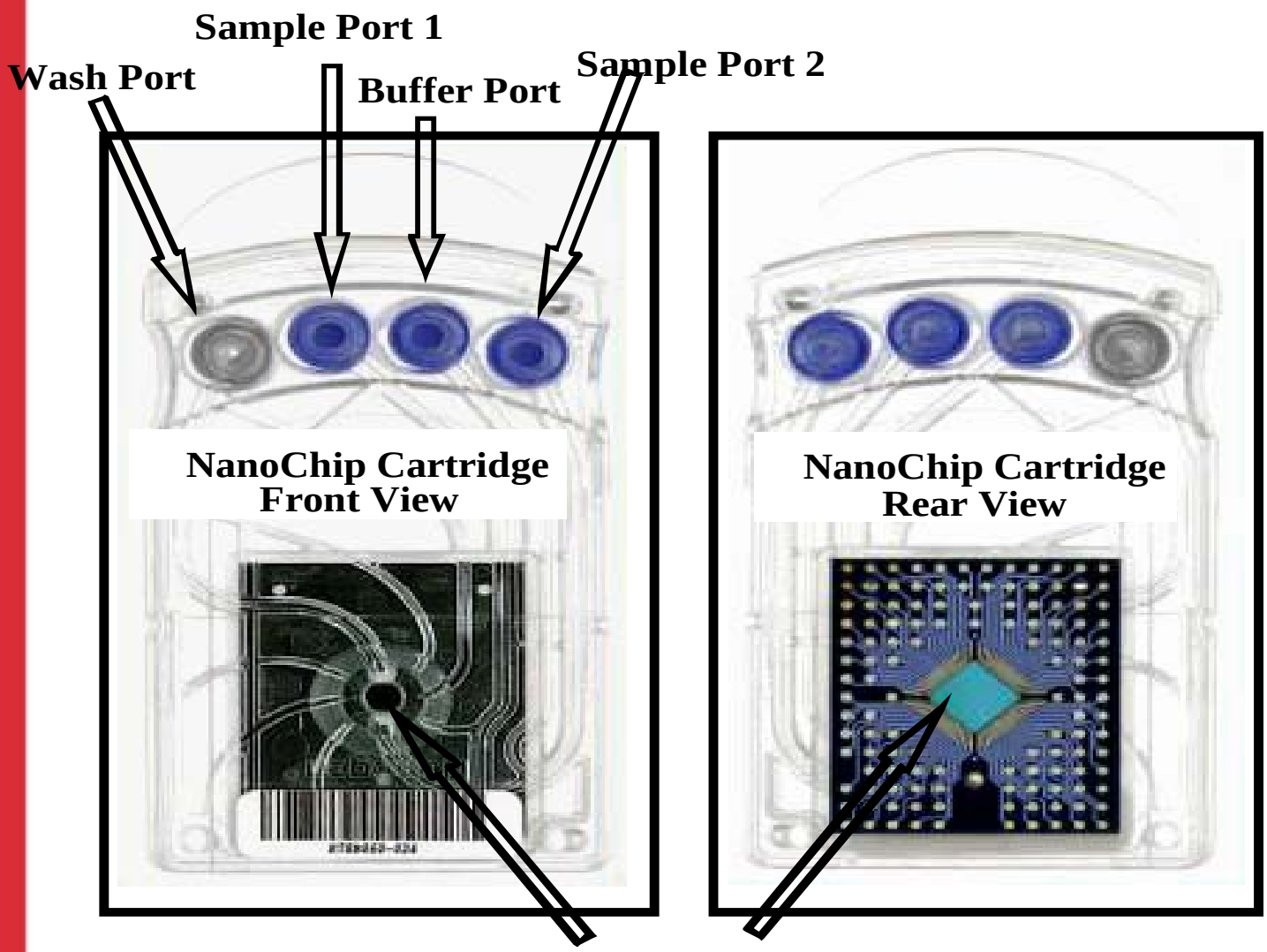
Nanochip to Detect Hypermethylation



Micro-array test sites
connected with platinium wires

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DNA Methylation Detection by Hybridization of Bisulphite DNA

EPIGENOMICS, Inc.

Nimblegen

Qiagen

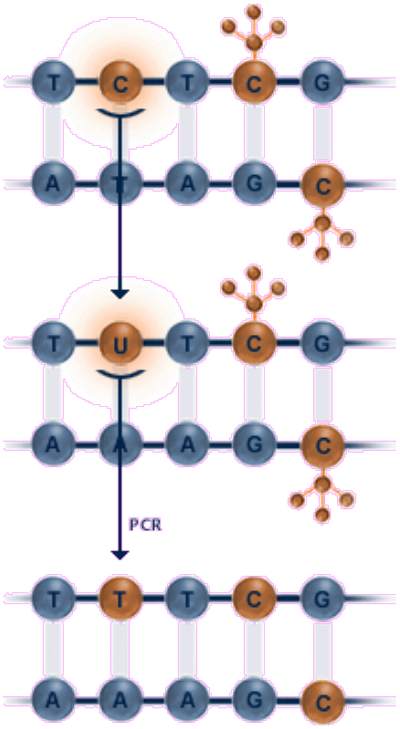
Roche 454

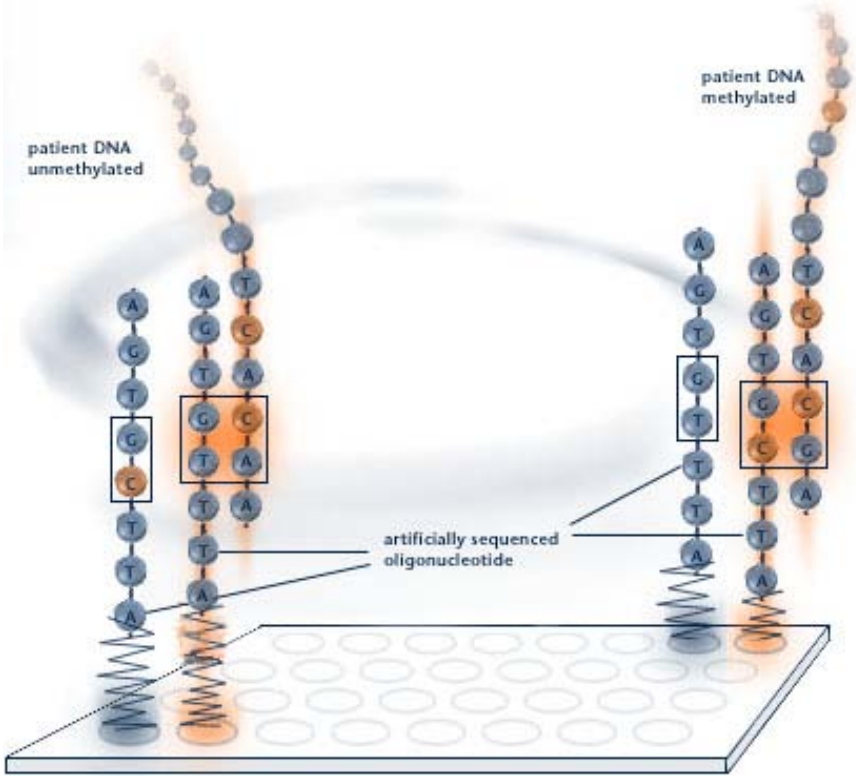
Illumina

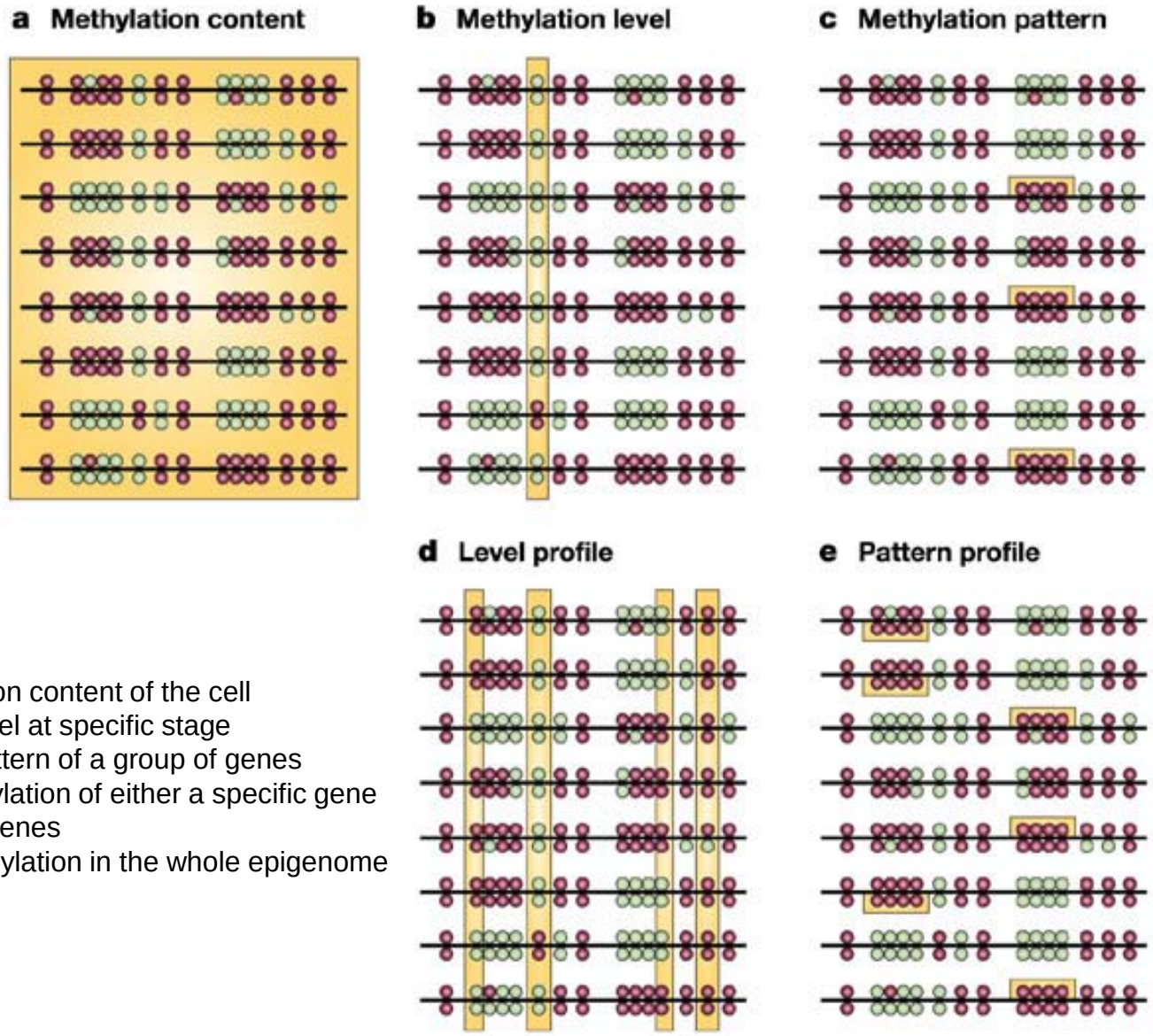
Solexa

Biotage, Inc.

Agilent





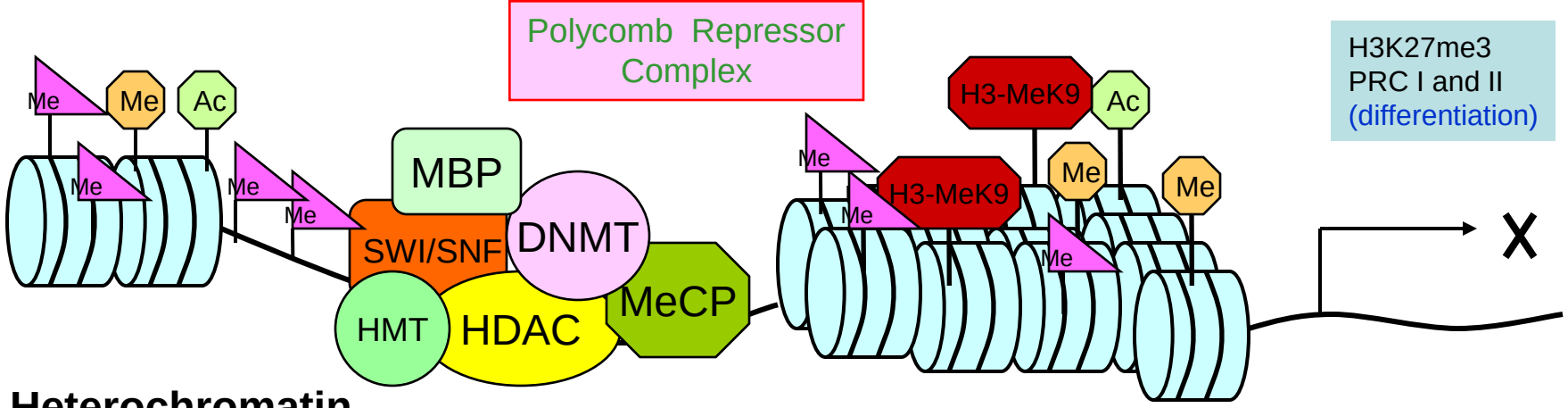


- Total methylation content of the cell
- methylation level at specific stage
- methylation pattern of a group of genes
- profile of methylation of either a specific gene or a number of genes
- pattern of methylation in the whole epigenome

To reduce
- false negative
- false positives

Laird *Nat Rev Cancer* 3:253

Nature Reviews | Cancer



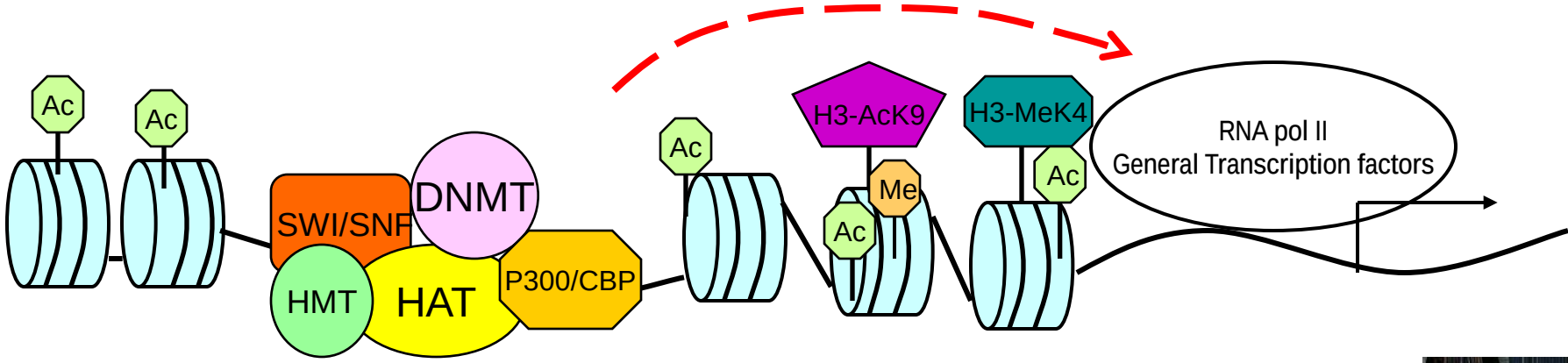
Heterochromatin

Silenced histone code (SHC)

Histone coding for silencing

Histone coding for transcription

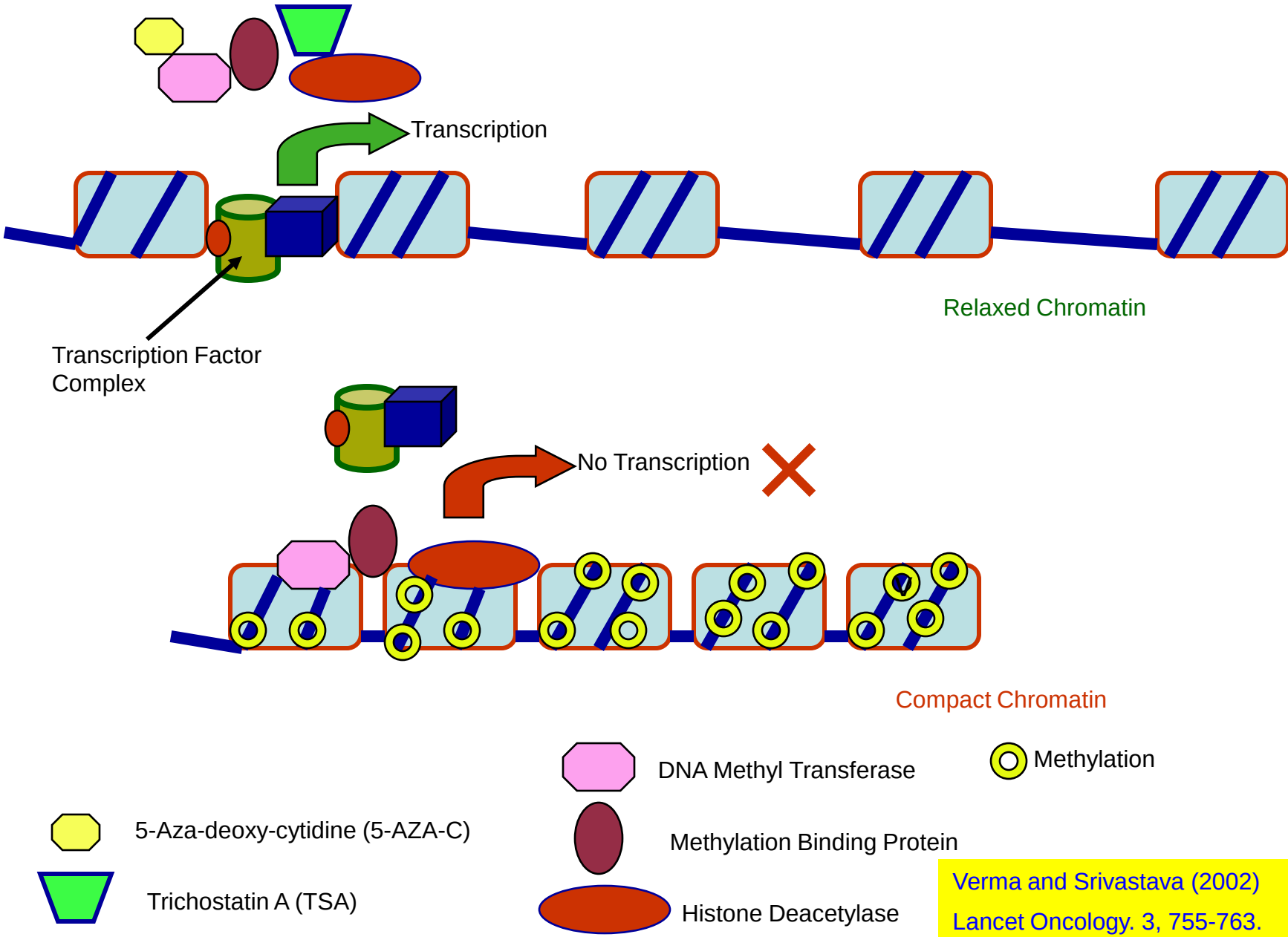
H3K9me
Next amino acid is ser -
Upon phosphorylation,
Change is permanent



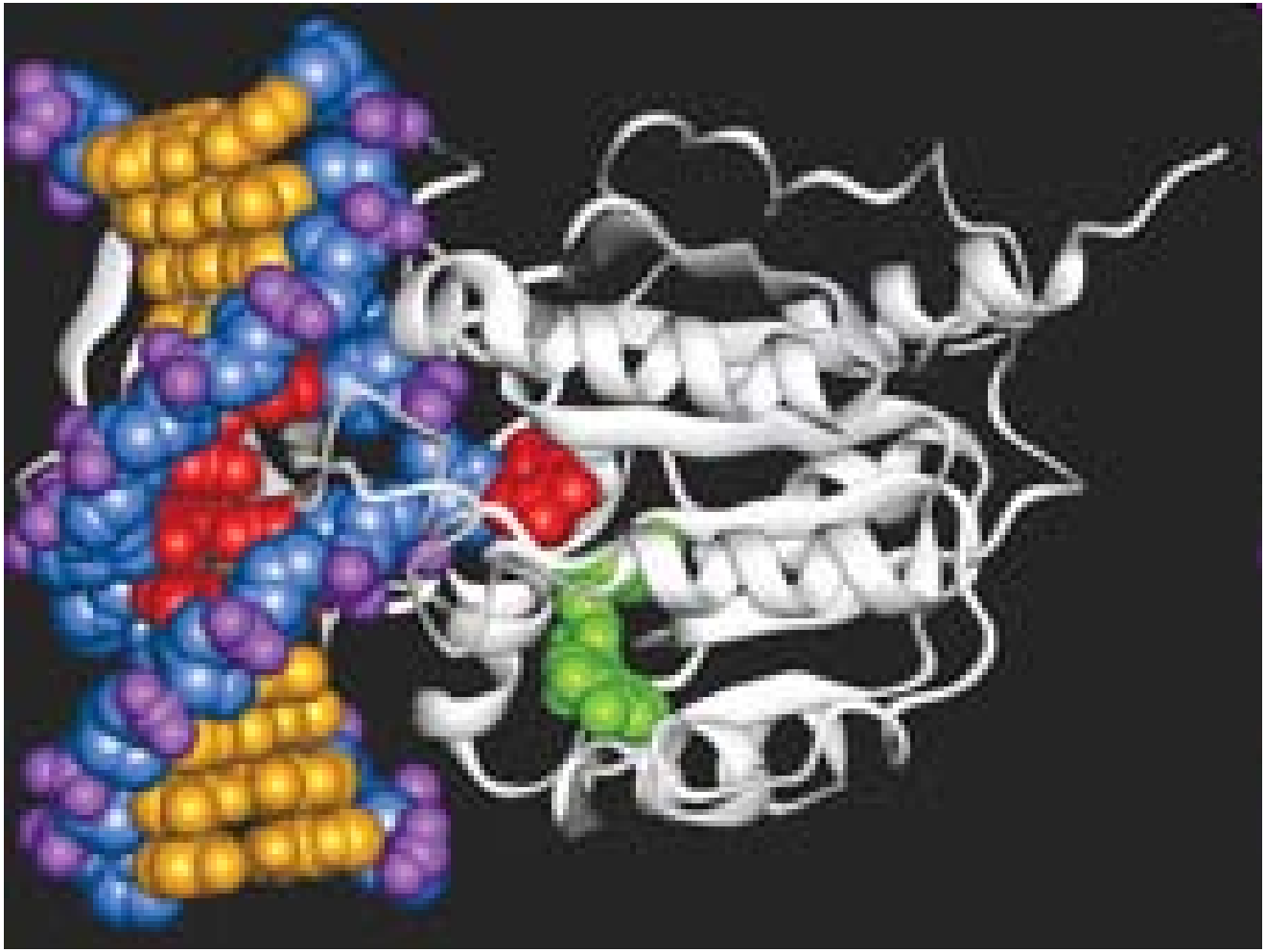
Euchromatin

Active histone code (AHC)

Inhibitors of DNA Methyltransferase (5-AZA-C) and Histone Deacetylase (TSA) can Restore Gene Activation



Verma and Srivastava (2002) Lancet Oncology. 3, 755-763.



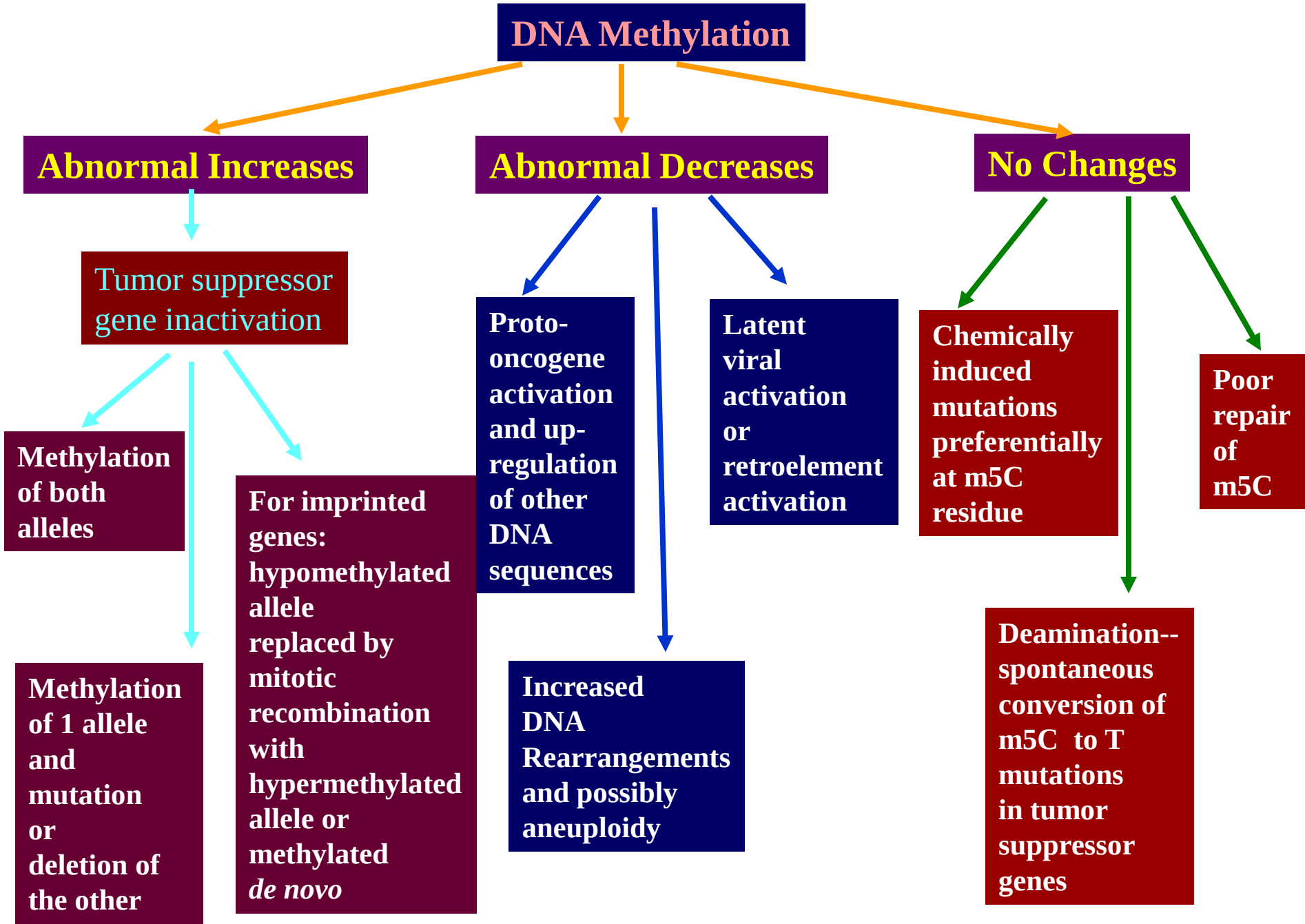
- Merck
- GlxSmith
- J & J
- Hoffman LaRoche
- Novartis
- Astra-Zeneca

A methylating enzyme (white)

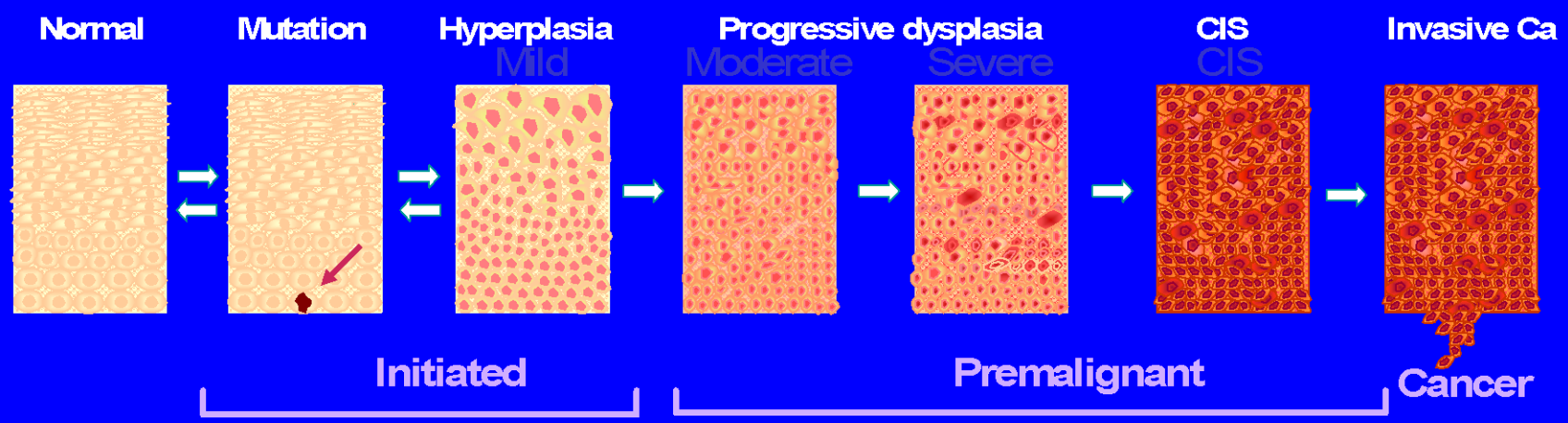
binds to its target site (red) on DNA; the methyl donor is shown in green.

Dennis NATURE 421: 686

DNA Methylation and Carcinogenesis



Cancer Development is a Multi-step Process



Breast	14-18 yrs →	6-10 yrs →
Cervix	9-13 yrs →	10-20 yrs →
Colon	5-20 yrs →	5-15 yrs →
Prostate	20 yrs →	3-15 yrs →
Lung	? yrs →	Many yrs →

Markers in Epidemiology Research

Currently Used:
Genetic markers
Biochemical (enzymatic or proteomic) markers

Unexplored:
Epigenetic markers

In epidemiology, biomarkers are used to follow disease prevalence by determining their level in cohort studies with potential of identifying the high risk population

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Examining Epigenetic Markers

Technology exists to utilize these markers for population studies

Easy to assay in small amount of sample (MS-PCR based assay)

Automation possible (nanochips)

Suitable for samples already collected for epidemiologic studies (tissue, blood, exfoliated cells)

Small Hurdle: Familiarity with technology

Tumor Types and Genes Regulated by Epigenetic Mechanism

TUMOR LOCATION	*GENE*
Breast	p16, BRCA1, GSTP1, DAPK, CDH1, TIMP-3
Brain	p16, $p14^{ARF}$, MGMT, TIMP-3
Bladder	p16, DAPK, APC
Colon	p16, $p14^{ARF}$, CRBP1, MGMT, hMLH1, DAPK, TIMP-3, APC
Endometrium	hMLH1
Esophagus	p16, $p14^{ARF}$, GSTP1, CDH1APC
Head and Neck	p16, MGMT, DAPK
Kidney	p16, $p14^{ARF}$, MGMT, GSTP1, TIMP-3, APC
Leukemia	p15, MGMT, DAPK1, CDH1, p73
Liver	p16, CRBP1, GSTP1, APC
Lymphoma	p16, p15, CRBP1, MGMT, DAPK, p73
Lung	p16, $p14^{ARF}$, CRBP1, MGMT, GSTP1, DAPK, FHIT, TIMP-3, RARbeta, RASSF1A
Ovary	p16, BRCA1, DAPK
Pancreas	p16, MGMT, APC
Prostate	GSTP1, p27(kip1)
Stomach	$p14^{ARF}$, P16, APC, hMLH1, MGMT
Uterus	p16, $p14^{ARF}$, hMLH1

Verma and Srivastava (2002). Lancet Oncol. 3: 755-363; Verma et al (2004) Crit. Rev. Clin. Sc. 41: 585-607; Verma and Manne (2006). Crit. Rev. Hematol. Oncol. 60: 9-18; Verma et al (2006). Mol. Diag. Therapy. 10: 1-15.

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Molecular Targets

Tumor suppressor genes

APC, p15, p16, p73, ARF/INK4A, VHL, ER, RARbeta, AR, HIC1, Rb

Invasive/Metastasis suppressor genes

E-cadherin, TIMP-3, mts-1, CD-44

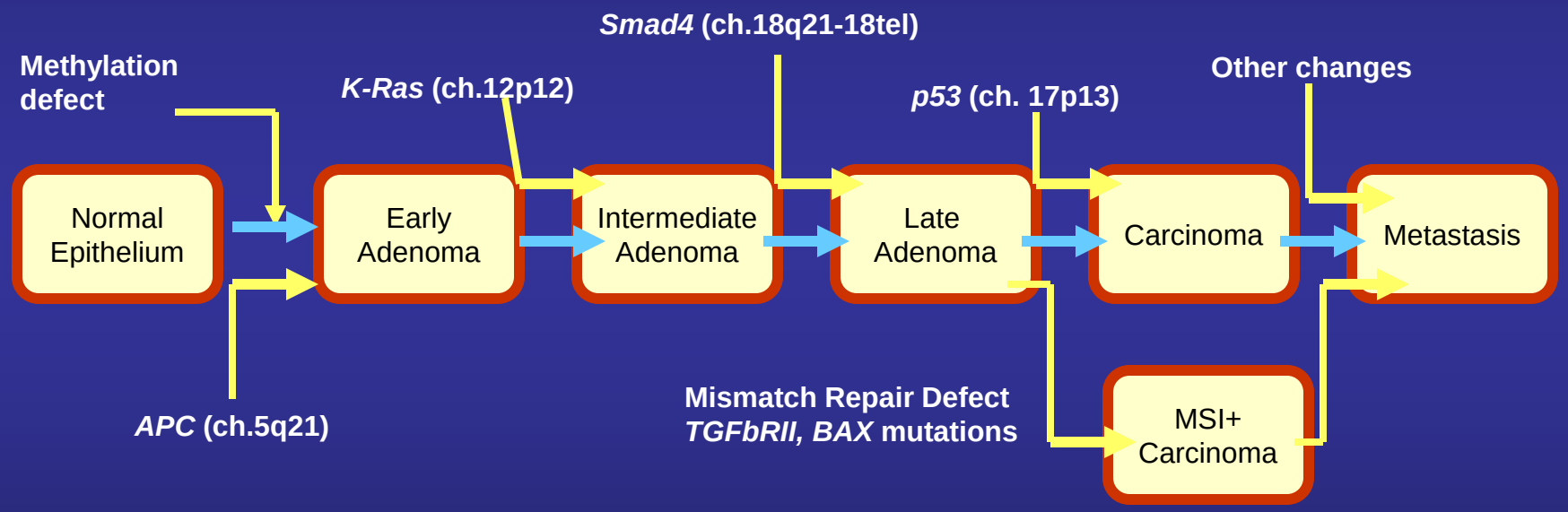
DNA repair genes

Methylguanine methyl transferase, hMLH1, BRCA1, GST

Angiogenesis

Thrombospondin-1 (TSP-1), TIMP-3

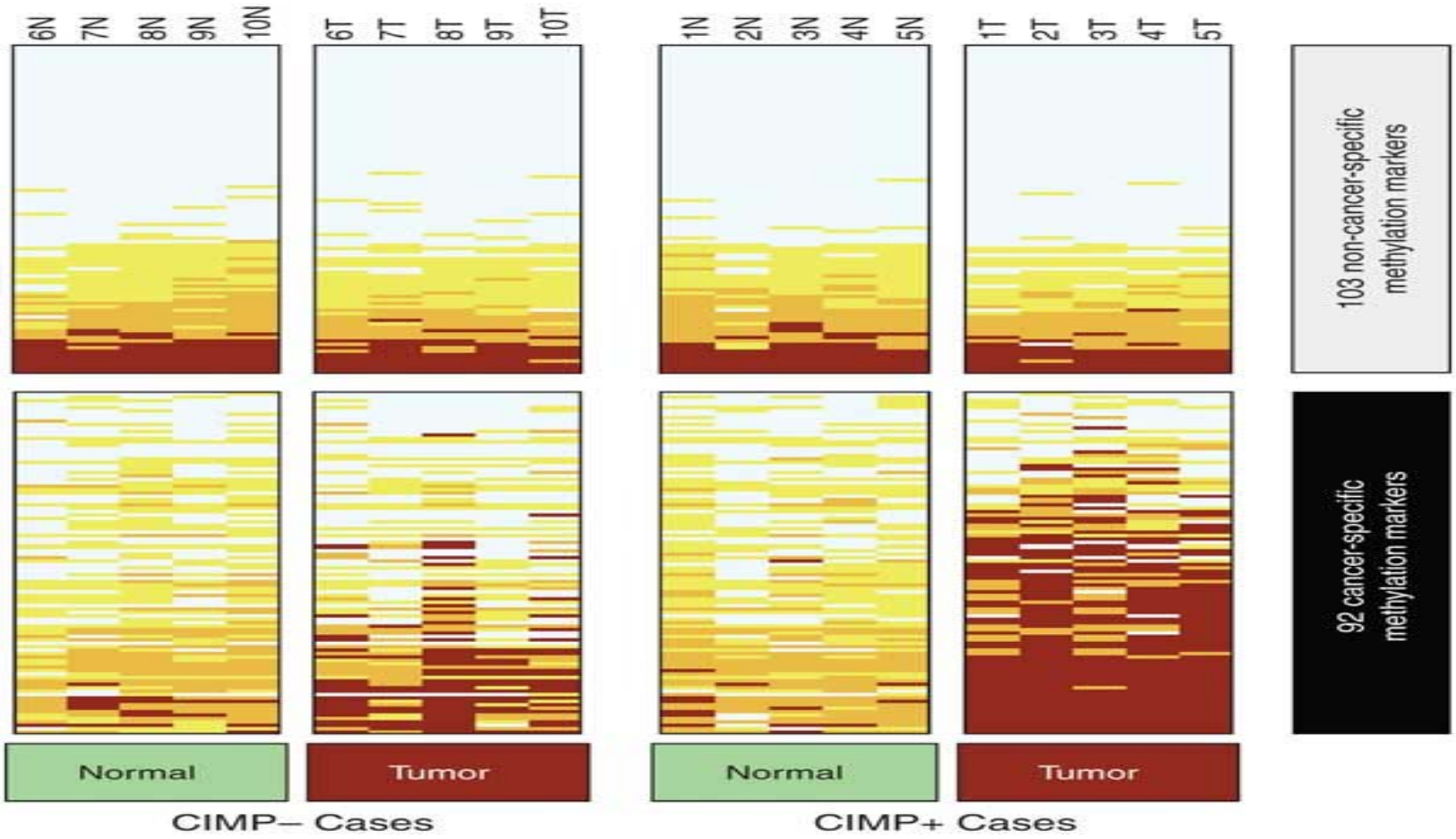
Potential Steps for Intervention



A Model for Colorectal Tumorigenesis

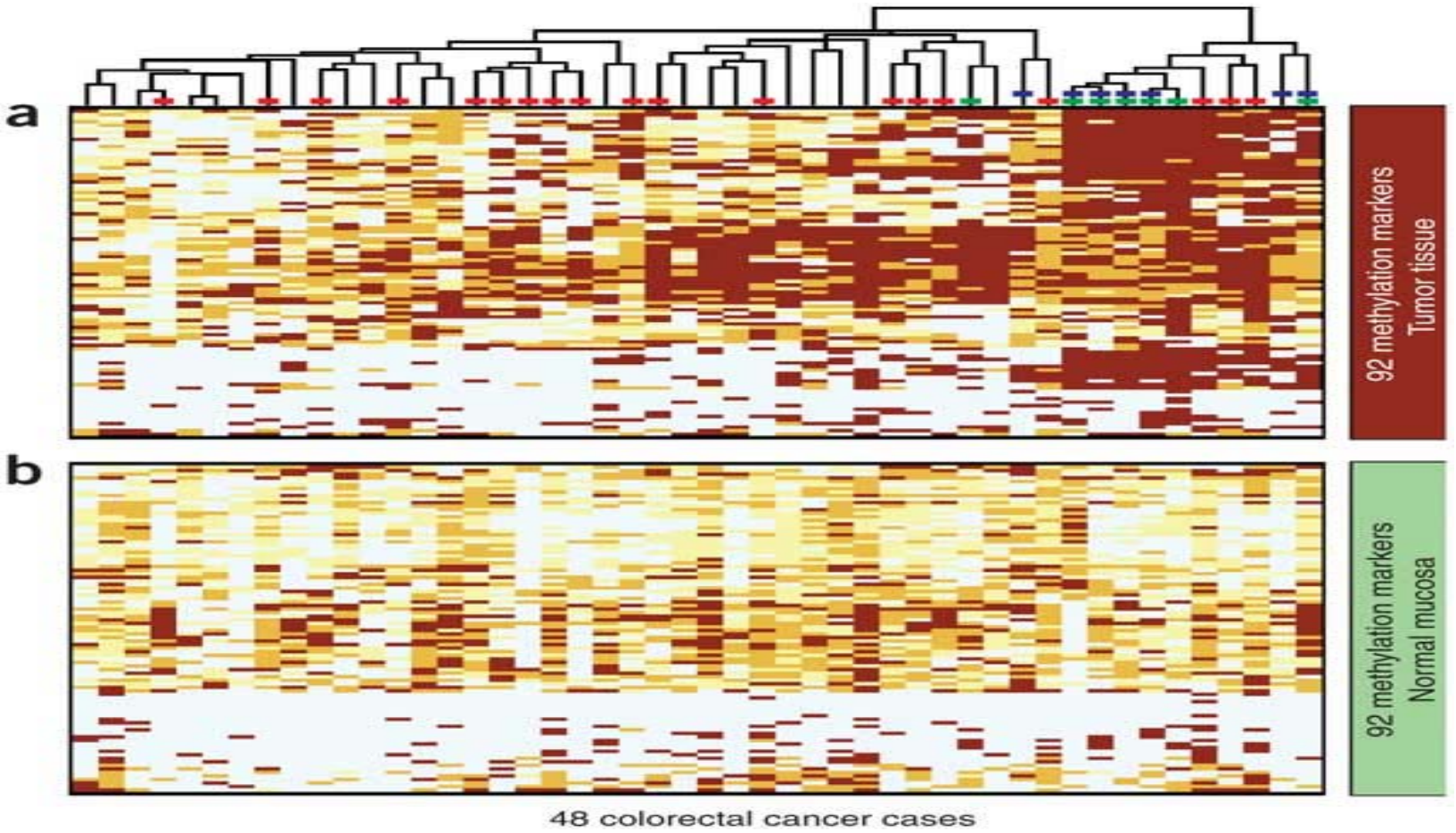
Modified from Jubb et al. *J Path*. 195: 111.

CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer



Nature Genetics ***38****, 787 - 793*

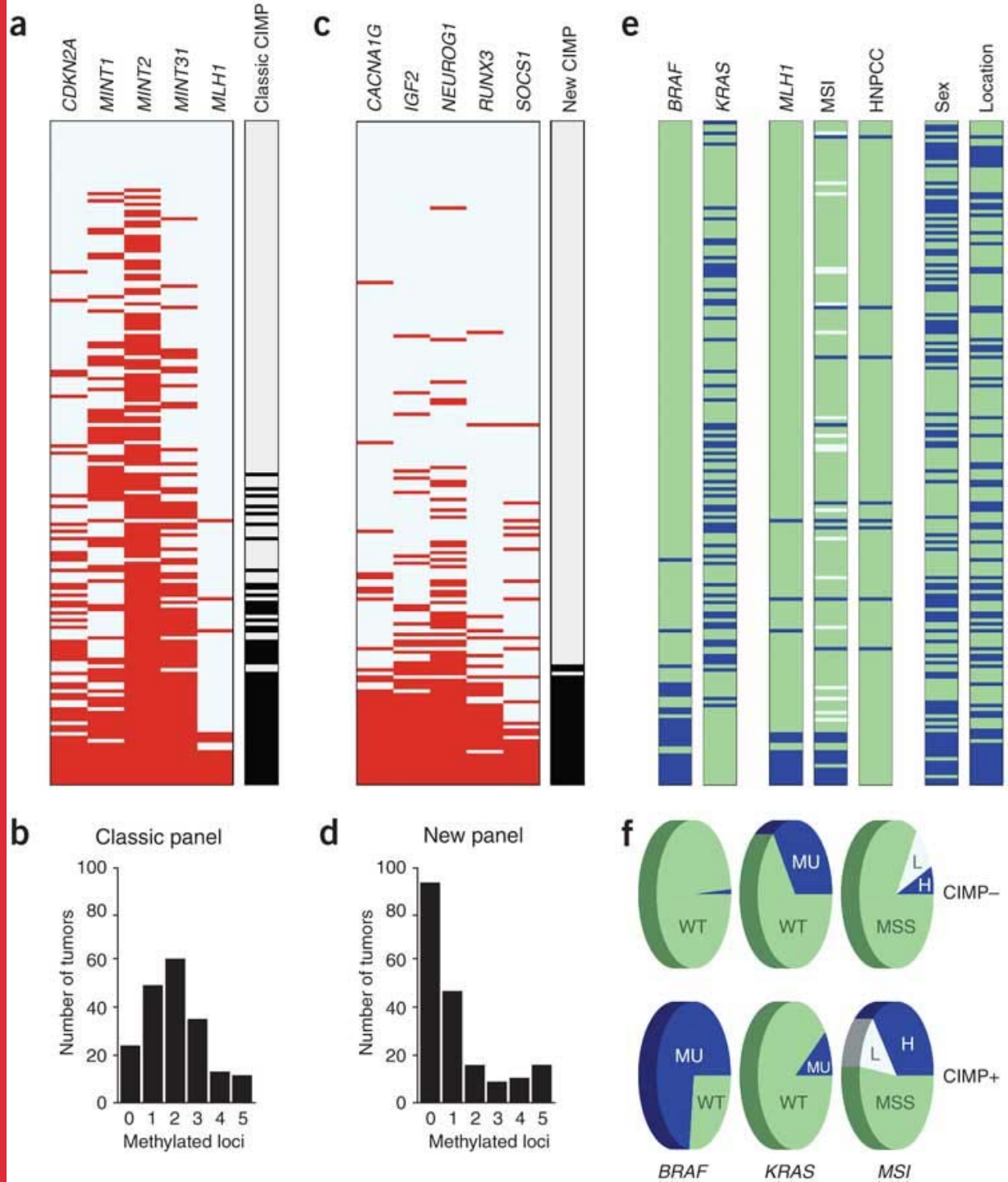
Identification of tumor clusters.



KRAS mutation indicated by a red rectangle overlaying the branch,
BRAF mutations indicated by a green rectangle
MSI-H cases designated with a blue rectangle.

48 Colorectal tumors

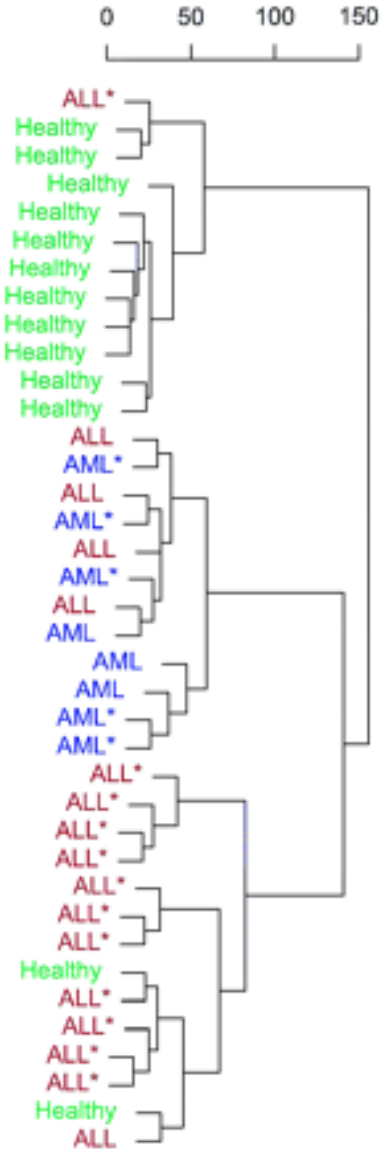
Nature Genetics ***38****, 787 - 793*



MSI-high (MSI-H) (blue),
MSI-low (MSI-L) (light blue)
or microsatellite stable (MSS) (green).

National Cancer Institute

Prediction of Tumor Class based on Methylation Analysis (AML and ALL)



AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia

Lymphoma

Adorjan et al *Nuc Ac Res*. 30: e21

Epigenetic Markers During Lung Cancer Progression

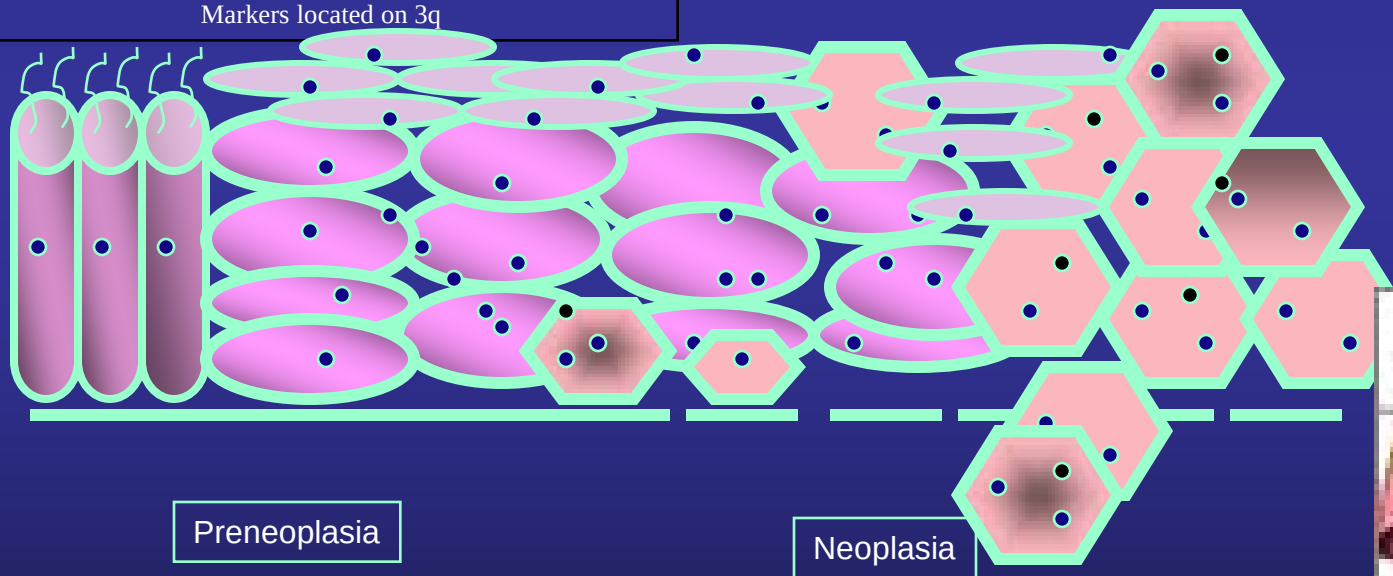
Annexin I and II

PIK3CA

p63

RARbeta,
DAPK, GSTP1, FHIT
RASSF1, MGMT

Markers located on 3q



Preneoplasia

Neoplasia

Small Cell Lung Carcinoma

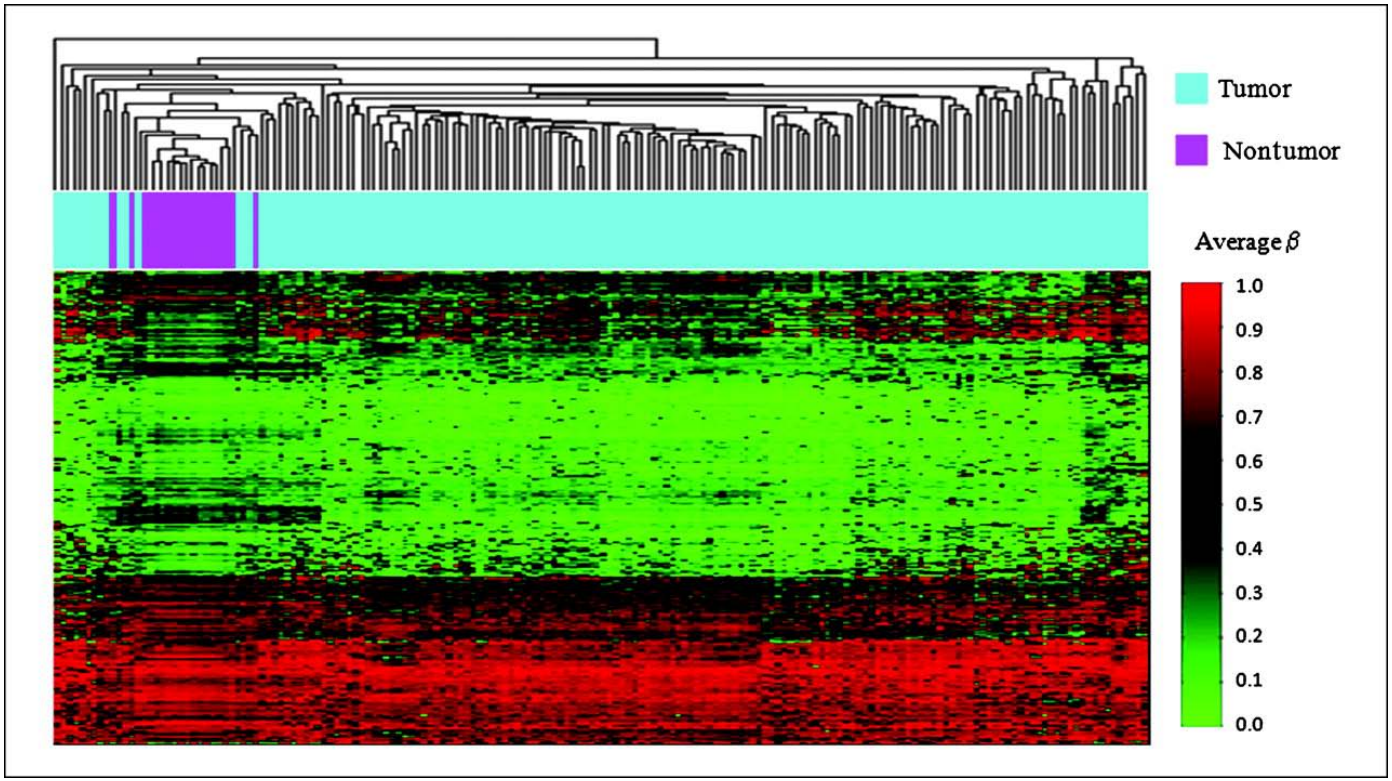
Progression of Lung Cancer

Figure 1. Unsupervised clustering of average {beta} values in tumor and nontumor pleura

ASBESTOS

MESOTHELIOMA

Non-Mutagenic carcinogen



803 cancer related genes

158 pleural mesothelioma with minimum mutation

18 normal pleura

Prediction of survival

Christensen, B. C. et al. Cancer Res 2009;69:227-234

Epigenetic Profiles Distinguish Pleural Mesothelioma from Normal Pleura and Predict Lung Asbestos Burden and Clinical Outcome

Cancer Research

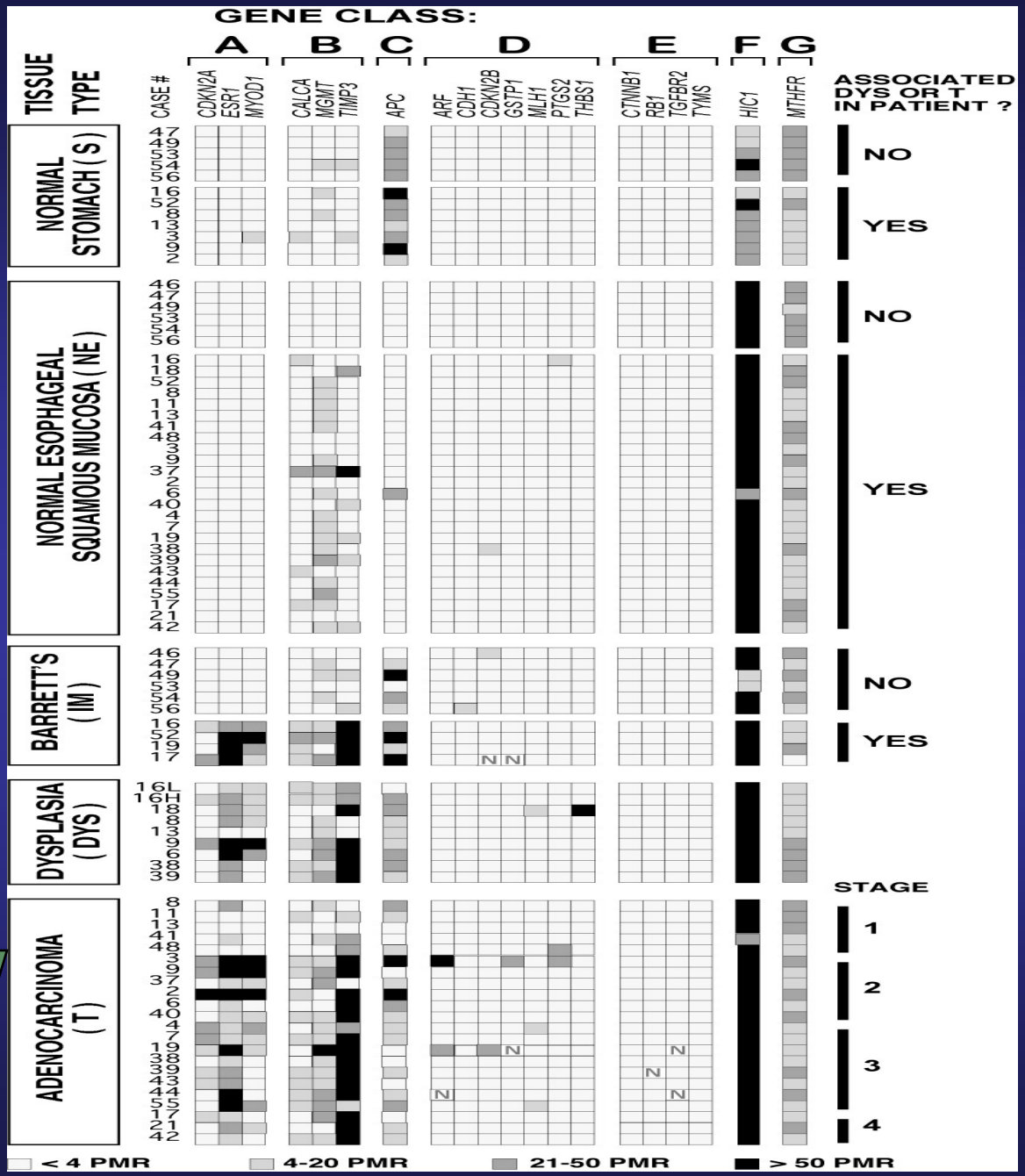
Copyright ©2009 Amer

Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

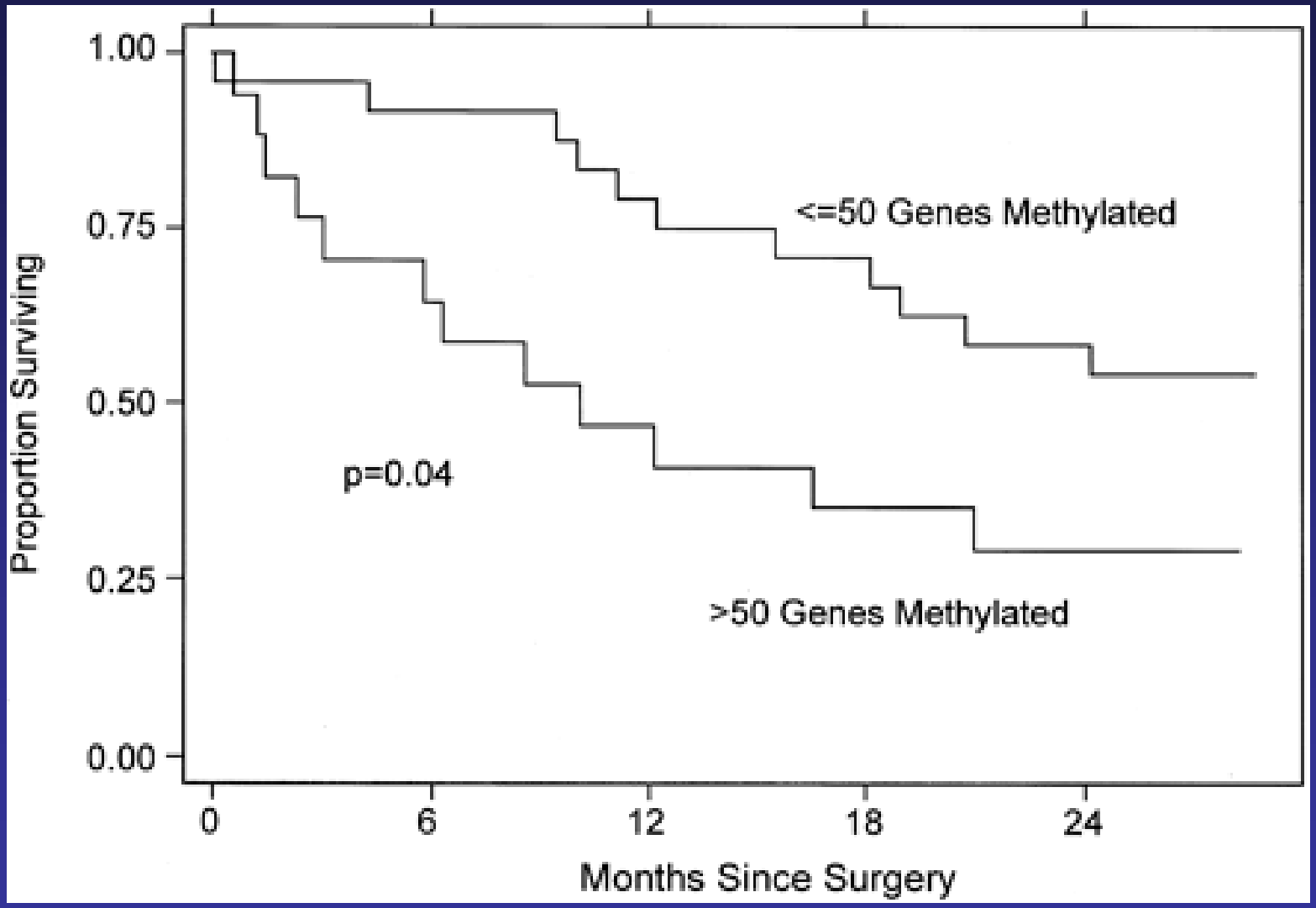
Cancer Research

61:3410

Cancer Progression

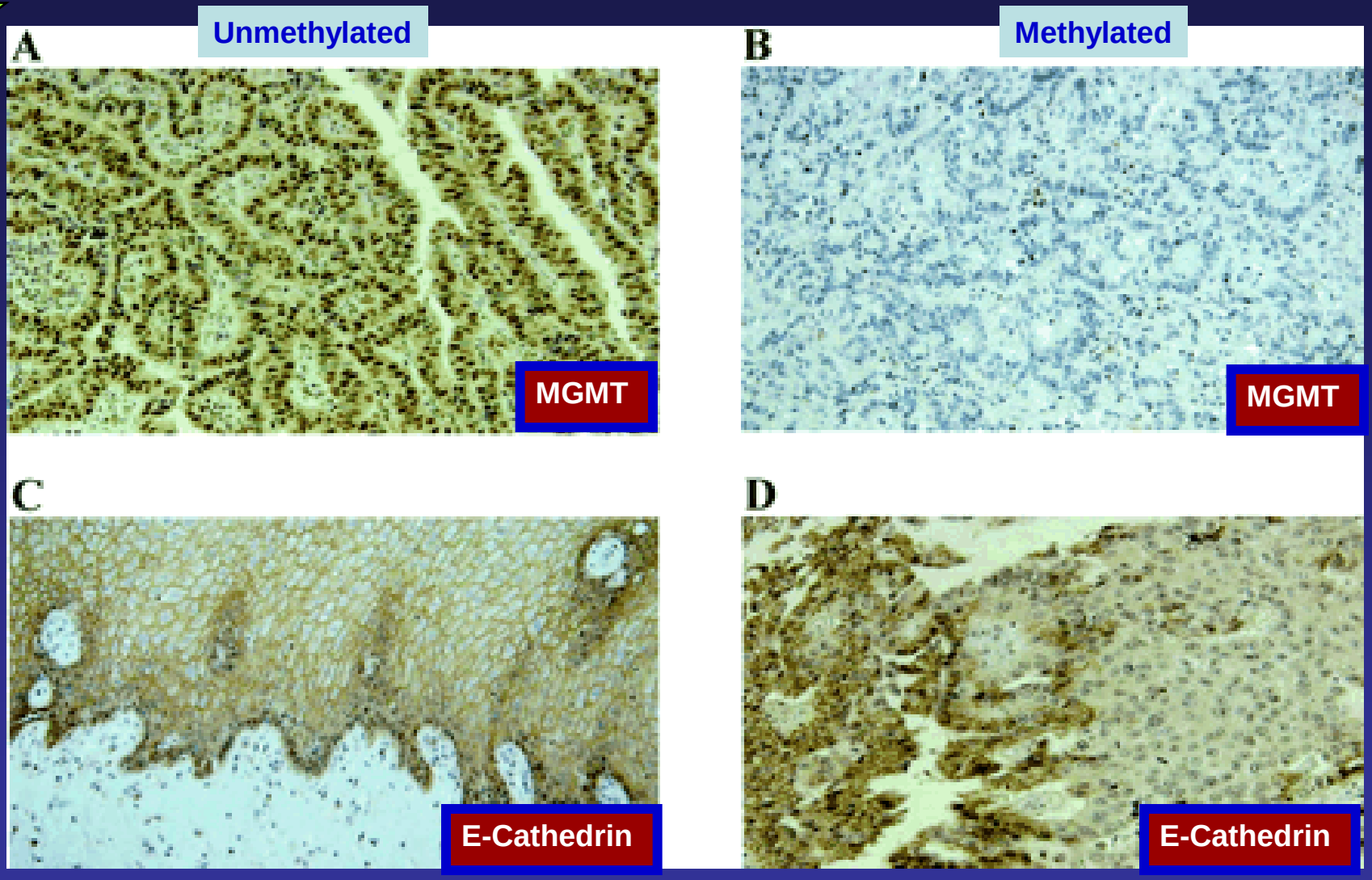


Esophageal Cancer: Probability of Survival



Brock et al. Clinical Cancer Research. 9: 2912

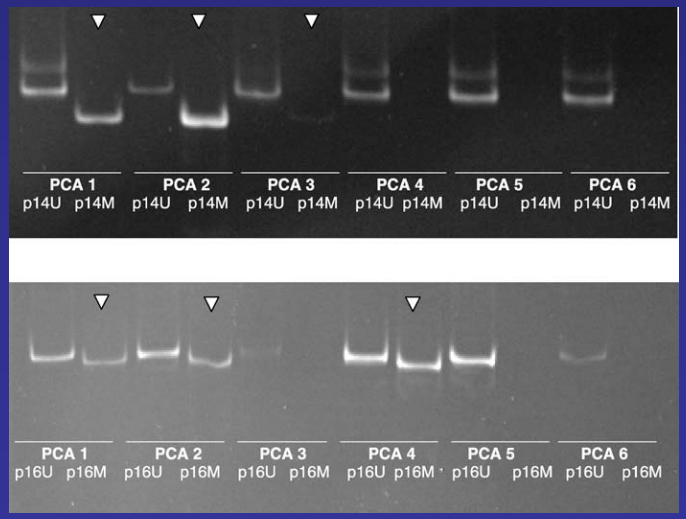
Esophageal Cancer: Immuno-histochemistry



Brock et al. Clinical Cancer Research. 9: 2912

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

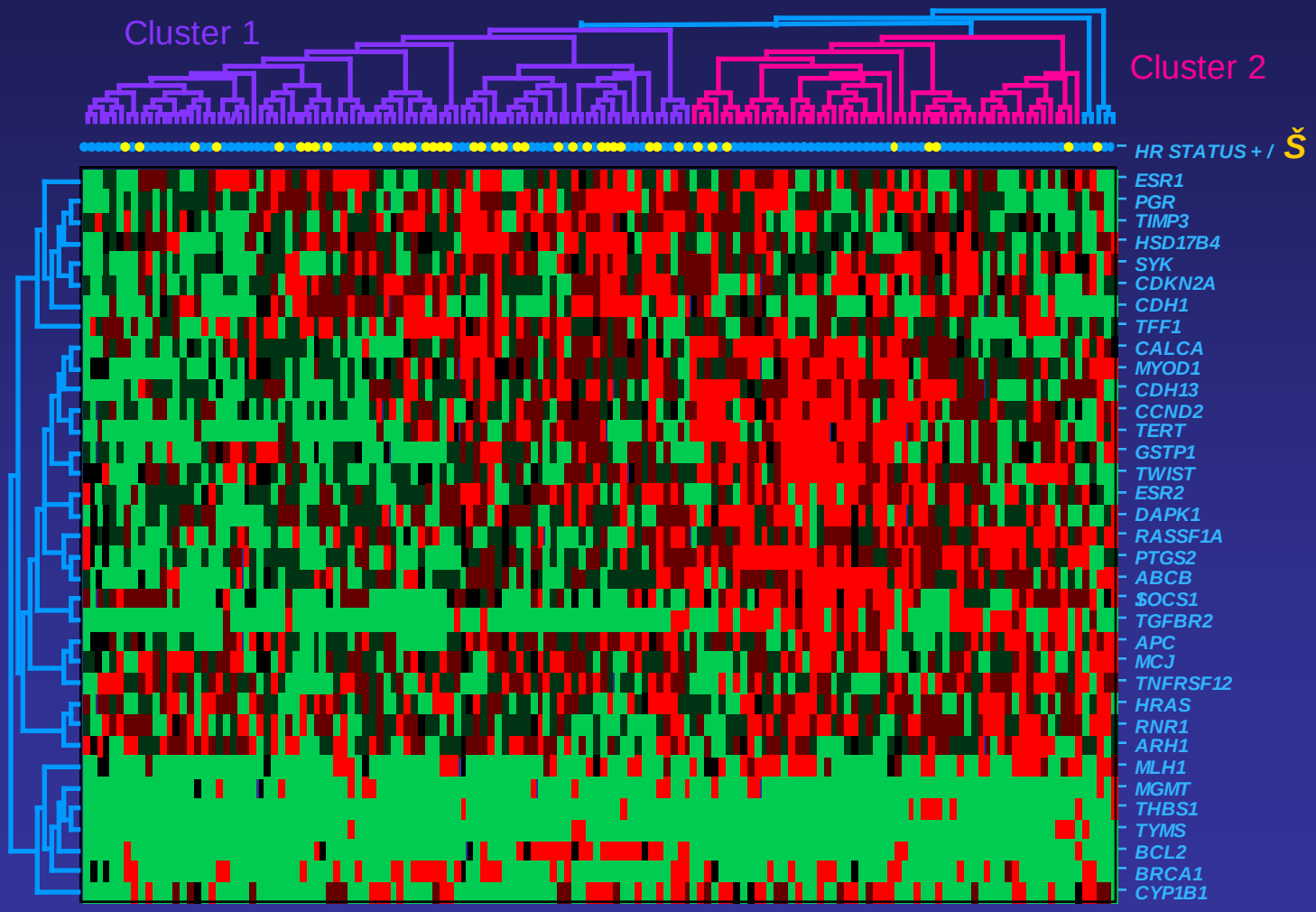
Pancreatic Carcinoma (PCA) : 39	19/39	p16INK4a
Chronic Pancreatitis (CP) : 16	0/16	p16INK4a
Normal Pancreatogram (NAD) : 6	0/6	p16INK4a



Sample: Pancreatic Fluid

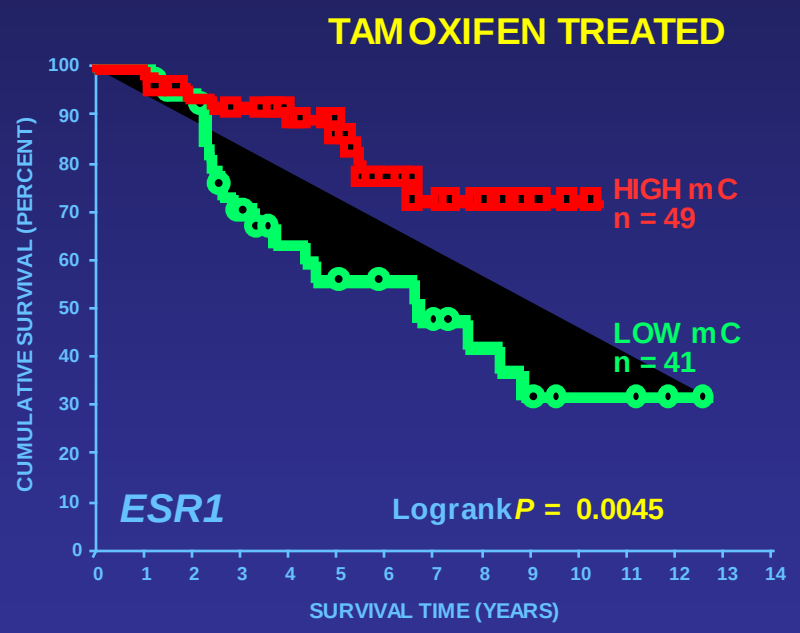
(Klump et al. *Mol Cell Path* 88: 217)

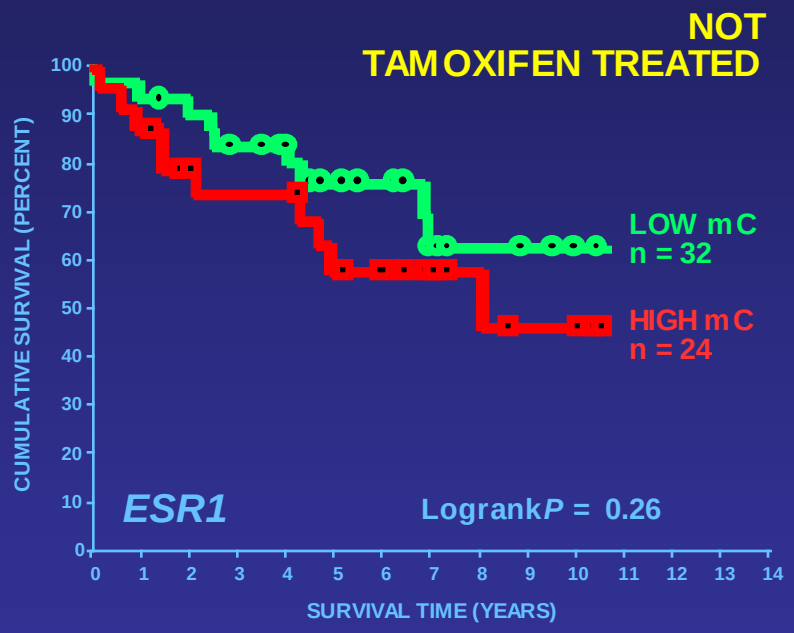
MethyLight Analysis of 35 Genes in 148 Human Breast Carcinomas



Widschwendter et al. *Cancer Research* 4, 3807

Breast Cancer Response to Tamoxifen Treatment by ESR1 Methylation





Widschwendter et al. *Cancer Research* 4, 3807

DNA **methylation** markers in Her-2/neu-positive and -negative cancers

Her_2	age	tumor size	LN status	HR status	E_CDH13	S_CDH13	E_MYOD1	S_MYOD1	E_PGR	S_PGR	E_BRCA1	S_BRCA1	E_HSD17B4	S_HSD17B4
+++	70	pT2	pos	pos		n.d.		n.d.		n.d		n.d.		n.d.
+++	51	pT1c	neg	pos		n.d.		n.d.		n.d.	n.d.	n.d.		
+++	48	pT1c	neg	pos										
+++	67	pT2	neg	neg										
+++	64	pT1c	neg	pos										
+++	57	pT1c	pos	pos										
+++	77	pT1c	pos	neg										
+++	57	pT1c	pos	pos										
0	58	pT1b	neg	pos										
0	63	pT2	pos	pos										
0	77	pT2	neg	neg										
0	60	pT1c	pos	pos										
0	60	pT1b	neg	pos										
0	64	pT1c	neg	pos										
0	81	pT2	n.d.	pos										
0	67	pT2	neg	pos		n.d.		n.d.		n.d.		n.d.		

Total
143 samples
E; epithelium
S, stroma

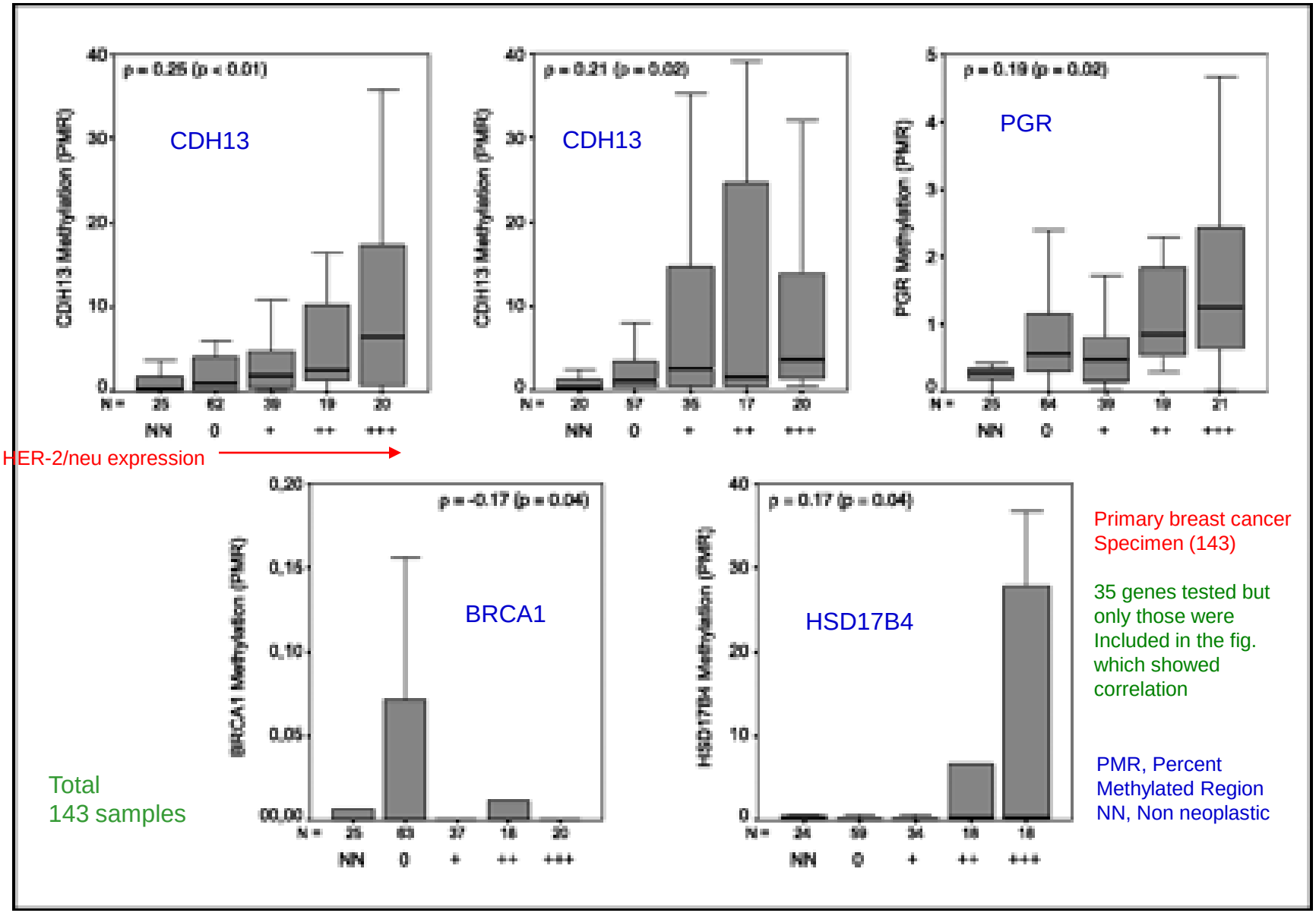
LN, lymph
Node status

LCM was
Used
To separate
Epithelium
and stroma

N.D.
Not Done

Fiegl et al. *Cancer Res*. 66: 29.

Association between DNA **methylation** and HER-2/neu expression (0, +, ++, +++) in primary breast cancer specimens



Fiegl et al. *Cancer Res*. 66: 29.

Methylation and Cancer Associated Infectious Agents

Viruses:

- p16 in HPV16/18 (Lung Cancer)
- RASSF1a in SV40 (Mesothelioma)
- HBV and HCV genes (Liver Cancer)
- EBV (Nasopharyngeal Carcinoma)
- H.pylori Infected Cells (Gastric Cancer)

asymptomatic healthy carriers

↓

chronically infected tissues

↓

pre-malignant lesions

↓

full-blown invasive tumor

LANA

EBNA

LANA, Latency Associated Nuclear Antigen
EBNA, Epstein-Barr Virus Nuclear Antigen

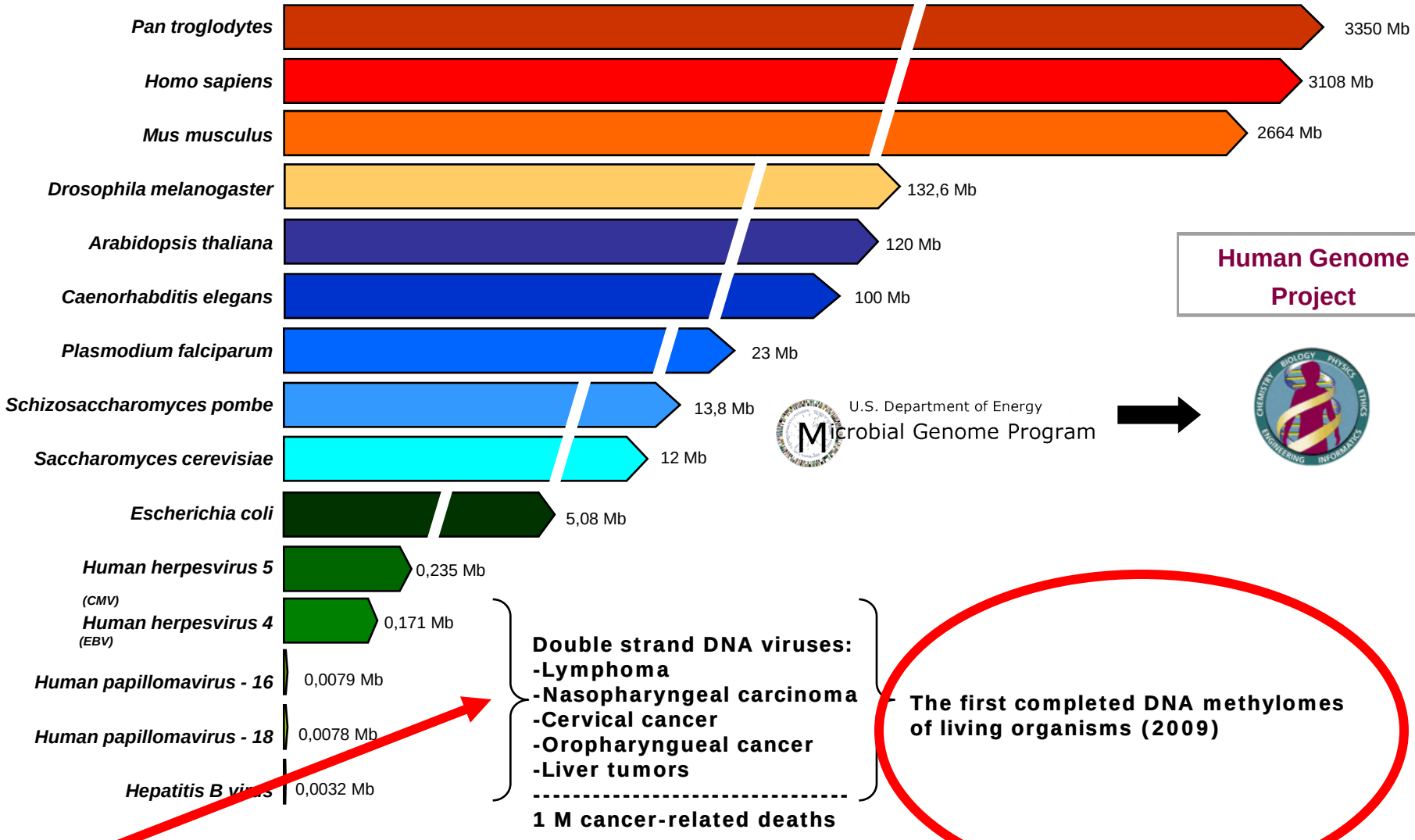
ylation in Human Papillomavirus-Infected

Chih Hsu[4], Jung-Ta Chen[5], Wen-Shan Liu[6], Ming-Chih Chiou[2,3],

[1]*Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan*

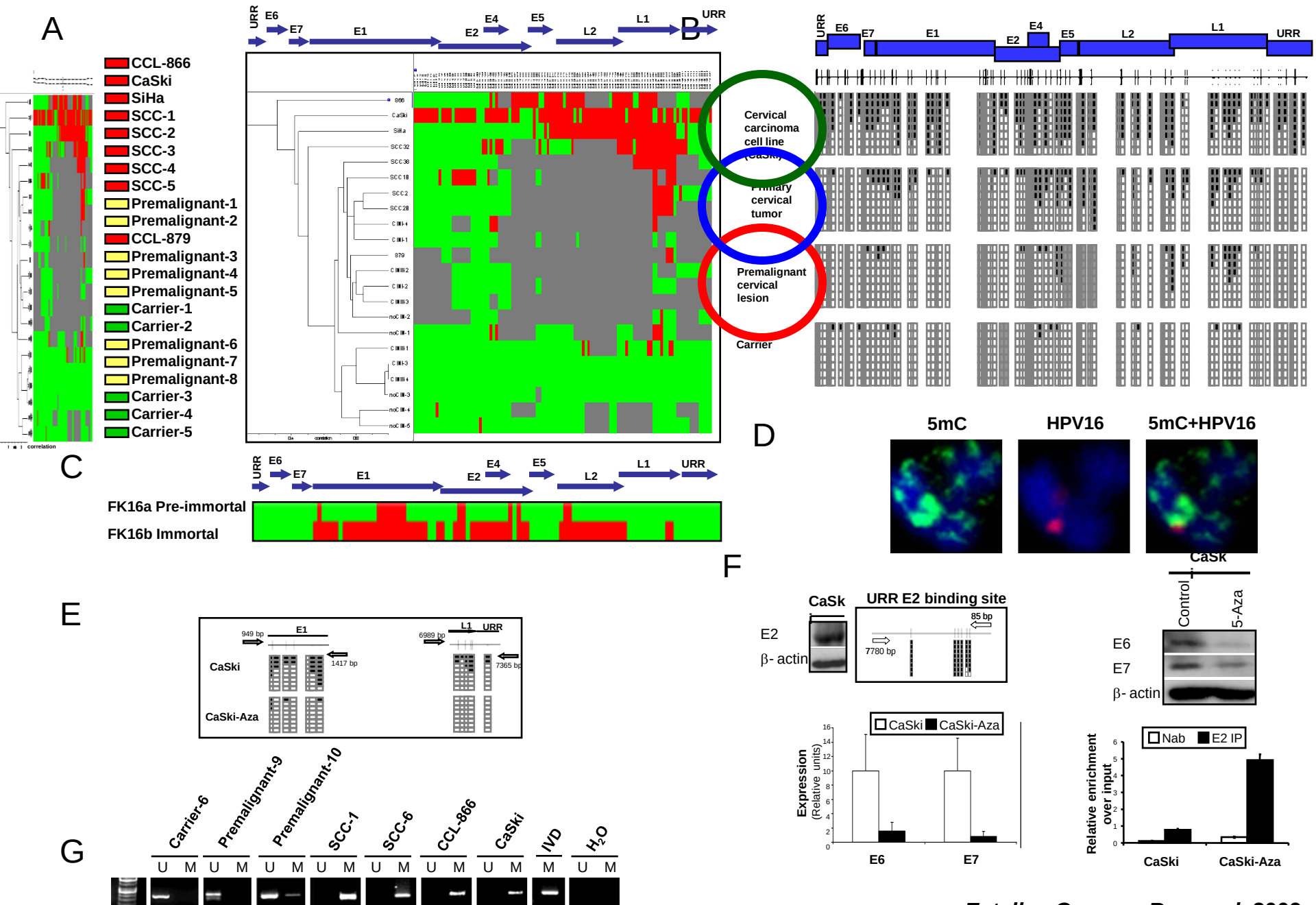
Complete methylome of HPV, EBV, and HBV. Esteller M Genome Research. 2009. 19: 438

Genomes



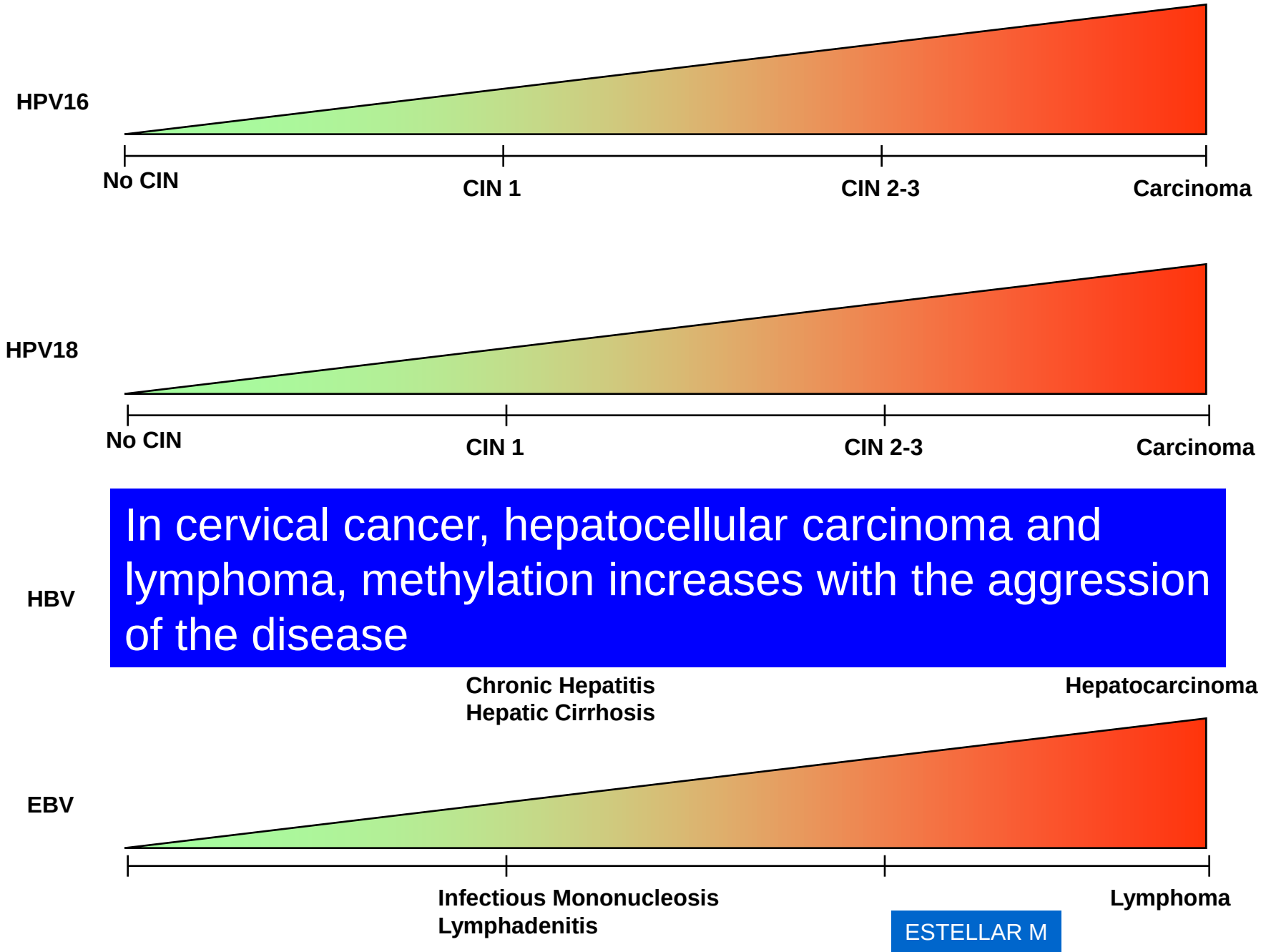
ESTELLAR

The DNA Methylome of the Human Papilloma Virus 16



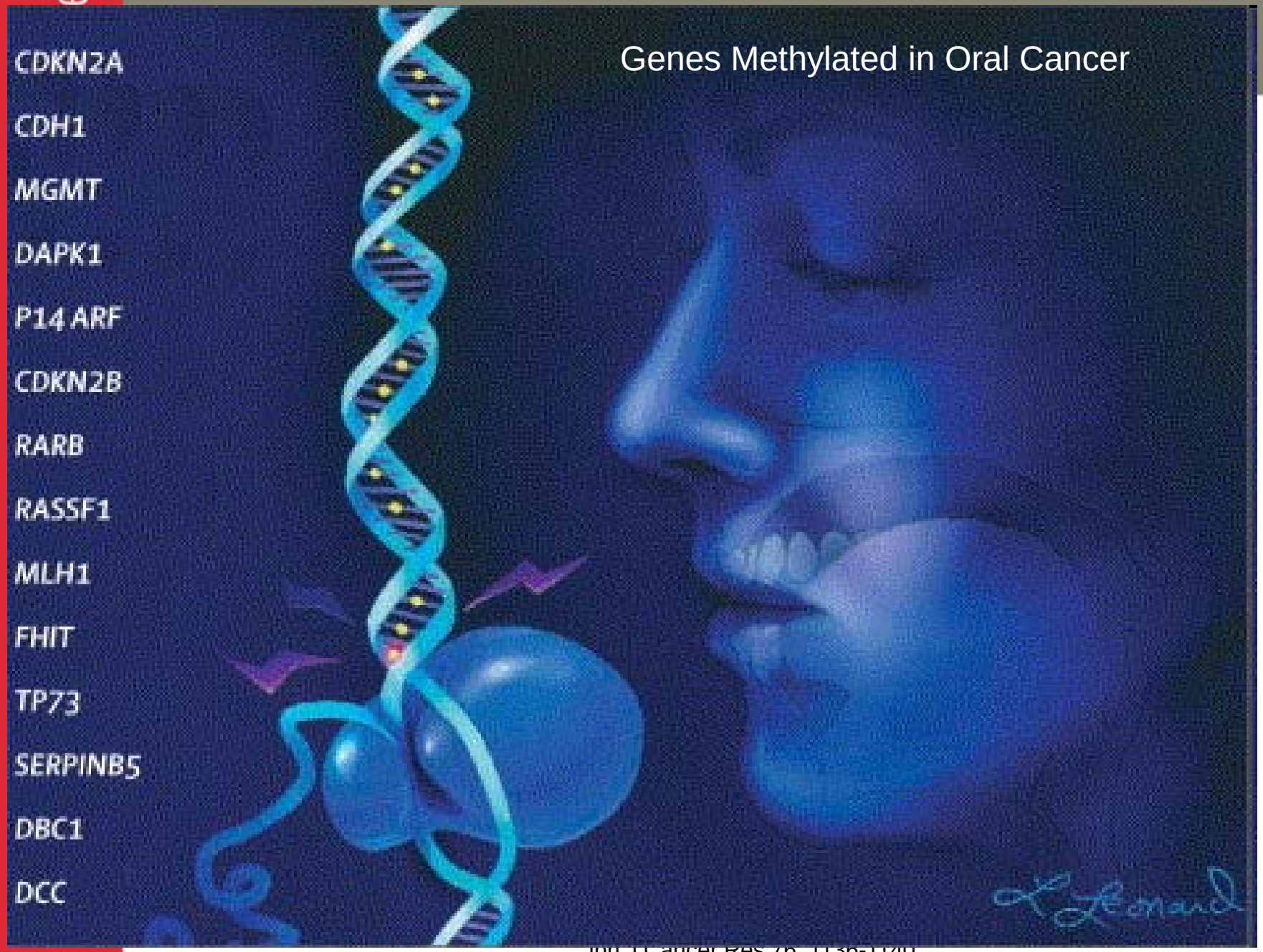
Esteller, Genome Research 2009

A Heat-Map for the DNA Methylomes of Double-Strand DNA Viruses Associated with Human Cancer



In cervical cancer, hepatocellular carcinoma and lymphoma, methylation increases with the aggression of the disease

ESTELLAR M



Jpn J Cancer Res 76, 1136-1140

Immunology and Epigenetics

1: Shin HJ, Park HY, Jeong SJ, Park HW, Kim YK, Cho SH, Kim YY, Cho ML, Kim HY, Min KU, Lee CW.Related Articles,
Links STAT4 expression in human T cells is regulated by DNA methylation but not by promoter polymorphism.
J Immunol.175(11):7143-50.

2: Espinoza CR, Feeney AJ.
The extent of histone acetylation correlates with the differential rearrangement frequency of individual VH genes in pro-B cells.
J Immunol. 175(10):6668-75.

Epigenomics Grants Predictive Biosciences Rights to Use a Biomarker in a Prostate Cancer Test

Epigenomics (www.epigenomics.com) granted Predictive Biosciences (www.predictivebiosci.com) a nonexclusive license to use its prostate cancer DNA methylation biomarker, mGSTP1, for the development and commercialization of a laboratory test to help in the diagnosis and management of prostate cancer. The agreement follows a similar deal covering mGSTP1 signed with Quest Diagnostics (www.questdiagnostics.com) in February 2009.

ion in Prostate Cancer

rug detoxification enzyme which

Seattle, WA, U.S.A., February 25,

G (Frankfurt, Prime Standard: ECX),

agnostics company, today announced

a non-exclusive licensing agreement

arker

Quest Diagnostics Incorpora

leading provider of diagnost

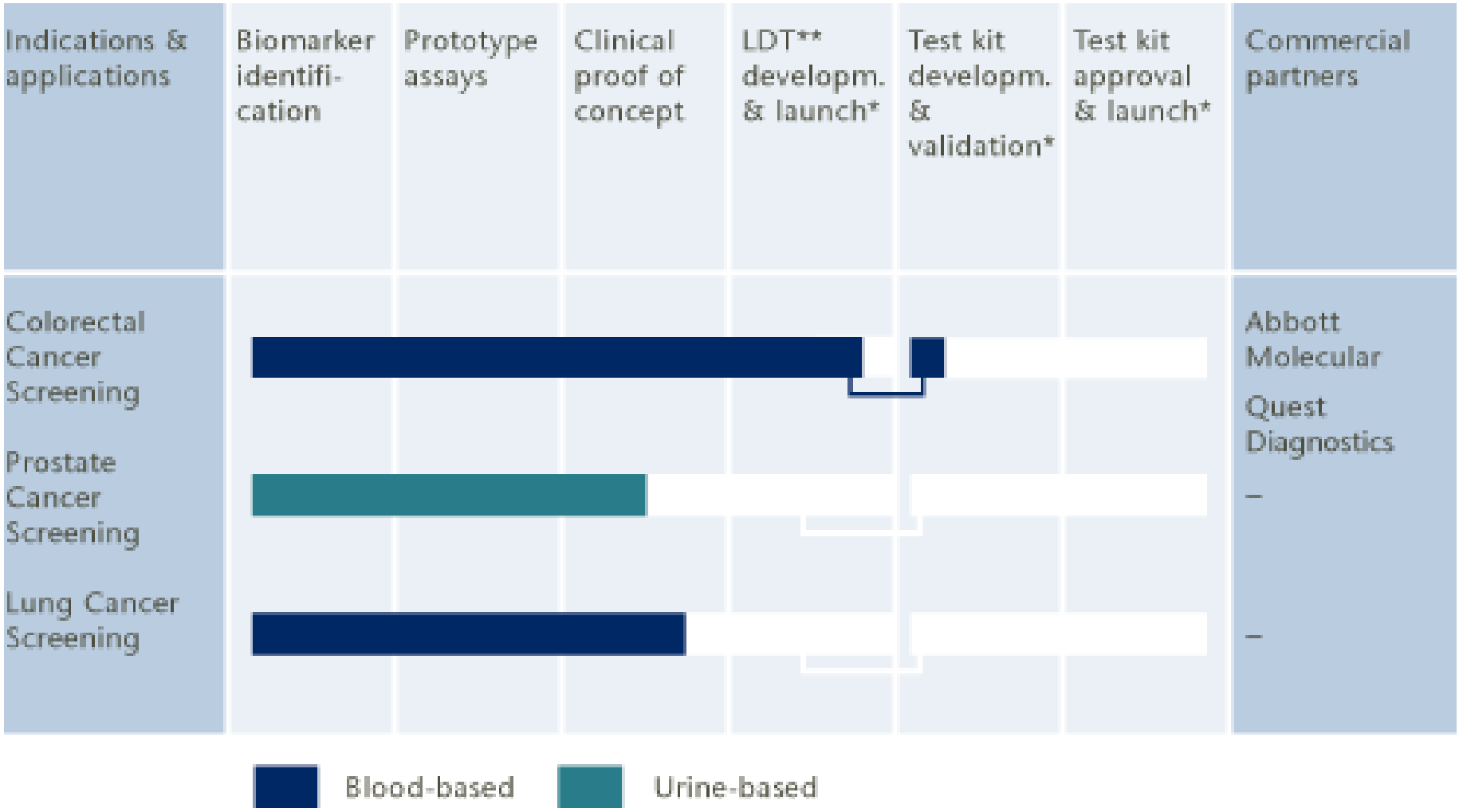
services.

Methyl-Profiler™ DNA Methylation PCR ARRAYS

Product*	Catalog #	Price*
Human Breast Cancer - *Signature Panel*	MeAH-011	$ 499
Human Gastric Cancer - *Signature Panel*	MeAH-021	$ 499
Human Liver Cancer - *Signature Panel*	MeAH-031	$ 499
Human Lung Cancer - *Signature Panel*	MeAH-041	$ 499
Human Prostate Cancer - *Signature Panel*	MeAH-051	$ 499
Human Stem Cell Transcription Factors - *Signature*	MeAH-511	$ 499
Human Inflammatory Response - *Signature Panel*	MeAH-521	$ 499
Human T Cell Activation - *Signature Panel*	MeAH-531	$ 499
Human Cytokine Production - *Signature Panel*	MeAH-541	$ 499
Custom Methyl-Profiler PCR Arrays	Inquire	Inquire

* *Methyl-Profiler PCR Arrays are available in Signature Panels (24 genes) & Complete Panels (96 genes).*

www.epigenomics.com

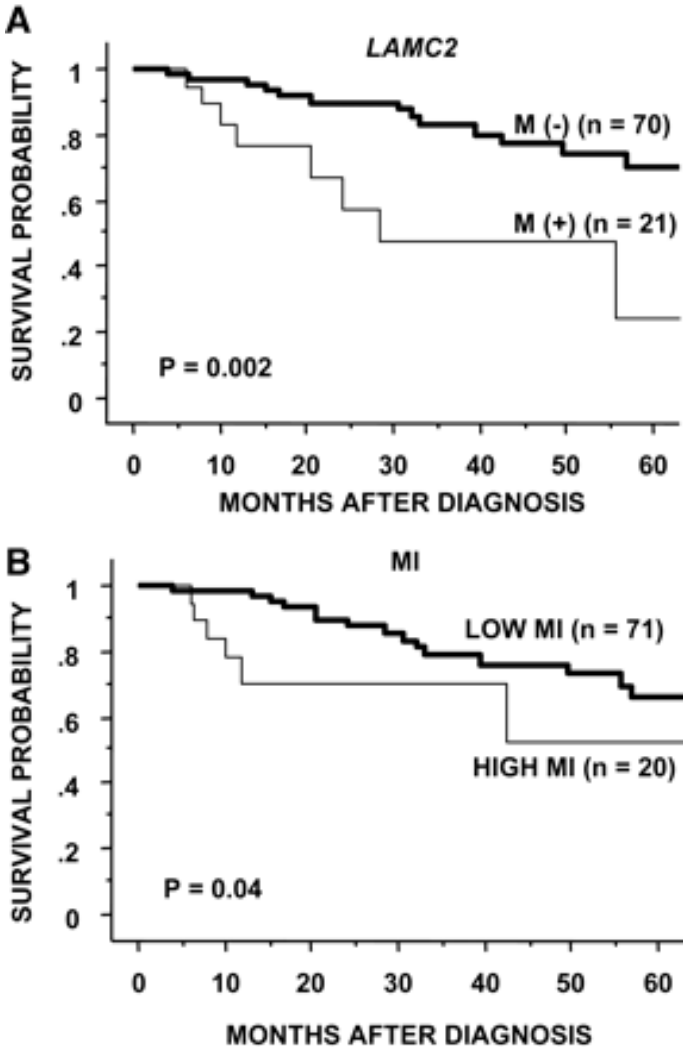


* By Epigenomics' commercial partners

** Laboratory-developed test

Bladder Cancer

Methylation of LAMC2 in Exfoliated Cells Isolated from Urine

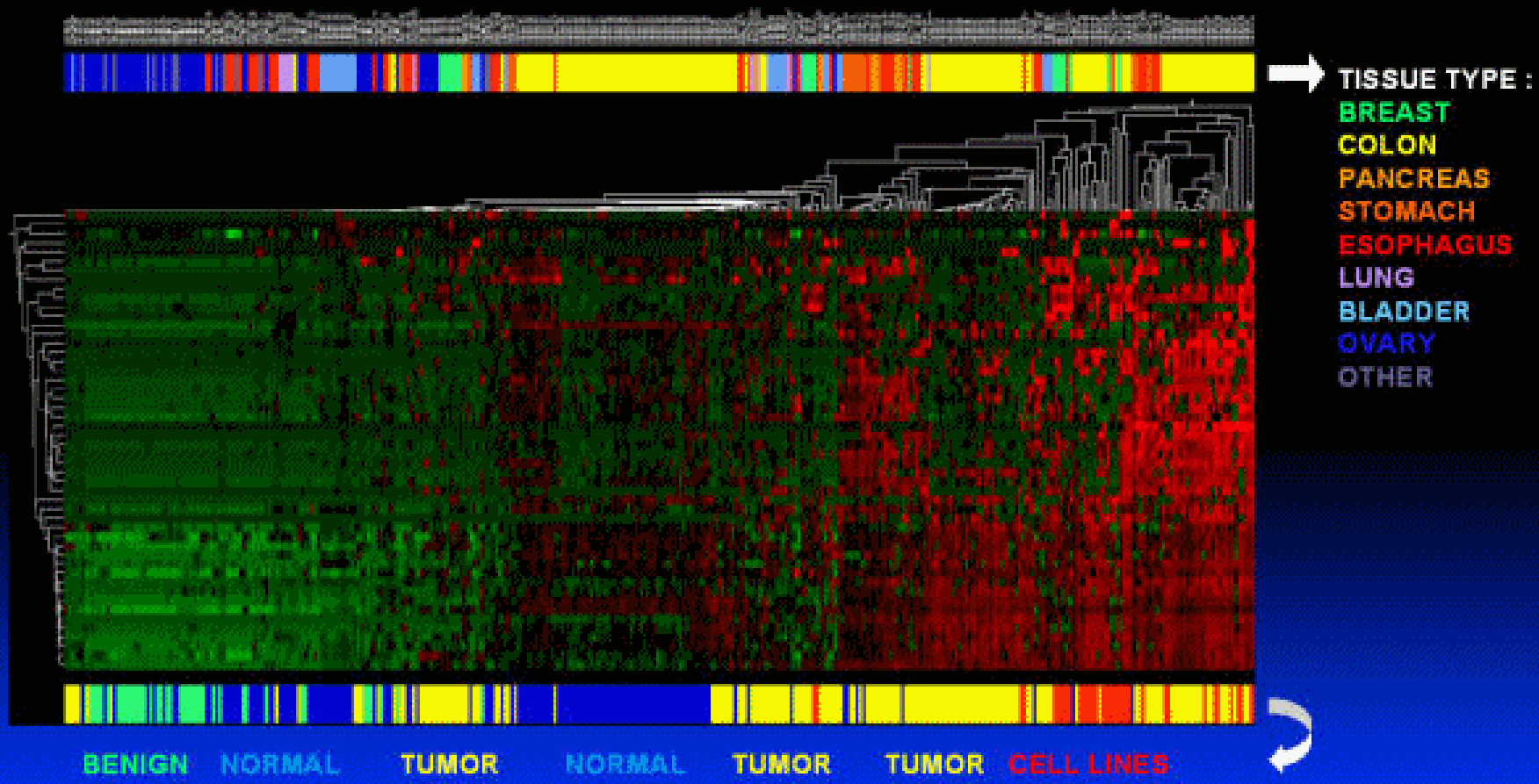


Another Study:
Schistosomes and Bladder Cancer

MI, Methylation Index

(Sathyanarayana et al. *Can Res* 64: 1425)

Clustering of Sample Type by CpG Island Hypermethylation



Cluster Analysis of 365 Human Samples with 50 DNA Methylation Markers

EPIGENETIC REMODELING BY ENVIRONMENTAL ARSENICALS

CA127989-01

Investigate the mechanisms and phenotypic consequences of epigenetic activation of Wnt5a gene in arsenical-induced malignant transformation.

Identify the decisive changes in the epigenome over the time course of arsenical induced bladder cancer.

Identify epigenetic targets of arsenic in human populations exposed to known levels of arsenic in their drinking water.

ROLE OF EPIGENETIC CHANGES INDUCED BY DIETARY AND ENVIRONMENTAL FACTORS IN CANCER

CA122396-01 (INTERNATIONAL AGENCY FOR RES ON CANCER)

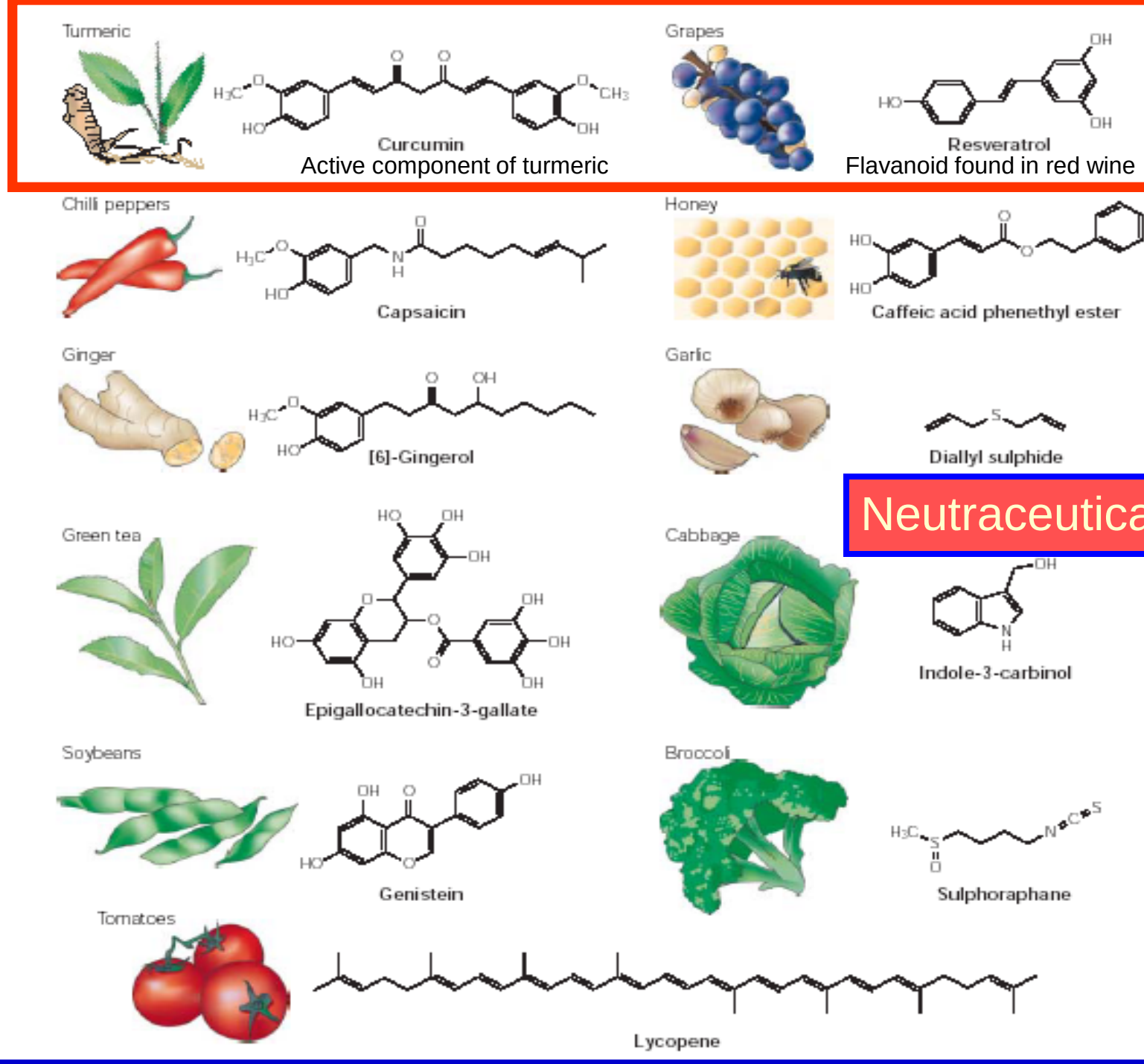
Large case-control studies on lung and upper-aero digestive tract (UADT) cancers in Europe

Analyze DNA methylation profiles, histone modifications and to profile their genomic locations

Study their association with dietary and environmental exposures

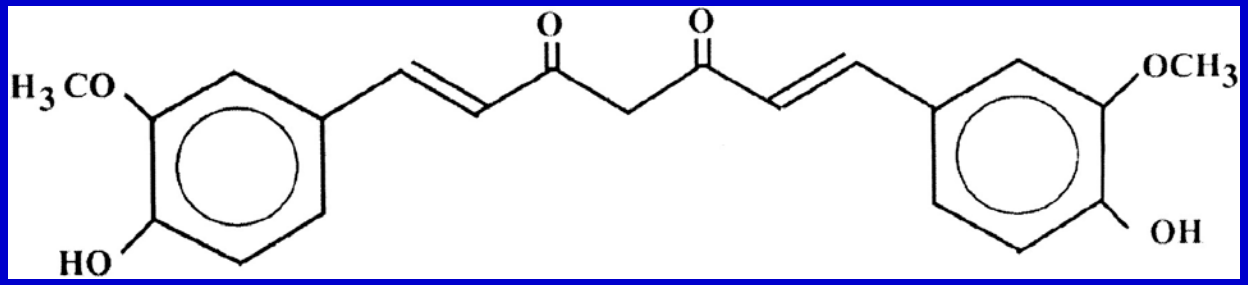
Compare the profile of DNA methylation and histone modifications between cases and controls and assess whether it can be a cancer susceptibility marker.

Dietary Polyphenols and Chromatin Remodeling



Irfan Rahman
Surh Y-J

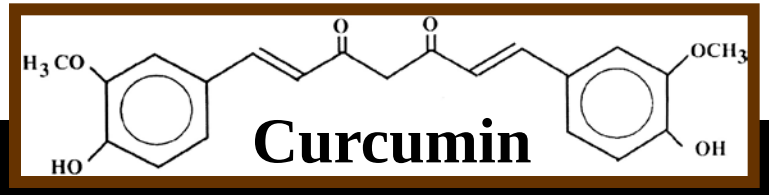
Structure and functions of curcumin

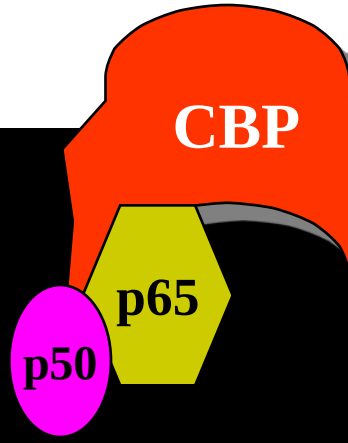


Active ingredient-diferuloyulmethane

- Derived from rhizome of the plant *Curcuma longa* (known as spice turmeric)
- Dietary phenolic antioxidant
- Used in the Ayurvedic system of medicine
- Antioxidant
- Anticancer
- Antiseptic

HAT inhibition at conc >10 μM





J Biol Chem 2004
Balasubramanyam et al

Curcumin, a novel p300/CBP specific inhibitor of acetyltransferase, represses the acetylation of histones and HAT dependent chromatin transcription

---

Biochem Pharmacol 2005
Kang et al

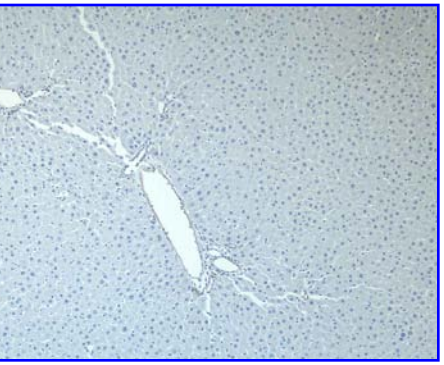
Curcumin-induced histone hypoacetylation: The role of reactive oxygen species

Khan, 2007

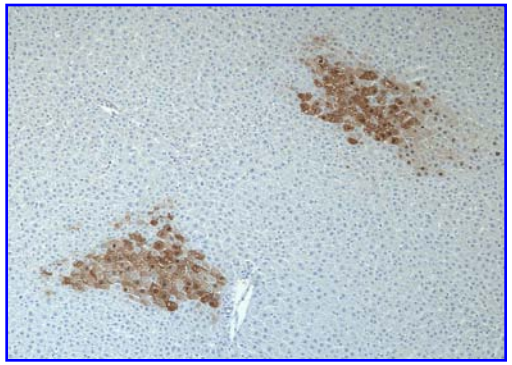
METHYL-DEFICIENT MODEL OF ENDOGENOUS HEPATOCARCINOGENESIS

- **Chronic deficiency in the methyl donors methionine, choline, folic acid and vitamin B_{12}**
- **No exogenous carcinogen added**
- **No genetic manipulation**
- **Hepatocellular carcinoma in 14-16 months in male rats and certain mouse strains**
- **Sequence of pathological changes similar to the development of hepatocellular carcinoma in humans**

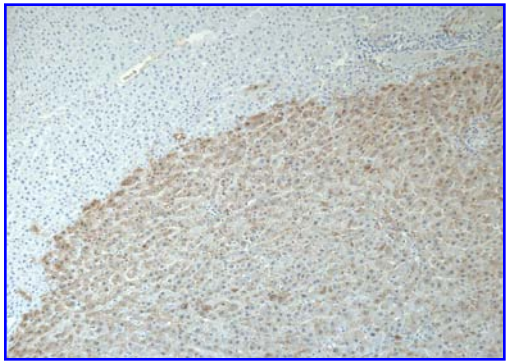
Normal tissue



36 weeks, GSTπ-foci



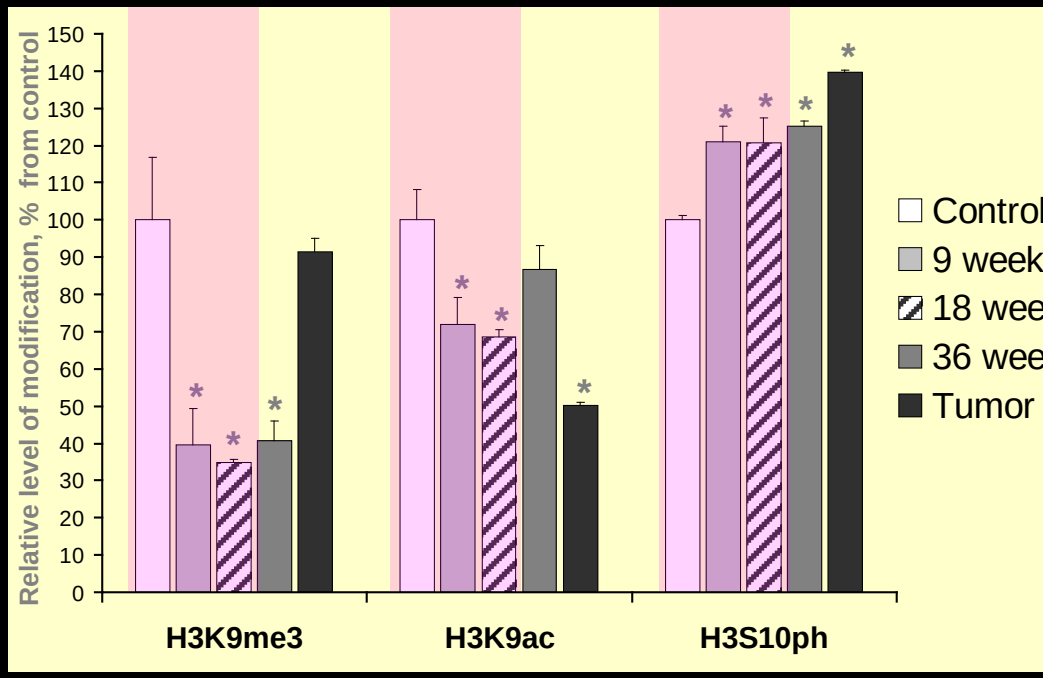
>54 weeks, GSTπ-tumor



Liver tumor

Igor et al. 2007 (personal communication)

ALTERATIONS OF HISTONE H3 MODIFICATIOINS IN LIVER OF F344 RATS DURING METHYL DEFICIENCY



N-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKP-…-C

Interplay between H3K9me3, H3K9Ac, and H3S10ph

National Cancer Institute

Clinical Specimens Currently Used for Methylation Analysis

Tissue
- Fixed
- Fresh

Blood
- Lymphocytes
- Serum

Biofluids
- Nipple aspirate
- Pancreatic secretions
- Urine

Exfoliated Cells
- Buccal cells from oral rinse

Cytological Smears
- Cervical cancer

Needle Biopsy

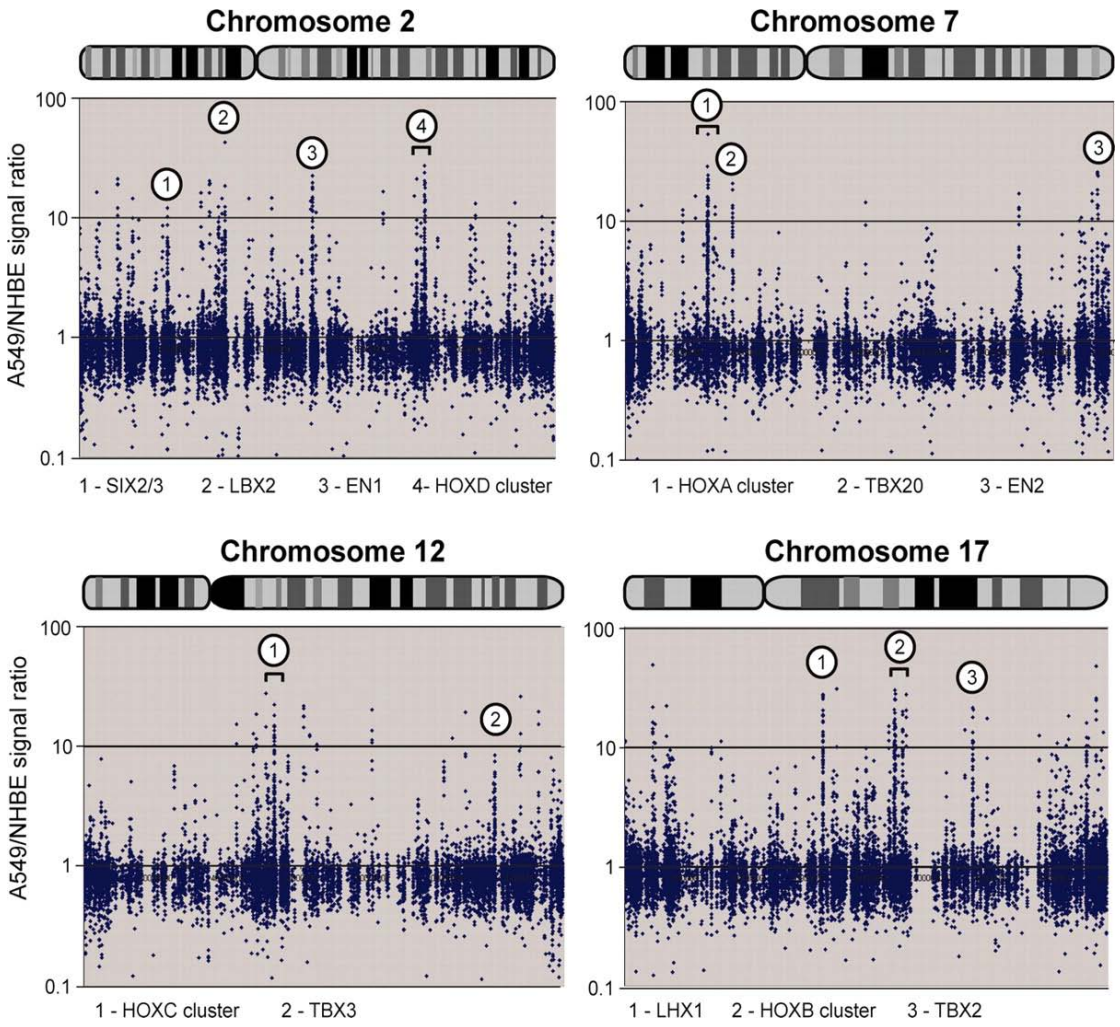
CpG Island Distribution in Chromosome 20, 21, and 22

Chromosome	20	21	22	Total
Length (Mb)	62	44	47	153
Mapped Genes	1244	611	1034	2889
First Coding Exon	204	104	226	524
Other Exon	282	127	241	650
Alu	187	76	194	457

CpG Island Searcher:

http://www.cpgislands.com
http://www.uscnorris.com/cpgislands/cpg.cgi.

Fig. 3. DNA methylation profiles of chromosomes 2, 7, 12, and 17 that carry the HOXA, HOXB, HOXC, and HOXD gene clusters



Rauch, Tibor et al. (2007) Proc. Natl. Acad. Sci. USA 104, 5527-5532

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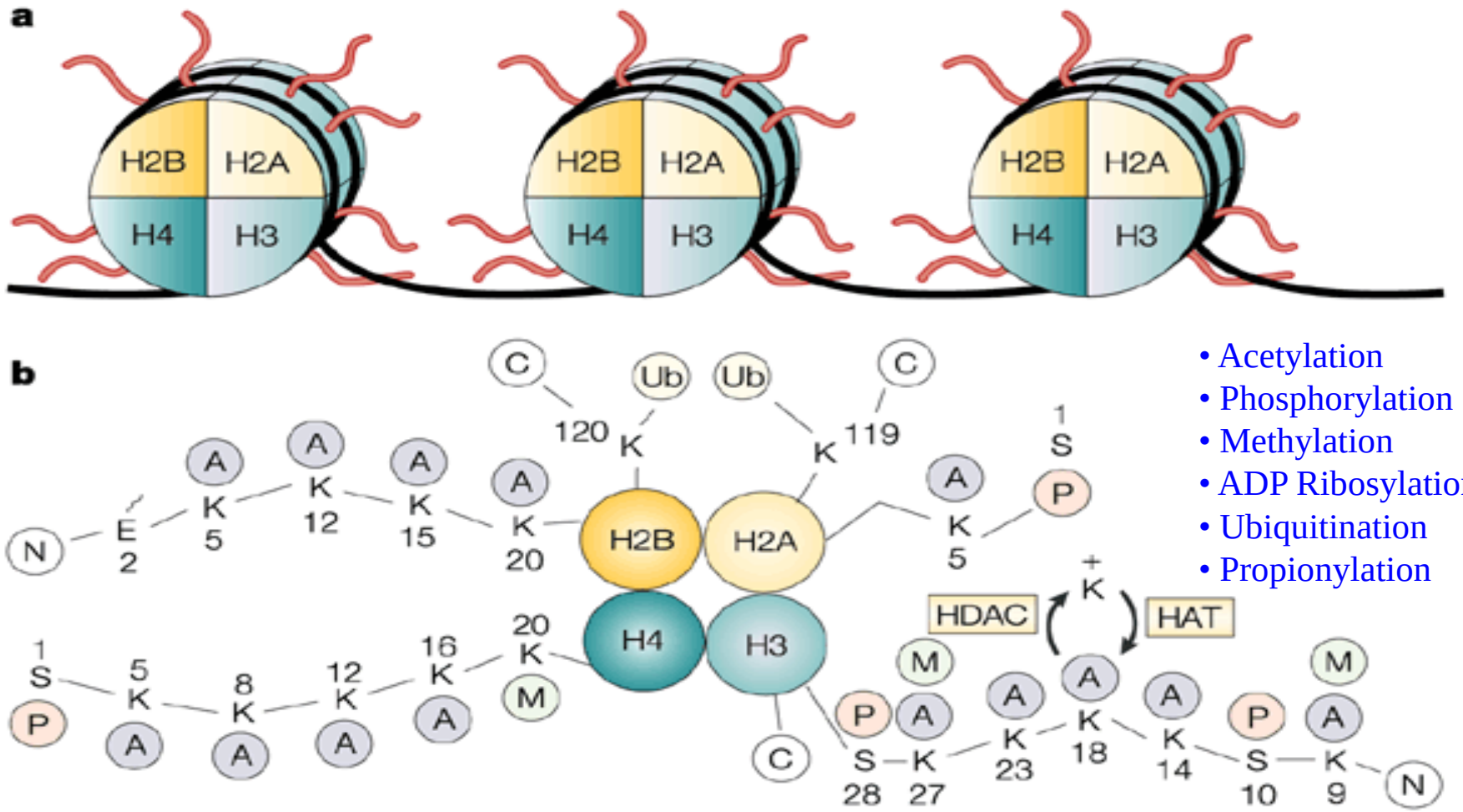
Histone Modifications



- Acetylation
- Phosphorylation
- Methylation
- ADP Ribosylation
- Ubiquitination
- Propionylation

Nature Reviews | Cancer

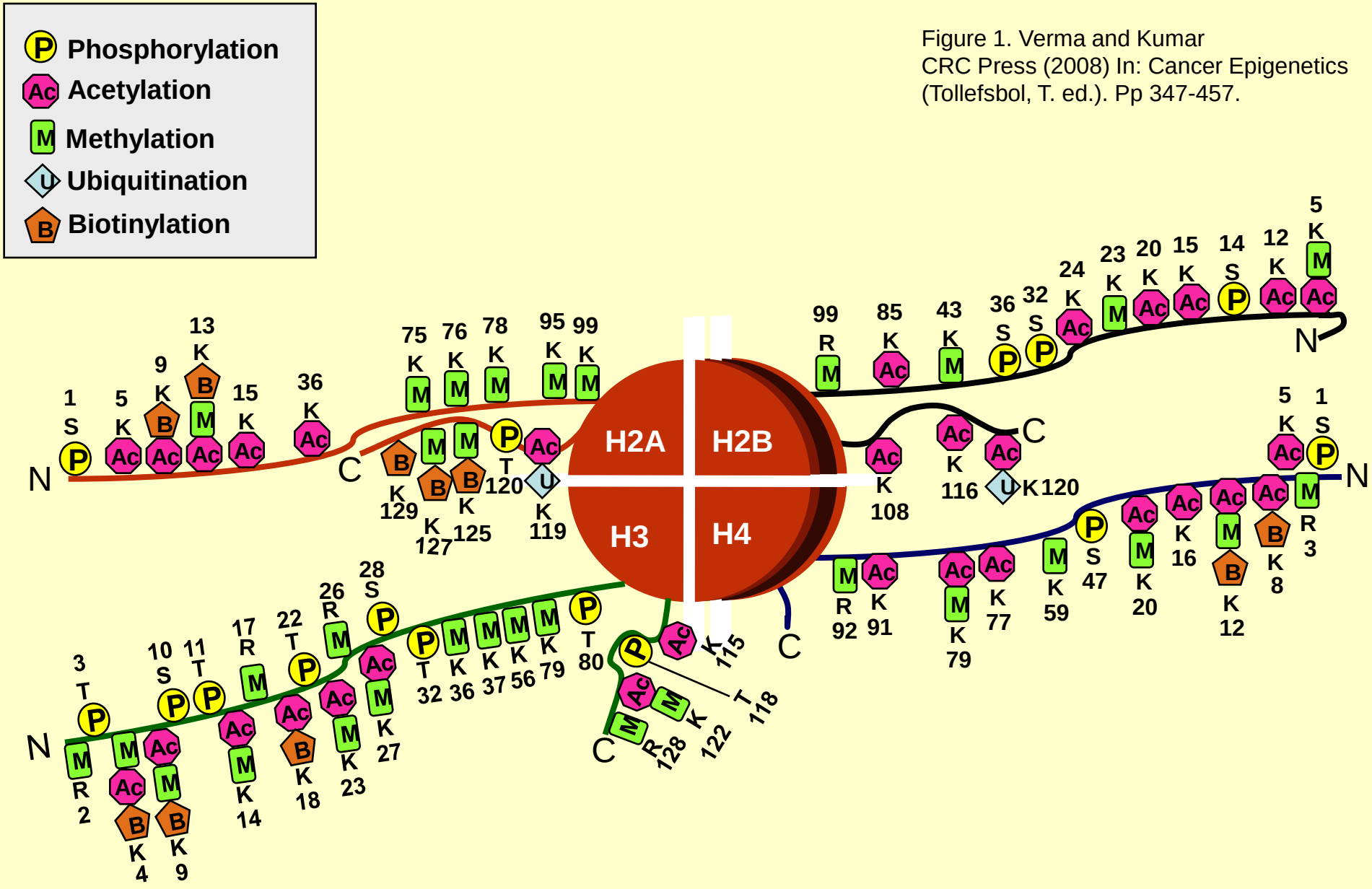


Figure 1. Verma and Kumar
CRC Press (2008) In: Cancer Epigenetics
(Tollefsbol, T. ed.). Pp 347-457.

Based on http://www.histone.com
and J. Nutr. 136:1763-1765

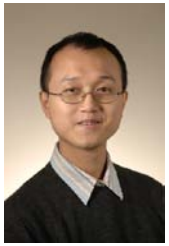
p53 is regulated by the lysine demethylase LSD1

Key Observation:

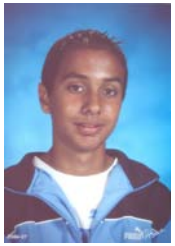
Histone lysine methylation has recently been shown to be reversible; however, it is not known whether non-histone proteins are substrates for demethylation.

LSD1 interacts with p53 to repress p53-mediated transcriptional activation and inhibit the role of p53 in promoting apoptosis.

Histone lysine-specific demethylase LSD1 removes both monomethylation (K370me1) and dimethylation (K370me2) at K370



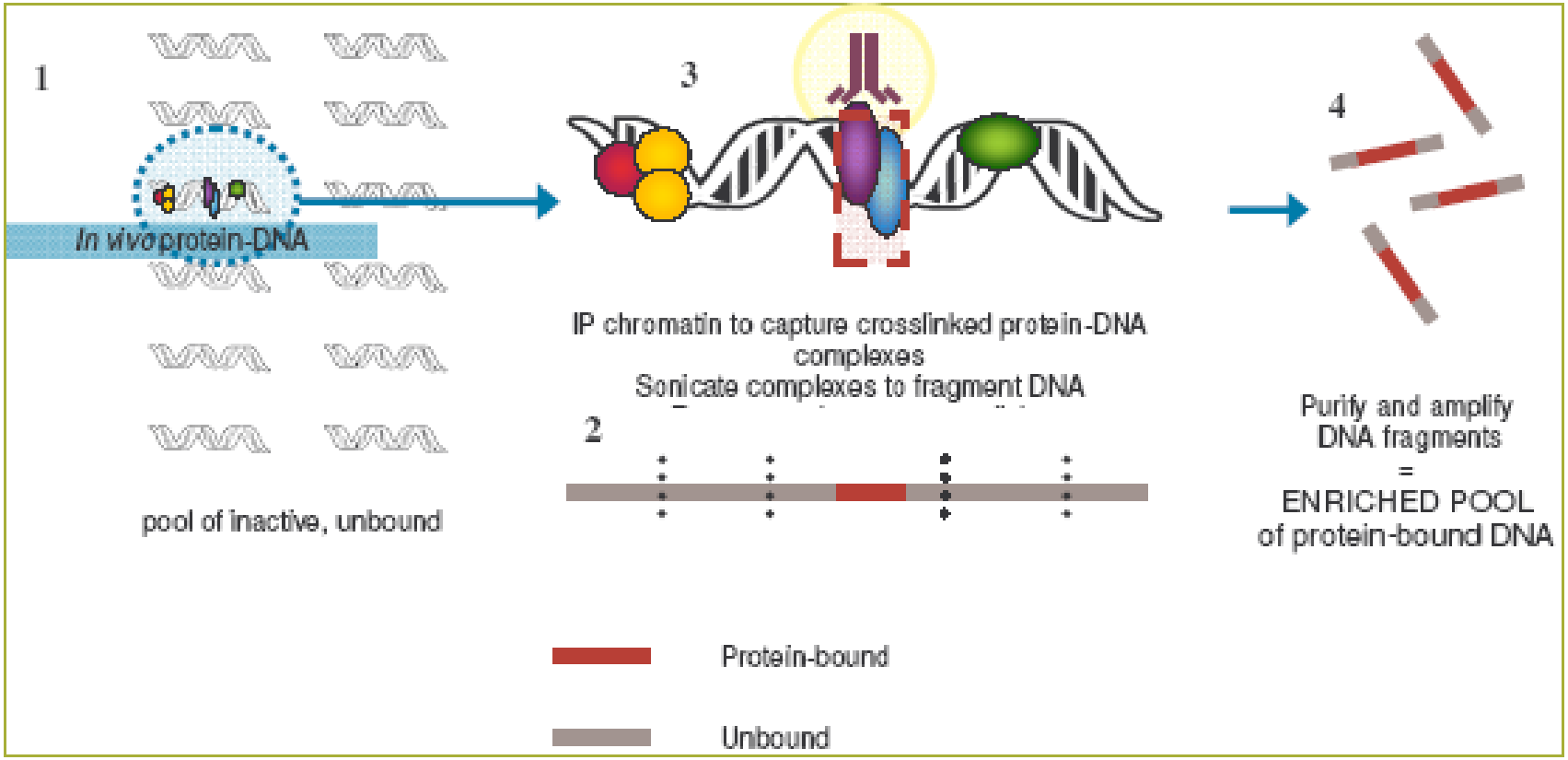
Jing Huang



Mudit Verma

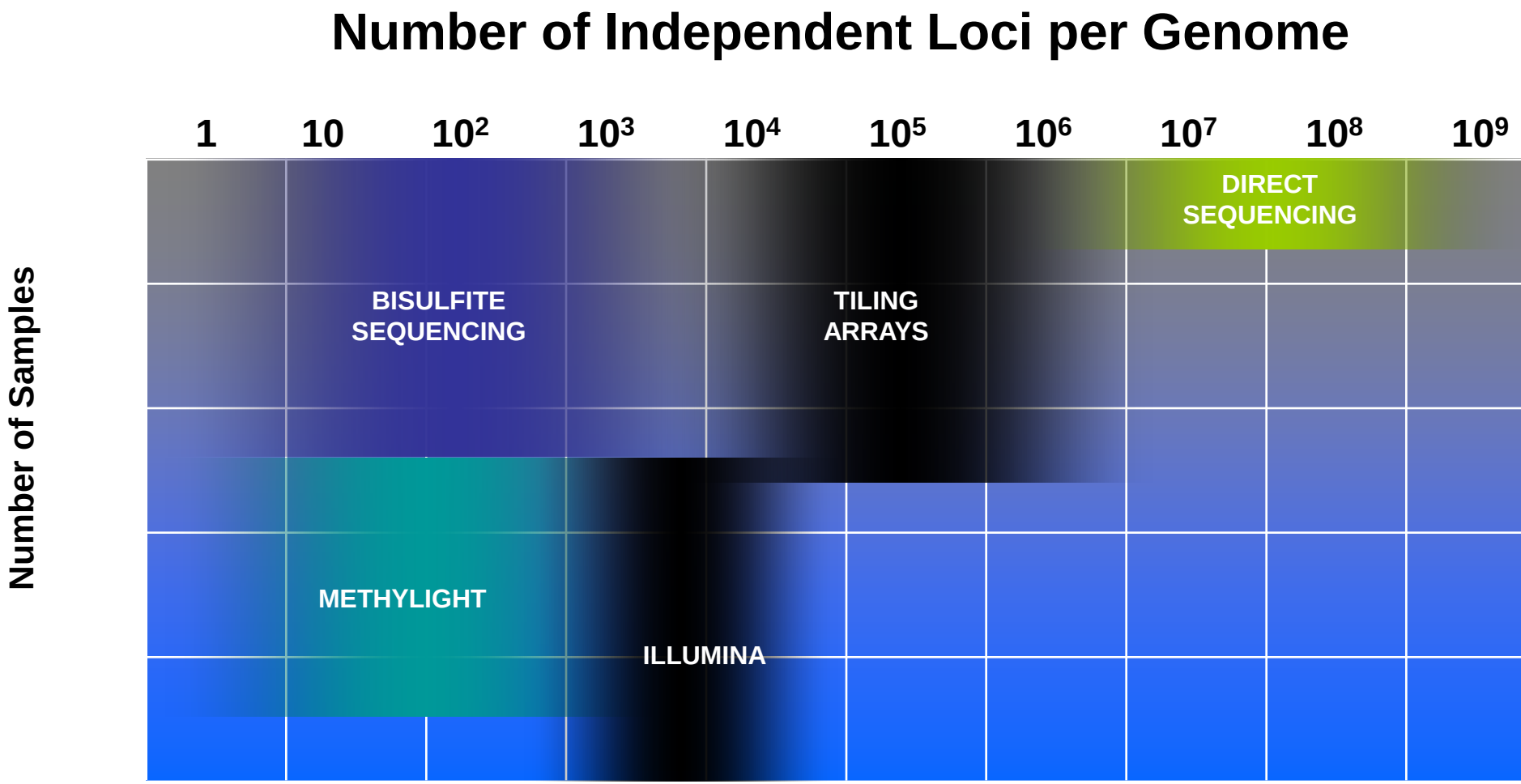
Huang et al. Nature. 449:105-8

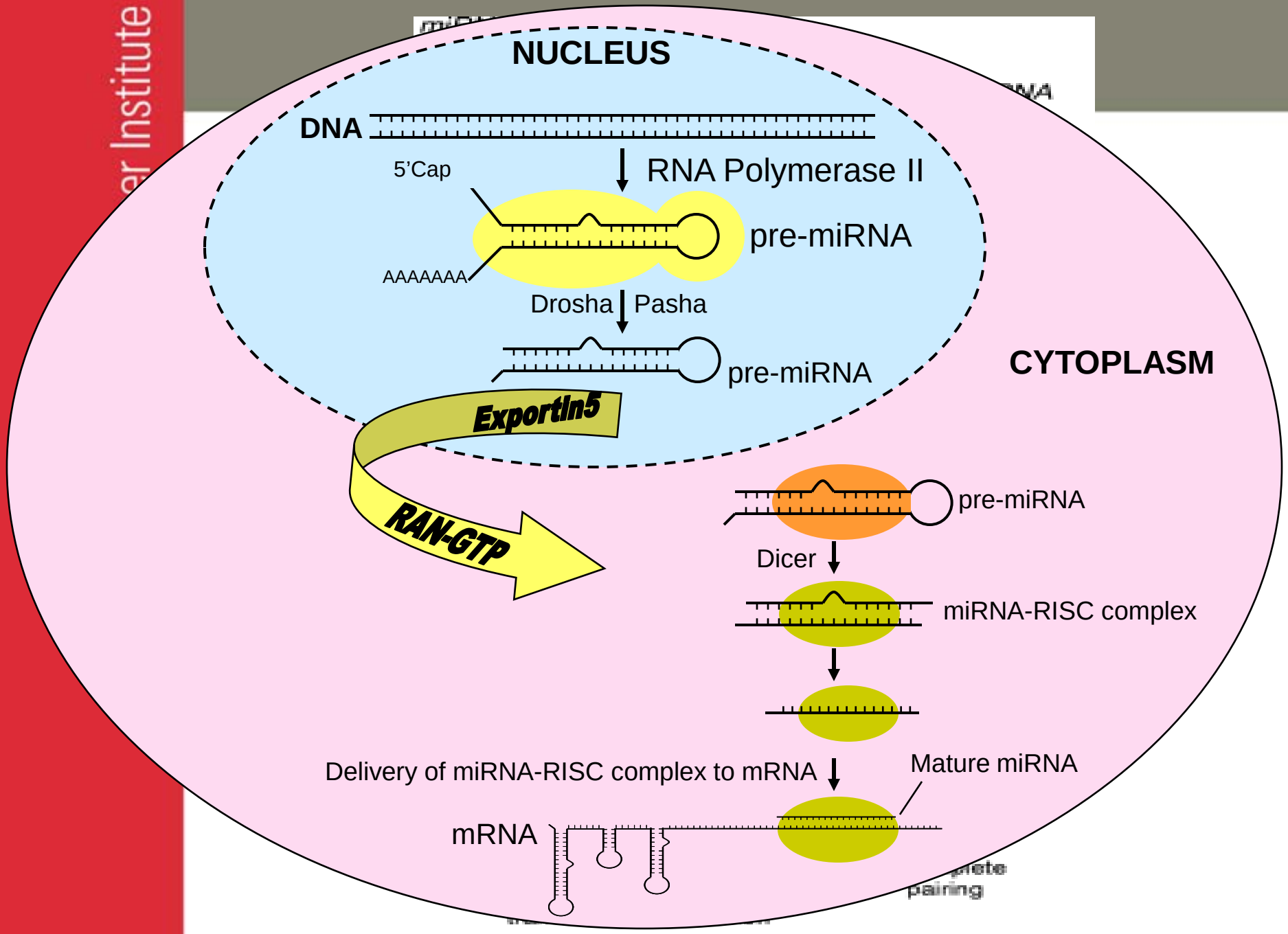
The sample preparation step of the general microarray workflow contains CHIP-on-chip-specific elements, as follows:



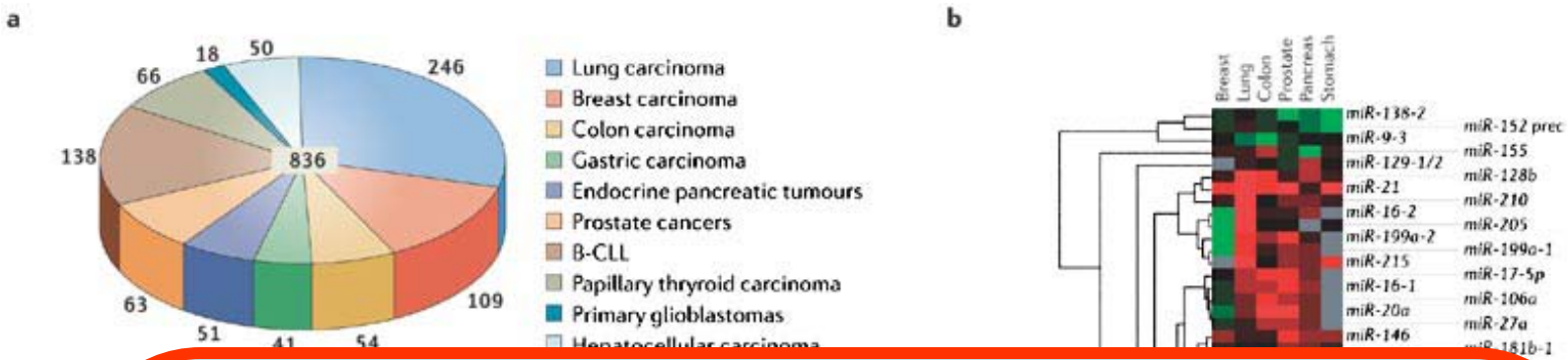
1. Cross-link protein-DNA complexes
2. Lyse cells and sonicate DNA: isolate unbound and bound fragments
3. IP chromatin to capture and purify bound DNA
4. Release and amplify bound DNA fragments
5. Prepare unbound (control) and amplified bound (target) DNA fragments for array application.

Epigenome Analysis




NUCLEUS
DNA
RNA Polymerase II
5'Cap
pre-miRNA
AAAAAAA
Drosha
Pasha
pre-miRNA
CYTOPLASM
Exportin5
RAN-GTP
pre-miRNA
Dicer
miRNA-RISC complex
Delivery of miRNA-RISC complex to mRNA
Mature miRNA
mRNA

Mirco RNA Signatures in Human Cancers



c

Micro RNA Polymorphism to Identify High Risk Populations

Mir-31 inhibits metastasis in breast cancer

nature REVIEWS CANCER

A microRNA DNA methylation signature for human cancer metastasis

Used a pharmacological and genomic approach to reveal aberrant epigenetic silencing program by treating lymph node metastatic cancer cells with a DNA demethylating agent followed by hybridization to an expression microarray.

Among the miRNAs that were reactivated upon drug treatment, miR-148a, miR-34b/c, and miR-9 were found to undergo specific hypermethylation-associated silencing in cancer cells compared with normal tissues.

These findings indicate that DNA methylation-associated silencing of tumor suppressor miRNAs contributes to the development of human cancer metastasis.

Amaia Lujambioa,b, Manel Estellera,b,j
PNAS March 27, 2008

National Cancer Institute

Target	Drug	Clinical Trial
DNA Methylation	5-Azacytidine	Phase I/II/II
	5-Aza-2'deoxycytidine	Phase I/II/II
	FCDR	
	Zebularine	
	Procainamide	
	EGCG	Phase I
	Psamaplin A	
	Antisense Oligomers	Phase I
Histone deacetylase	Phenylbutyric acid	Phase I/II
	SAHA (Suberoylanilide hydroxamic acid) or Vorinostat	PhaseI/II
	Depsipeptide	Phase I/II
	Valproic Acid	Phase I/II

Adverse Experiences SAHA
Duvic et al. 2007.
Am S Hem 109:31.

- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting

Eggr et al Nature. 429:457.

- HDAC inhibitors are a novel class of anticancer drugs that mainly leads to an accumulation of acetylated proteins

Thereby inducing

- Cell cycle arrest
- Differentiation
- Migration
- apoptosis in cancer and transformed cells

- Few HDAC inhibitors act as radiation-sensitizing drugs resulting in better radiation therapy (head and neck cancer) responsiveness

HDAC 1, 2, 3, 8, 11 have been characterized (Khan, I , 2007)

Phase I study of epigenetic modulation with 5-azacytidine and valproic acid in patients with advanced cancers.

Braiteh F, Soriano AO, Garcia-Manero G, Hong D, Johnson MM, Silva Lde P, Yang H, Alexander S, Wolff J, Kurzrock R. Clin Cancer Res. 2008 Oct 1;14(19):6296-301. (colorectal cancer, melanoma and breast cancer)

5 Azacytidine
S.C. daily for 10 days

+

Valproic Acid
Orally daily to titrate to 75-100 ug/ml

28 Days Cycle

55 people with Advanced cancer
Median age 60

→

Peripheral blood
- Pyrosequencing
- Chip

Analysis
Day 1, 10 and 28

- **The maximum tolerated dose was 75 mg/m(2) of 5-AZA in combination with valproic acid.**
- **Dose-limiting toxicities were neutropenic fever and thrombocytopenia, which occurred at a dose of 94 mg/m(2) of 5-AZA.**
- **Stable disease lasting 4 to 12 months (median, 6 months) was observed in 14 patients (25%).**

A significant decrease in global DNA methylation and induction of histone acetylation were observed.

The combination of 5-AZA and valproic acid is safe at doses up to 75 mg/m(2) for 5-AZA in patients with advanced malignancies.

Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome.

Soriano et al. Blood. 2007. 110(7):2302-8.

- **Combination of 5-azacitidine (5-AZA), valproic acid (VPA), and ATRA in patients with acute myeloid leukemia or high-risk myelodysplastic syndrome.**

- **A total of 53 patients were treated.**

- **The overall response rate was 42%.**

- **A significant decrease in global DNA methylation and induction of histone acetylation were achieved.**

- **VPA blood levels were higher in responders.**

- **The combination studied is safe and has significant clinical activity.**

This clinical trial was registered at www.clinicaltrials.gov as no. NCT00326170.

Histone Inhibitors in Clinical Trials (Clinicaltrials.gov)

STATUS	STUDY
Recruiting	Safety Study of the Histone Deacetylase Inhibitor, CHR-3996, in Patients With Advanced Solid Tumours
Recruiting	Phase II Study of Histone-Deacetylase Inhibitor ITF2357 in Refractory/Relapsed Lymphocytic Leukemia
Recruiting	phII Study of an HDAC Inhibitor in Very High-Risk Relapsed/Refractory Hodgkin's Lymphoma Patients
Recruiting	Phase IIA Study of the HDAC Inhibitor ITF2357 in Patients With JAK-2 V617F Positive Chronic Myeloproliferative Diseases
Recruiting	Phase II Trial of the Histone-Deacetylase Inhibitor ITF2357 Followed by Mechlorethamine in Relapsed/Refractory Hodgkin's Lymphoma Patients
Recruiting	HDAC Inhibitor Vorinostat (SAHA) With Capecitabine (Xeloda) Using a New Weekly Dose Regimen for Advanced Breast Cancer
Recruiting	Valproic Acid, Temozolomide, and Radiation Therapy in Treating Patients With Glioblastoma Multiforme
Recruiting	Study of Vorinostat (MK0683) an HDAC Inhibitor, or Placebo in Combination With Bortezomib in Patients With Multiple Myeloma
Recruiting	Study of Vorinostat (MK0683), an HDAC Inhibitor, in Combination With Bortezomib in Patients With Relapsed or Refractory Multiple Myeloma
Completed	A Phase II Study of Epigenetic Therapy to Overcome Chemotherapy Resistance in Refractory Solid Tumors
Recruiting	Sorafenib and LBH589 in Hepatocellular Carcinoma (HCC)
Recriting	Phase II Study of Valproic Acid With FEC100 for Patients With Locally Advanced Breast Cancer

Total : 84 studies

http://clinicaltrials.gov/ct2/results?term=histone+inhibitors&pg=4

Methylation Inhibitors in Clinical Trials (Clinicaltrials.gov)

STATUS	STUDY
Completed	A Phase II Study of Epigenetic Therapy to Overcome Chemotherapy Resistance in Refractory Solid Tumors
Active Not Recruiting	Azacytidine and Valproic Acid in Patients With Advanced Cancers
Recruiting	Azacitidine With or Without MS-275 in Treating Patients With Myelodysplastic Syndromes, Chronic Myelomonocytic Leukemia, or Acute Myeloid Leukemia
Active Not Recruiting	PhII 5-Azacytidine Plus Valproic Acid and Eventually Atra in Intermediate II and High Risk MDS
Recruiting	Decitabine With or Without Interferon Alfa-2b in Treating Patients With Unresectable or Metastatic Solid Tumors
Recruiting	Hydralazine Valproate for Cervical Cancer
Recruiting	Hydralazine Valproate for Ovarian Cancer
Recruiting	Decitabine in Treating Patients With Previously Untreated Acute Myeloid Leukemia
Recruiting	Chronic Hepatitis C Non-Responder Study With AdoMet and Betaine
Recruiting	Azacitidine, Docetaxel, and Prednisone in Treating Patients With Metastatic Prostate Cancer That Did Not Respond to Hormone Therapy
Recruiting	Low Dose Decitabine + Interferon Alfa-2b in Advanced Renal Cell Carcinoma

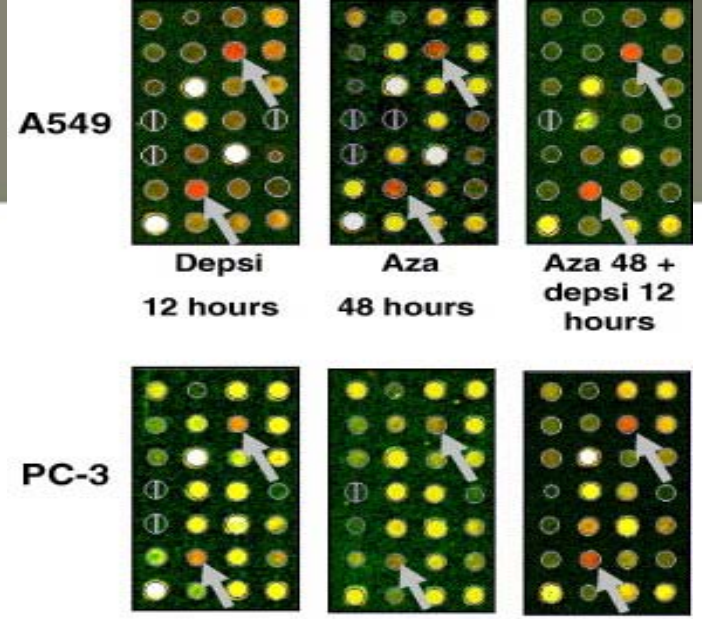
Schering-Plough (Decitabine {5-aza-Deoxycytadine} Trial for melanoma) (8 hrs to inactivate DNMT1)

Bristol-Myers Squibb (other compounds)

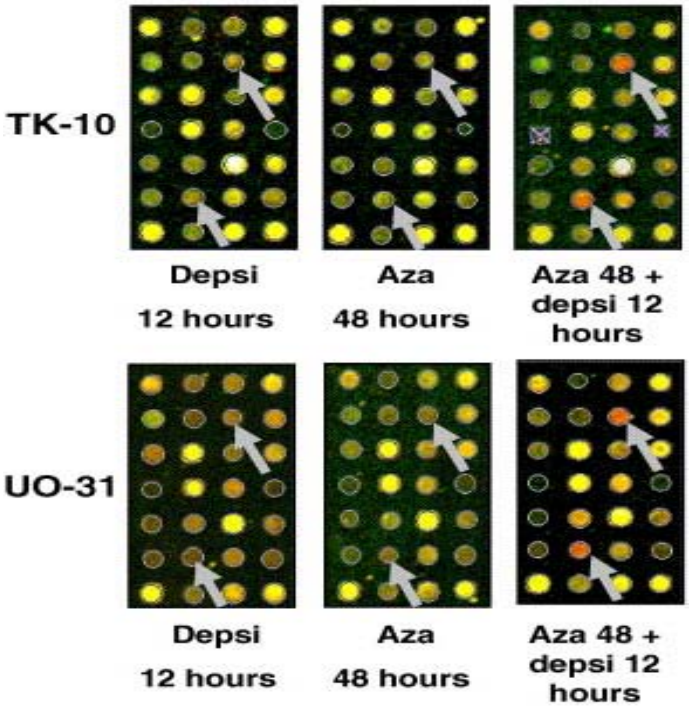
http:// ibitors

National Cancer Institute

Gene Reactivation



Pharmacoepigenomics is acquired during persons life



The pro-apoptotic BIK (Bcl2-interacting killer)

Dai et al. 2006, BBRC 351:455-461

Exfoliated Cells are Good Source of DNA to Study Epidemiology

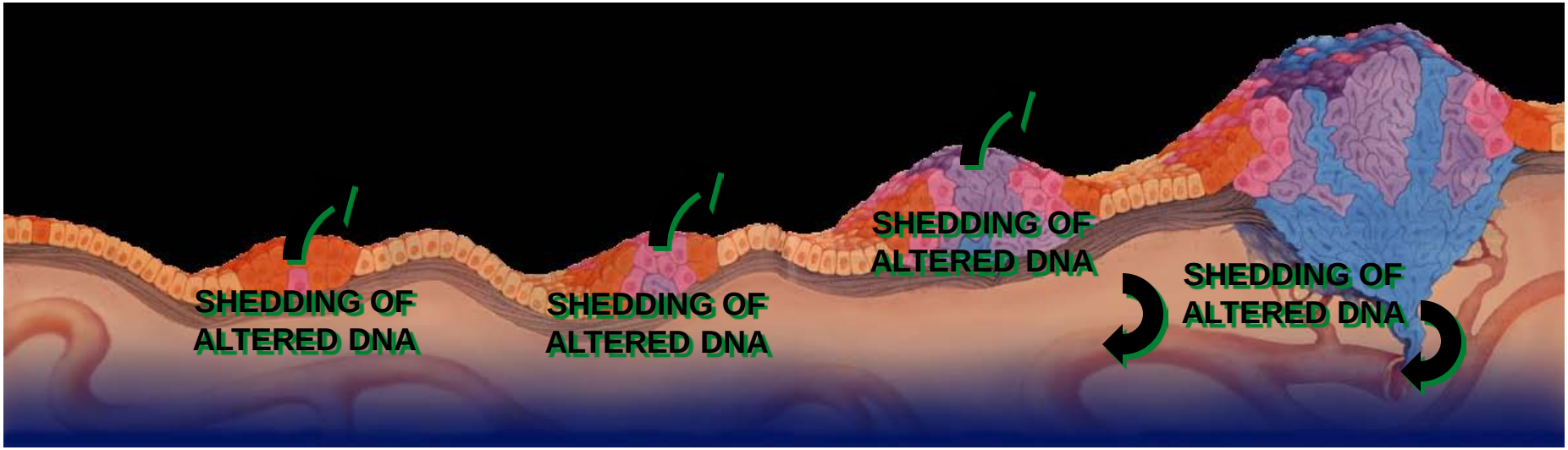
CANCER RISK ASSESSMENT

PROGRESSION RISK ASSESSMENT

EARLY DETECTION

DIAGNOSIS, PREDICTION & PROGNOSIS

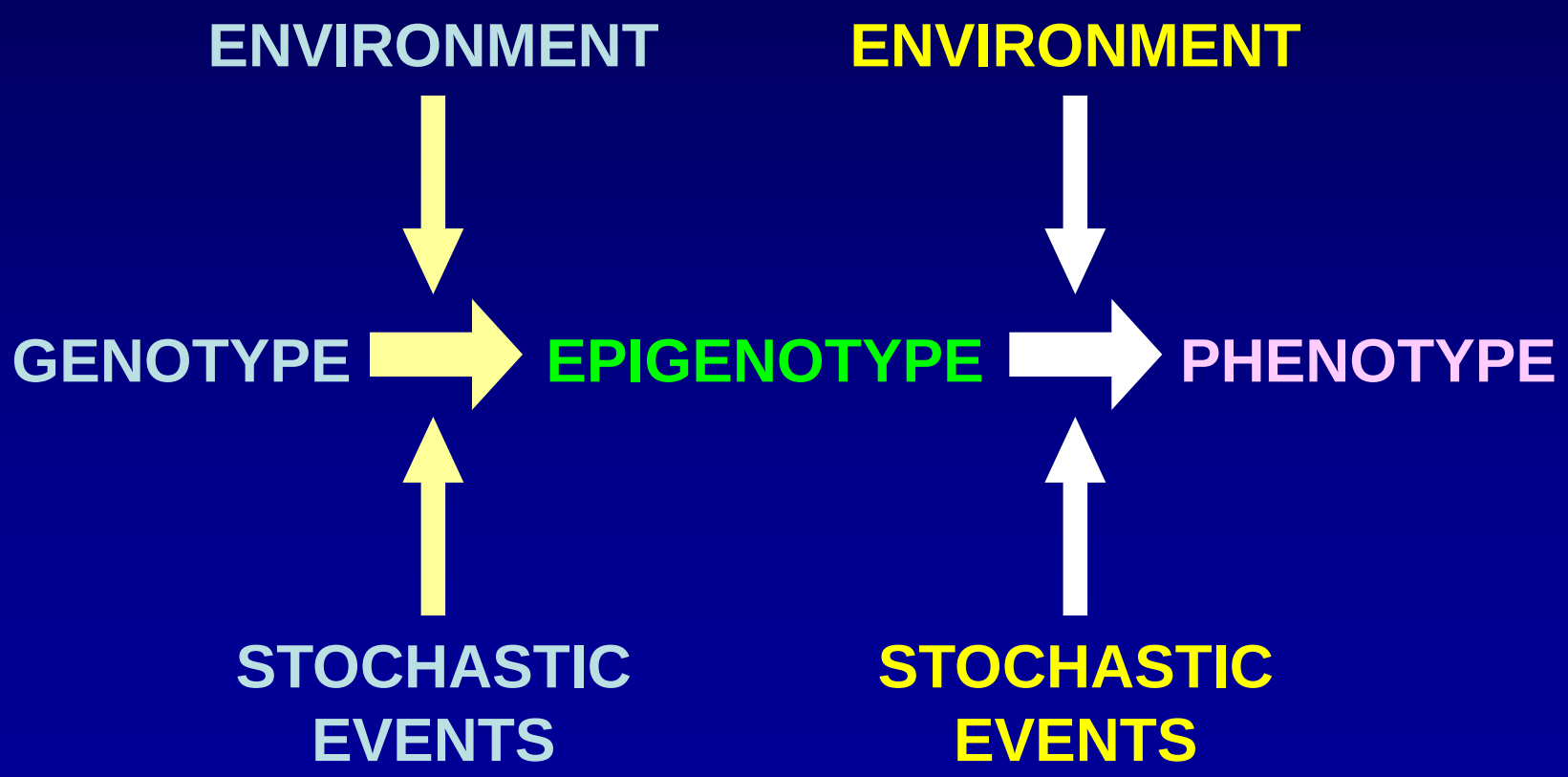
MONITORING FOR RECURRENCE



PREDISPOSITION → HYPERPLASIA → DYSPLASIA → CARCINOMA → METASTASIS

Adapted from Weinberg 1996

Epigenotype as an Intermediate Phenotype



National Cancer Institute

Research Opportunities

Will inclusion of epigenetic markers help in identification of new risk factors (modifiable factors and host factors) in different races and ethnic groups?

Will epigenetic markers in cohort and case control studies improve sensitivity and specificity of markers and help in identifying high-risk populations?

Are genetic and epigenetic events correlated during cancer development?

Are there race/ethnicity specific miRNAs?

National Cancer Institute

EPIGENETICS

AND

CANCER

Editors

Mukesh Ve

Barbara K. D

Asad Umar

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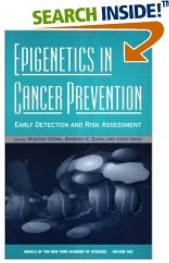

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Epigenetics in Cancer Prevention: Early Detection and Risk Assessment (A
New York Academy of Sciences) (Paperback)

by Mukesh Verma (Editor), Barbara K. Dunn (Editor), Asad Umar (Editor) "The transition to malignancy requires reconfiguration of the genome's expression program that does not result entirely from actual changes in primary D sequence-i.e.,..." (more)

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Editorial Reviews

Book Description

This volume addresses the question of how knowledge of epigenetic phenomena like DNA methylation and acetylation can be applie assessment.The objectives of the papers include defining the terminology used in epigenetics, identifying and prioritizing areas of r technology available for quantitation of methylation and high-throughput assays, and discussing clinical correlates to epigenetic ch

Book Info

(New York Academy of Sciences) Presents material from the conference entitled Epigenetics in Cancer Prevention: Early Detection Cancer Institute, held in Bethesda, MD, on December 3-4, 2001.

See all Editorial Reviews

Product Details

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Publisher: New York Academy of Sciences (January 27, 2004)

Language: English

ISBN-10: 080187999X

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New York Academy of Sciences

2003

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Issue 3 (July/August/Sept 2006)
Issue 4 (Oct/Nov/Dec 2006)

Volume 2

Issue 1 (Jan/Feb/Mar 2007)
Issue 2 (April/May/June 2007)

Volume 2, Issue 2
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National Cancer Institute

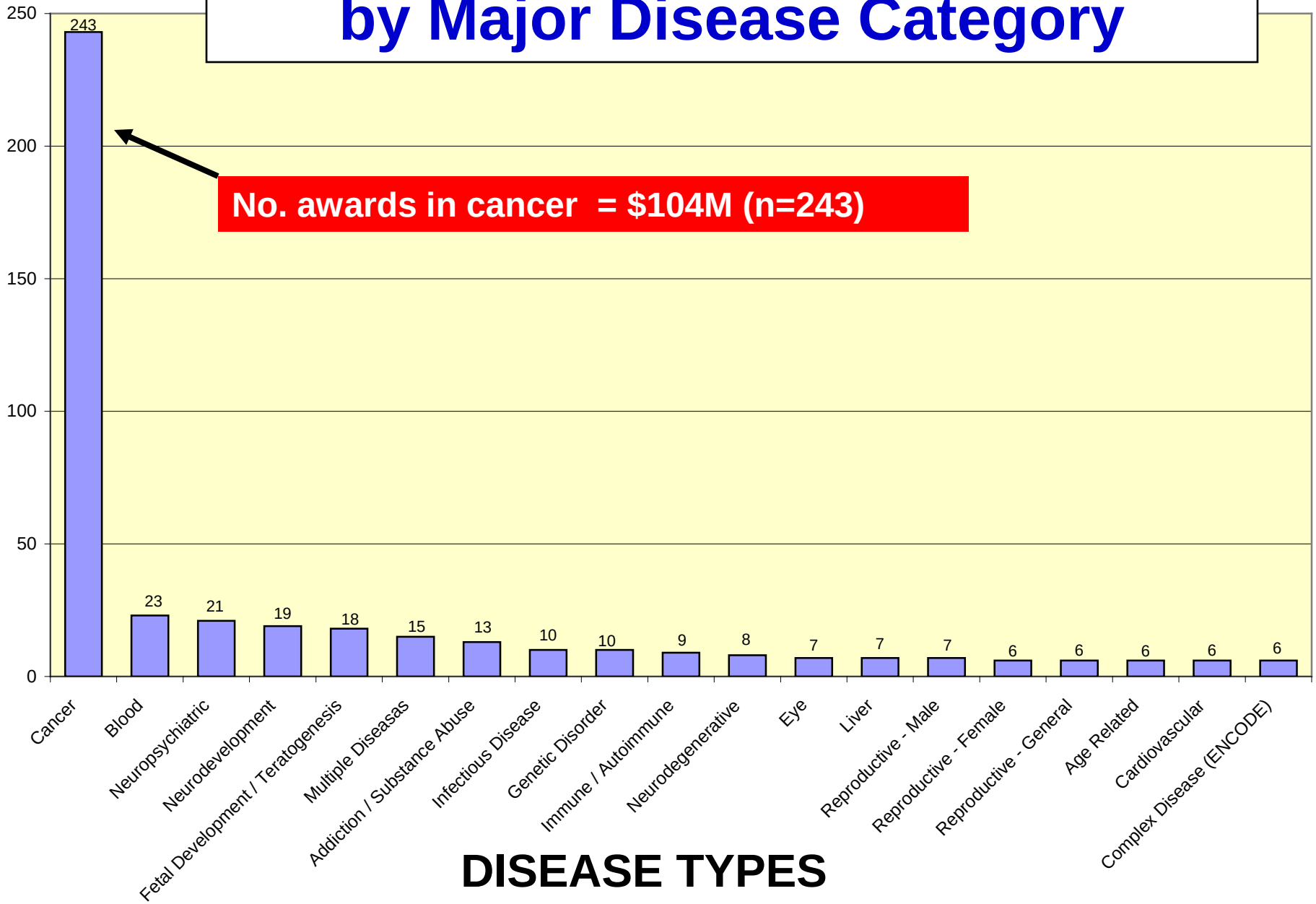
NIH Roadmap 1.5

http://nihroadmap.nih.gov/epigenomics/

- **Incubator space (5-10 year projects)**
- **Test bed for high risk, enabling, or emerging scientific opportunities**
- **Accelerate the pace of discovery**
- **Must be transformative**
- **Trans-NIH, all Institutes/Centers participate**
- **NIH Common Fund**

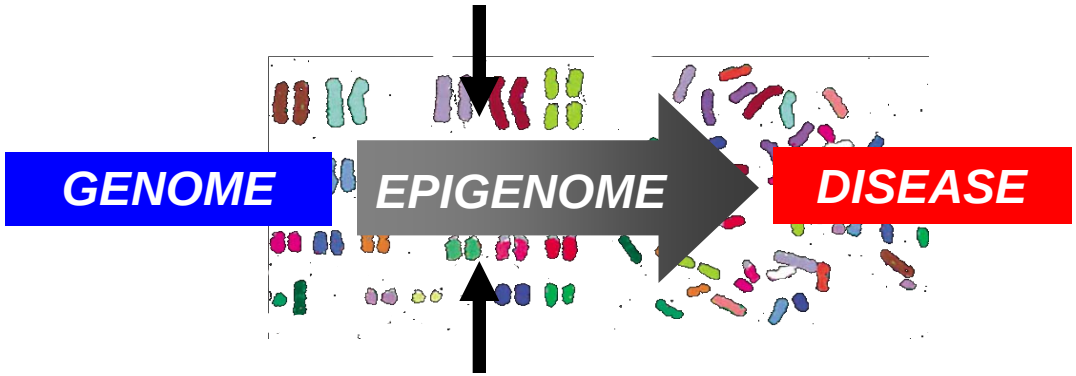
Epig
Several
and neurobehavioral and cognitive dysfunctions

Extramural Epigenetics Portfolio by Major Disease Category



Epigenomic Changes Have Been Implicated in a Wide Variety of Human Diseases

Development
Cell Differentiation
Aging



Environmental exposures
Nutrition
Chemical toxins
Metals
Mediators of stress
Drugs of abuse
Infection (including HIV)

Cancer
Cardiopulmonary disease
Autoimmune disease
Obesity
Diabetes
Neurodevelopmental disorders
Schizophrenia
Addiction
Depression

Roadmap 1.5: Translation to Clinical Practice

FY08 **FY17**

- **Reference epigenomes**
- **Developmental biology**
- **Technology development**
- **Data infrastructure**

- **Application of epigenetics to disease and common conditions**
- **Biomarker development – imaging and predictive biology**

NIH Roadmap Epigenomics Program

- Mapping Centers initiate processing of hematopoietic progenitor subsets.

  These include lymphoid, myeloid, and erythroid progenitors routinely isolated from total CD34+.

- All hematopoietic cell samples will be processed for

  Chromatin structural mapping by Digital DNaseI at mapping centers

  Concurrently, crosslinked chromatin, total RNA, and purified DNA will be made available to the consortium for parallel studies of histone modifications, RNA, and DNA methylation by other Centers.

Epigenetics Working Group

Co-Chairs: Sam wilson (NIEHS), Nora Volkow (NIDA)

***Ex officio* Chairs: Ting-Kai Li (NIAAA), James Battey (NICHD)**

Brenda Weis	NIEHS
Jerry Heindel	NIEHS
Fred Tyson	NIEHS
John Satterlee	NIDA
Joni Rutter	NIDA
Christine Colvis	NIDA
Jonathan Pollock	NIDA
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Johanna Dwyer	OD/ODS
Bruce Howard	NICHD
Rebecca Lipsitz	NIH/OD
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Nasrin Nabavi	NIAID
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Susan Old	NHLBI
Deborah Olster	OD/OBSSR
Jim Ostell	NLM/NCBI
Suzana Petanceska	NIA
Robert Riddle	NINDS
Sharon Ross	NCI
Rochelle Small	NIDCR
Philip Smith	NIDDK
Mukesh Verma	NCI
Ashley Xia	NIAID
Samir Zakhari	NIAAA
Keji Zhao	NHLBI

Timeline for Epigenomics Program

FY07 FY08 FY09 FY10 FY11 FY12 FY13 FY14 FY15

JUMPSTART

- International Committee
- Standardization; Database
- Reagents (antibodies)

RFA 1
Mapping Cent.

RFA RFA?
- Comprehensive reference epigenomes
- Incorporate new tech/targets as available
- Public resource (cells/tissue, data)

RFA 2
RM/IC Projects

RFA RFA RFA RFA RFA
- Co-fund RM/IC-based projects

RFA 3
Data Analysis/ Coord Center

RFA RFA
- Contribute to public data resource (via NCBI)
- Cross-cutting data integration, analysis, visualization

RFA 4
Tech Develop

RFA RFA RFA
- New tools/tech, application to mapping, RM/IC projects

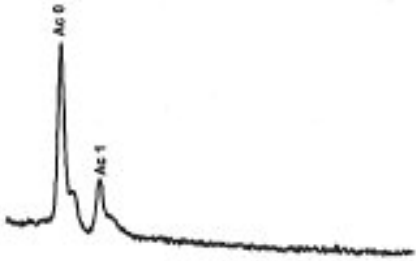
RFA 5
Discovery of Novel Marks

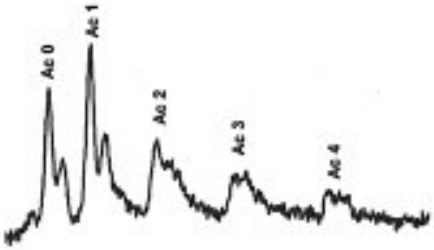
RFA RFA?
- New marks, application to mapping, RM/IC projects

TRANSITION to ICs

Transition to ICs

A Model for a Standarized Treatment of Oncological Patients Using HDAC Inhibitors





Quantification of Histone Acetylation Levels in Blood: Baseline

Administration of HDAC Inhibitor

Quantification of Histone Acetylation Levels in Blood: Surrogate Marker of HDAC Action

CANCER PATIENT

Time: How Long?

BIOLOGICAL EFFECT

Time: How Long?

CLINICAL EFFECT

Personalized Medicine

Viller-Garea and Estellar. 2004. .Int. J. Cancer 112, : 171-178

Conclusion: Epigenetics in Cancer Management

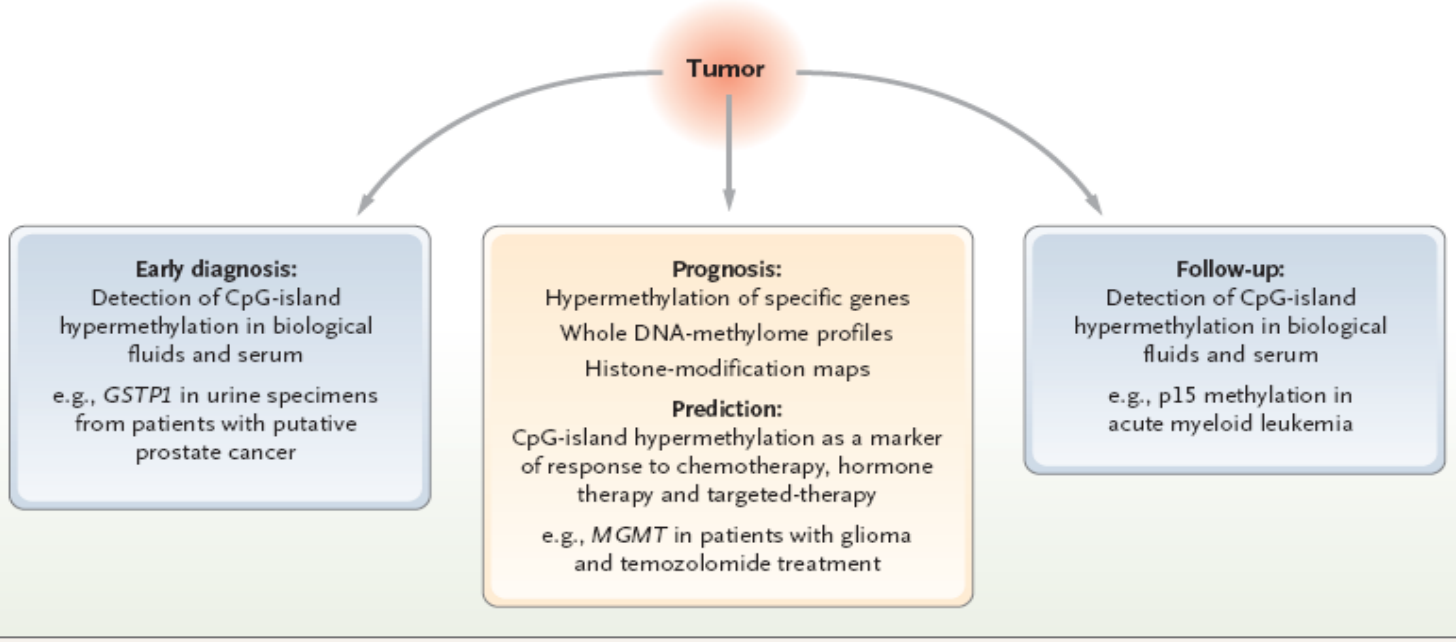


Figure 4. Epigenetics in Cancer Management.
DNA methylation techniques permit the sensitive and quantitative detection of hypermethylated tumor-suppressor genes in all types of biologic fluids and biopsy specimens. The establishment of DNA methylation and histone-modification profiles of the primary tumor specimen itself might be a valuable tool in determining the prognosis and predicting the patient's response to therapies.

Esteller, NEJM, 358:1148-59, 2008