

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

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Chief

Methods and Technologies Branch (MTB)

Program Director

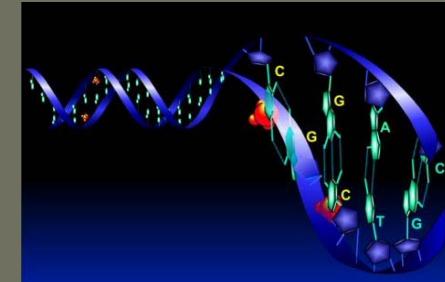
Epidemiology and Genetics Research Program (EGRP)

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)



Outline

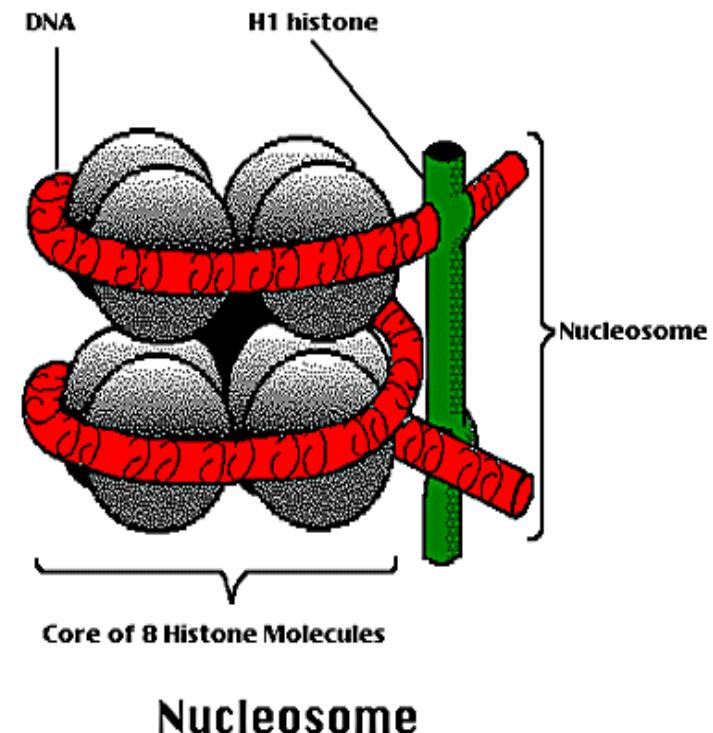
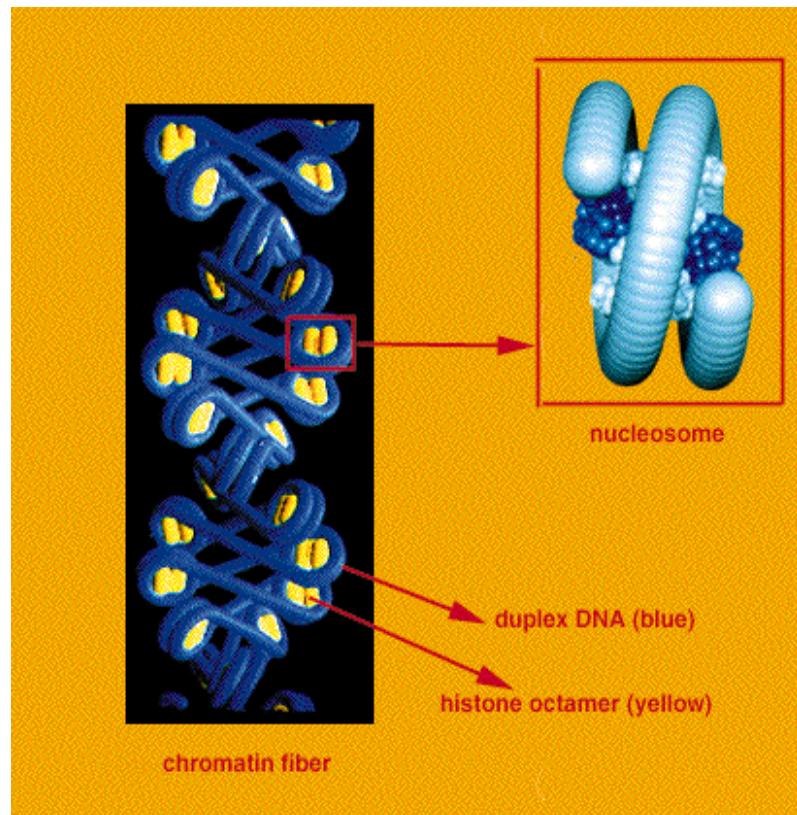
- Molecular Basis of Epigenetics
- Epigenetic Profiling: Technology
- Examples from Clinical Studies (Cancer)
- Implication in Personal Health
- NIH Roadmap: Epigenomics

Nucleosomes (Units of Chromatin)

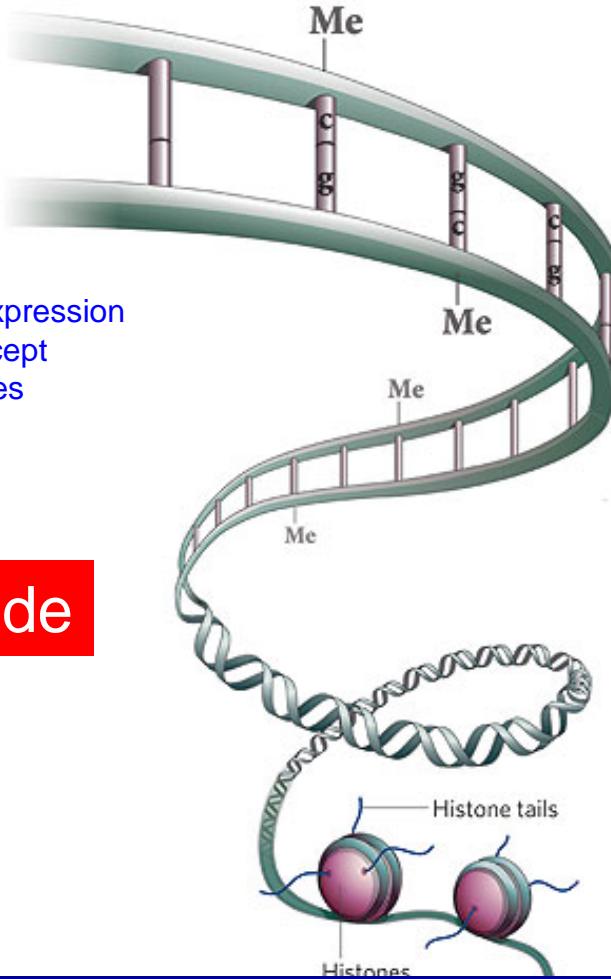
DNA
Histones H2a, H2b, H3, H4

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



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



The two main components of the epigenetic code

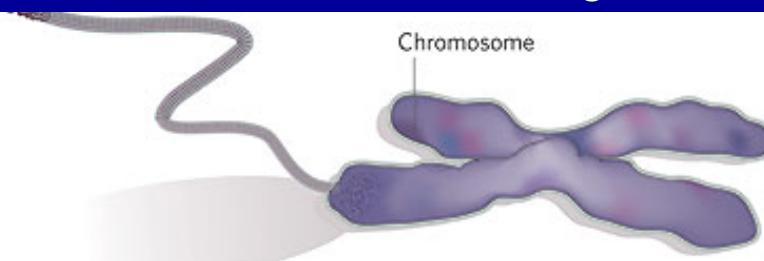
DNA methylation

Methyl marks added to certain DNA bases repress gene activity.

Methylation 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.

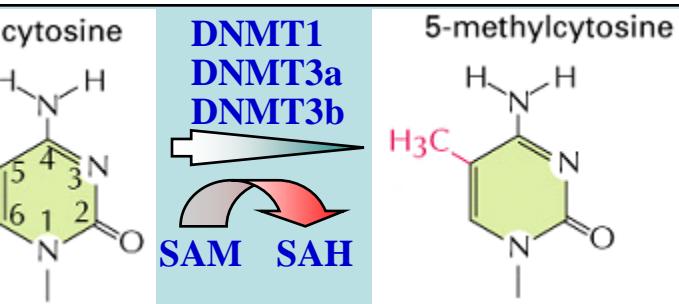


EPIGENETICS

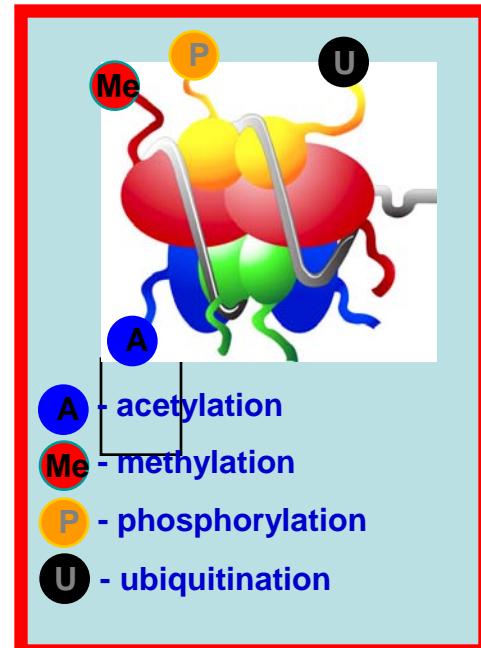
Epigenetic alterations – changes induced in cells that alter expression of the information on transcriptional, translational, or post-translational levels without change in DNA sequence



Methylation of DNA

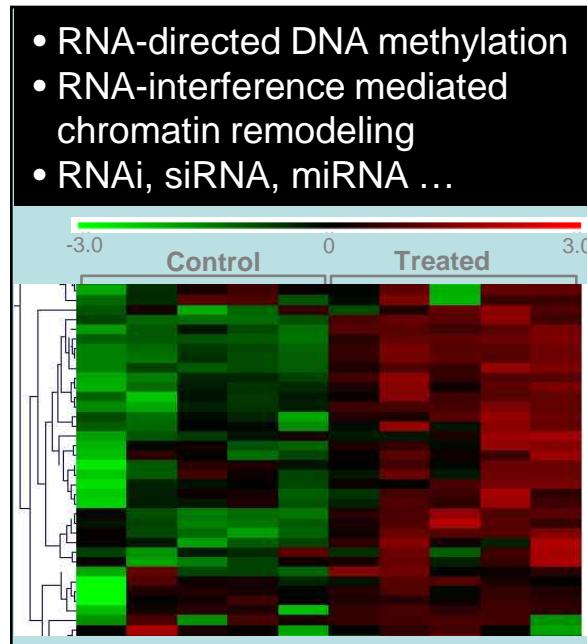


Modifications of histones



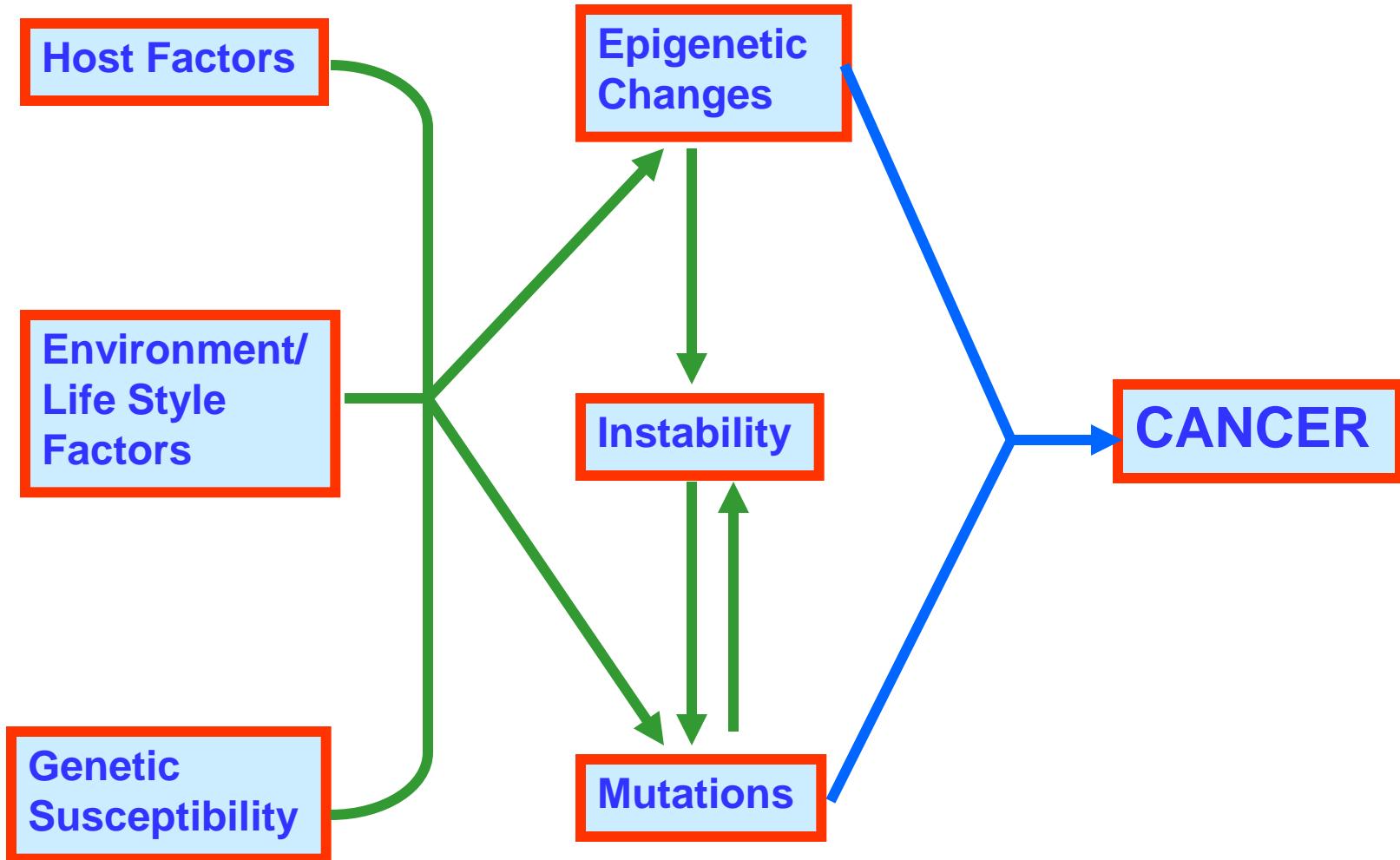
RNA-mediated modifications

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



Genomic Imprinting

Background



A multistage model of carcinogenesis in skin

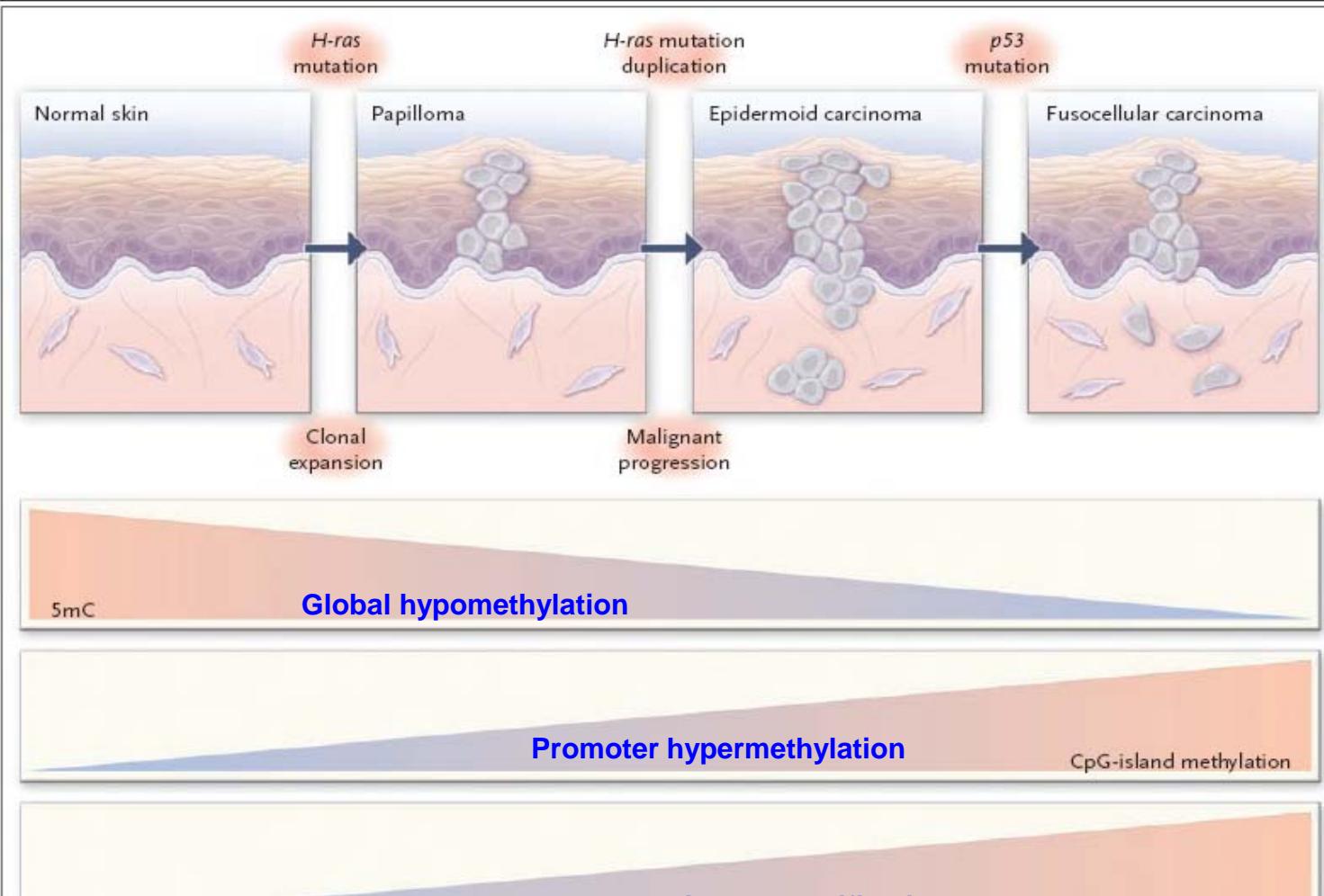
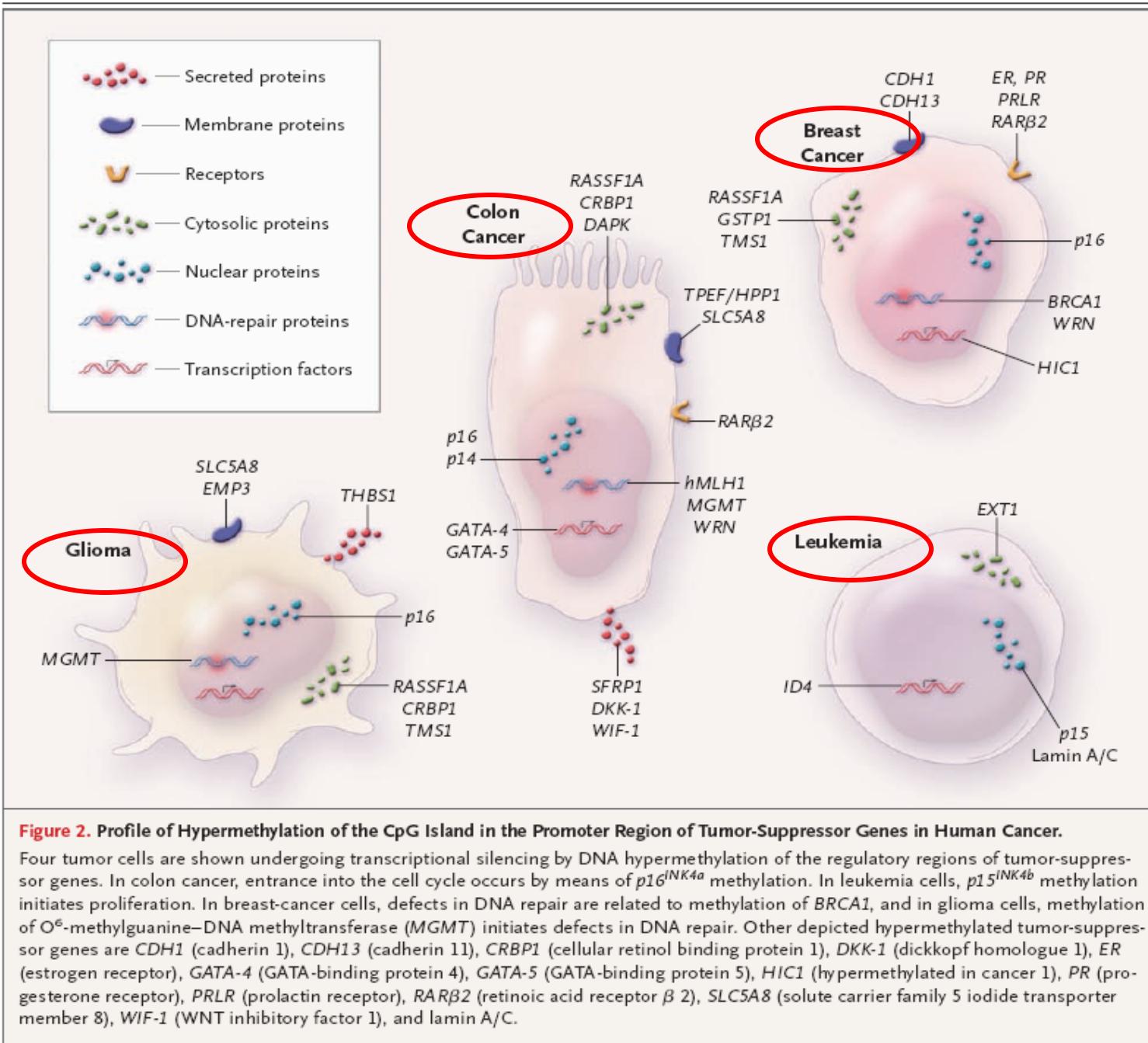


Figure 1. Epigenetic Alterations in Tumor Progression.

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.



Epigenetics



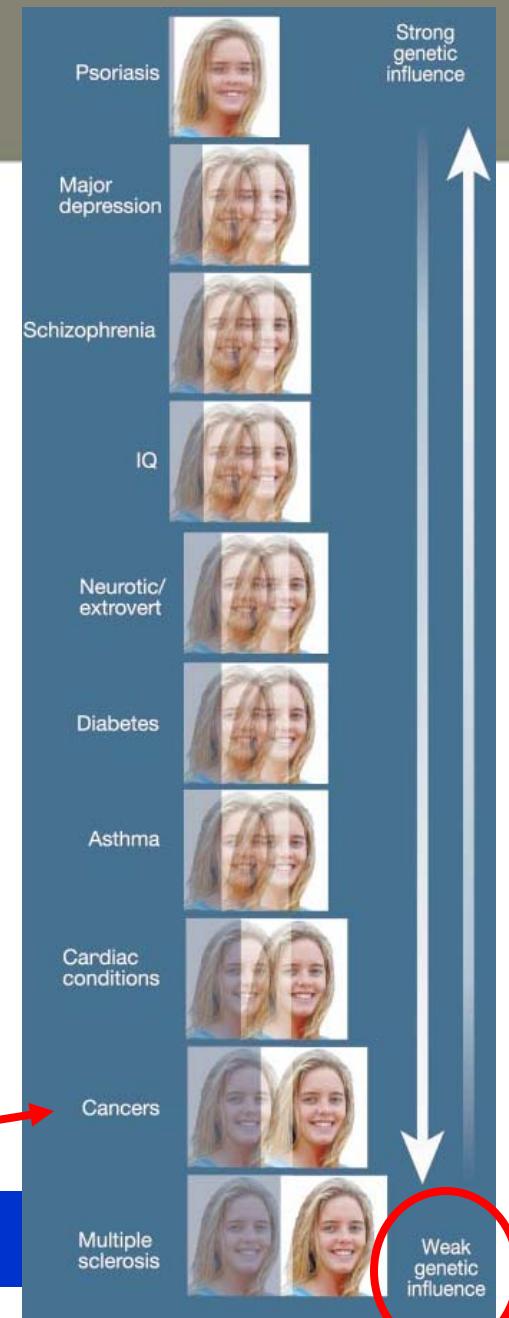
Epigenetic events are alterations in gene expression **without changes in the DNA coding sequence** that are **heritable through cell division**

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



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 phenotypic discordance may be observed, such as **differences in susceptibilities to disease** and a wide range of anthropomorphic features.

Manel Esteller 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 epigenetics is missing from our understanding of how different phenotypes can be originated from the same genotype.



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

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

Genetic

Epigenetic

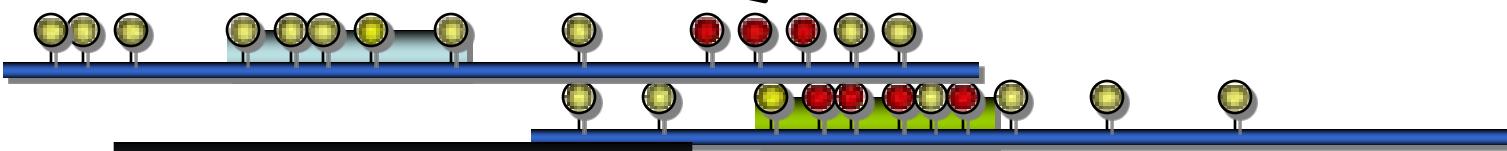
**Transcription
Factor
Changes**

**Cell-cycle
Regulated
Changes**



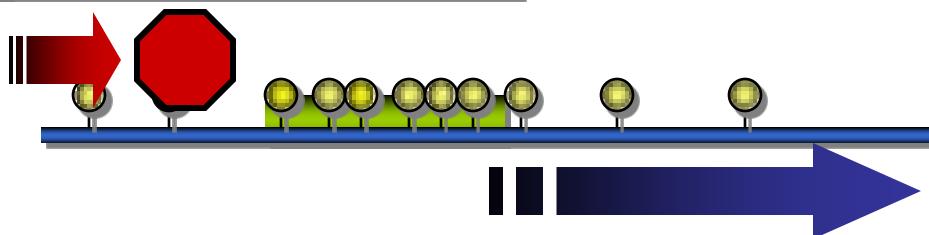
Unmethylated CpG

Methylated CpG



Hypermethylation

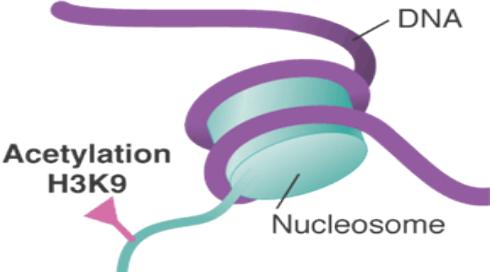
Hypomethylation



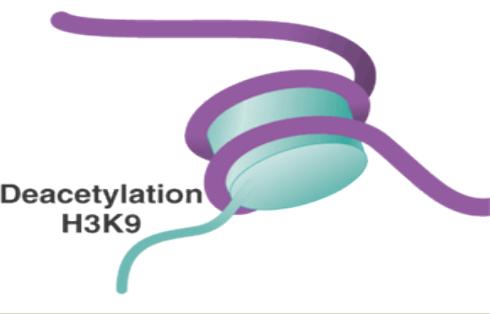
Inactivated Tumor Suppressors Activated Oncogenes

Transcription

Active



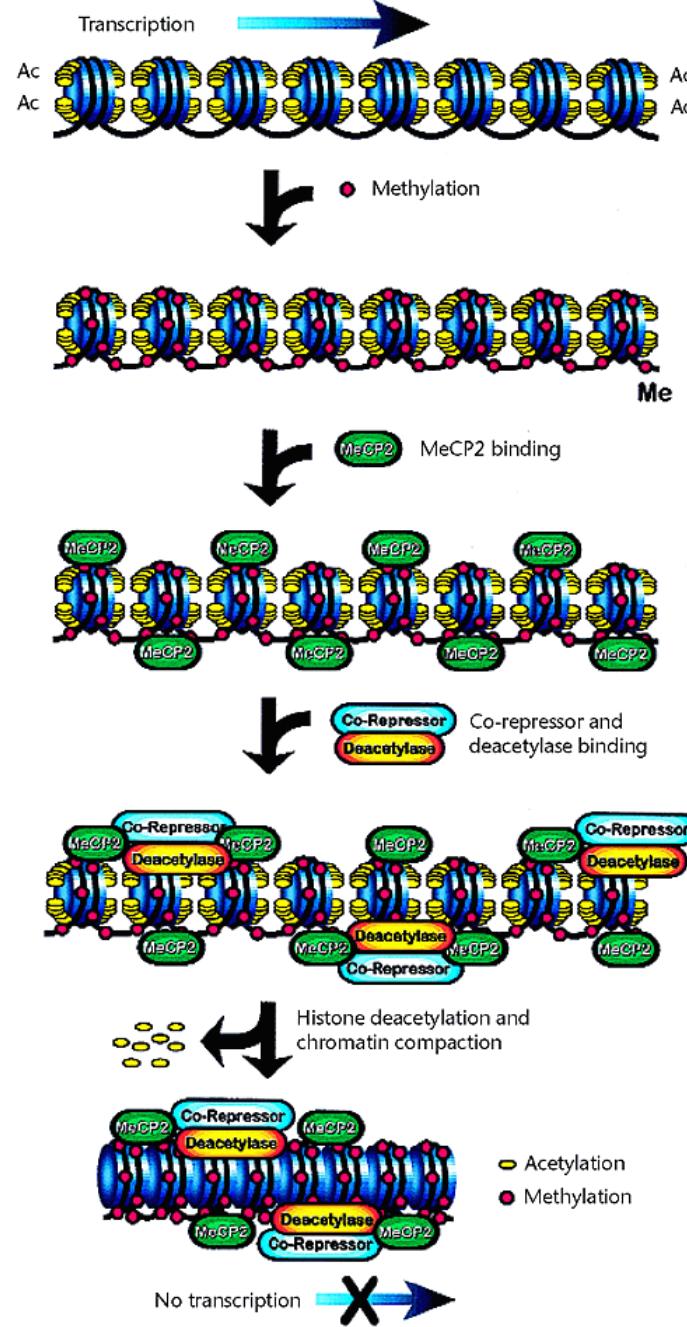
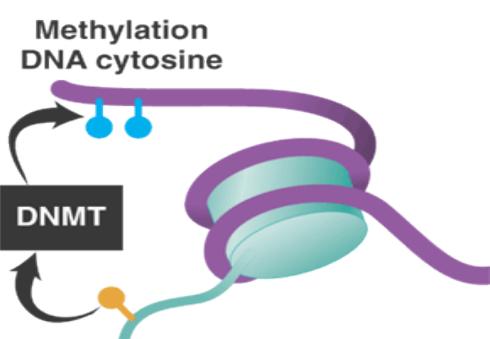
Inactive



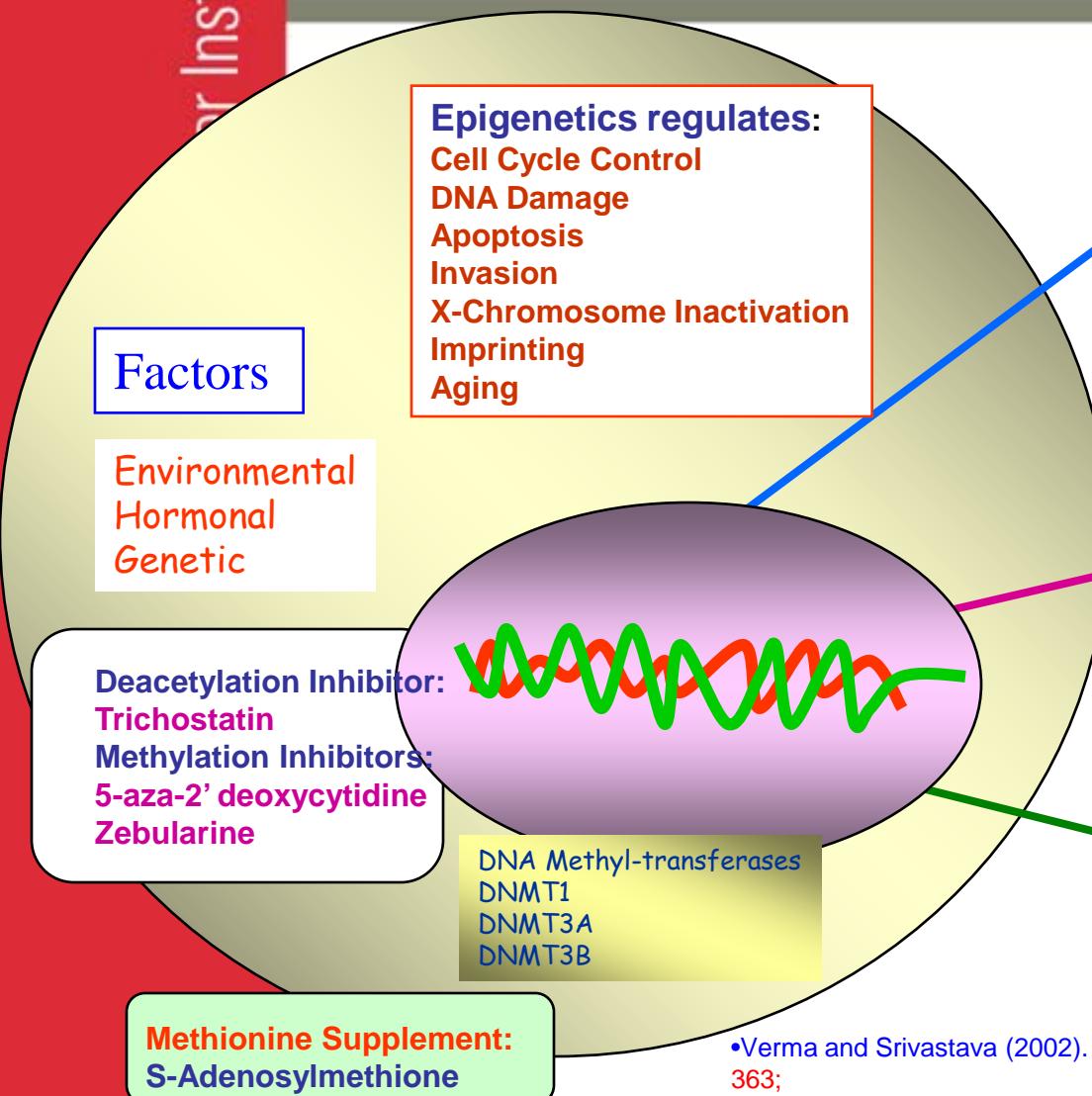
Silenced



Stably
silenced

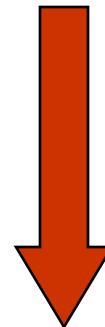


Epigenetic Markers and Targets



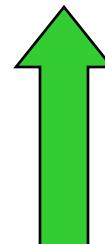
Hypermethylation

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.

Cancer Institute - N

U.S. National Institut



May 3, 2005 • Volum

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Deaths Remain Low
Years

Director's Update

Cancer Centers: Pro
Leadership and New
Opportunities

Spotlight

Cancer Epigenetics:
Genetic Mutations

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NCI Grand Rounds

Epigenetics and Cancer Prevention

Some cancers involve the inappropriate 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 epigenetic changes are stable, they may change over time by environmental factors or drugs.

Epigenetic changes are essential for normal development and can lead to cancer. But the changes can also potentially prevent cancer. For example, a diet rich in folic acid, folate, and genistein, which are found in many fruits and vegetables, may help prevent prostate tumors. By adding methyl groups to DNA, these substances at Johns Hopkins University helped develop an experimental test to detect this DNA methylation, the most common epigenetic 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 DCCP's Early Detection Research Network are engaged in analytical validation of *GSTP1* methylation for prostate cancer, as well as

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

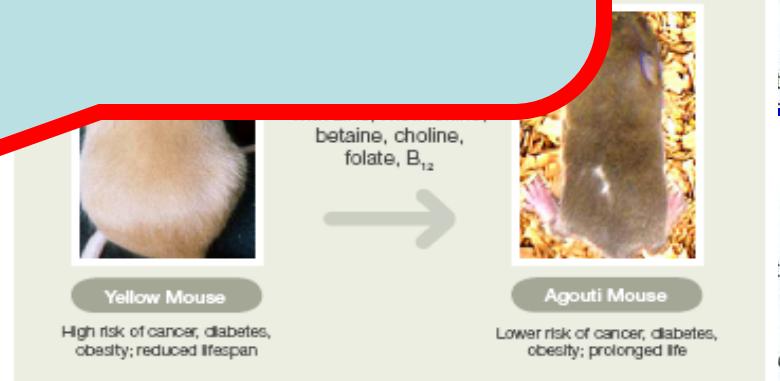
"We cannot reverse genetic mutations, but by using drugs we may be able to reverse changes in methylation," said Dr. Verma. "This approach has promise for prevention."

Diet also may affect methylation. Giving pregnant mice diets rich in folic acid, folate, and genistein, which are found in many fruits and vegetables, can modify the methylation of a certain gene that may cause a tumor. This tumor may be associated with increased cancer risk.

In presumably healthy mice, a diet that's high in folic acid, folate, and genistein may reduce the risk of cancer. This is intriguing because DCCP is working to understand how epigenetic changes influence disease.*

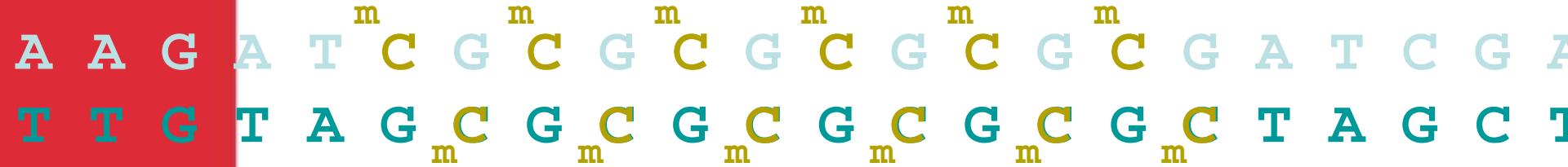
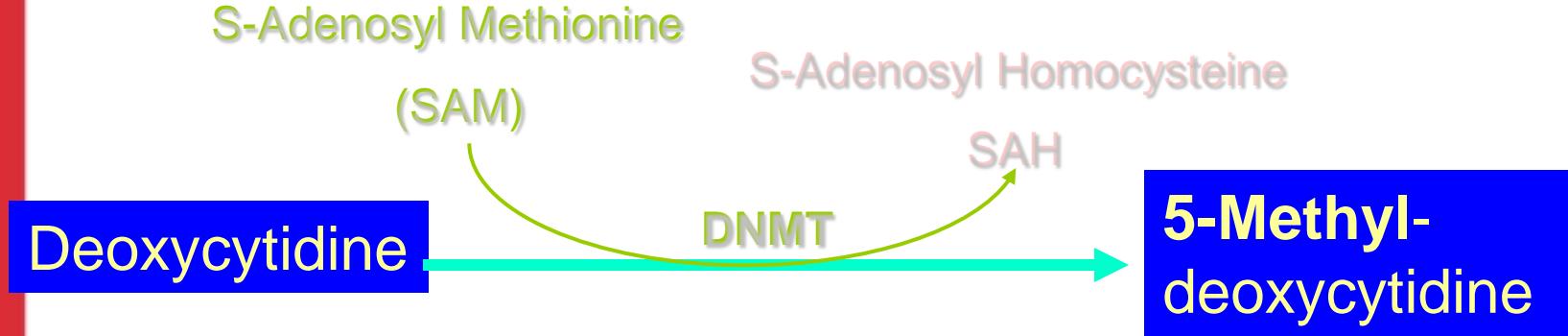
"Cancer epigenetics is important for prevention because we may be able to use methylation markers to identify people at higher risk of cancer and perhaps detect cancer earlier"

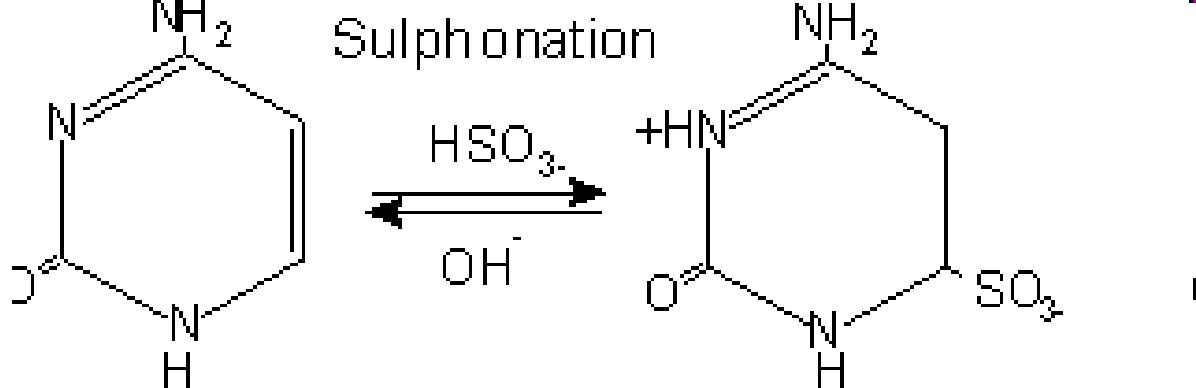
- Mukesh Verma, DCCPS



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

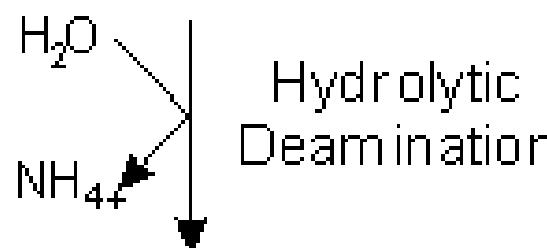
Mechanism of DNA methylation



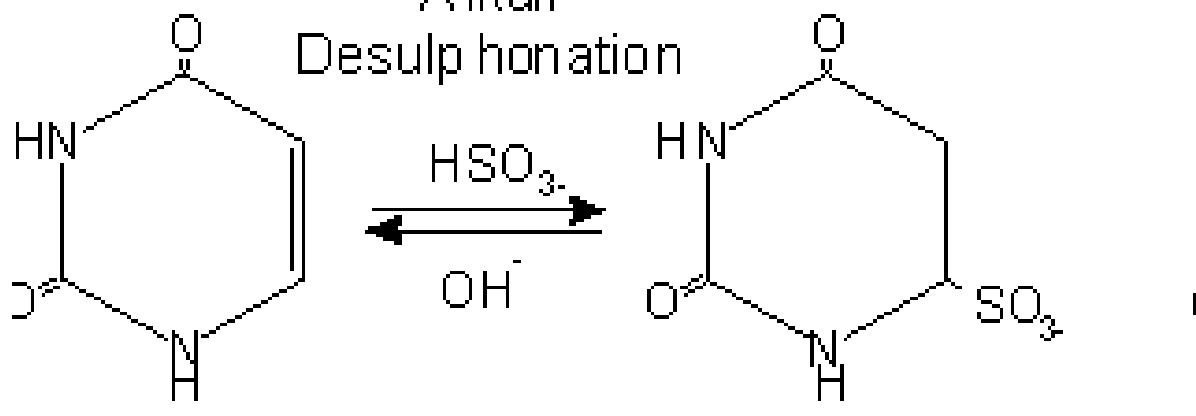


Cytosine

Cytosine
sulphonate



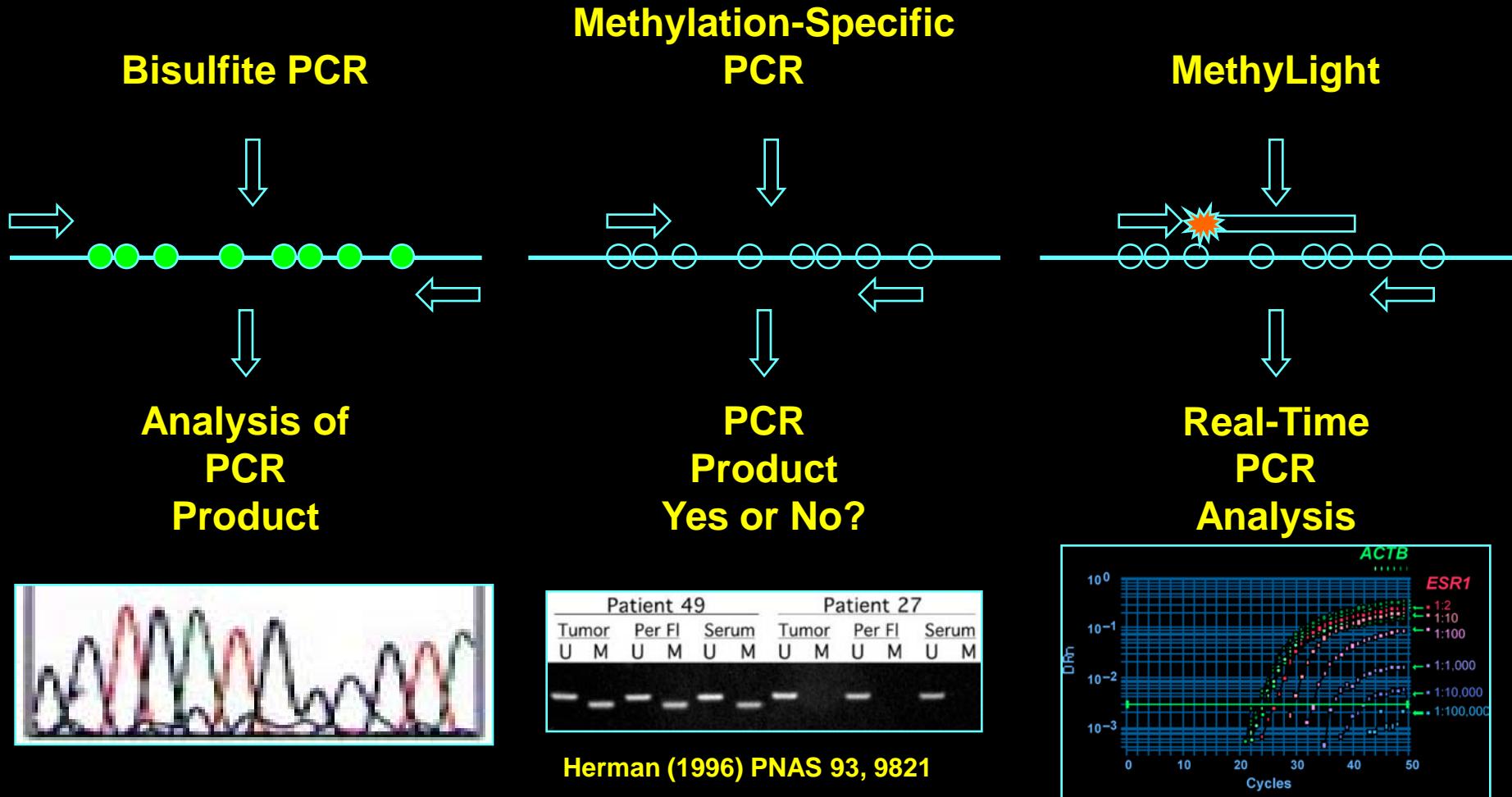
Alkali
Desulphonation



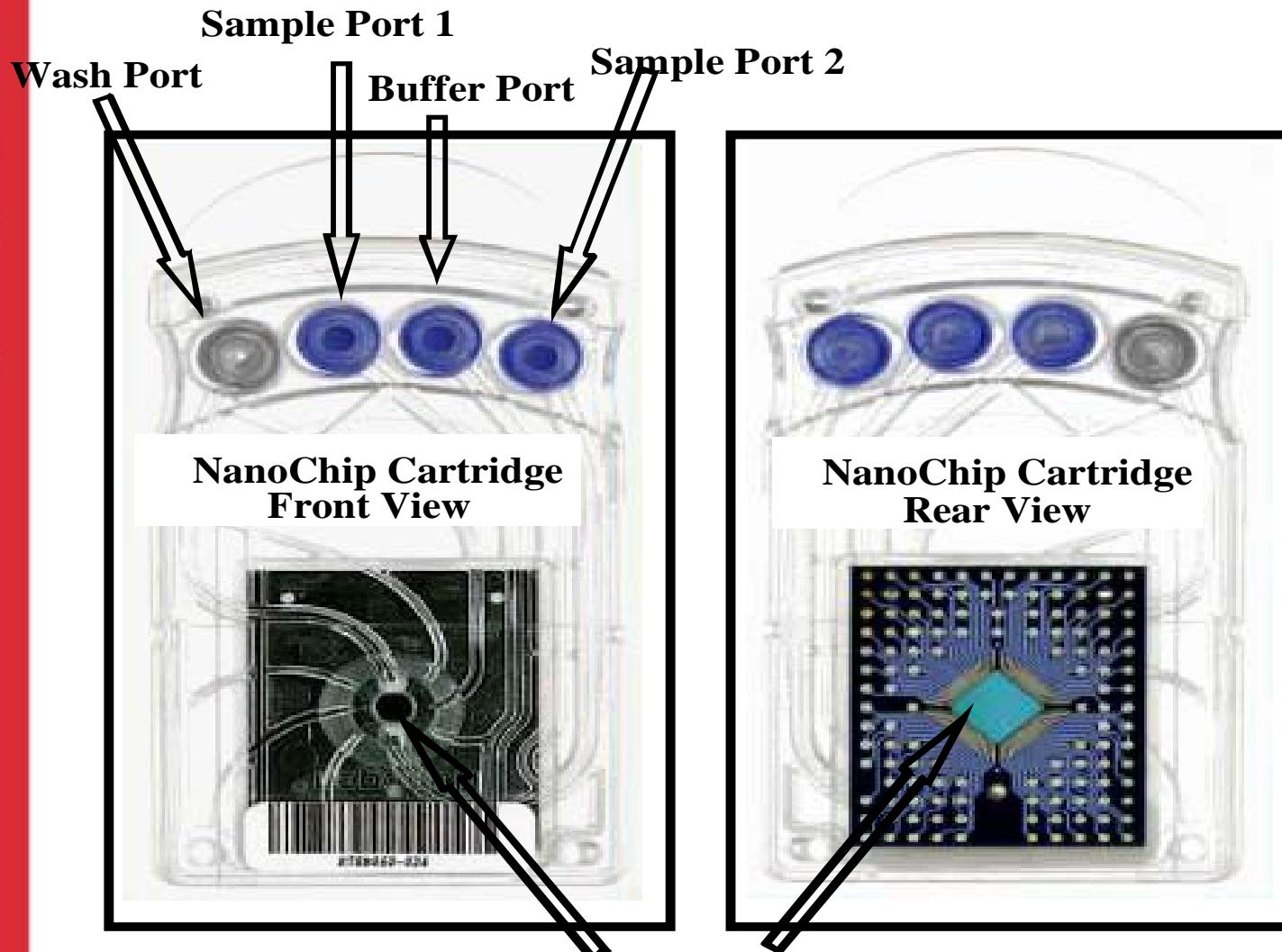
Uracil

Uracil
sulphonate

Bisulfite-Dependent DNA Methylation Analysis



Nanochip to Detect Hypermethylation



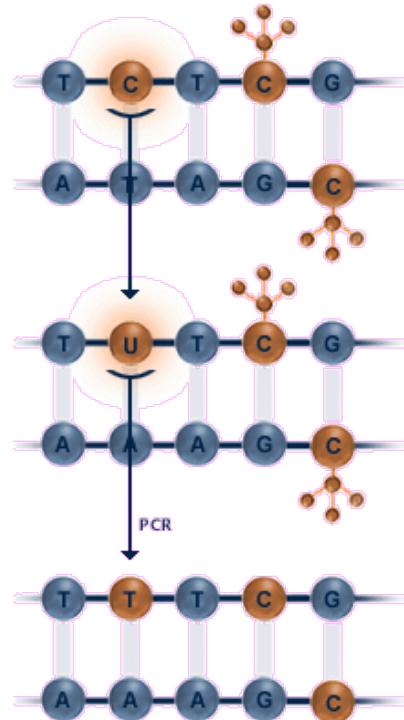
**Micro-array test sites
connected with platinum wires**

DNA Methylation Detection by Hybridization of Bisulphite DNA

EPIGENOMICS, Inc.

Roche 454

Agilent



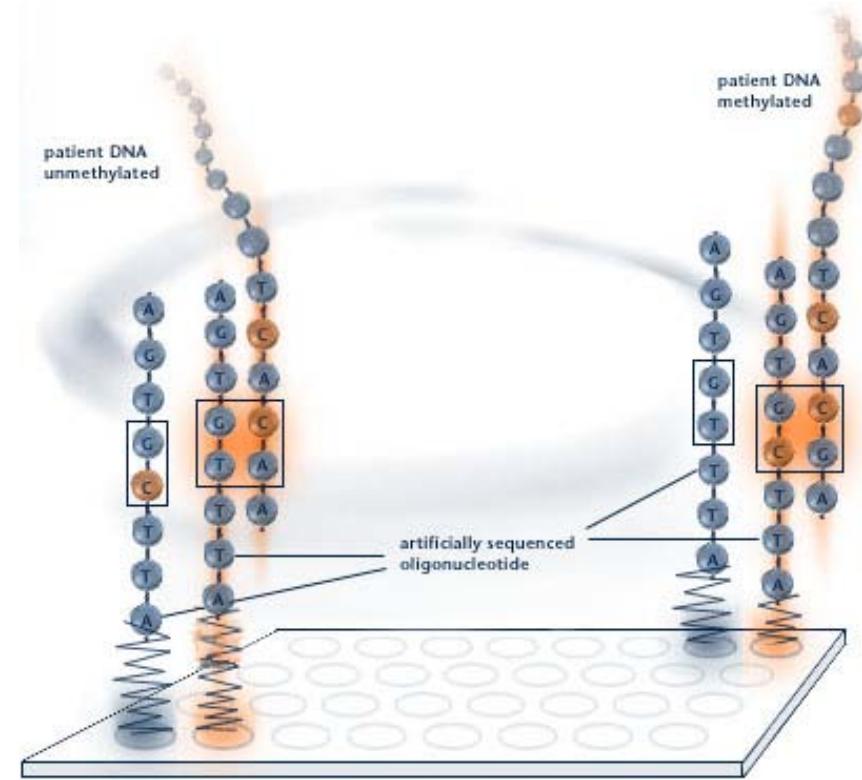
Nimblegen

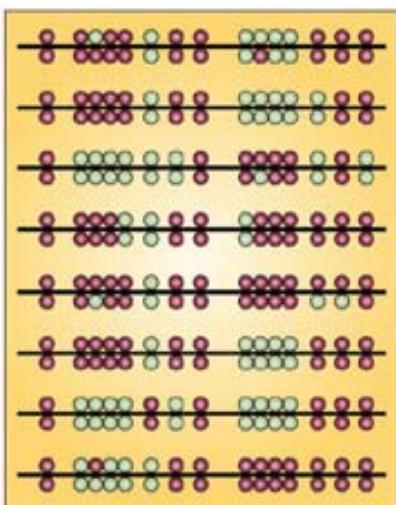
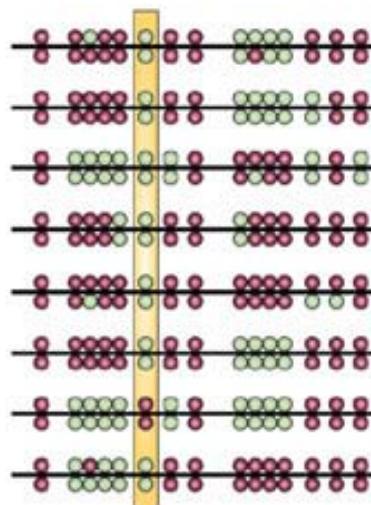
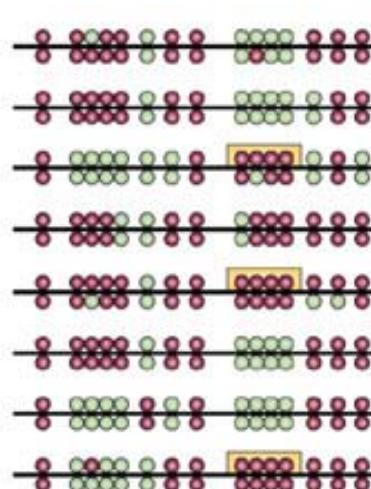
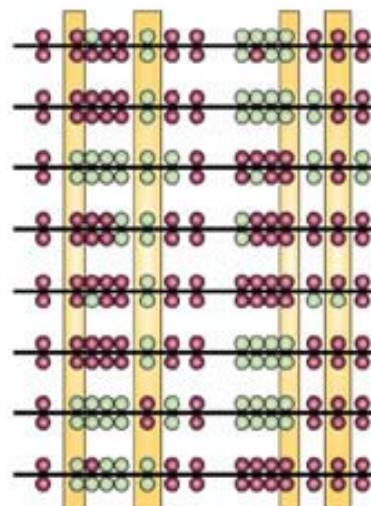
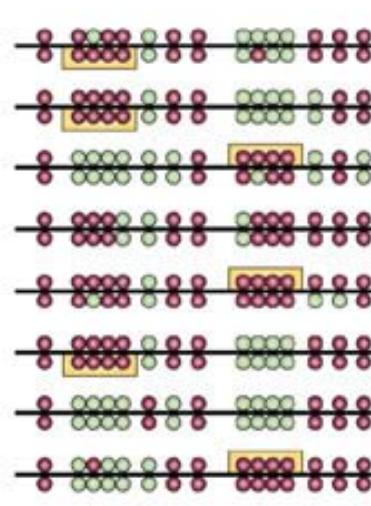
Illumina

Solexa

Qiagen

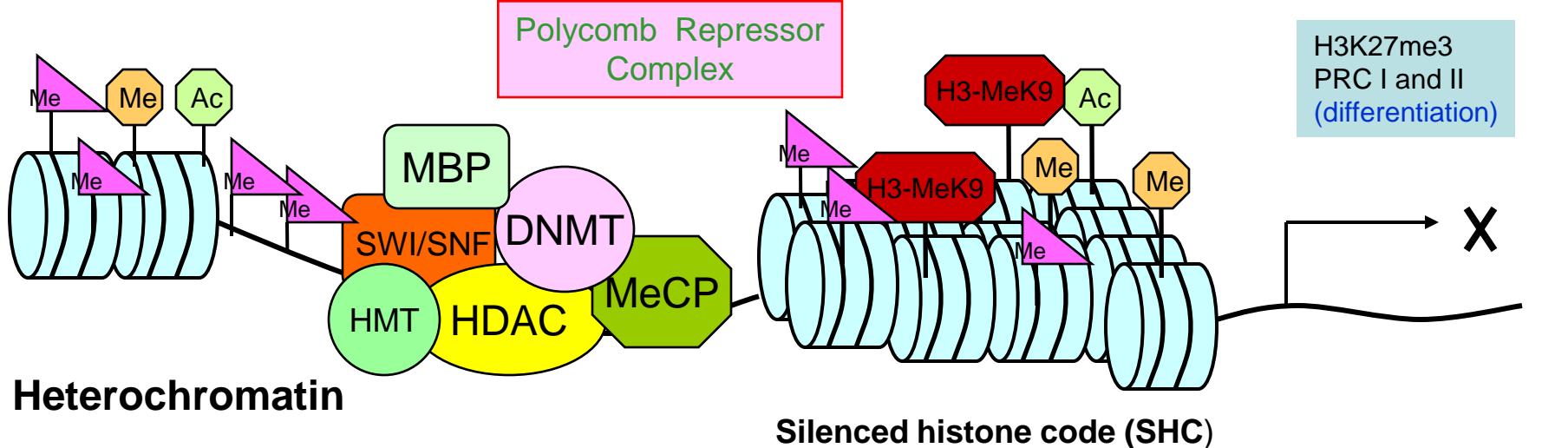
Biotage, Inc.



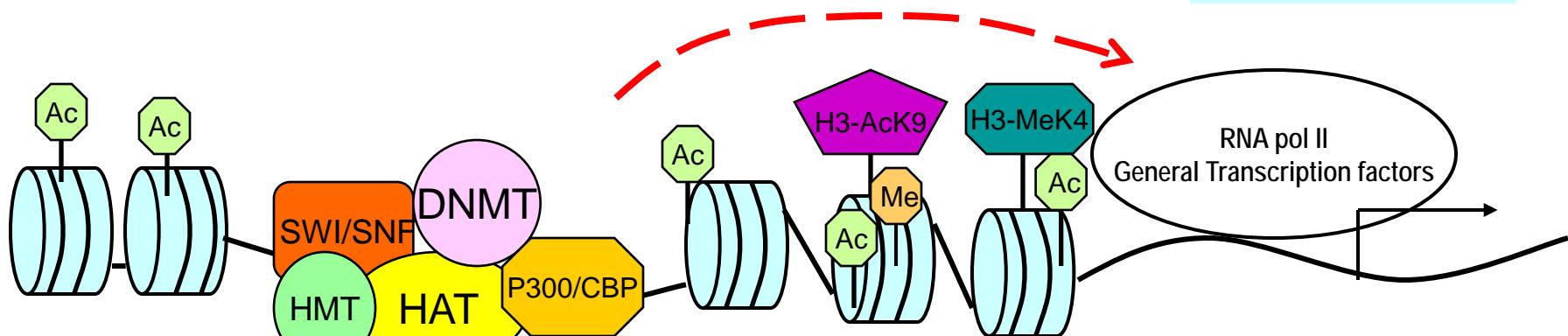
a Methylation content**b Methylation level****c Methylation pattern****d Level profile****e Pattern profile**

- 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

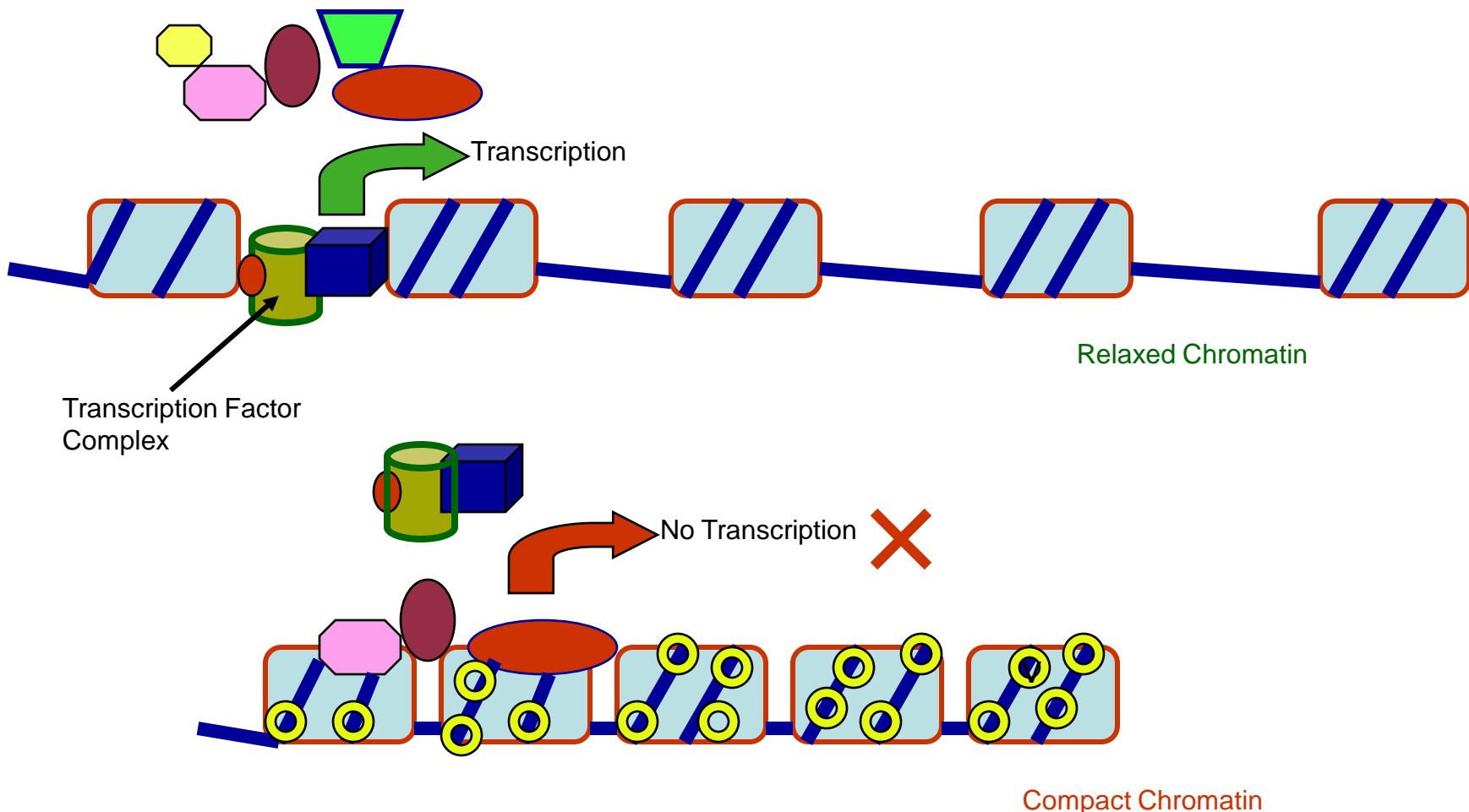
To reduce
• false negative
• false positives



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



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



5-Aza-deoxy-cytidine (5-AZA-C)



Trichostatin A (TSA)



DNA Methyl Transferase



Methylation Binding Protein

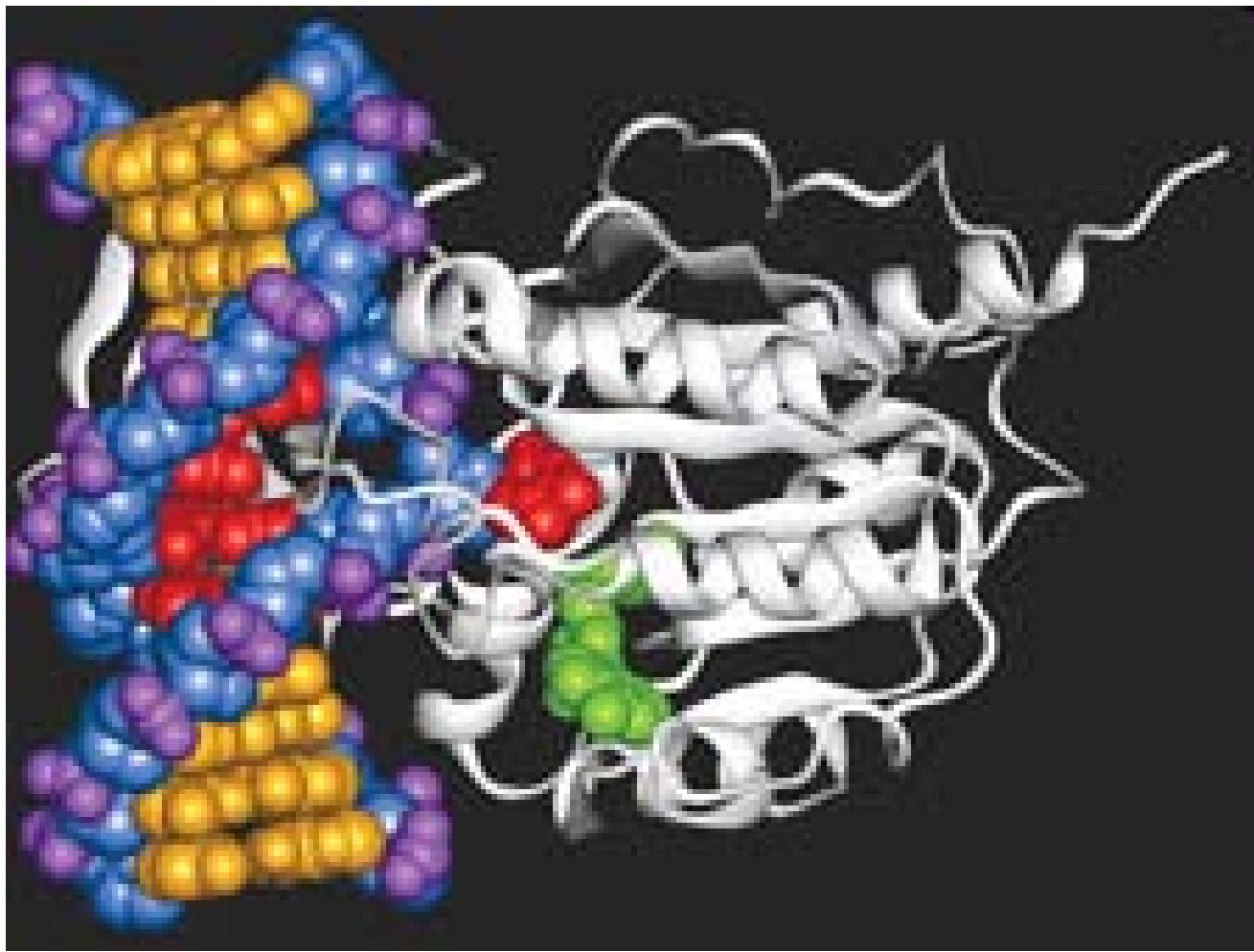


Histone Deacetylase



Methylation

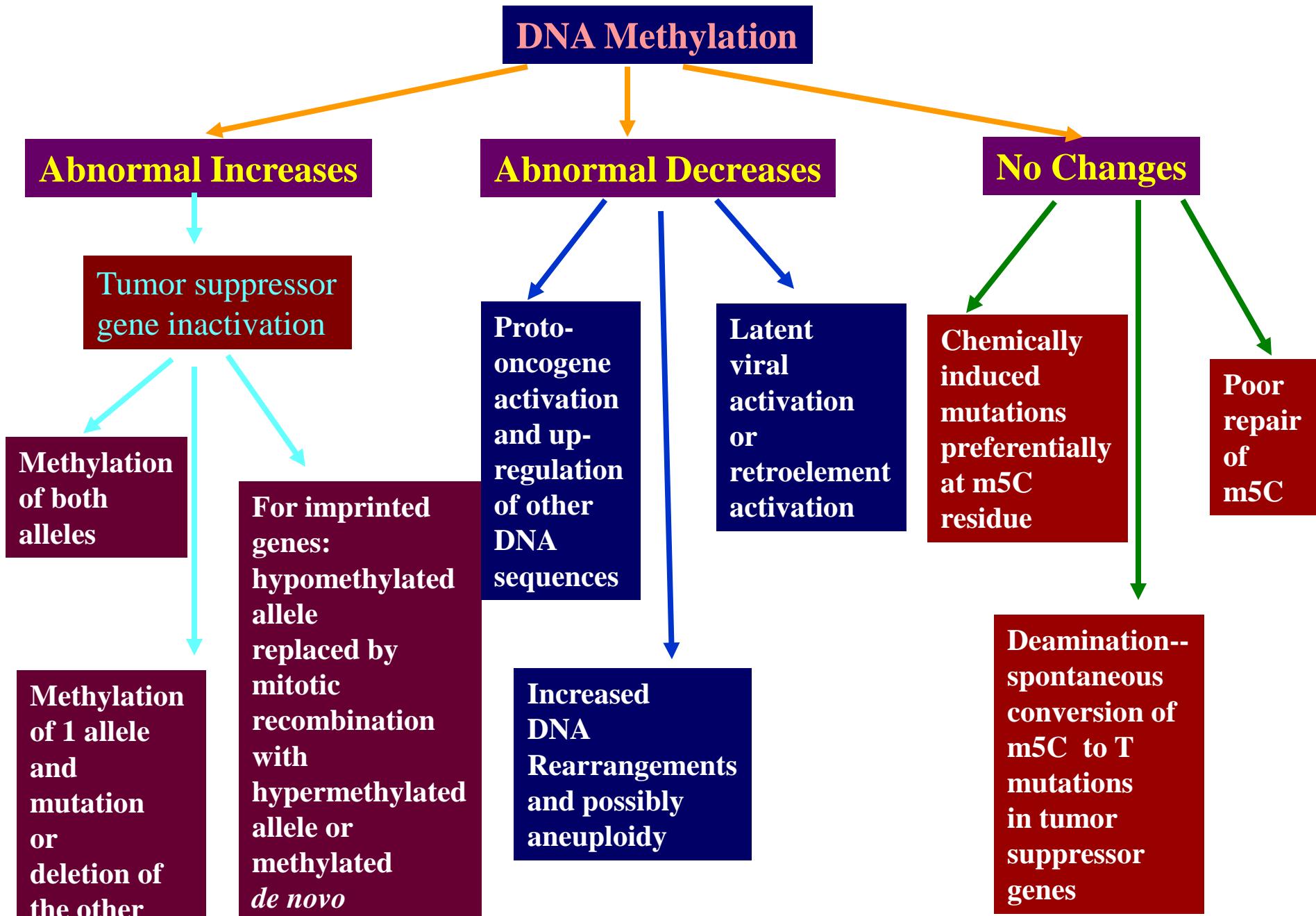
Verma and Srivastava (2002)
Lancet Oncology, 3, 755-763.



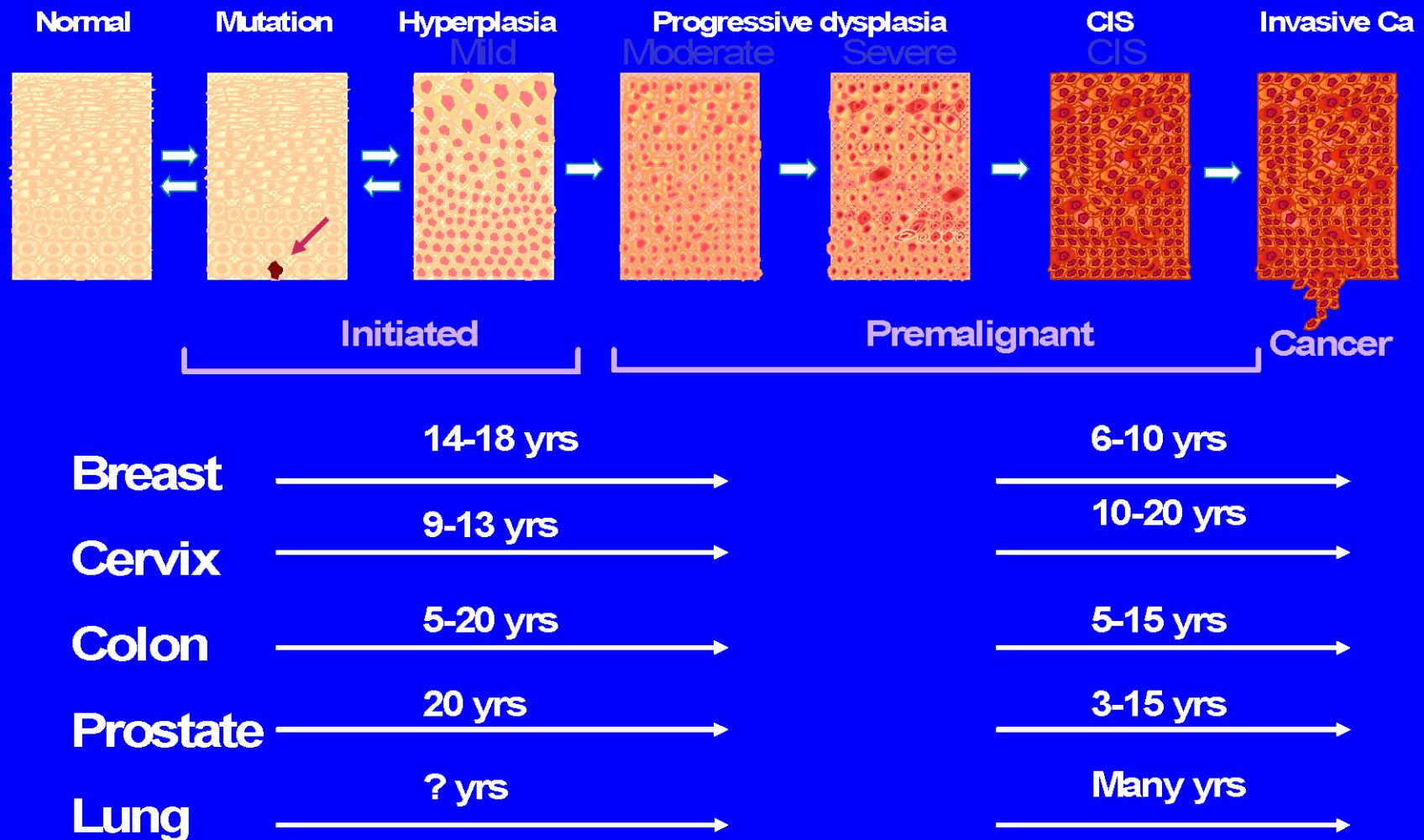
- Merck
- GlxSmith
- J & J
- Hoffman LaRoche
- Novartis
- Astra-Zeneca

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

DNA Methylation and Carcinogenesis



Cancer Development is a Multi-step Process



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

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	Verma and Srivastava (2002). <i>Lancet Oncol.</i> 3: 755-363;
Pancreas	p16, MGMT, APC	Verma et al (2004) <i>Crit. Rev. Clin. Sc.</i> 41: 585-607;
Prostate	GSTP1, p27(kip1)	Verma and Manne (2006). <i>Crit. Rev. Hematol. Oncol.</i> 60: 9-18;
Stomach	p14 ^{ARF} , P16, APC, hMLH1, MGMT	Verma et al (2006). <i>Mol. Diag. Therapy.</i> 10: 1-15.
Uterus	p16, p14 ^{ARF} , hMLH1	

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

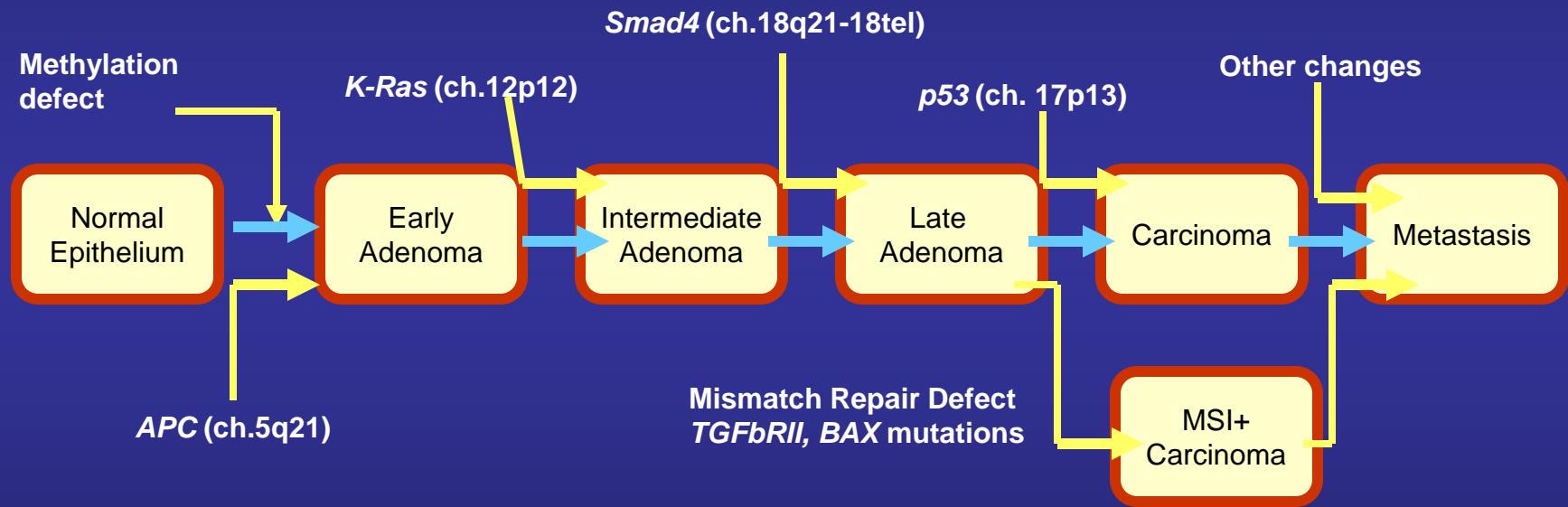
DNA repair genes

Methylguanine methyl transferase, hMLH1, BRCA1, GST

Angiogenesis

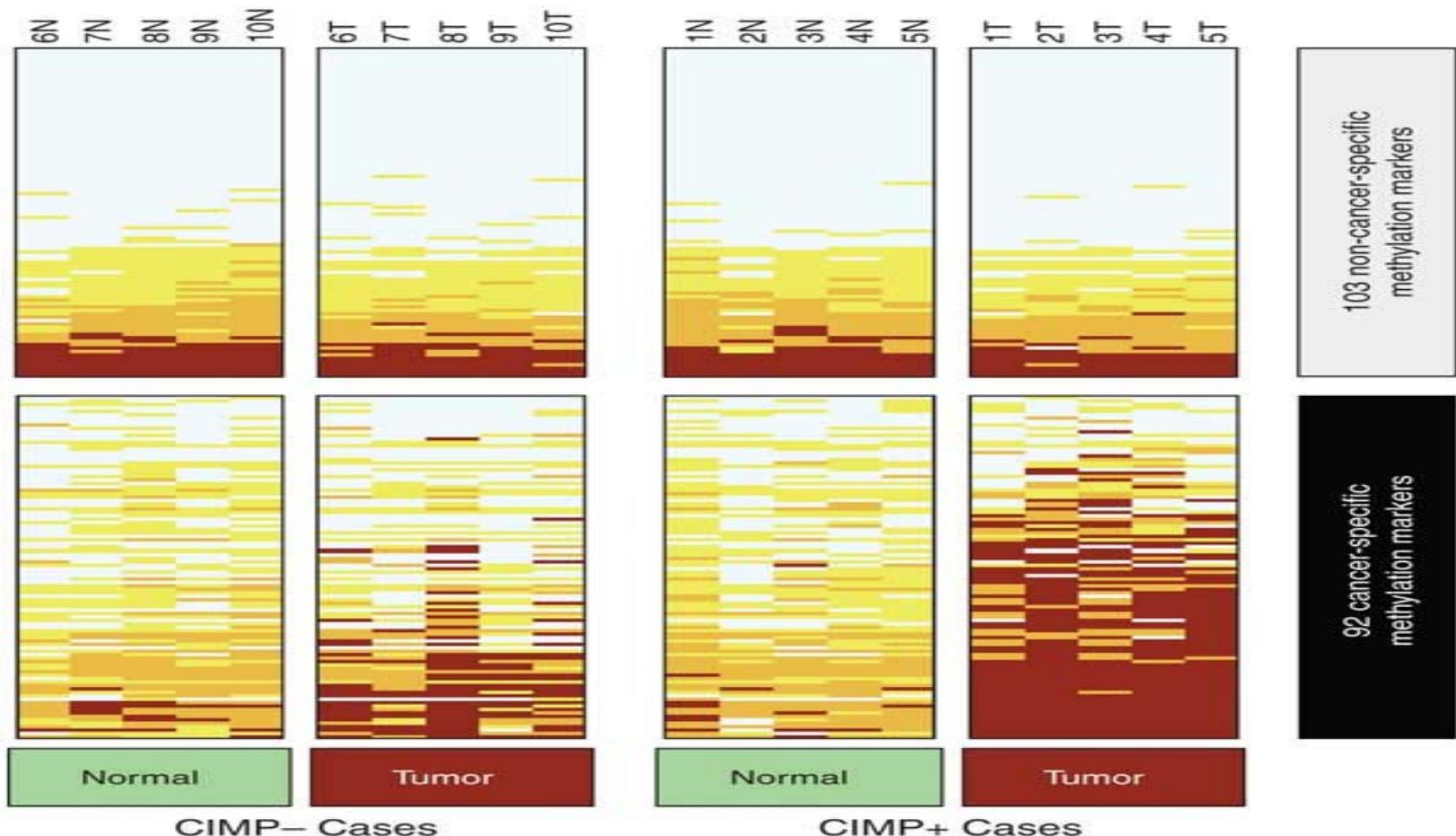
Thrombospondin-1 (TSP-1), TIMP-3

Potential Steps for Intervention

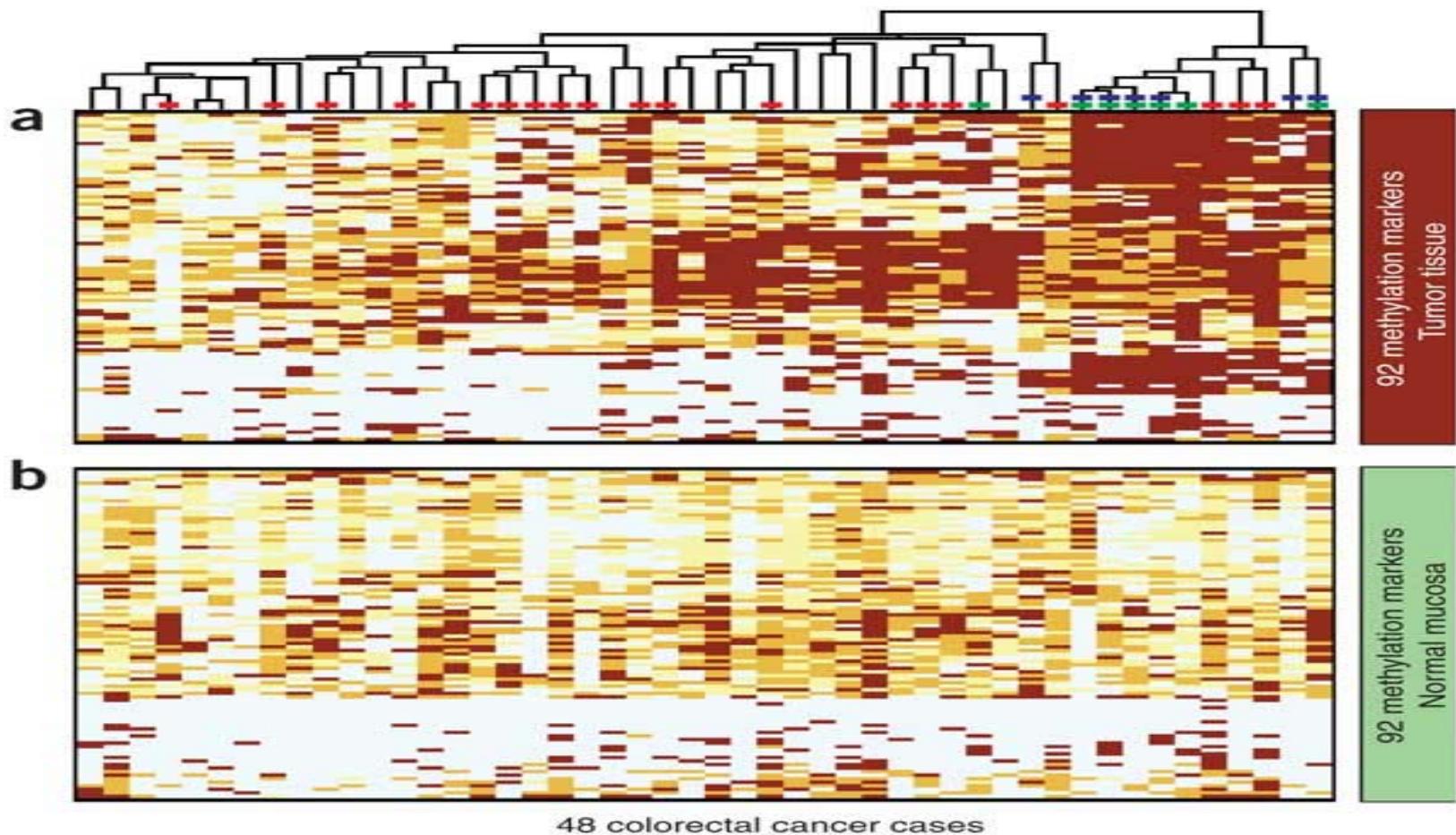


A Model for Colorectal Tumorigenesis

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

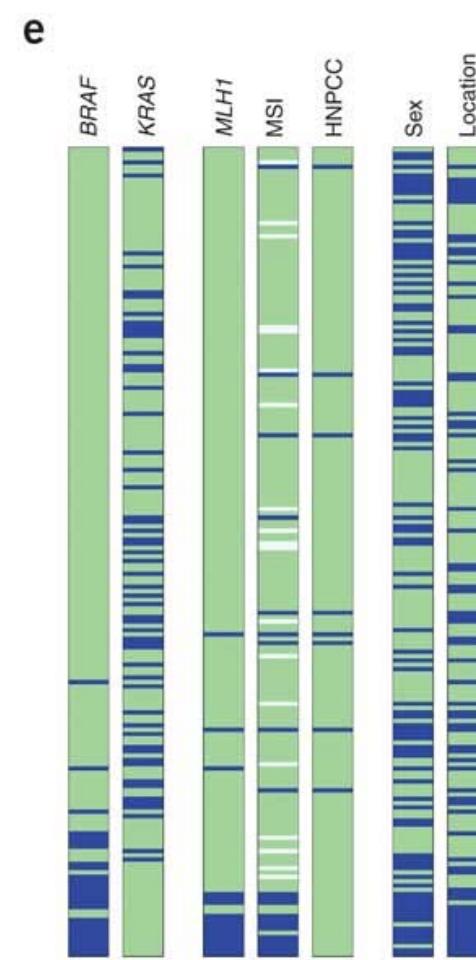
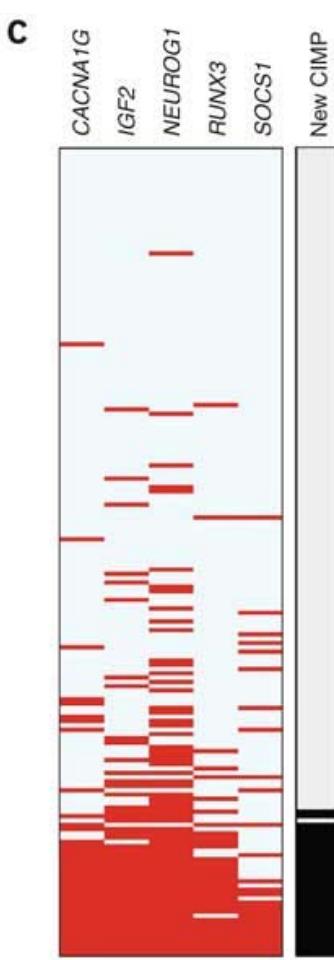
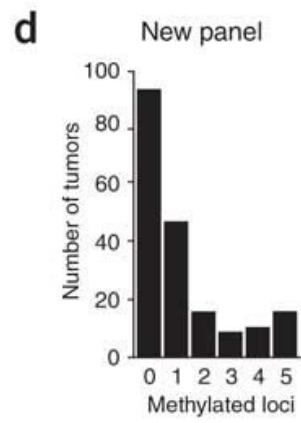
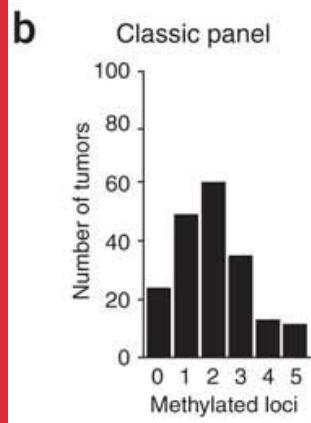


Identification of tumor clusters.

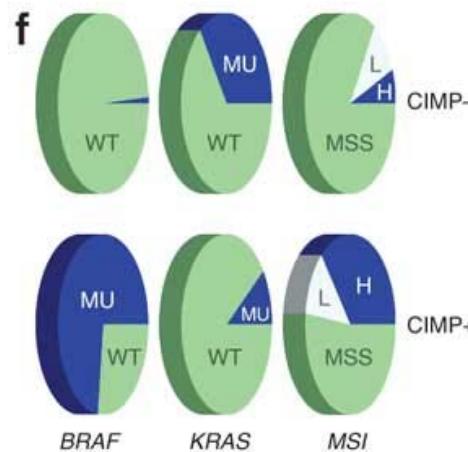


KRAS mutation indicated by a red rectangle overlaying the branch,
BRAF mutations indicated by a green rectangle
MSI-H cases designated with a blue rectangle.

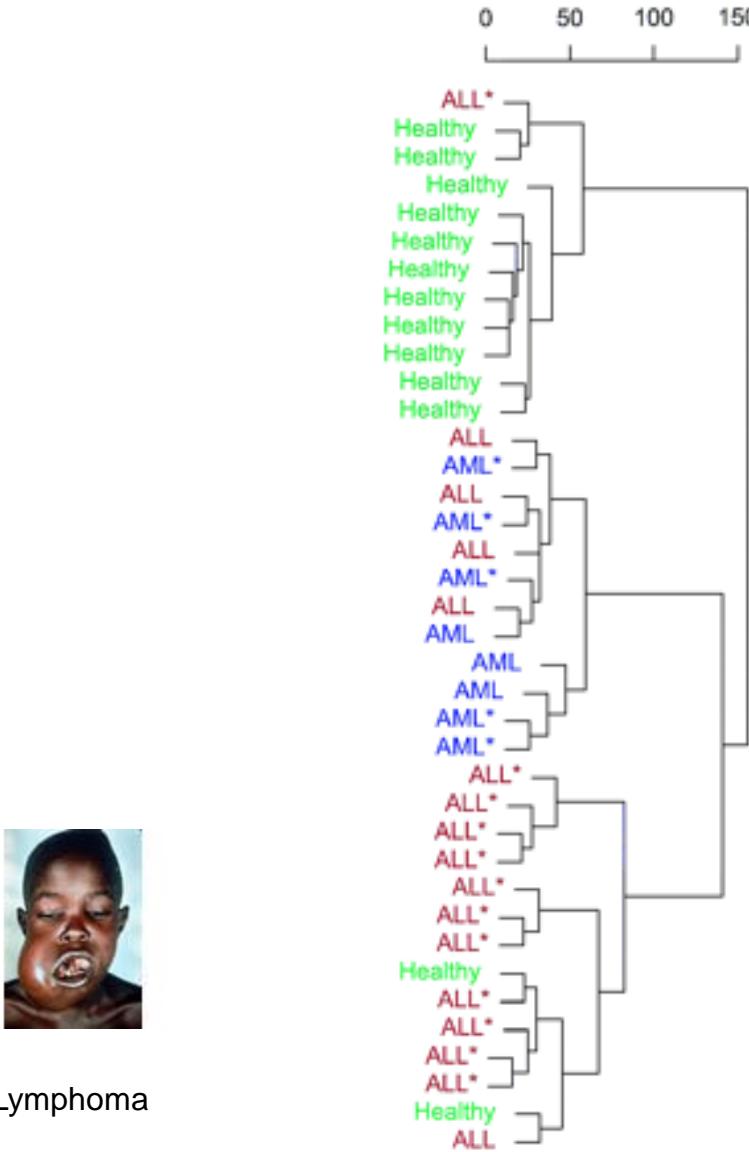
48 Colorectal tumors



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



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



AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia



Lymphoma

Epigenetic Markers During Lung Cancer Progression

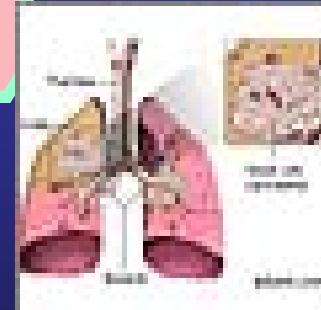
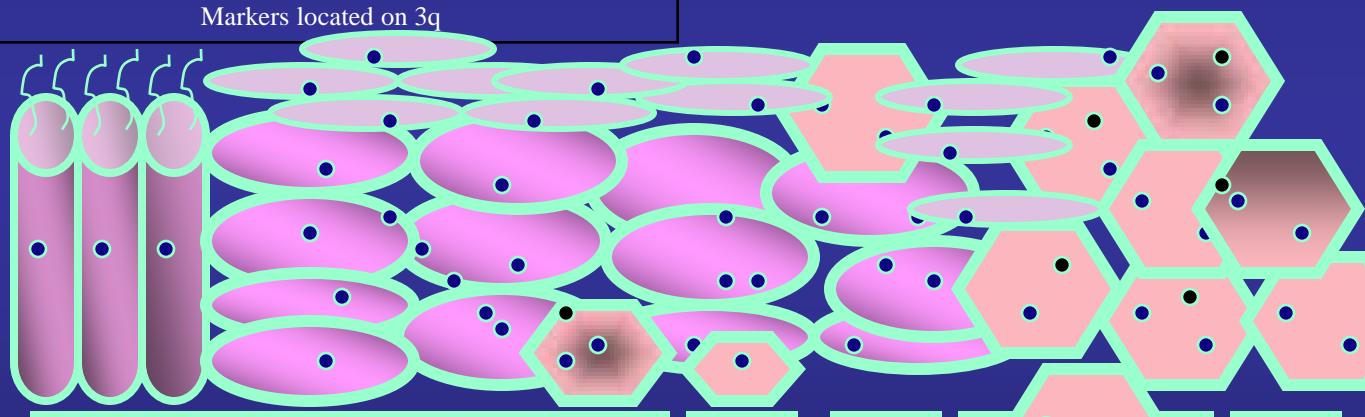
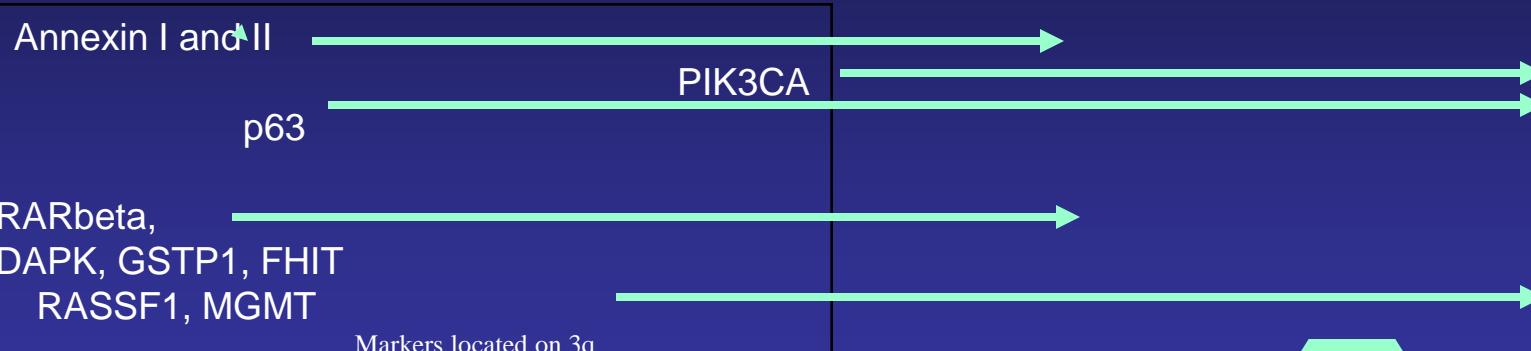
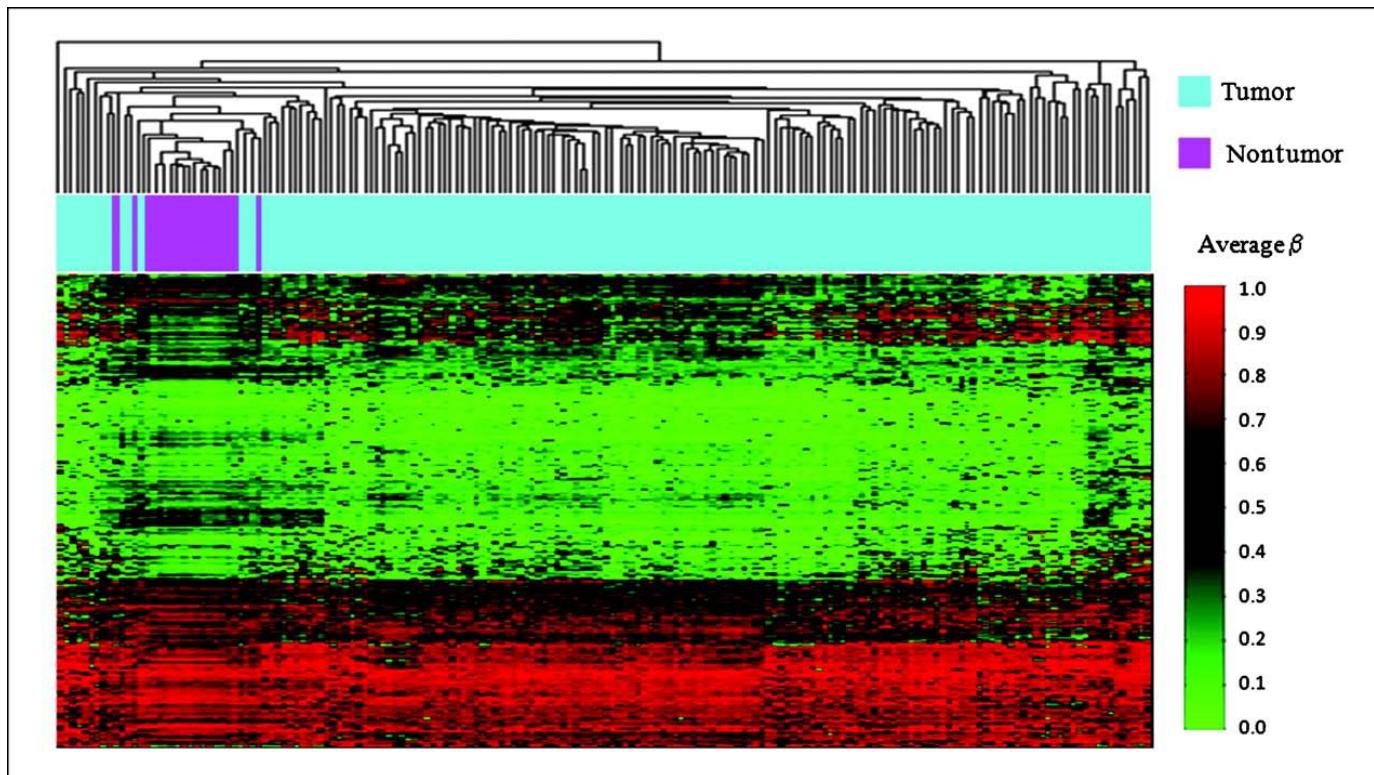


Figure 1. Unsupervised clustering of average {beta} values in tumor and nontumor pleura

ASBESTOS

MESOTHELIOMA

Non-Mutagenic carcinogen



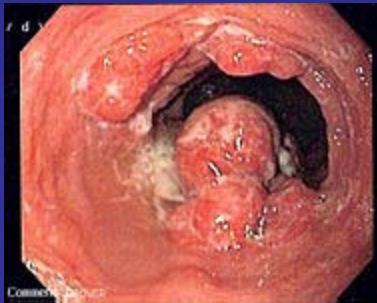
Christensen, B. C. et al. Cancer Res 2009;69:227-234

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

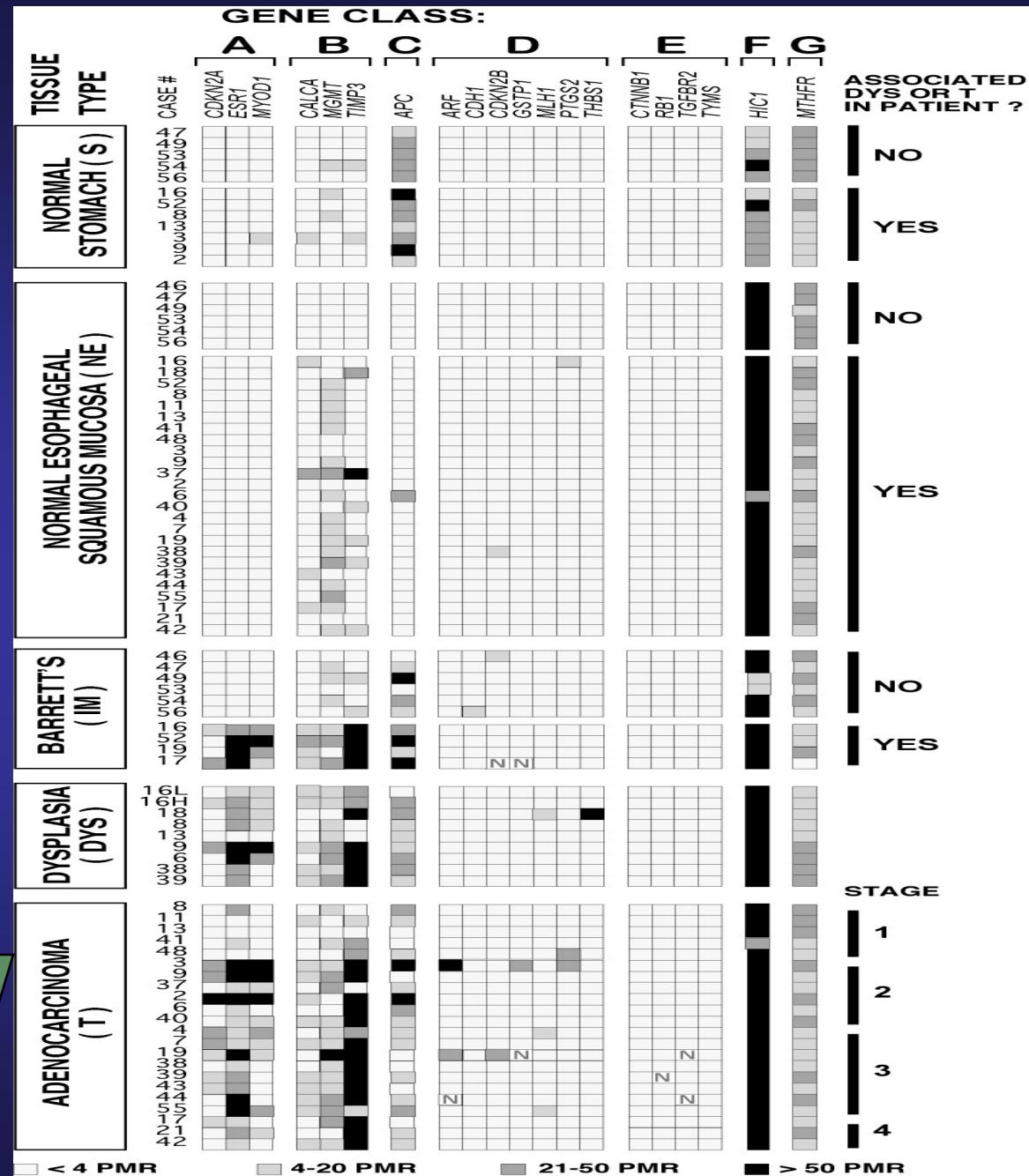
Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

Cancer Research

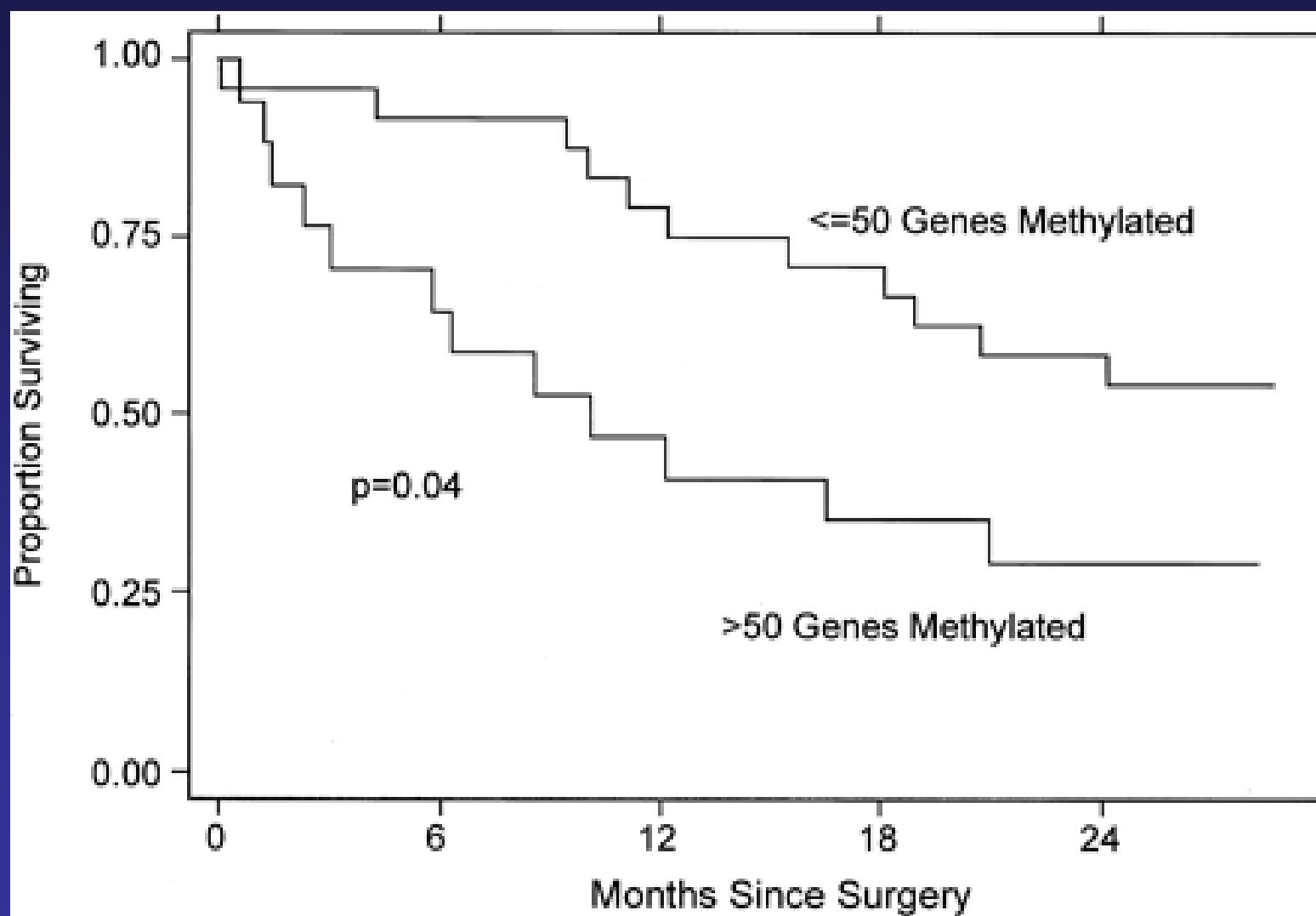
61:3410



Cancer Progression



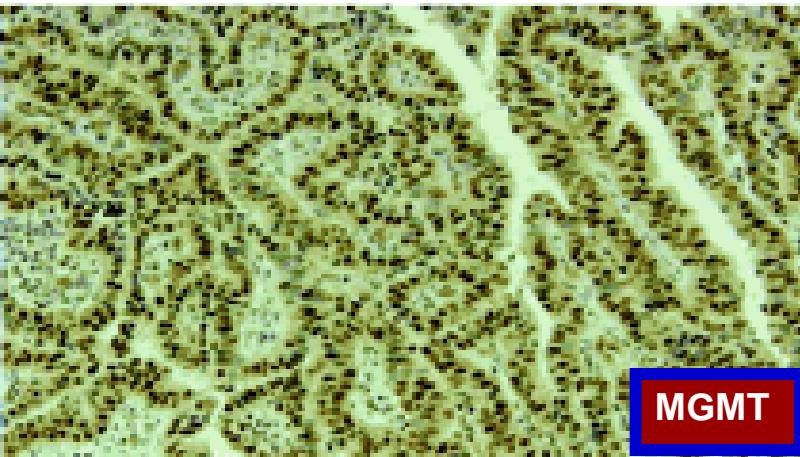
Esophageal Cancer: Probability of Survival



Esophageal Cancer: Immuno-histochemistry

A

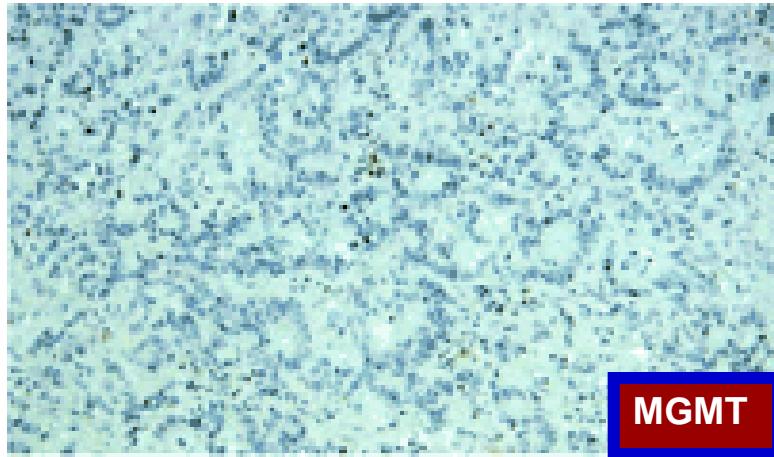
Unmethylated



MGMT

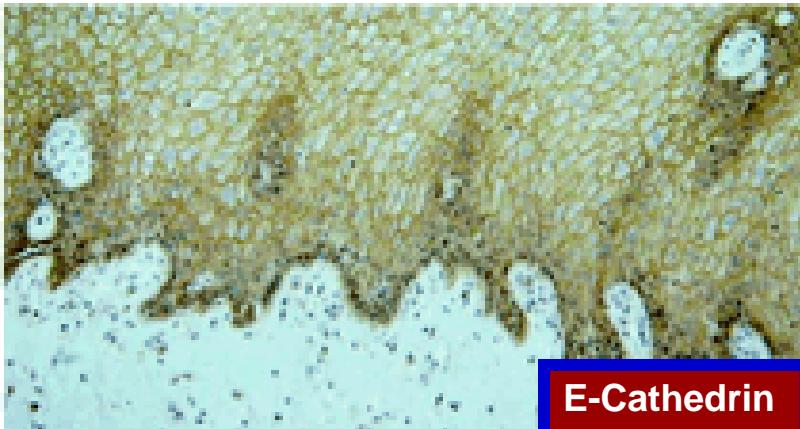
B

Methylated



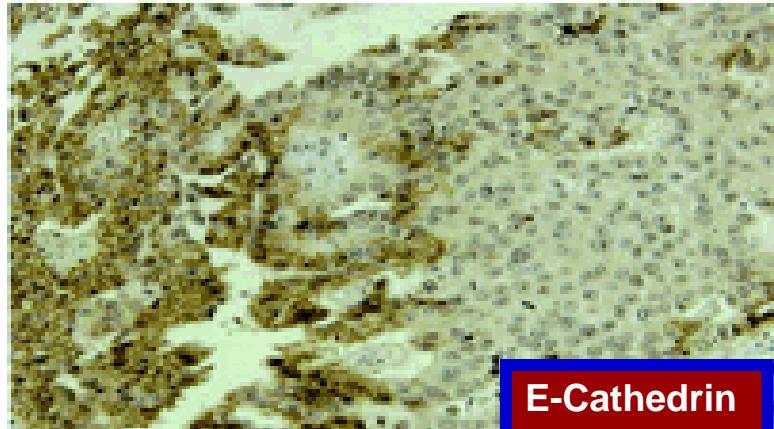
MGMT

C



E-Cathedrin

D



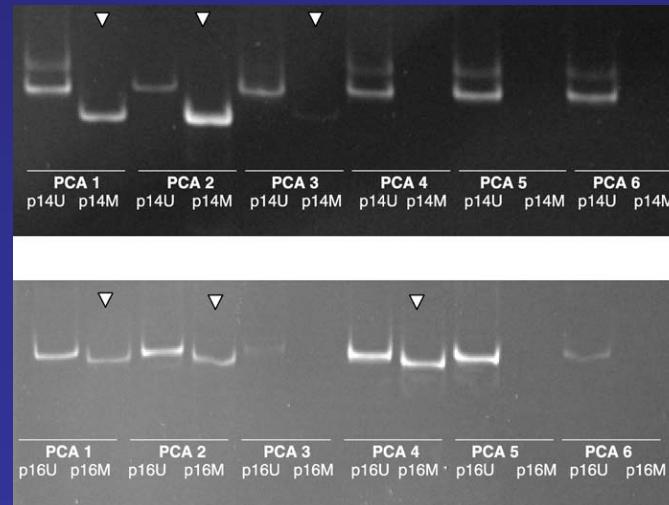
E-Cathedrin

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

Pancreatic Carcinoma (PCA) : 39 19/39 p16INK4a

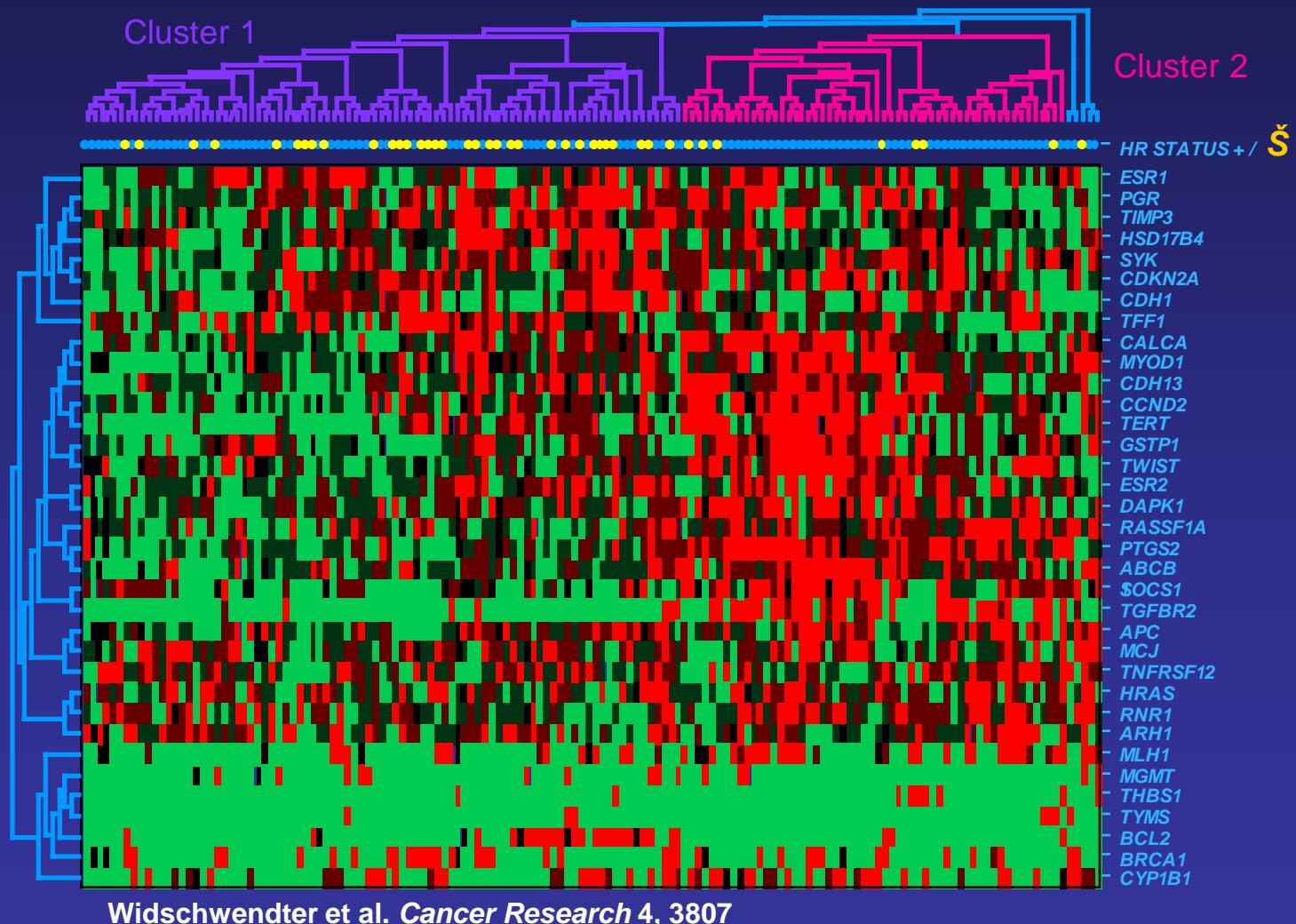
Chronic Pancreatitis (CP) : 16 0/16 p16INK4a

Normal Pancreatogram (NAD) : 6 0/6 p16INK4a

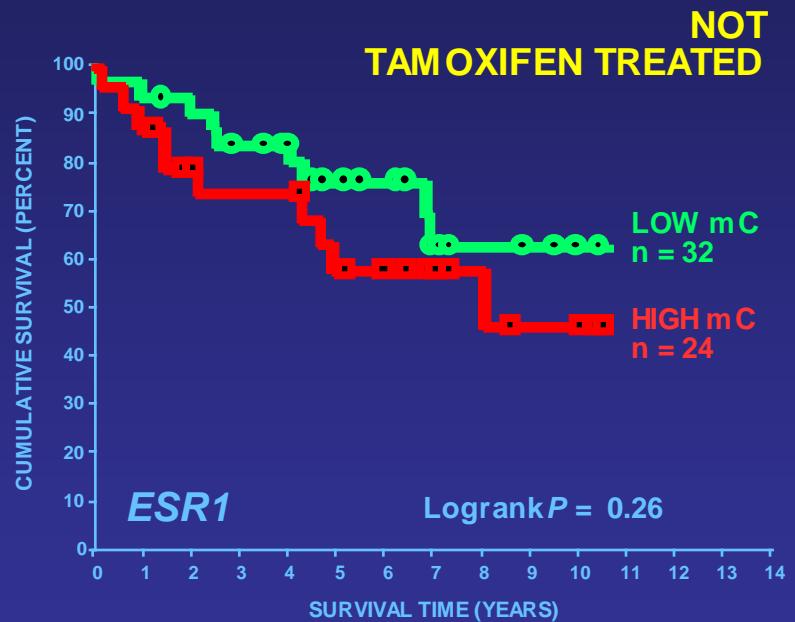
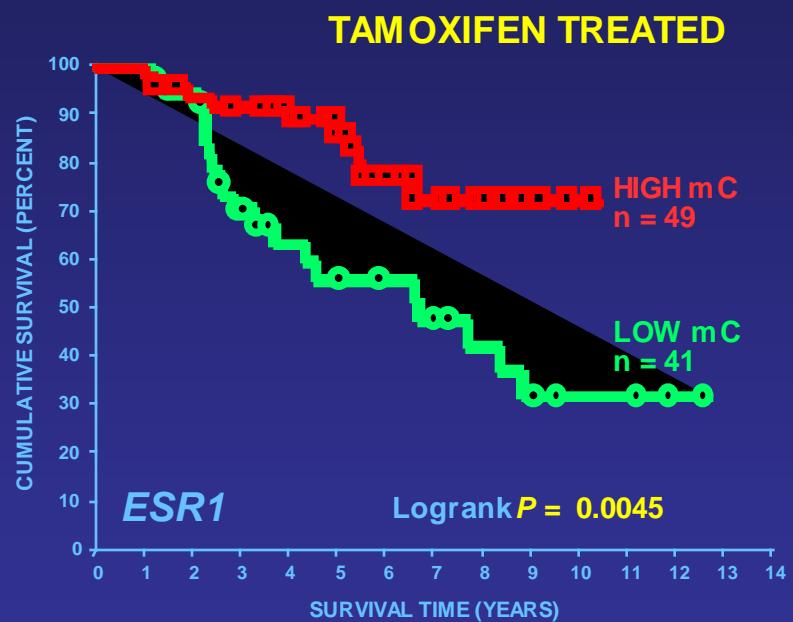


Sample: Pancreatic Fluid

MethyLight Analysis of 35 Genes in 148 Human Breast Carcinomas



Breast Cancer Response to Tamoxifen Treatment by ESR1 Methylation



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

Her-2	age	tumor size	LN status	HR status	e. conn3	s. conn3	e. MYO31	s. MYO31	e. PGR	s. PGR	e. BRCA1	s. BRCA1	e. HSPD1724	s. HSPD1724
+++	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.	n.d.		

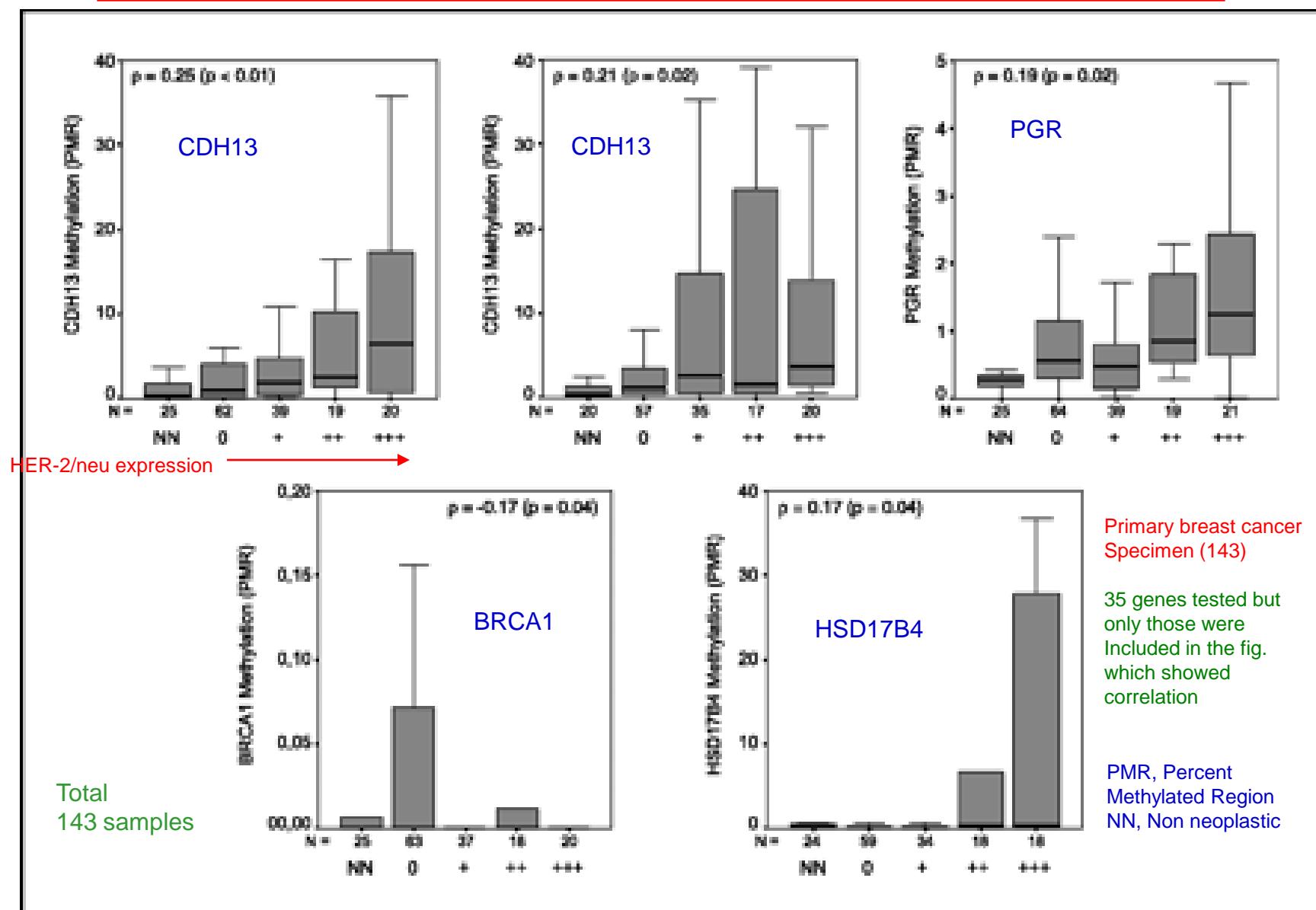
Total
143 samples
E; epithelium
S, stroma

LN, lymph
Node status

LCM was
Used
To separate
Epithelium
and stroma

N.D.
Not Done

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



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

Methylation in Human Papillomavirus-Infected

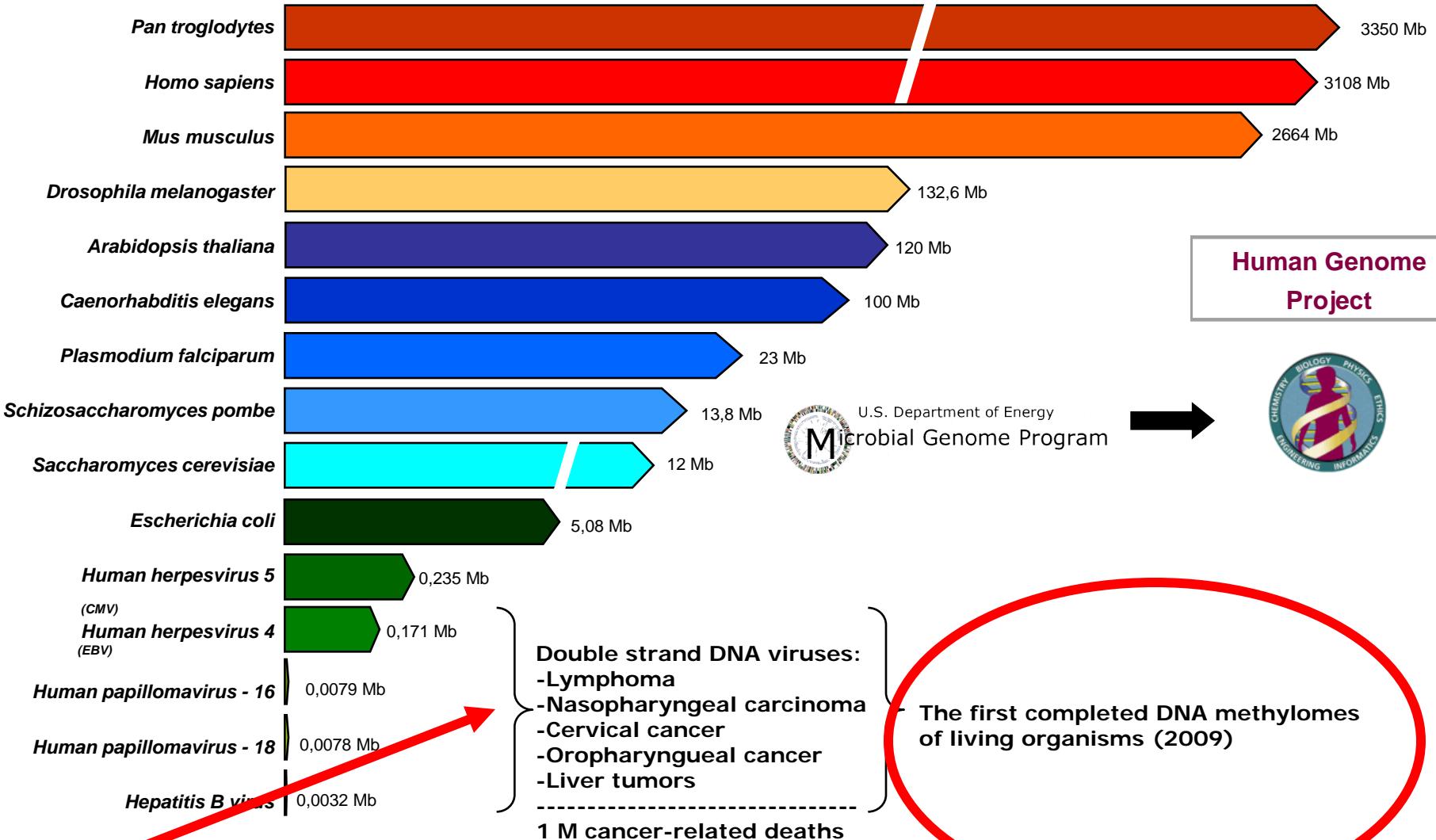
Chih Hsu⁴, Jung-Ta Chen⁵, Wen-Shan Liu⁶, Ming-Chih Chiou^{2,3},

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

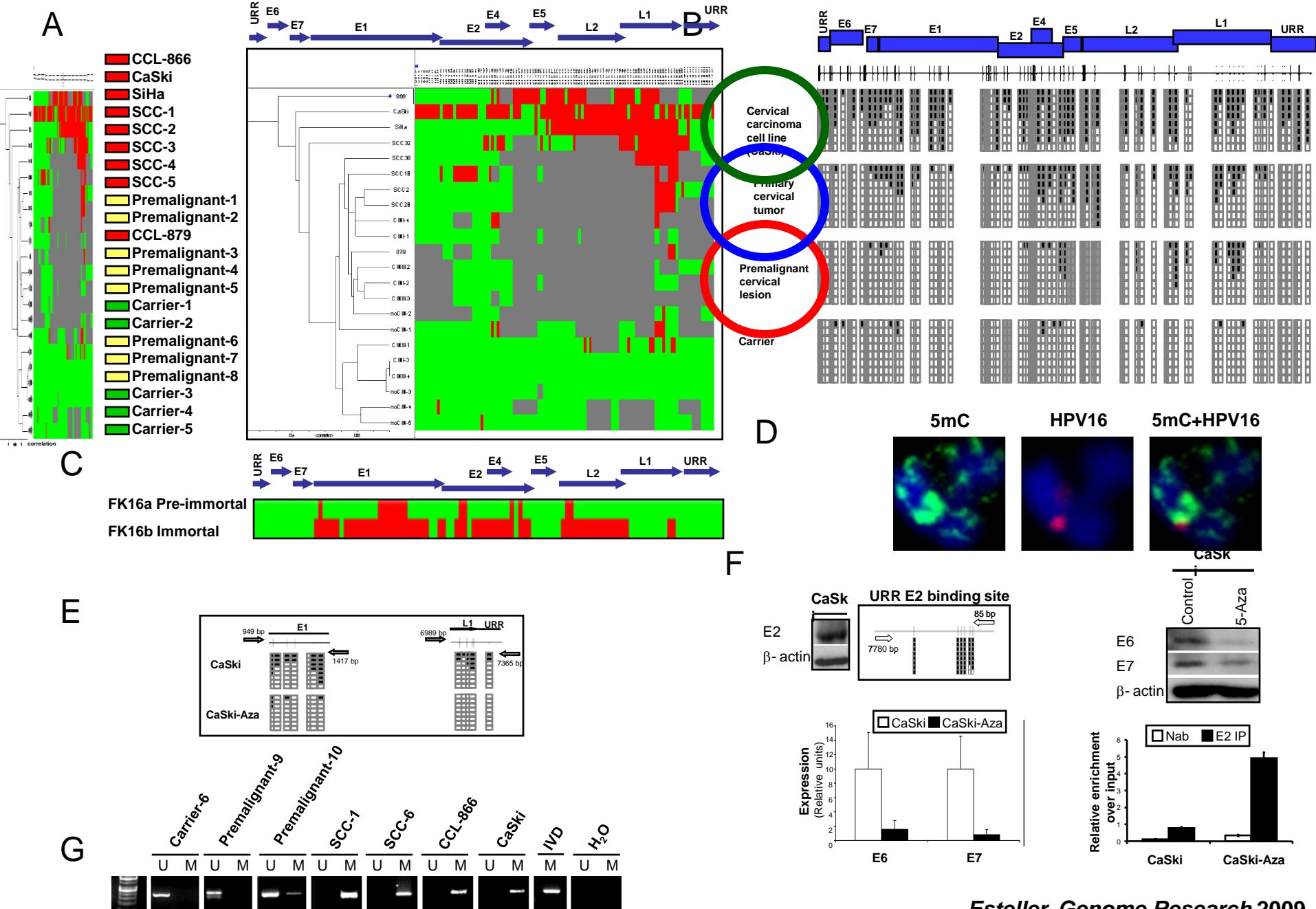
^{2,3}Institute of Biotechnology, National Chiao Tung University, Hsinchu, Taiwan

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

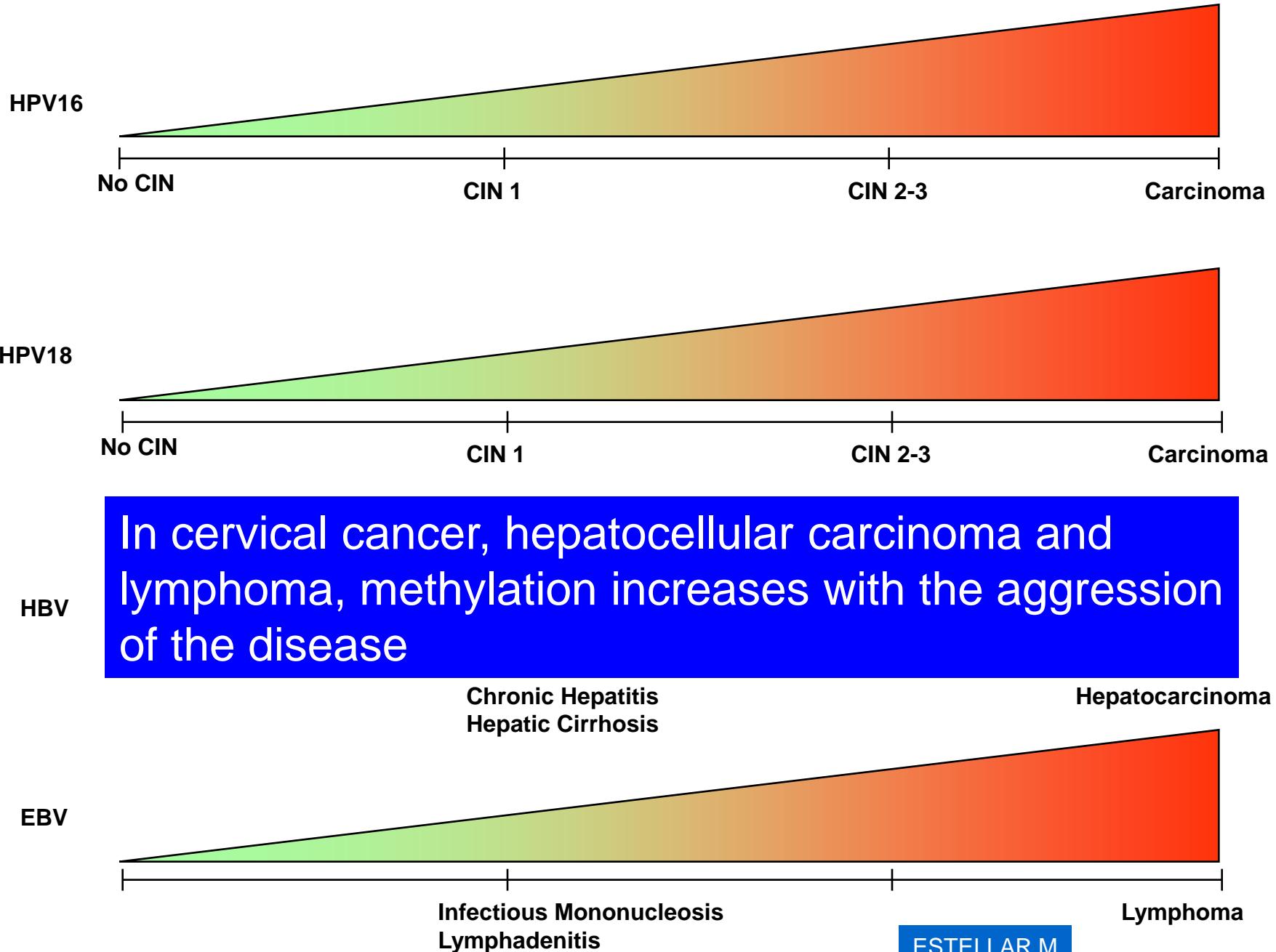
Genomes



The DNA Methylome of the Human Papilloma Virus 16



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



CDKN2A

CDH1

MGMT

DAPK1

P14 ARF

CDKN2B

RARB

RASSF1

MLH1

FHI1

TP73

SERPINB5

DBC1

DCC

Genes Methylated in Oral Cancer

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: Espinosa 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.

Quest Diagnostics Incorporated
leading provider of diagnostic services.

ion in Prostate Cancer

drug detoxification enzyme which

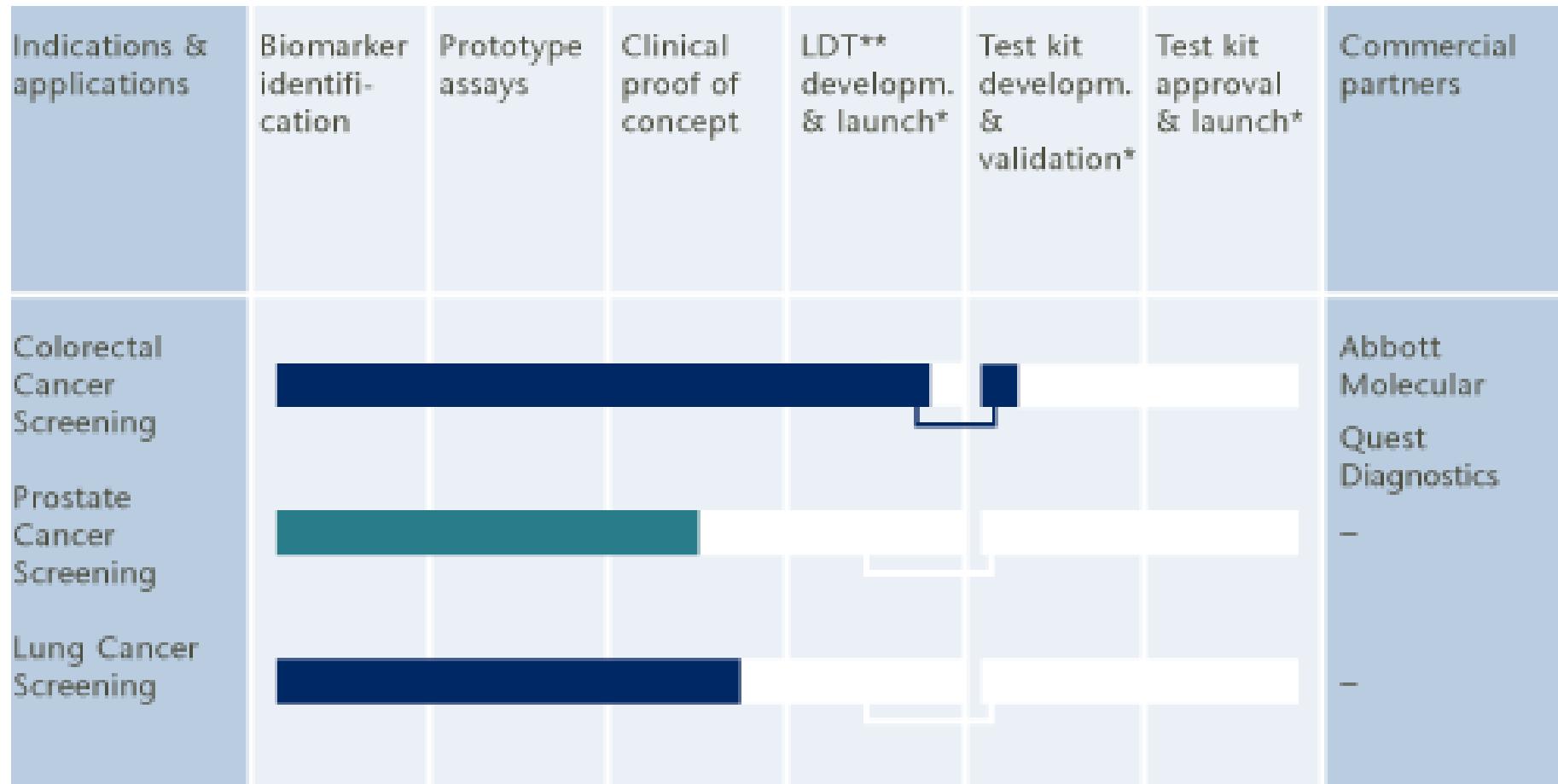
Seattle, WA, U.S.A., February 25, G (Frankfurt, Prime Standard: ECX), diagnostics company, today announced a non-exclusive licensing agreement

marker

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).



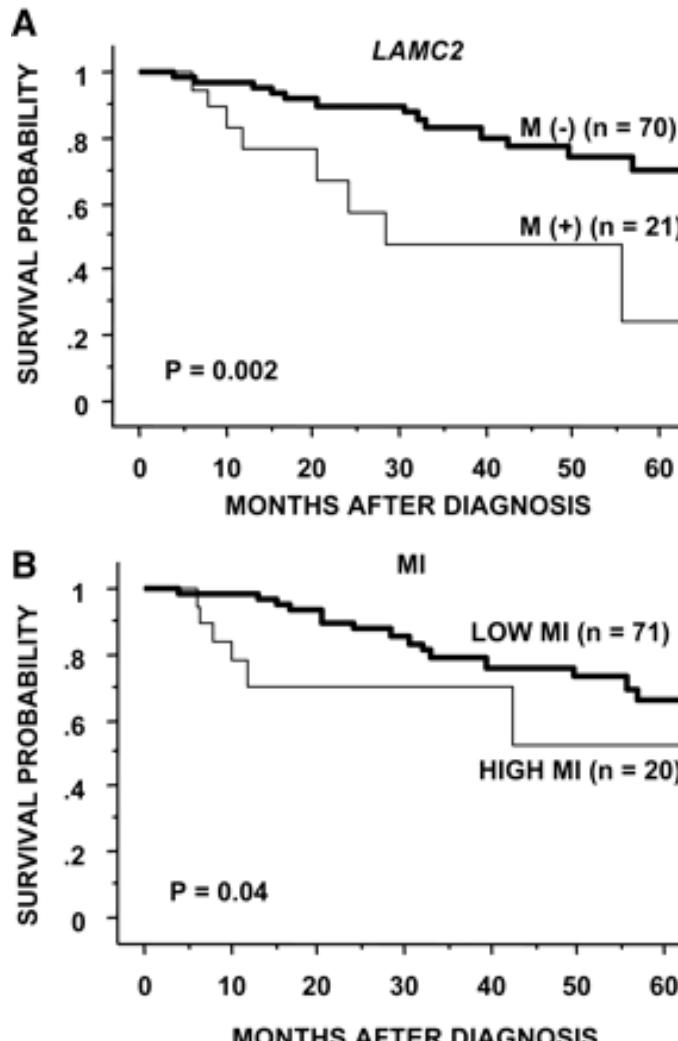
Blood-based Urine-based

* By Epigenomics' commercial partners

** Laboratory-developed test

Bladder Cancer

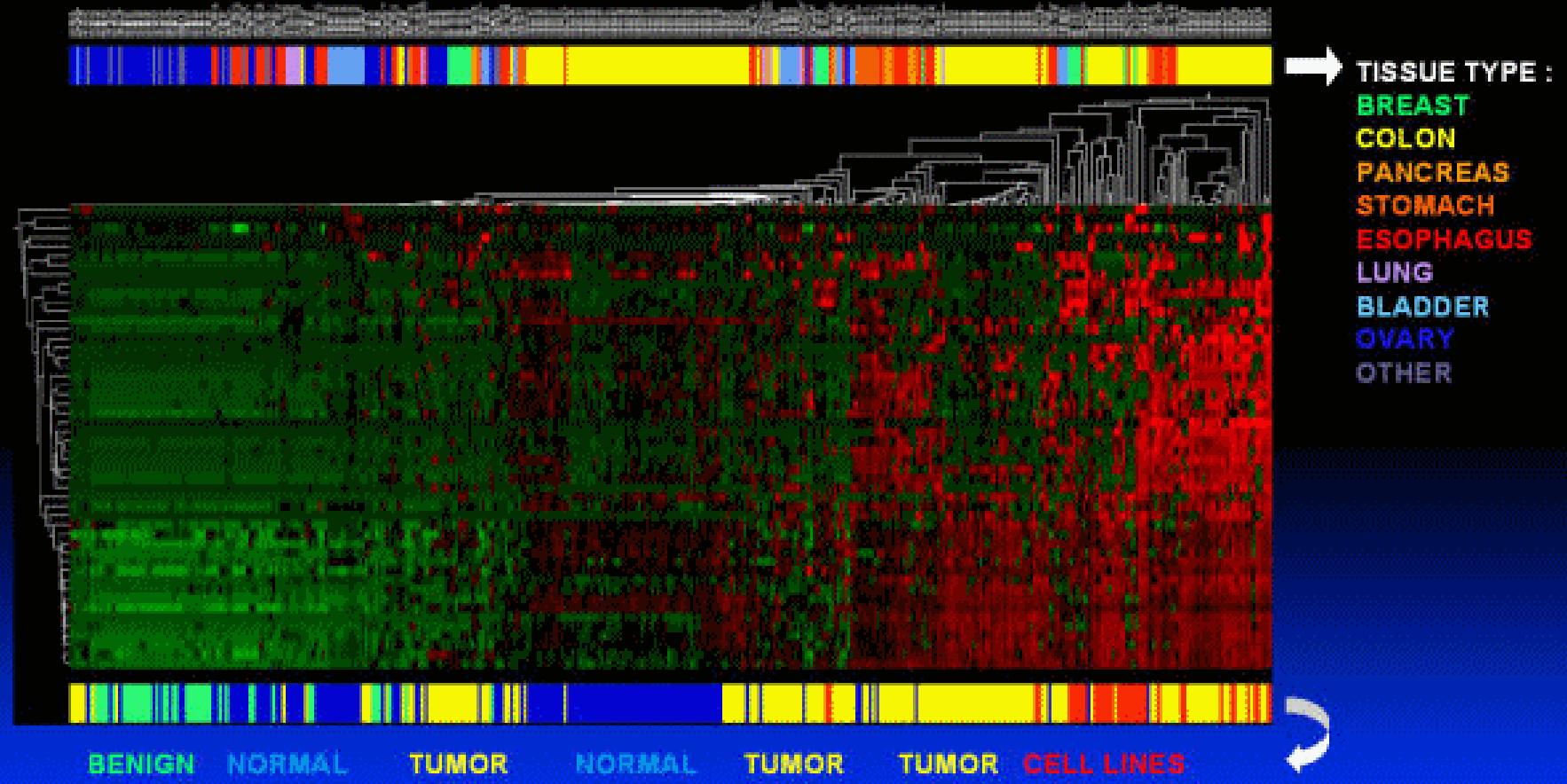
Methylation of LAMC2 in Exfoliated Cells Isolated from Urine



Another Study:
Schistosomes and Bladder Cancer

MI, Methylation Index

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)

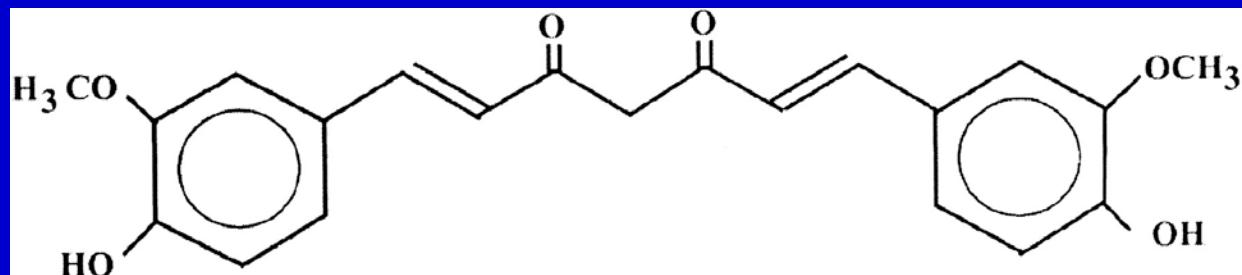
Large case-control studies on lung and upper-aero digestive tract (UADT) cancers in Europe

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.

Structure and functions of curcumin

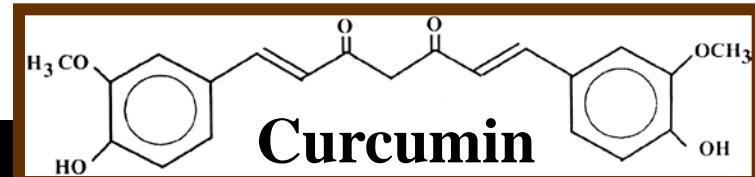


Active ingredient-diferuloylmethane

- 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

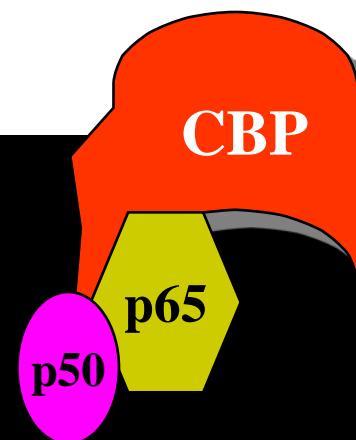


HAT inhibition at conc >10 μ M



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

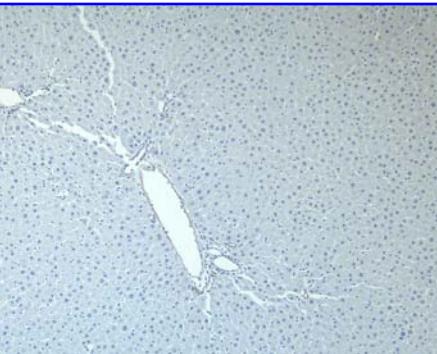
Kang et al

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

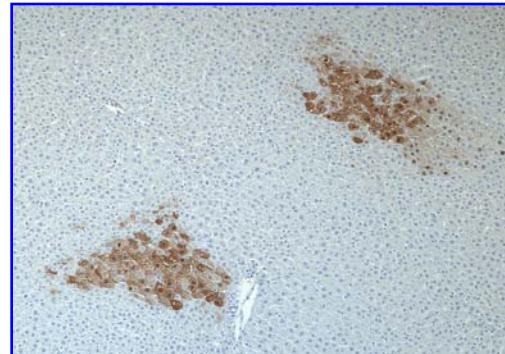
METHYL-DEFICIENT MODEL OF ENDOGENOUS HEPATOCARCINOGENESIS

- Chronic deficiency in the methyl donors methionine, choline, folic acid and vitamin B₁₂
- 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

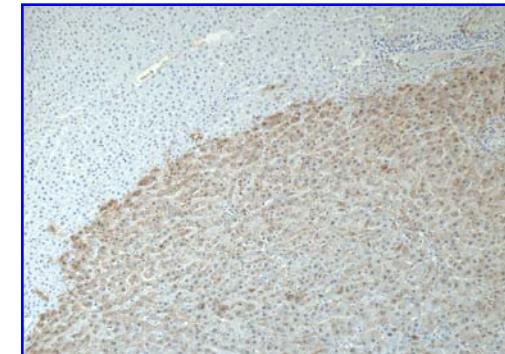
Normal tissue



36 weeks, GST π -foci



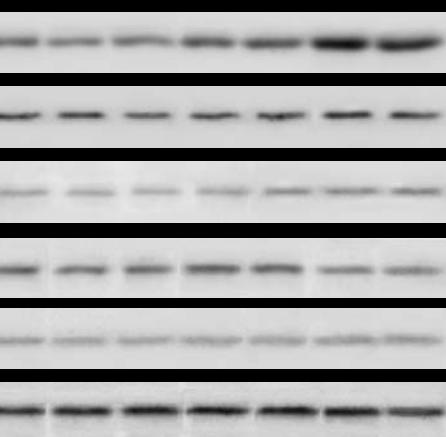
>54 weeks, GST π -tumor



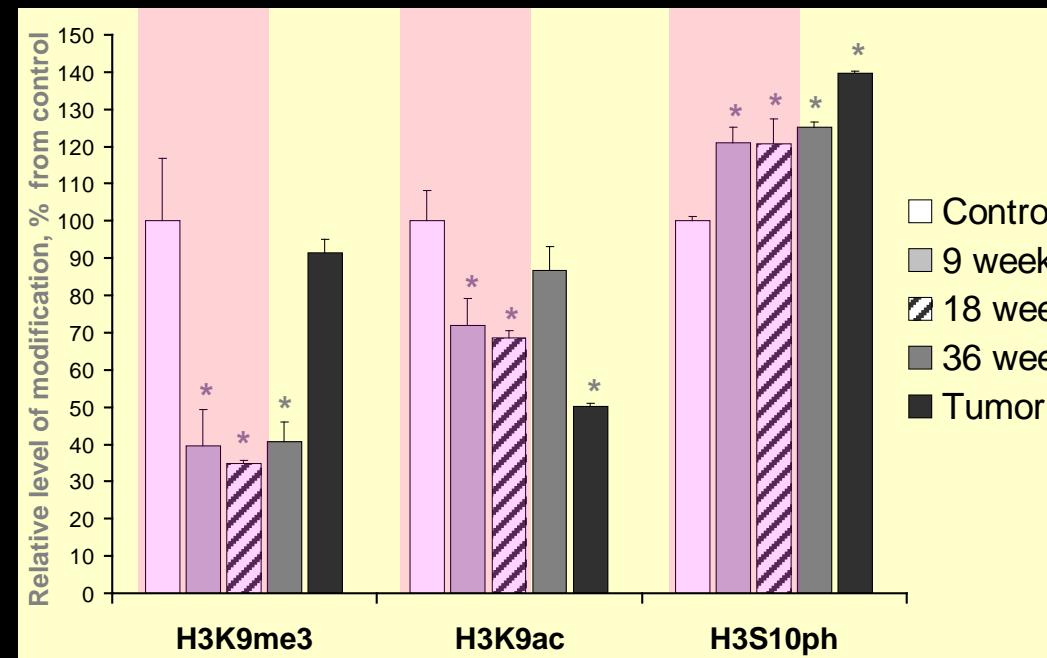
Liver tumor



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



H3K9me3
H3K9me2
H3K9me1
H3K9ac
H3S10ph
H3



N-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKP-...C

Interplay between H3K9me3, H3K9Ac, and H3S10ph

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

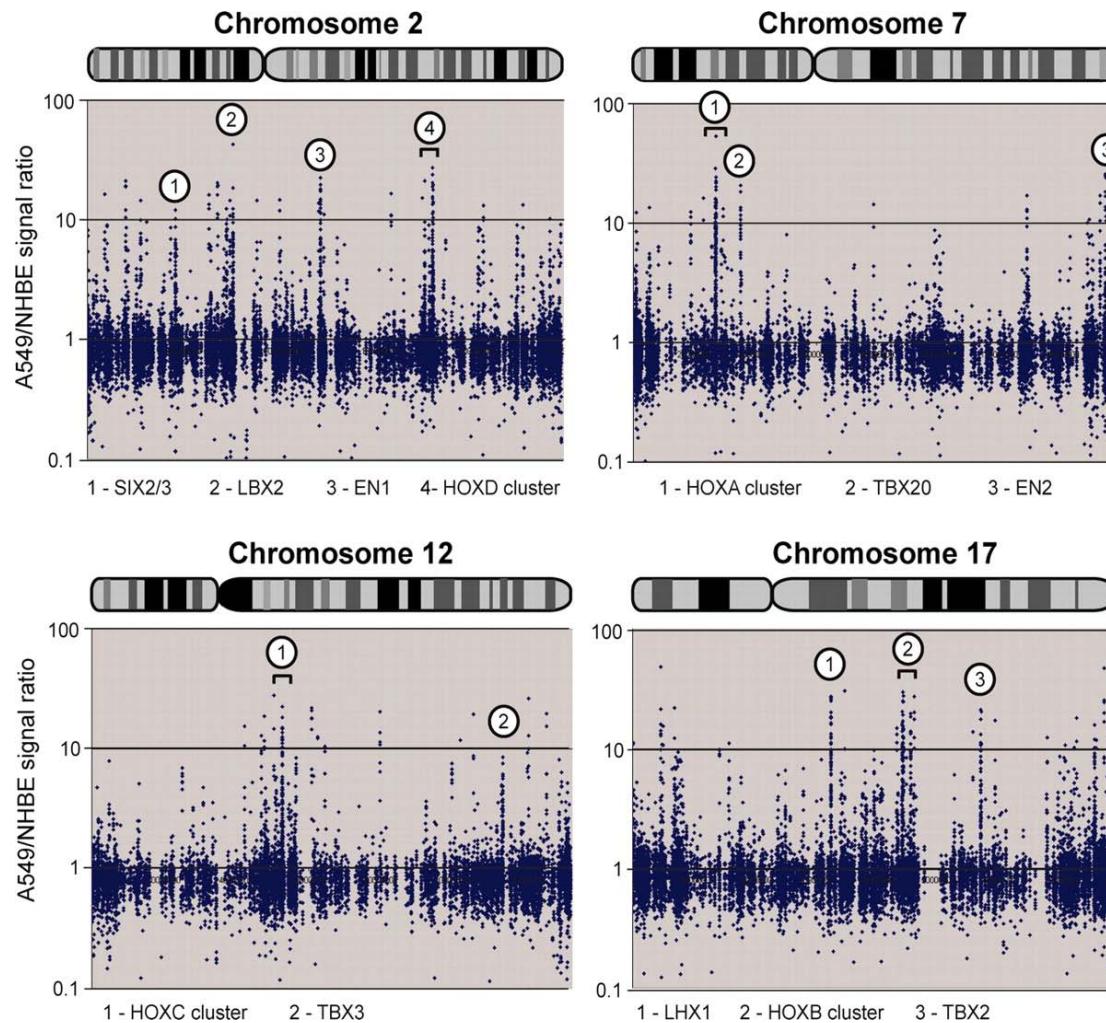
Chromosome	20	21	22	Total
Length (Mb)	62	44	47	153
Mapped Genes	1244	611	1034	2889
First Coding Exon	204	104	226	524
Other Exon	282	127	241	650
Alu	187	76	194	457

CpG Island Searcher:

<http://www.cpgislands.com>

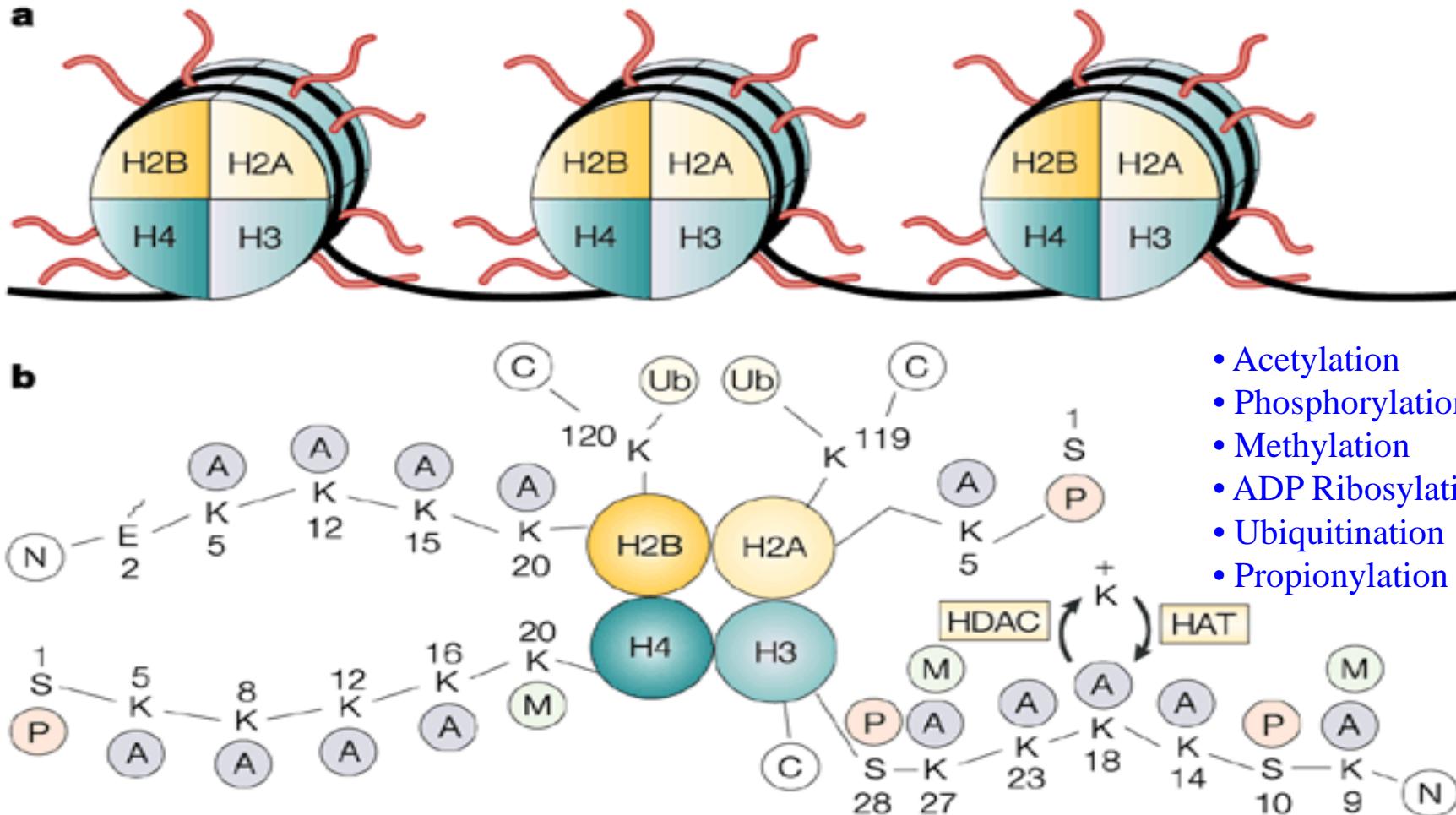
<http://www.uscnorris.com/cpgislands/cpg.cgi>.

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



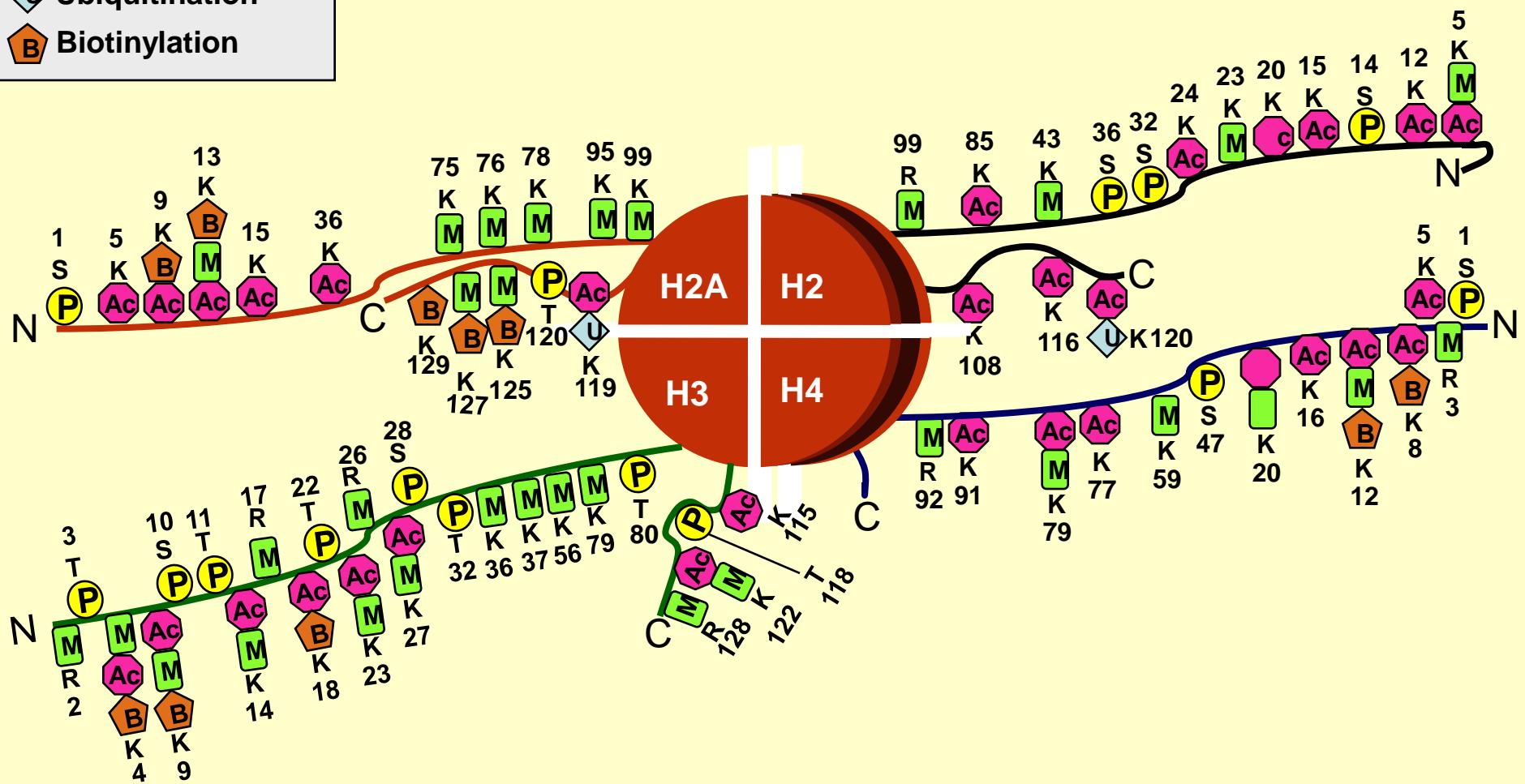
Rauch, Tibor et al. (2007) Proc. Natl. Acad. Sci. USA 104, 5527-5532

Histone Modifications



- P Phosphorylation
- Ac Acetylation
- M Methylation
- U Ubiquitination
- B Biotinylation

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

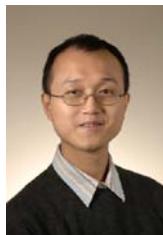
p53 is regulated by the lysine demethylase LSD1

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

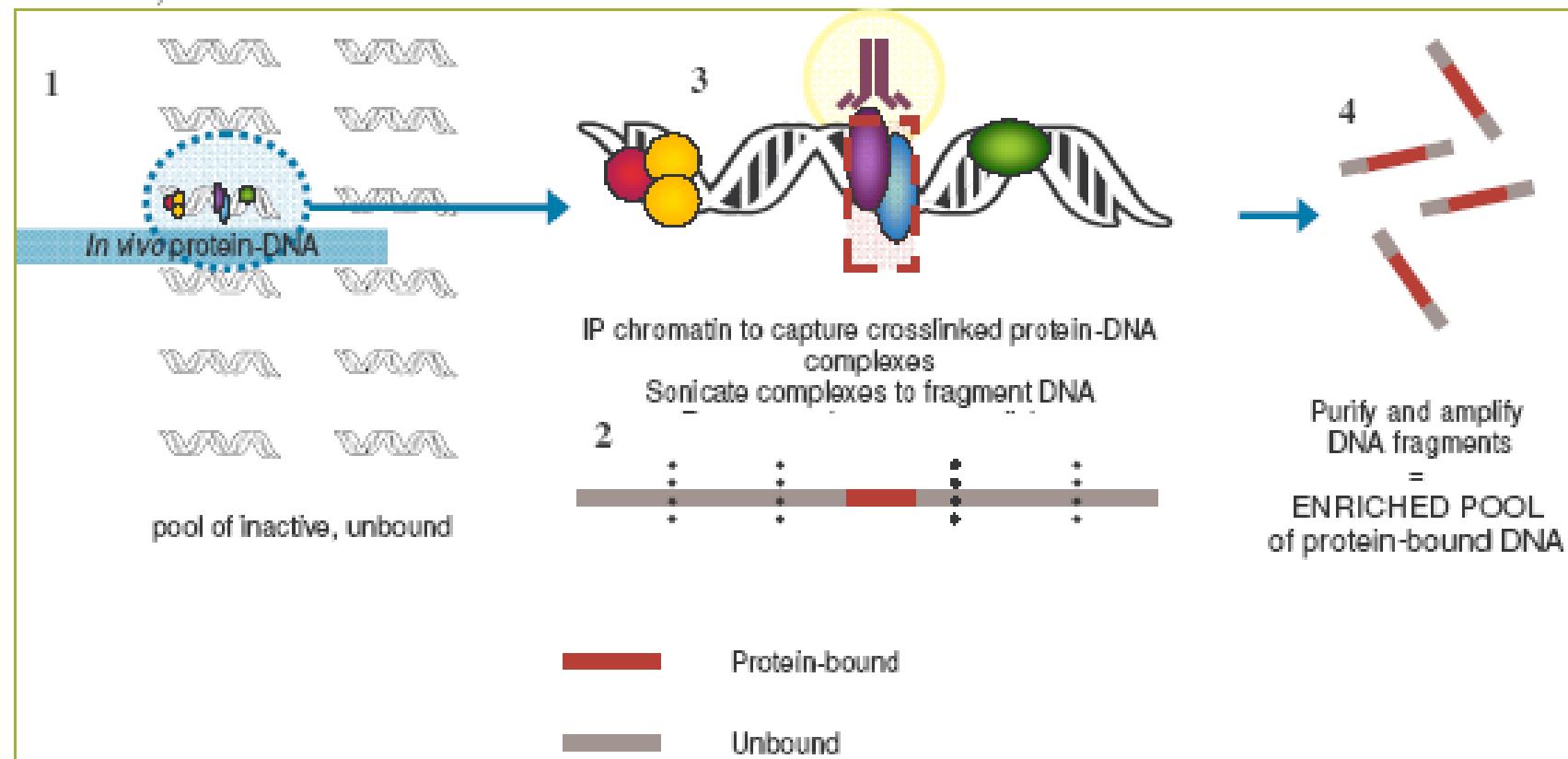


Jing Huang



Mudit Verma

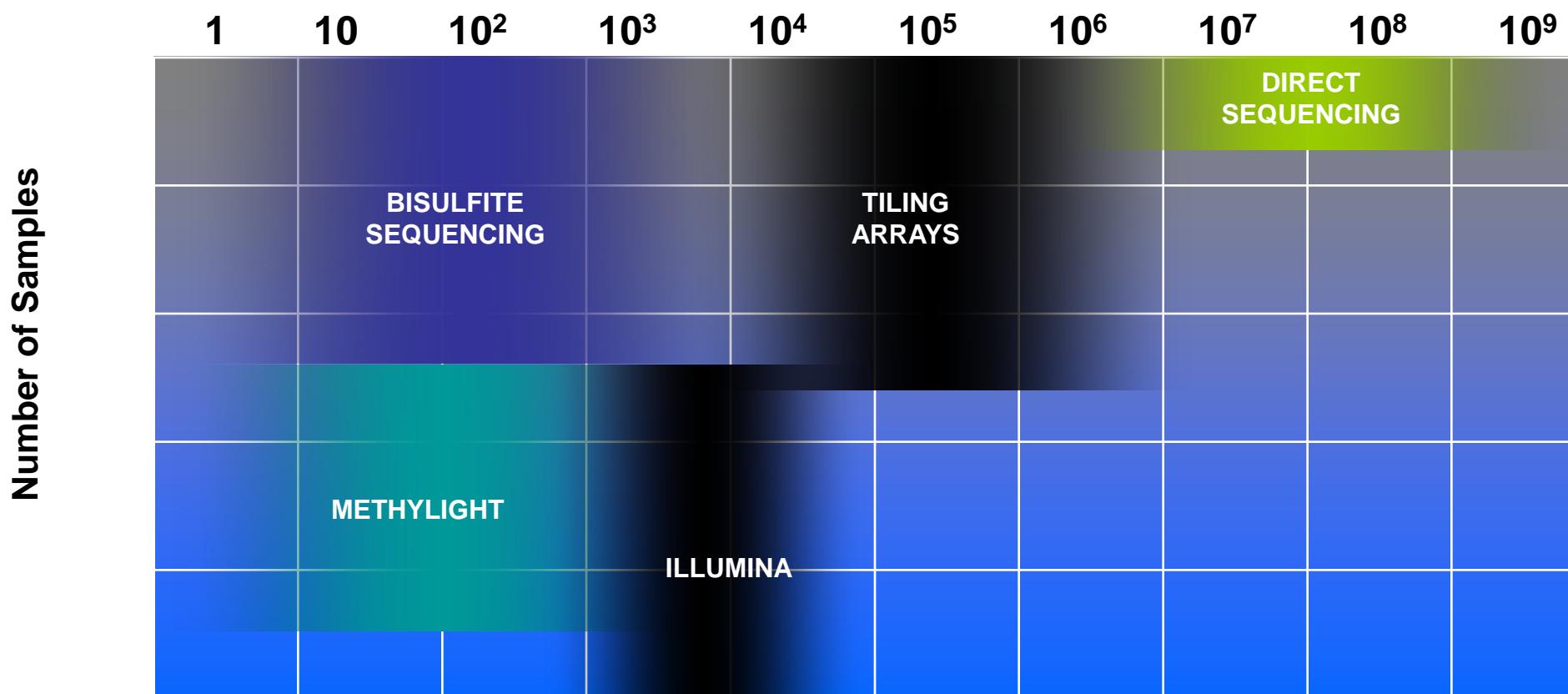
The sample preparation step of the general microarray workflow contains CHIP-on-chip-specific elements, as follows:

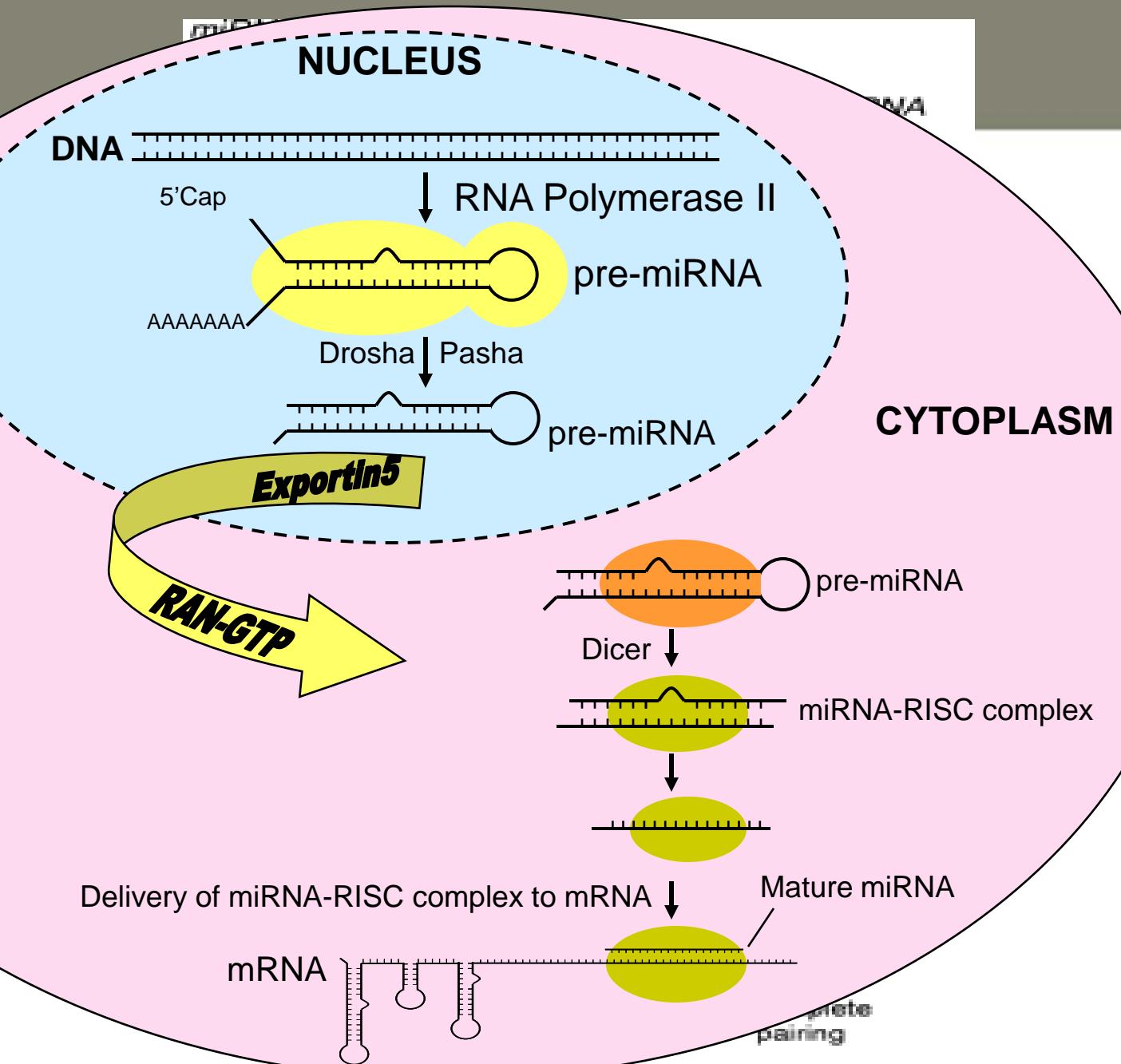


1. Cross-link protein-DNA complexes
2. Lyse cells and sonicate DNA: isolate unbound and bound fragments
3. IP chromatin to capture and purify bound DNA
4. Release and amplify bound DNA fragments
5. Prepare unbound (control) and amplified bound (target) DNA fragments for array application.

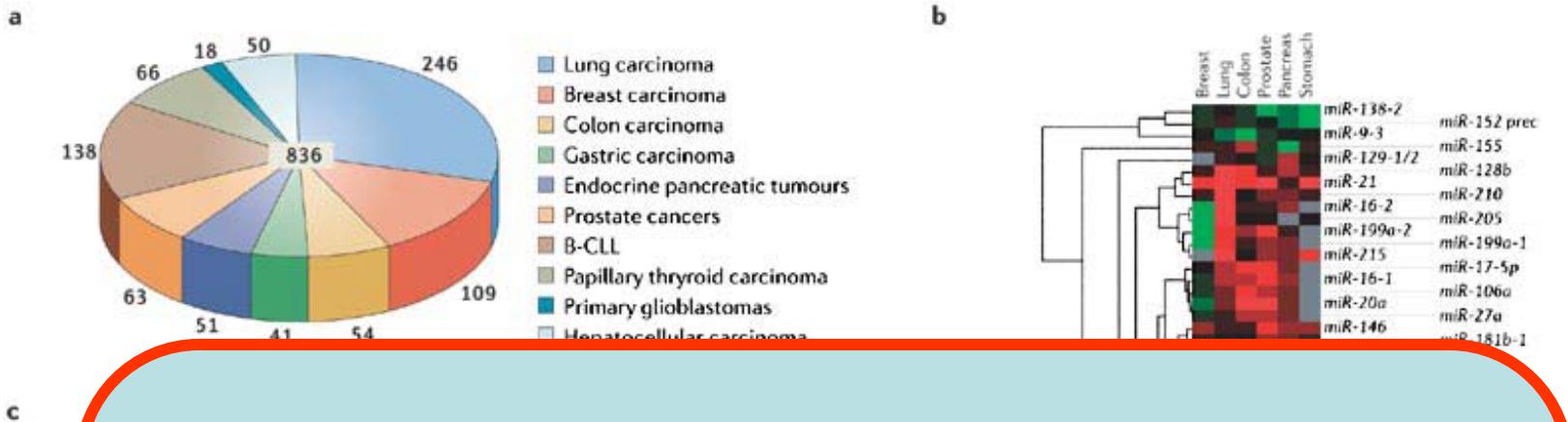
Epigenome Analysis

Number of Independent Loci per Genome





Mirco RNA Signatures in Human Cancers



Mir-31 inhibits metastasis in breast 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.

Target	Drug	Clinical Trial
DNA Methylation	5-Azacytidine	Phase I/II/II
	5-Aza-2'deoxyctydine	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	Phase I/II
	Depsipeptide	Phase I/II
	Valproic Acid	Phase I/II

Eggr et al Nature. 429:457.

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

- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting



- 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/m² of 5-AZA in combination with valproic acid.
- Dose-limiting toxicities were neutropenic fever and thrombocytopenia, which occurred at a dose of 94 mg/m² 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/m² 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)
Recruting	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>

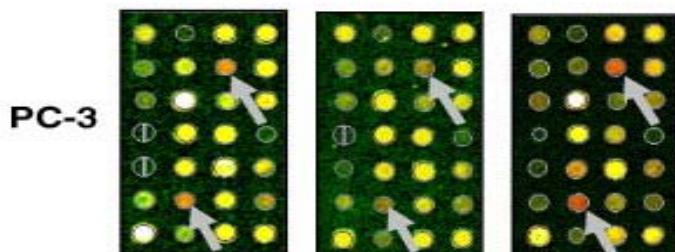
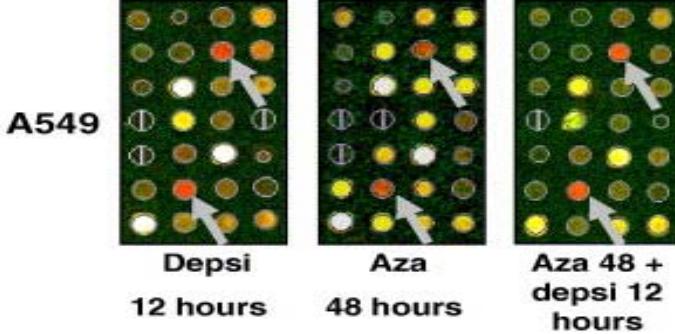
Methylation Inhibitors in Clinical Trials (Clinicaltrials.gov)

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

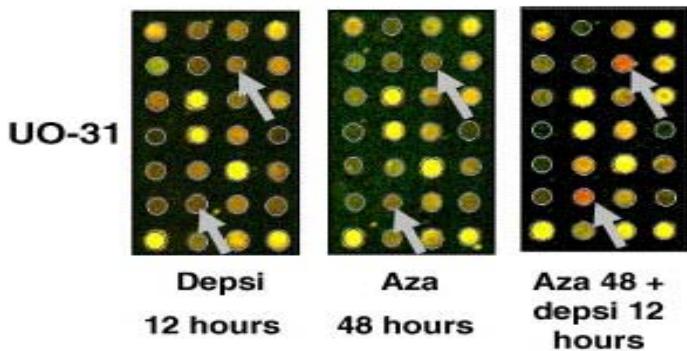
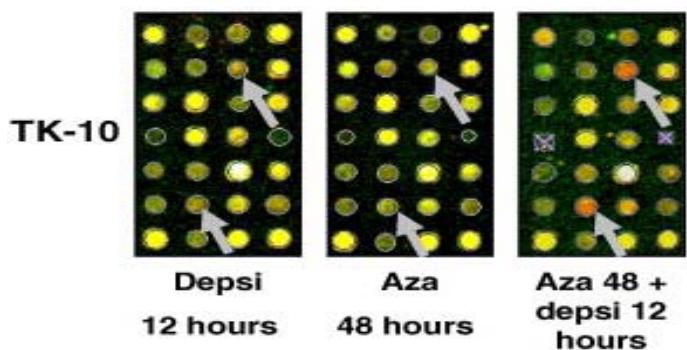
Schering-Plough (Decitabine {5-aza-Deoxycytidine} Trial for melanoma) (8 hrs to inactivate DNMT1)

<http://> Bristol-Myers Squibb (other compounds)

Inhibitors



Pharmacoepigenomics is acquired during persons life



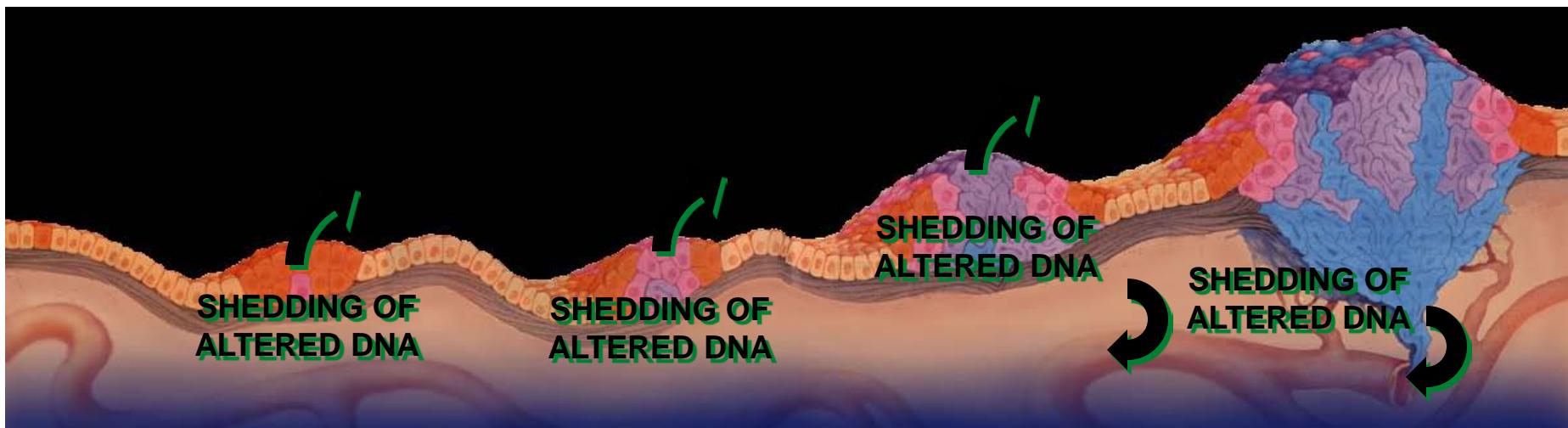
Gene Reactivation

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

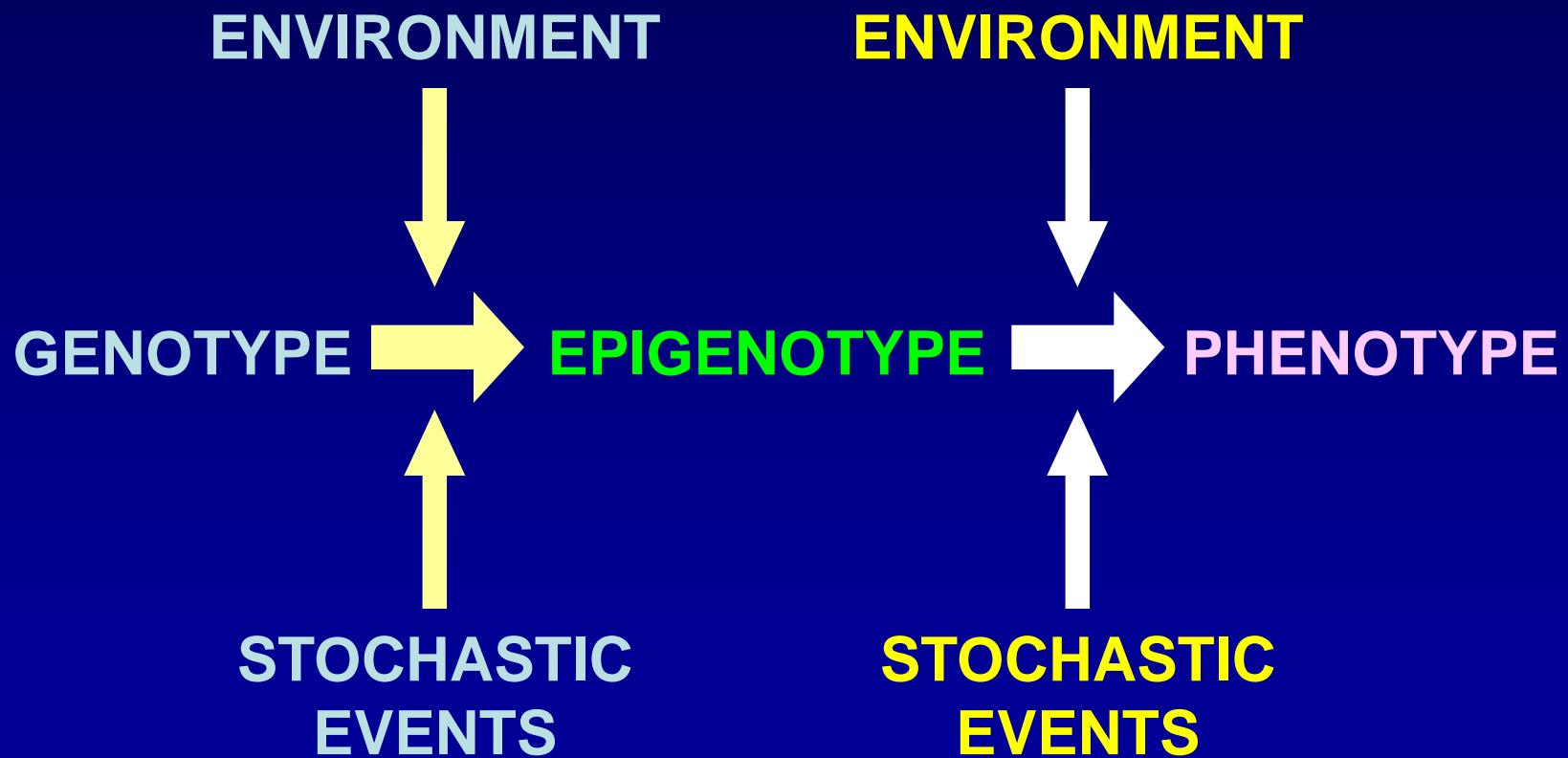
CANCER RISK ASSESSMENT PROGRESSION RISK ASSESSMENT EARLY DETECTION DIAGNOSIS, PREDICTION & PROGNOSIS MONITORING FOR RECURRENCE



PREDISPOSITION → HYPERPLASIA → DYSPLASIA → CARCINOMA → METASTASIS

Adapted from Weinberg 1996

Epigenotype as an Intermediate Phenotype



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?



EPIGENETICS AND CANCER



Editors
Mukesh Verma
Barbara K. Dunn
Asad Umar

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Epigenetics in Cancer Prevention

EARLY DETECTION AND RISK ASSESSMENT

Edited by Mukesh Verma, Barbara K. Dunn, and Asad Umar

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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 DNA sequence-i.e.,..." ([more](#))

Key Phrases: [epigenetic biomarkers](#), [bioactive food components](#), [novel imprinted genes](#), [Cancer Res](#), [New York Acad Sci](#), [Nucleic Acids Res](#) ([more...](#))

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Book Description

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

Book Info

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

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104 DNA Methylation Regulates the Growth Suppressor PRMT1 in Cancer Cells
112 Identification of Human SET-Point 4 and Exon 3 of Facilitates Chromatin Remodeling by the SET-Point 4 Protein
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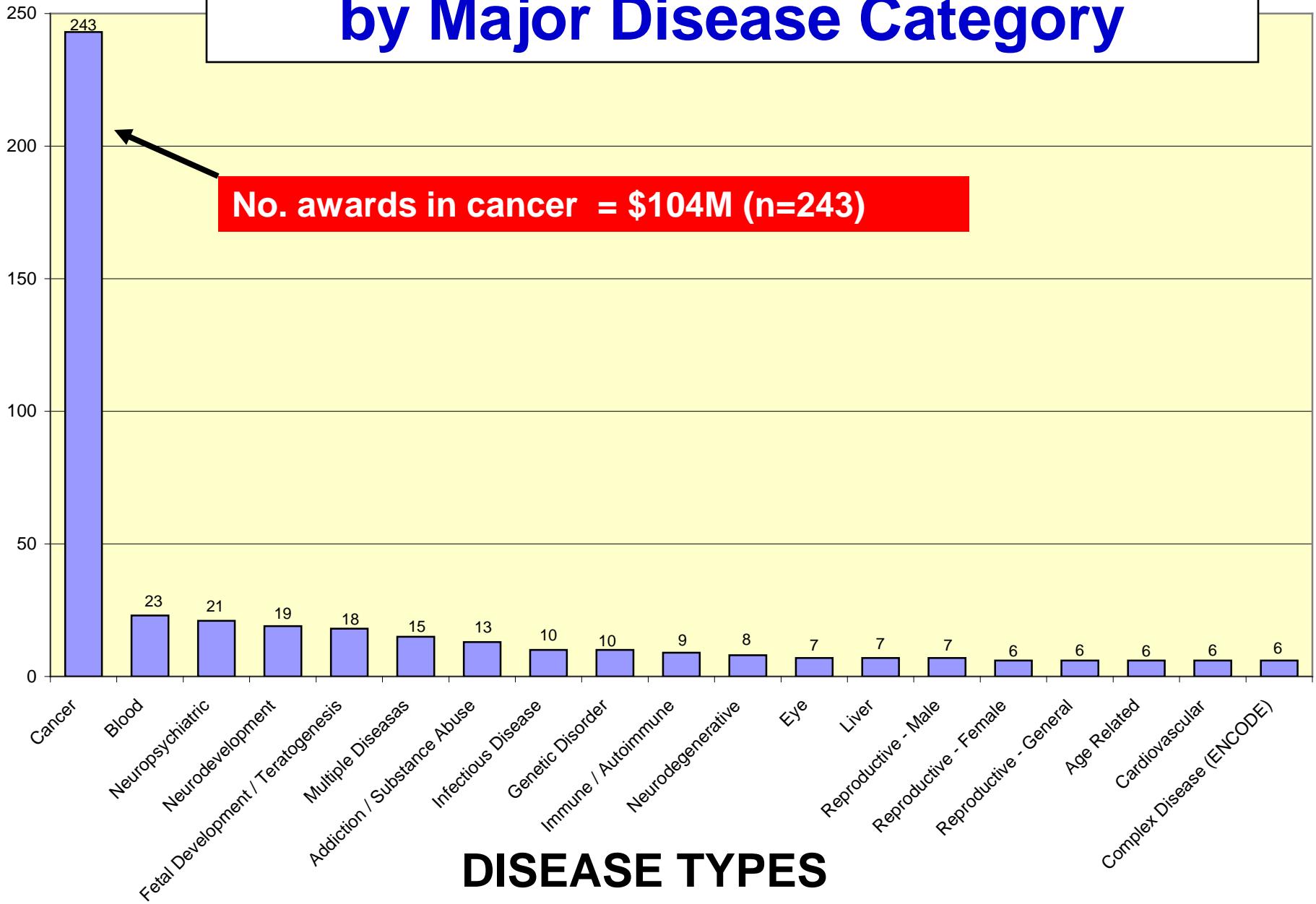


- Incubator space (5-10 year projects)
- Test bed for high risk, enabling, or emerging scientific opportunities
- Accelerate the pace of discovery
- Must be transformative
- Trans-NIH, all Institutes/Centers participate
- NIH Common Fund

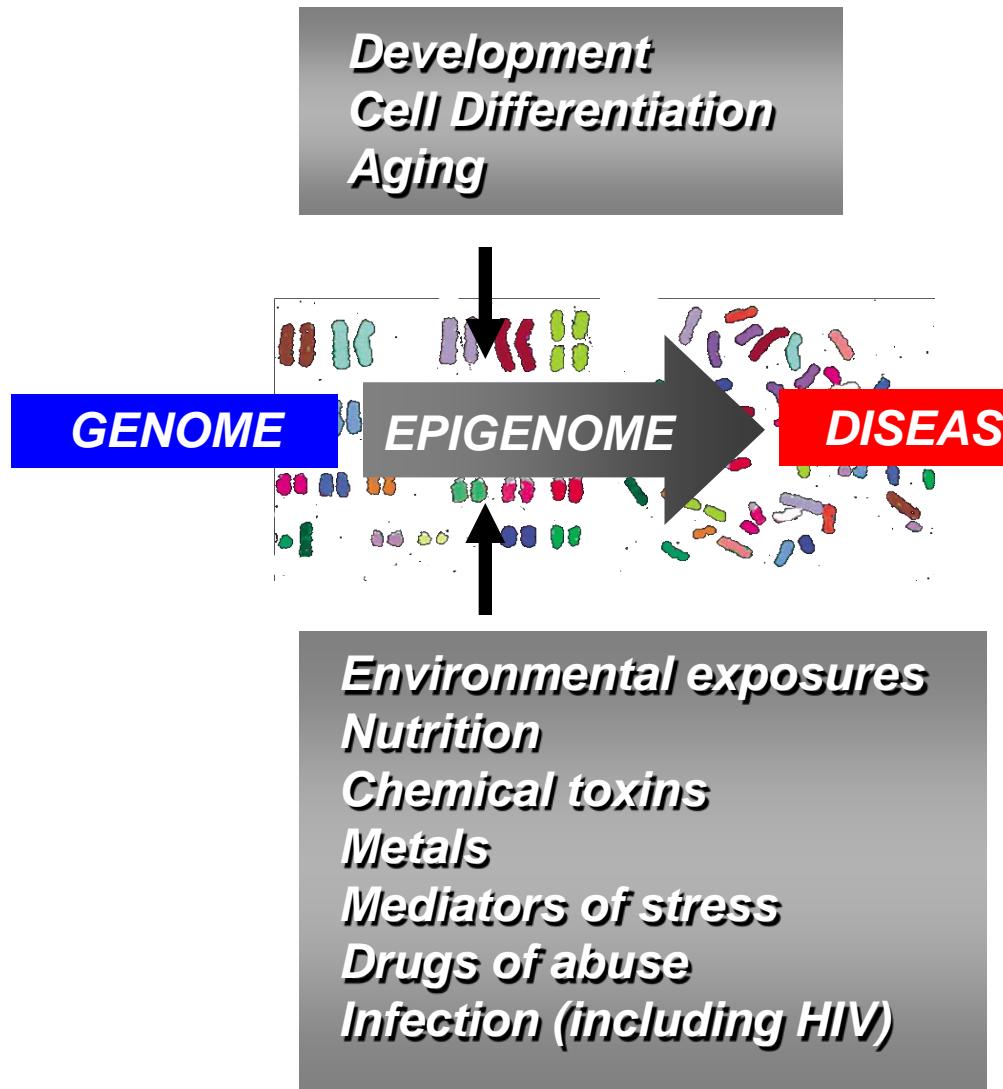
Epigenetically Regulated Diseases:

Several cancers, autoimmune disorders, reproductive disorders, and neurobehavioral and cognitive dysfunctions

Extramural Epigenetics Portfolio by Major Disease Category



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



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

Roadmap 1.5: Translation to Clinical Practice

FY08

Basic
Research



FY17

Clinical
Application

- 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.

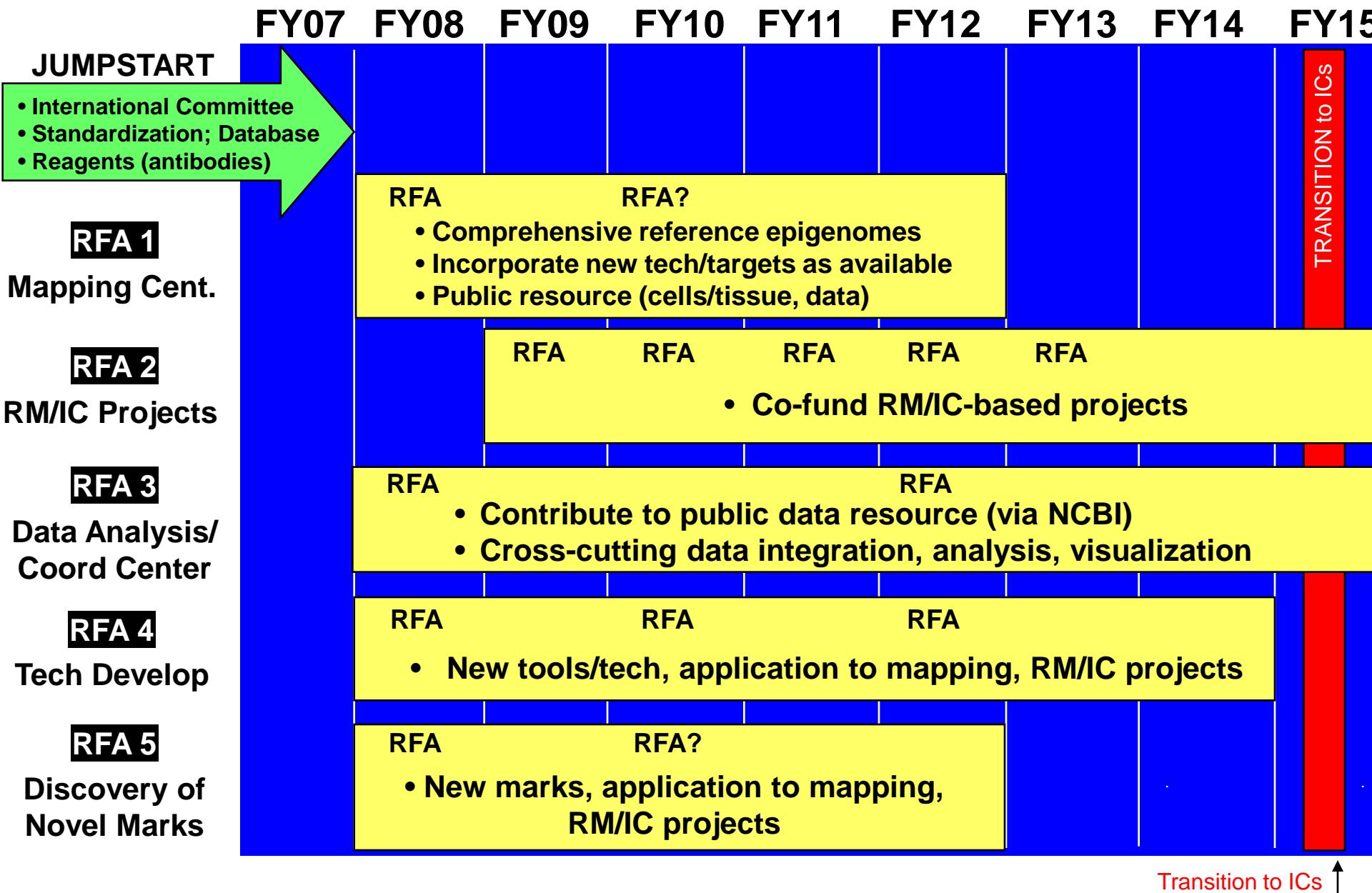
Epigenetics Working Group

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

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

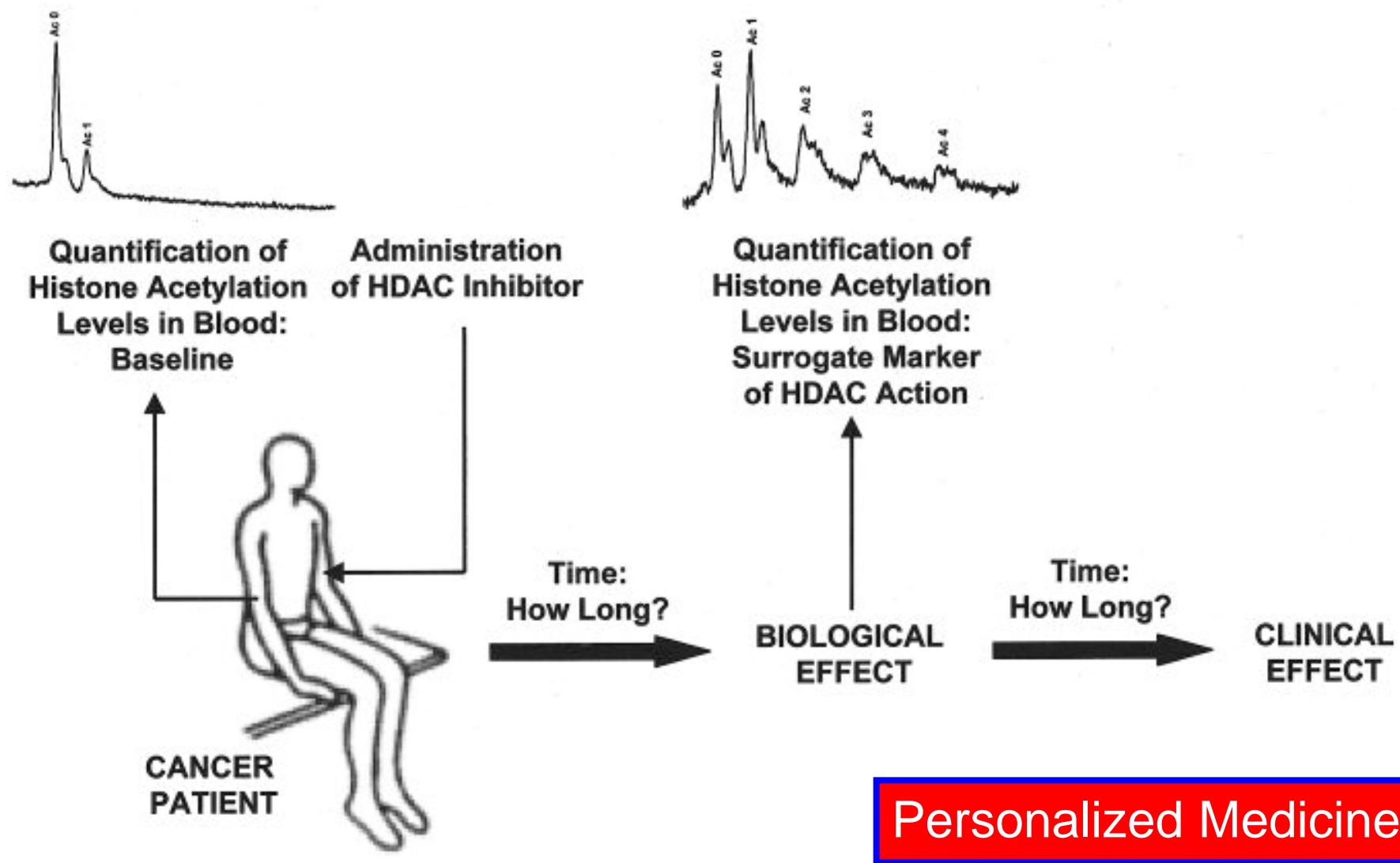
Brenda Weis	NIEHS	Roger Little	NIMH
Jerry Heindel	NIEHS	Conrad Mallia	NIAID
Fred Tyson	NIEHS	Anna McCormick	NIA
John Satterlee	NIDA	Nasrin Nabavi	NIAID
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Christine Colvis	NIDA	Susan Old	NHLBI
Jonathan Pollock	NIDA	Deborah Olster	OD/OBSSR
David Shurtleff	NIDA	Jim Ostell	NLM/NCBI
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James Batty	NICHD	Sharon Ross	NCI
Lisa Brooks	NHGRI	Rochelle Small	NIDCR
Anthony Carter	NIGMS	Philip Smith	NIDDK
Jennifer Couch	NCI	Mukesh Verma	NCI
Johanna Dwyer	OD/ODS	Ashley Xia	NIAID
Bruce Howard	NICHD	Samir Zakhari	NIAAA
Rebecca Lipsitz	NIH/OD	Keji Zhao	NHLBI

Timeline for Epigenomics Program



Transition to ICs

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



Conclusion: Epigenetics in Cancer Management

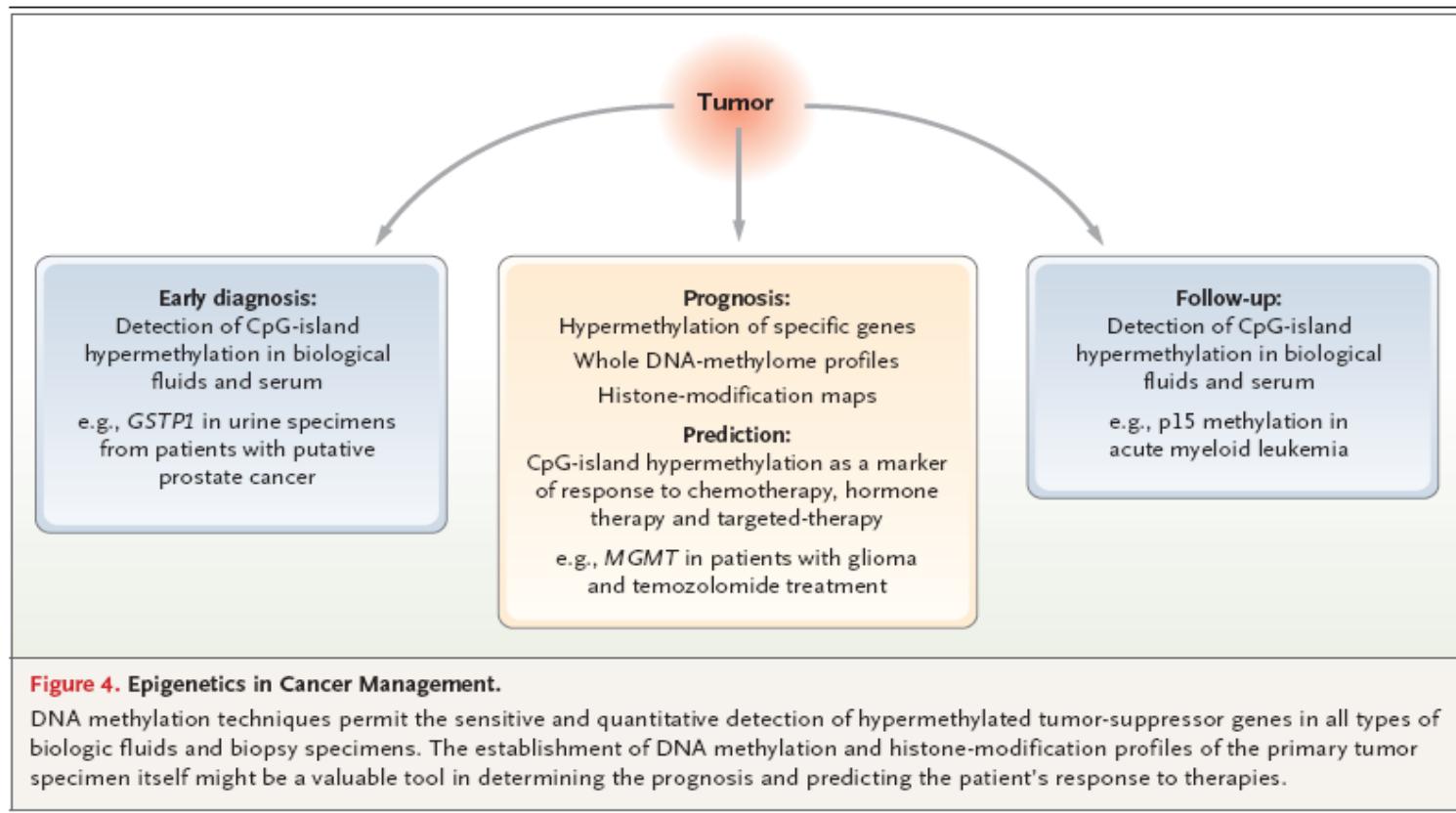


Figure 4. Epigenetics in Cancer Management.

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.