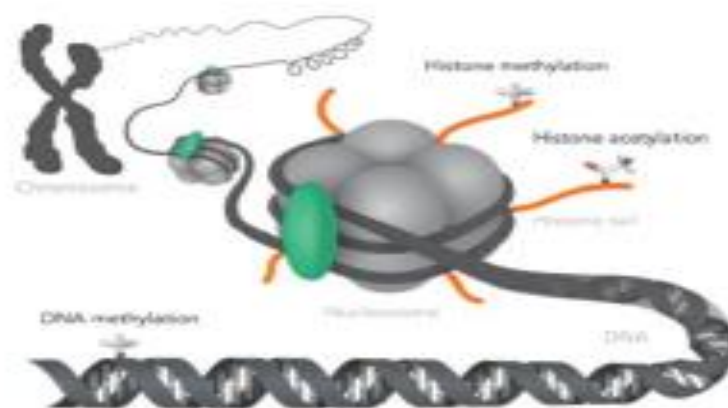


Epigenetics and cancer

National Cancer Institute

Epigenetics and Cancer



Mukesh Verma, Ph.D.

Chief, Methods and Technologies Branch
Program Director,
Epidemiology and Genomics Research Program
DCCPS, NCI, NIH

Hallmarks of cancer

Hallmarks of Cancer: New Dimensions



Nonmutational epigenetic reprogramming and polymorphic microbiomes both constitute distinctive enabling characteristics that facilitate the acquisition of hallmark capabilities

Epigenetics



Epigenetics

Epigenetics:

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

Genetic Code



The two main components of the epigenetic code

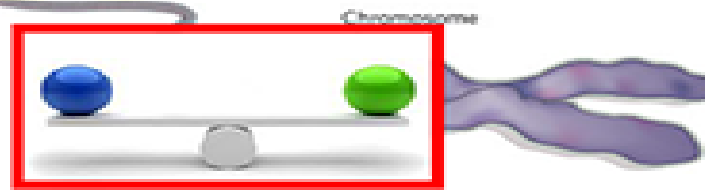
DNA methylation
Methyl marks added to certain DNA bases repress gene activity.

Methylation Code

Histone Code

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

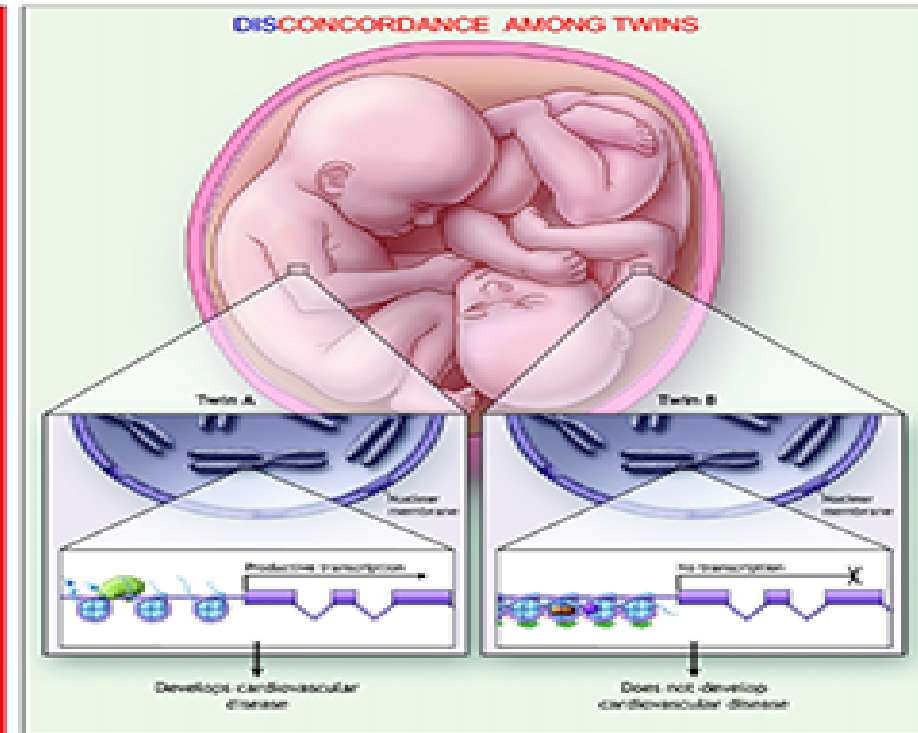
The genetic information provides the blue print for the manufacture of all the proteins necessary to create a living organism, whereas the epigenetic information provides the instructions on how, where and when the genetic information will be used.



DNA and destiny



The choices you make
can change your genes
— and those of your kids.



Epigenetic predisposition to angiogenesis? Individual? Populations?

Pharmacogenomics and pharmacoeigenomics (personalized medicine)

Microenvironment, microbiome, and gene expression

GWAS and EWAS

Global cancer deaths

GLOBAL CANCER DEATHS

In 2019, more men than women died from cancers caused by known risk factors, in part because males tend to smoke and drink alcohol more than females. Men are also more likely to work in jobs that expose them to risk factors.



©nature

Source: Ref 1.

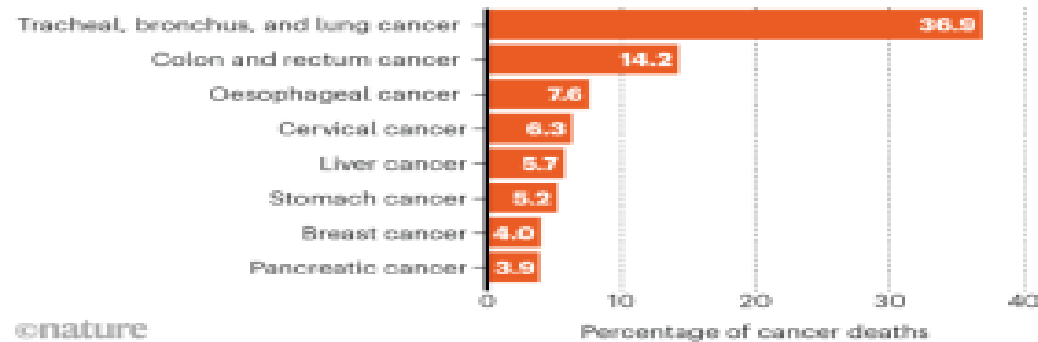
<https://www.nature.com/articles/d41586-022-02355-x>

GBD 2019 Cancer Risk Factors Collaborators *Lancet* **400**, 563–591 (2022).

Cancer tumor deaths

CANCER DEATHS BY TUMOUR TYPE

In men and women, among cancers caused by preventable risk factors, tumours of the lung, trachea and bronchus were the leading cause of death. Smoking was the biggest risk factor associated with those cancer deaths.



doi: <https://doi.org/10.1038/d41586-022-02355-5>

nature

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NEWS > 2022 > article

NEWS | 21 August 2022

Almost half of cancer deaths are preventable

Data show that smoking, drinking alcohol and obesity are the biggest contributors to cancer worldwide.

Georgia Siufolet

nature briefing

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Cancer continuum

DCCPS covers cancer continuum



Prevention

Tobacco, physical activity, diet, sun, environment, HPV immunization



Early Detection

Breast, cervical, colorectal cancer screening



Diagnosis

Incidence, Stage at diagnosis



Treatment

Trends in cancer treatment



Life After Cancer

Financial burden of cancer care, Cancer survivorship



End of Life

Mortality, Person – years of life lost

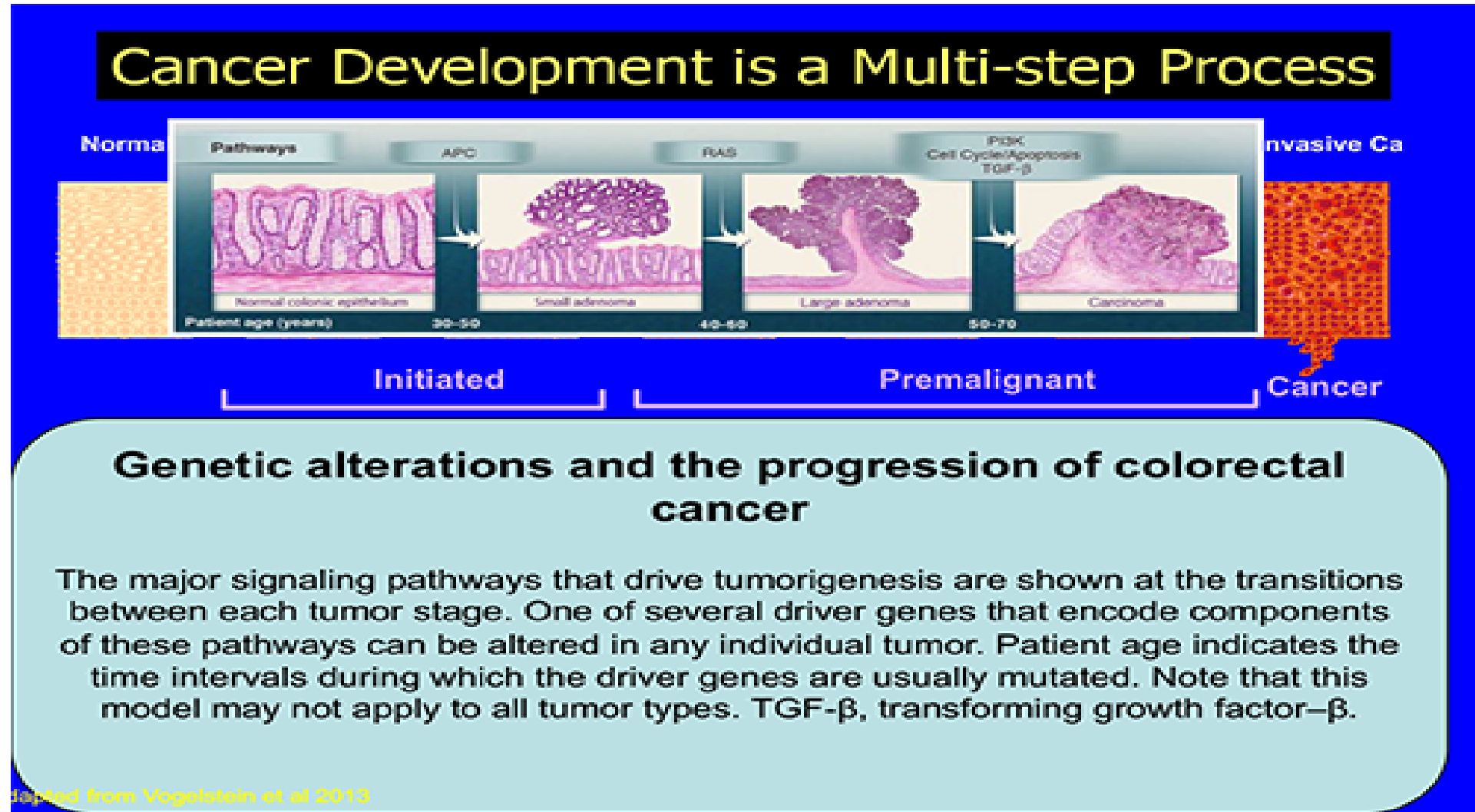
Prevention



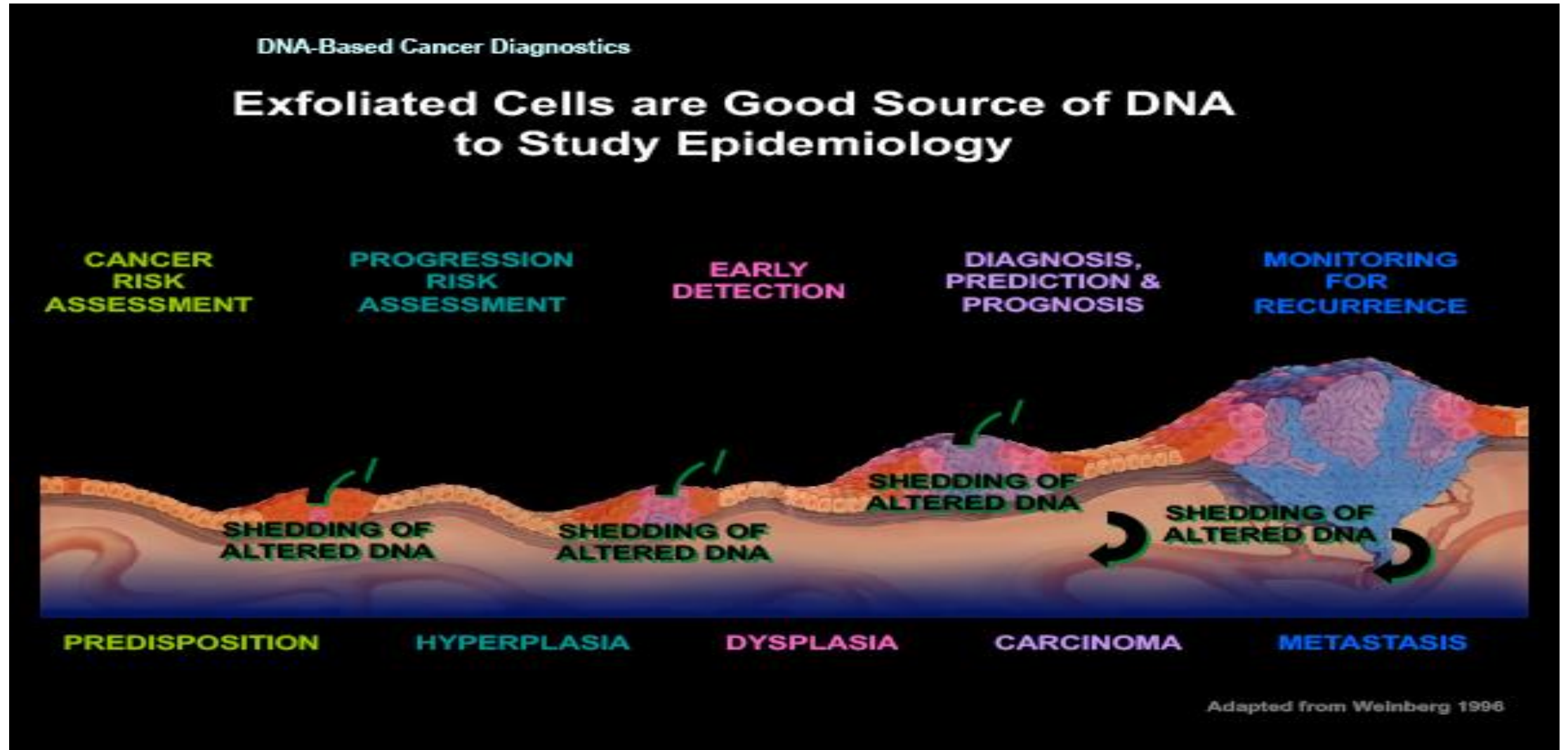
Cancer recurrence
Secondary cancer

Prevention: restoring transcription, halting progression, or stopping metastasis

Cancer development



DNA sources



Paradigm shift

Paradigm shifts in genetics

1850 -1900 : Proto-genetics

*Mendelian inheritance
Darwin, natural selection*

1900 -1950 : Age of genetics

*gene concept, mutation,
genotype-phenotype*

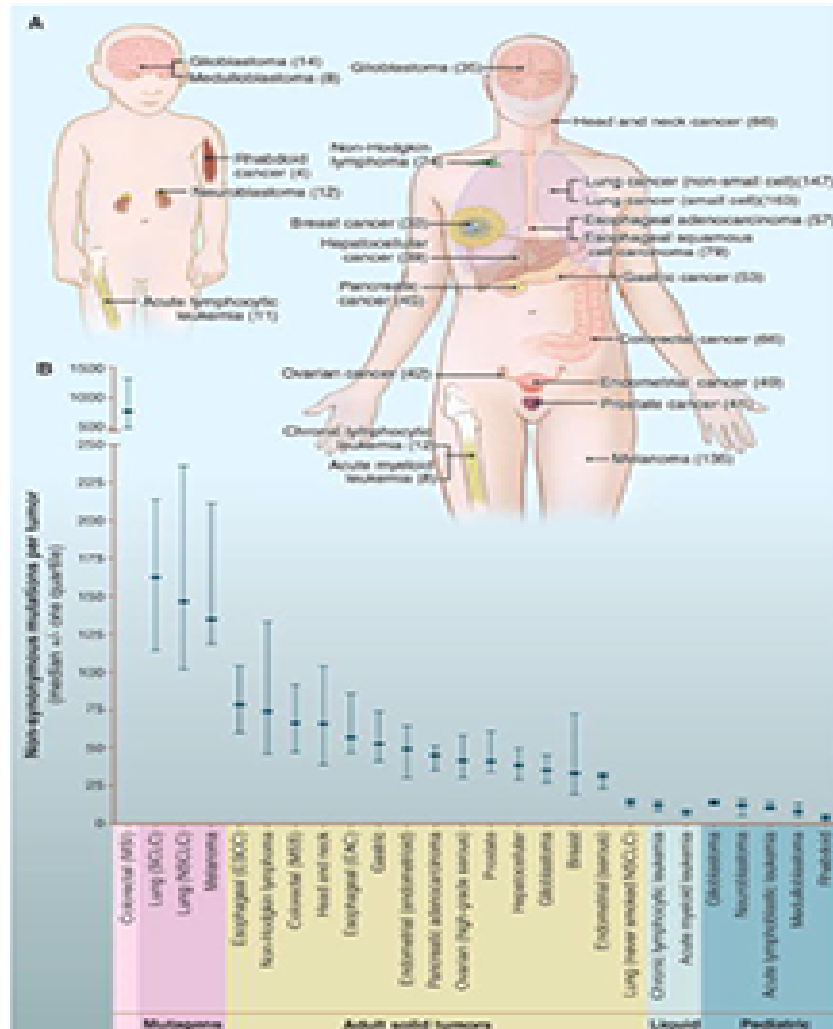
1950-2000 : Age of DNA

*structure, genetic code,
genome sequence*

2000 - : Age of epigenetics

*epigenetic code, epigenome,
epigenetic medicine*

Genome landscape



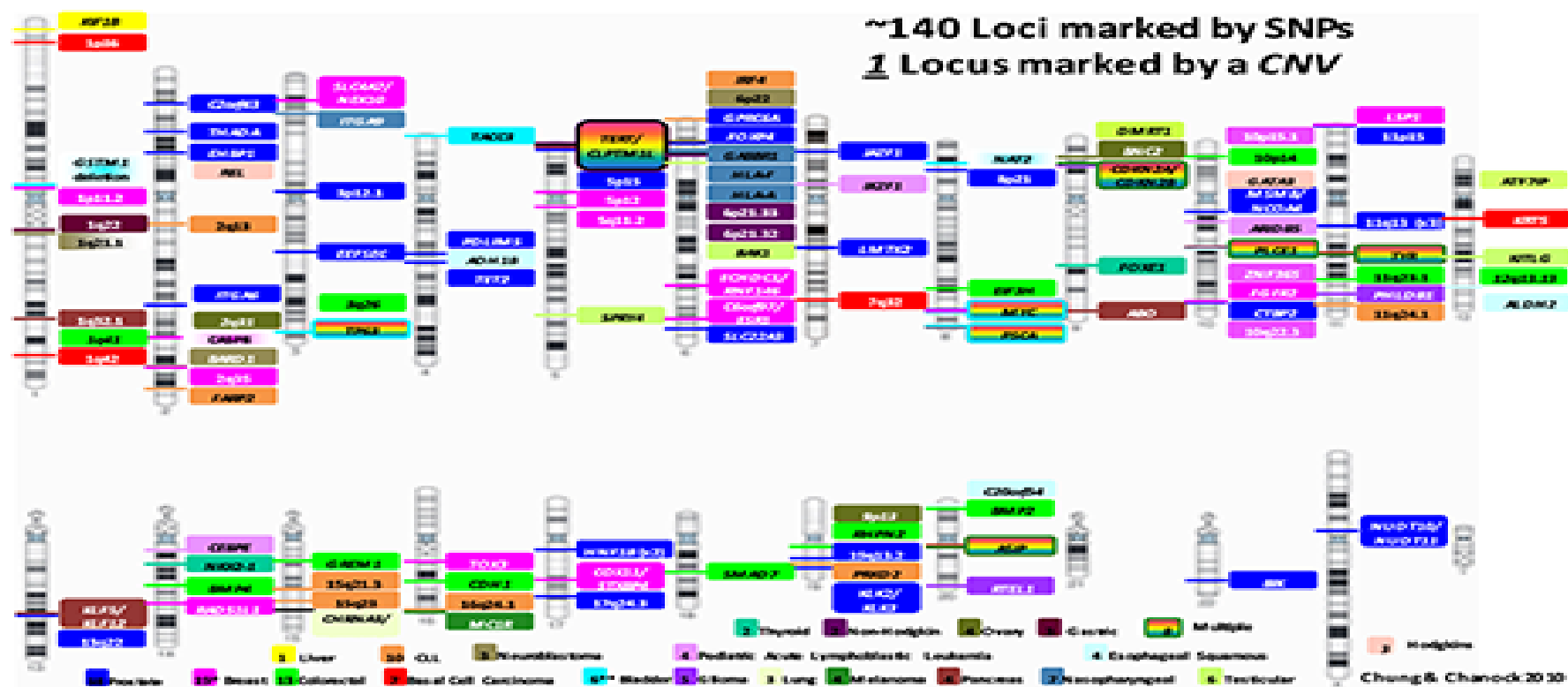
CANCER GENOME LANDSCAPE
 Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies



Adapted from Vogelstein and Kinzler (Science 2013)

GWAS hits

Published GWAS Etiology Hits (2010)



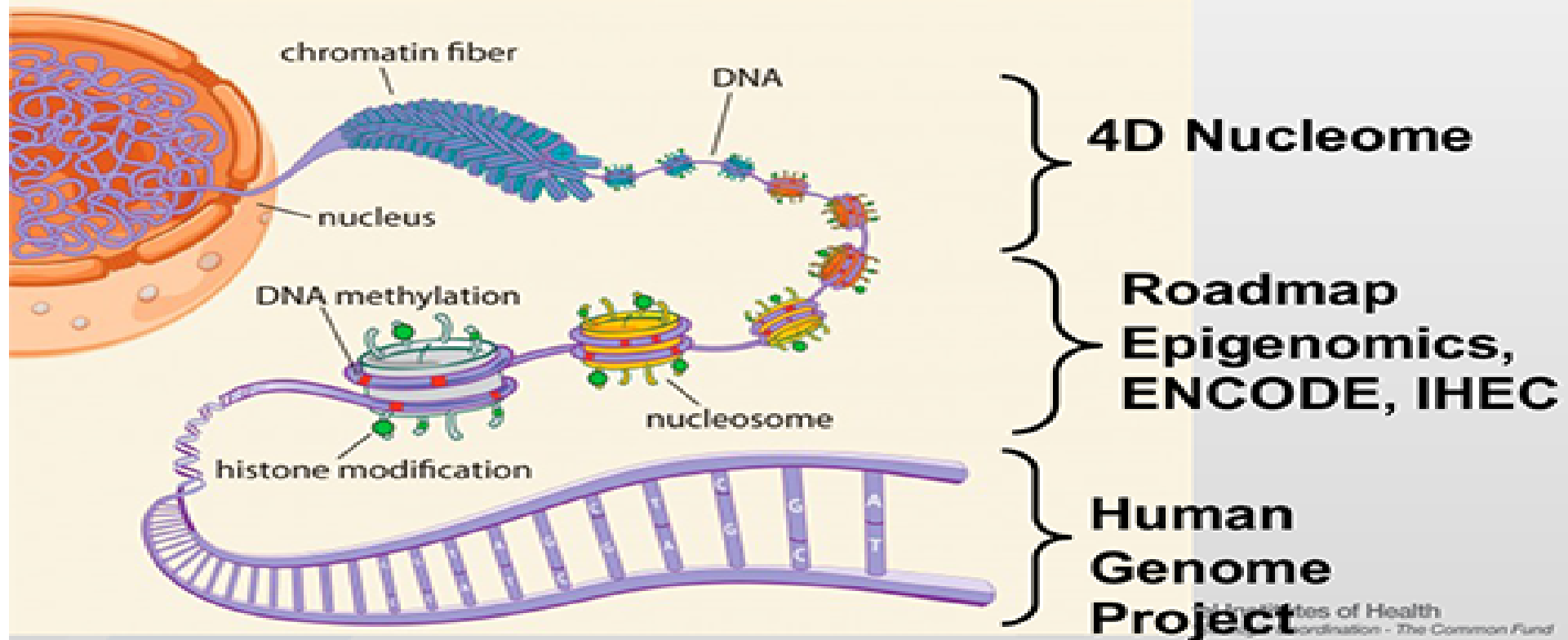
Genome associations

Published Genome-Wide Associations through 12/2013
Published GWA at $p \leq 5 \times 10^{-8}$ for 17 trait categories



Genome sequence

There's more to the genome than its sequence



Kornberg and nucleosome

Nucleosomes (Units of Chromatin)

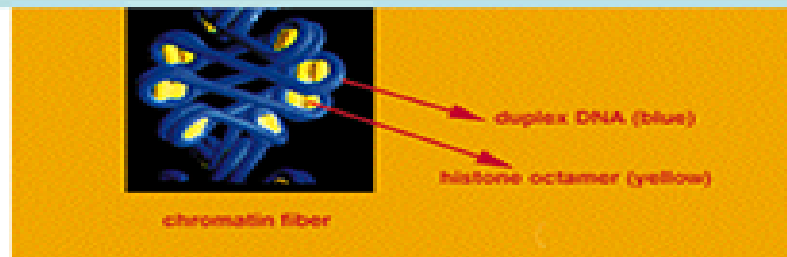
DNA
Histones H2a, H2b, H3, H4

To neutralize charge and provide stability

H1 is a linker histone which binds to the DNA linking two adjacent nucleosomal cores

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

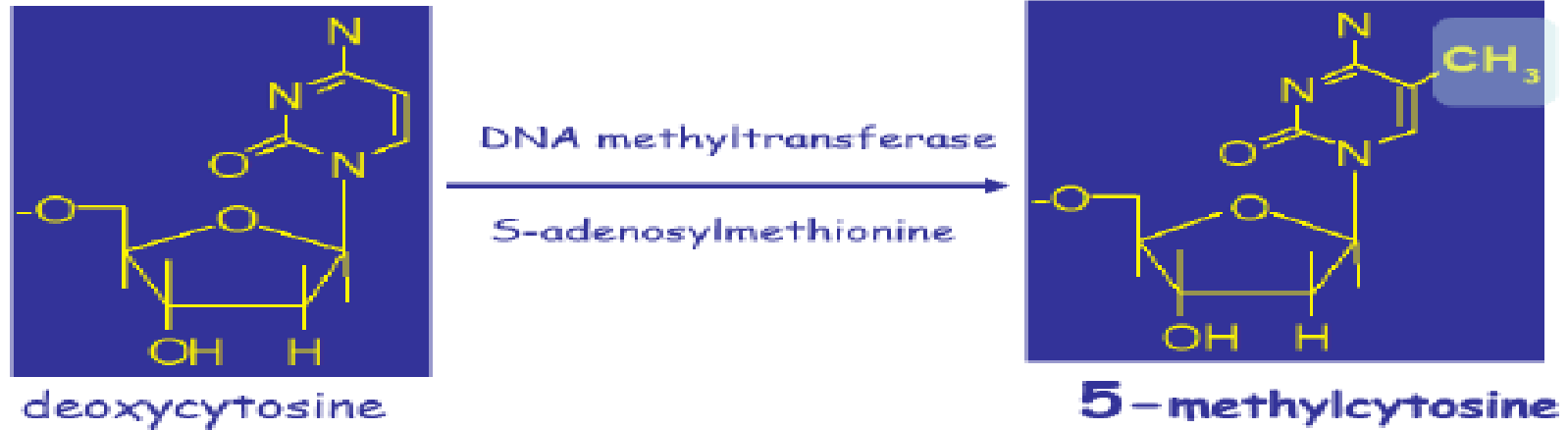
1974: Roger Kornberg discovers nucleosome who won Nobel Prize in 2006.



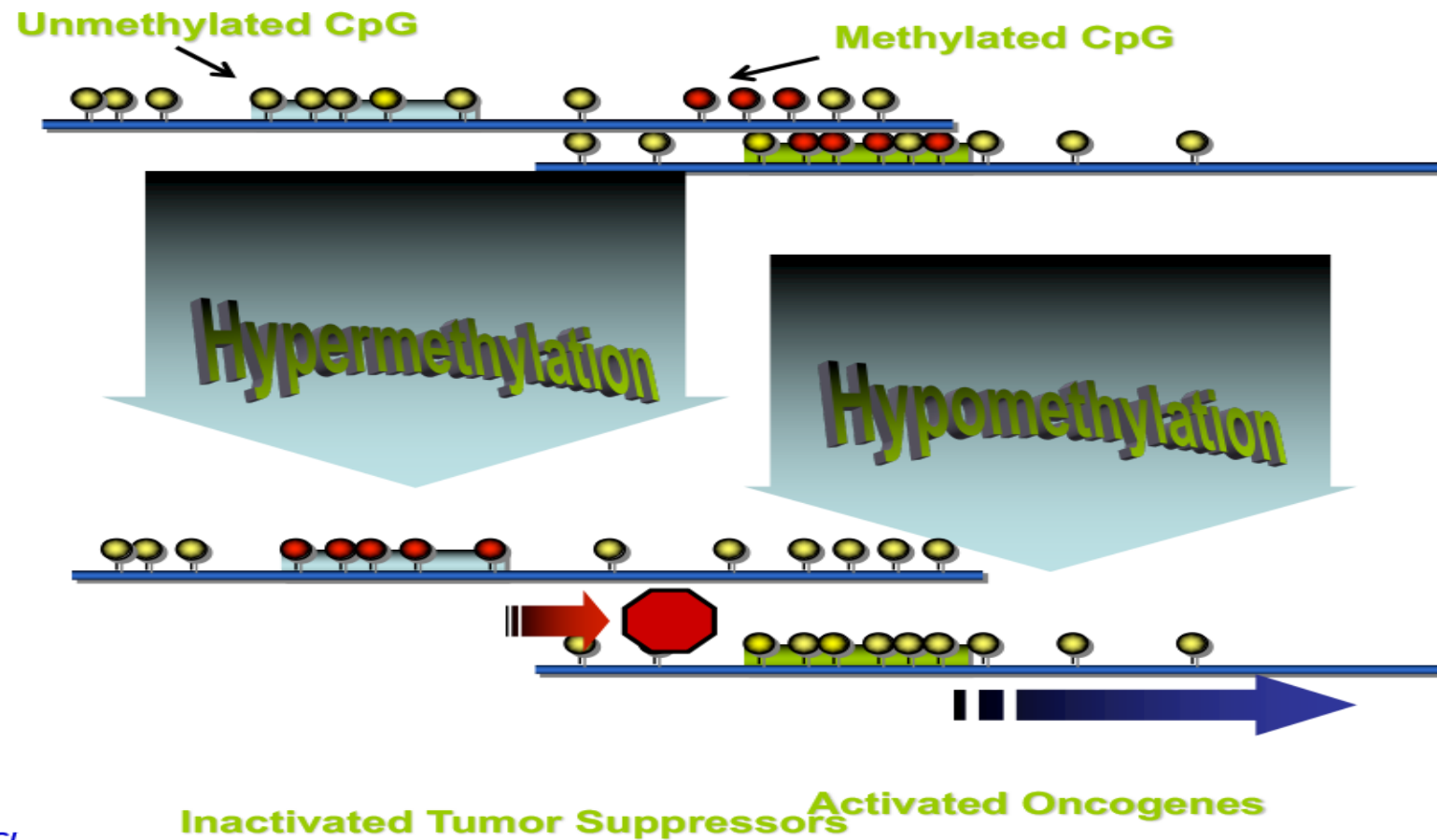
Shores are 0-2kb from islands
Shelves are 2-4 kb and enhancers are beyond shelves

DNA methylation

DNA Methylation



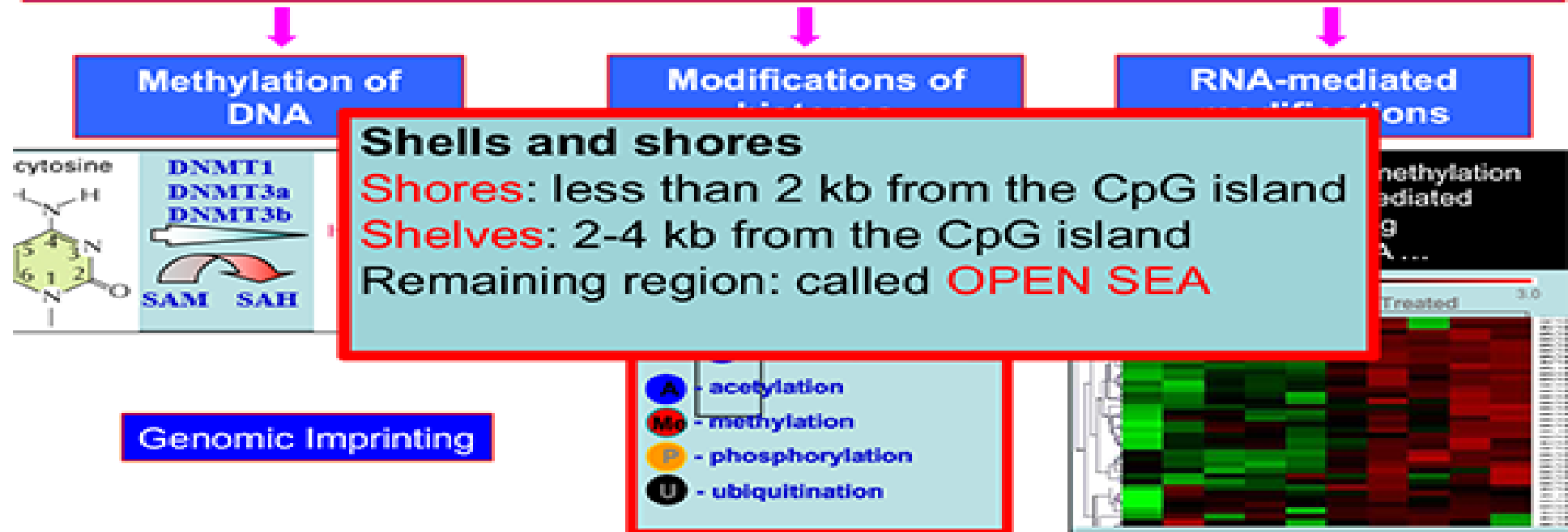
DNA methylation



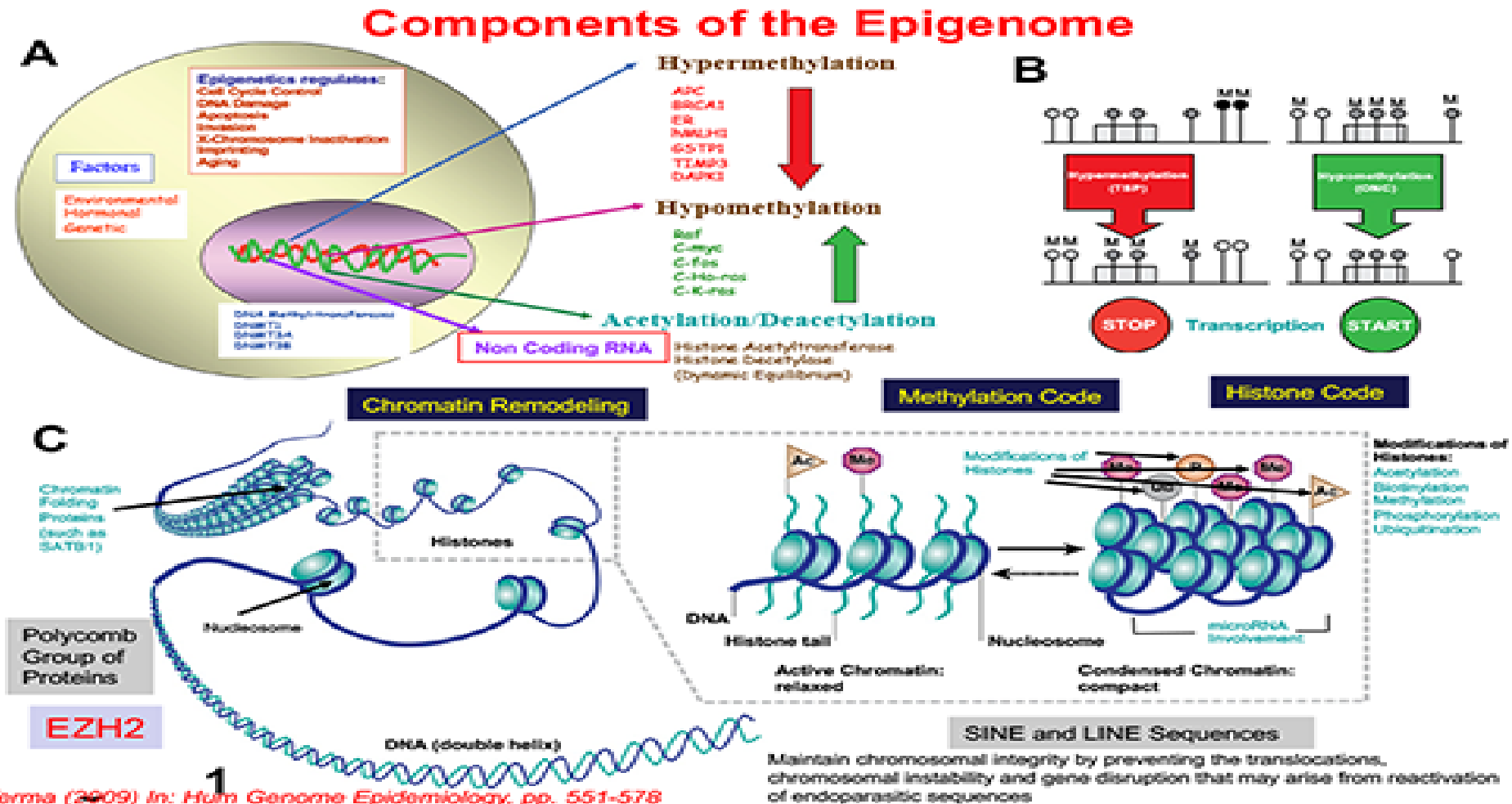
Epigenetics

EPIGENETICS

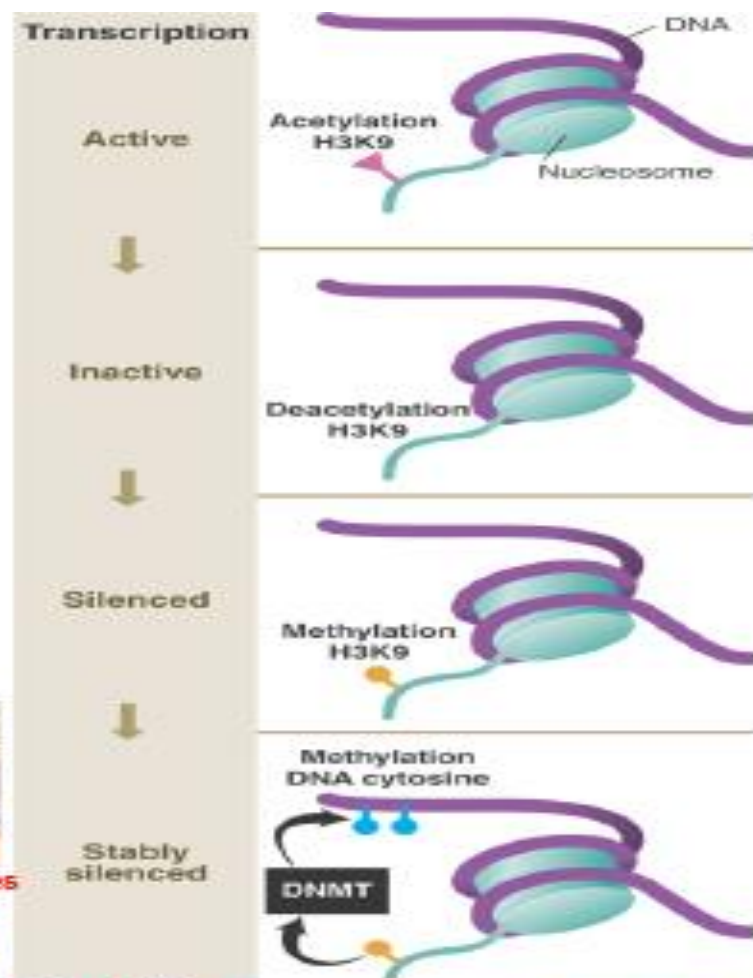
Epigenetic alterations – changes induced in cells that alter expression of the information on transcriptional, translational, or post-translational levels without change in DNA sequence



Epigenome components



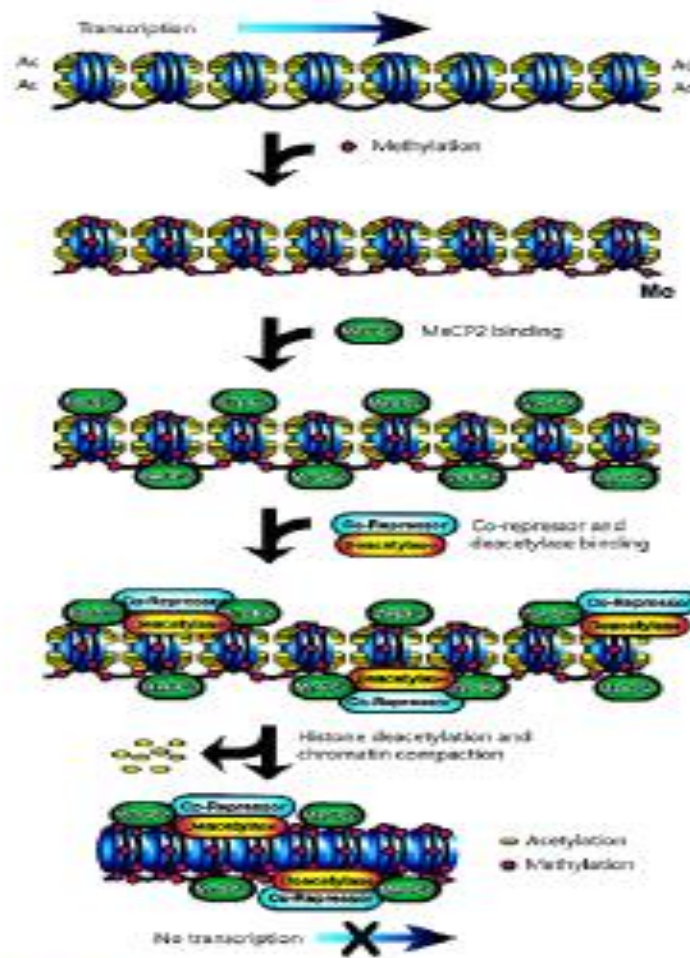
Methylation



Peter Jones



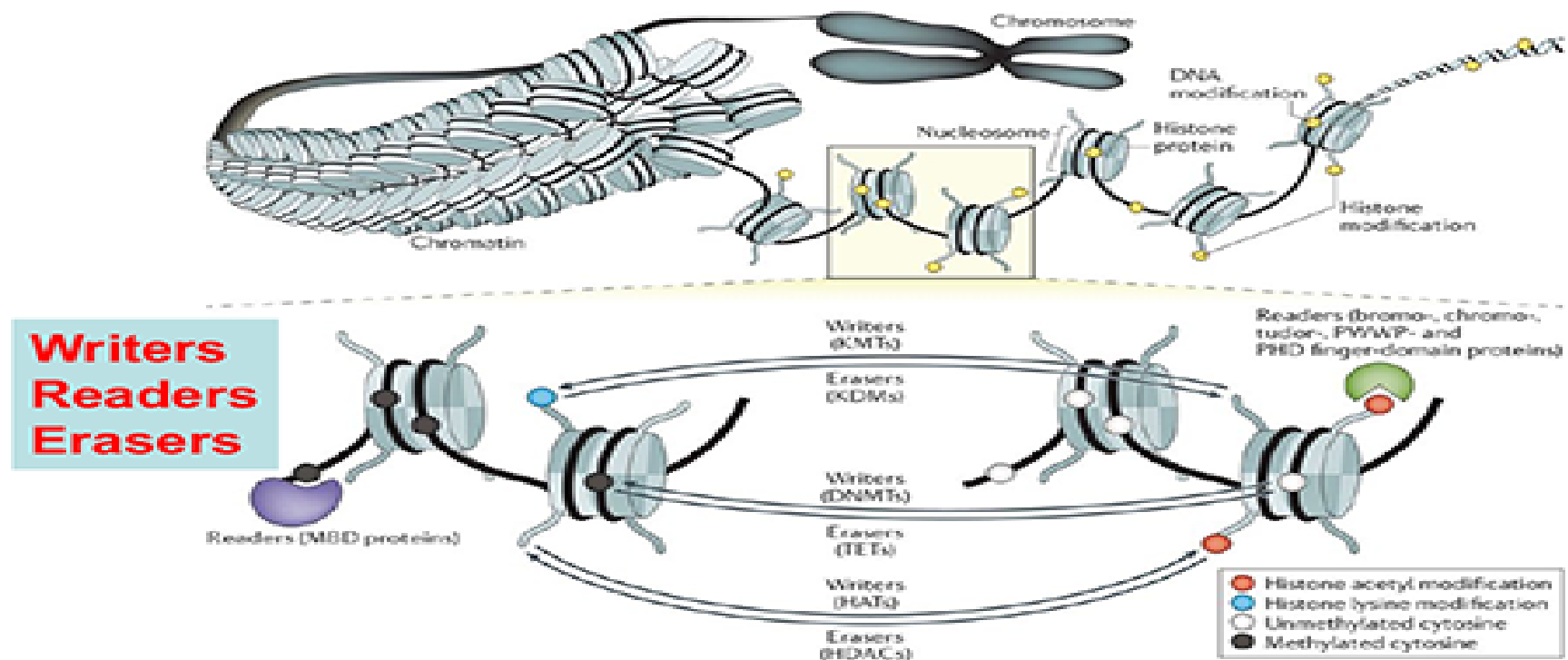
Andrew Feinberg



Chromatin modifications

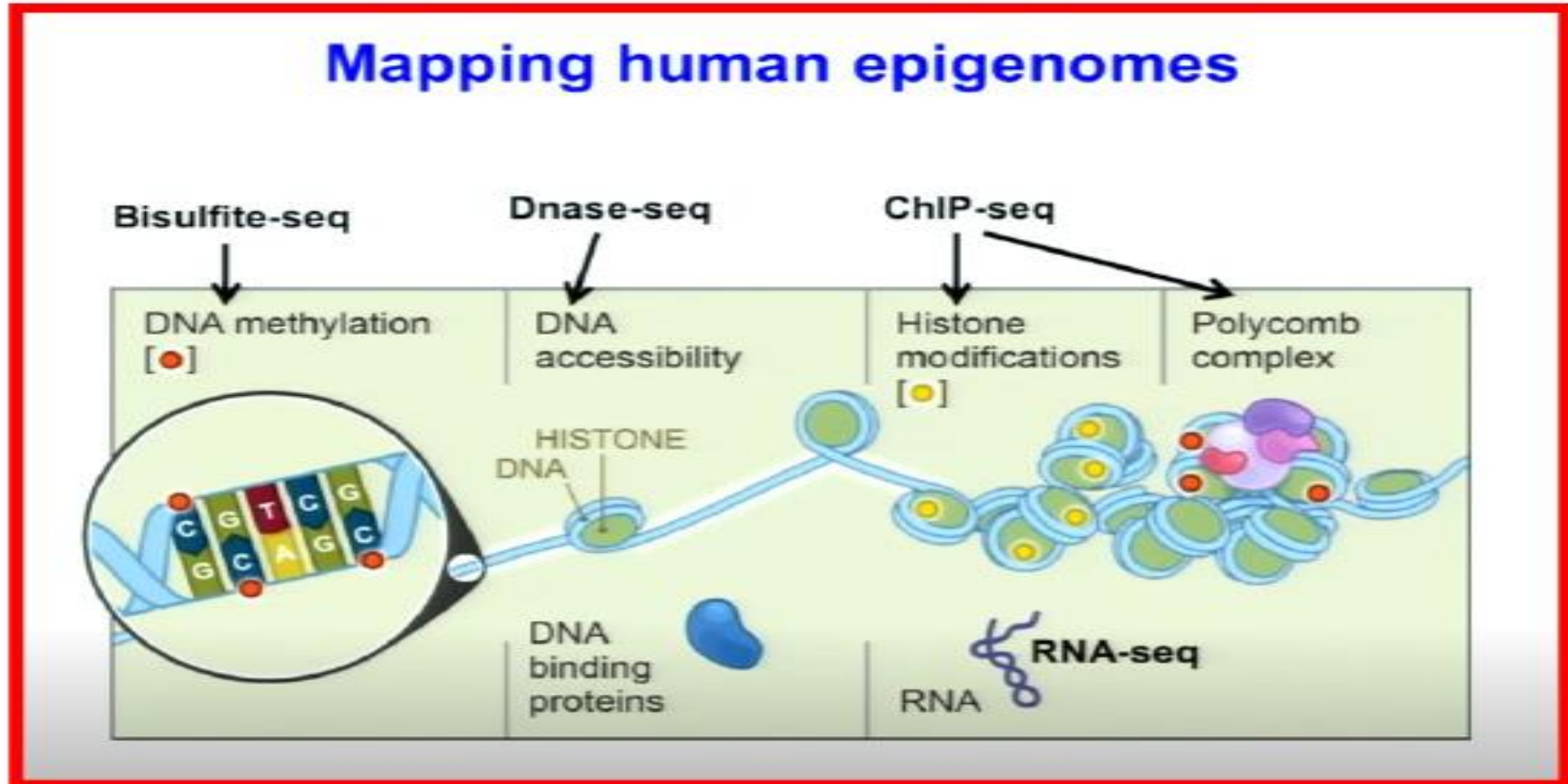
Figure 1 : Modulation of covalent modifications on chromatin.

From Targeting the cancer epigenome for therapy



Ten-eleven translocation (TET) family of 5-methylcytosine oxidases.

Epigenomes



Genome versus epigenome



Genome vs. epigenome – why is it important?



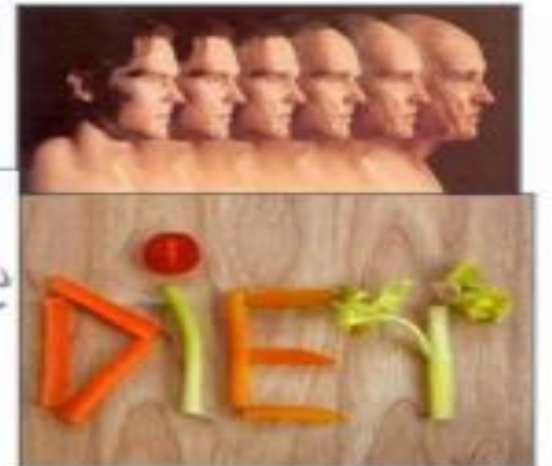
• Genome is generally constant; epigenome changes

- Age
- Diet
- Disease
- Lifestyle
- Environment



• Areas of interest:

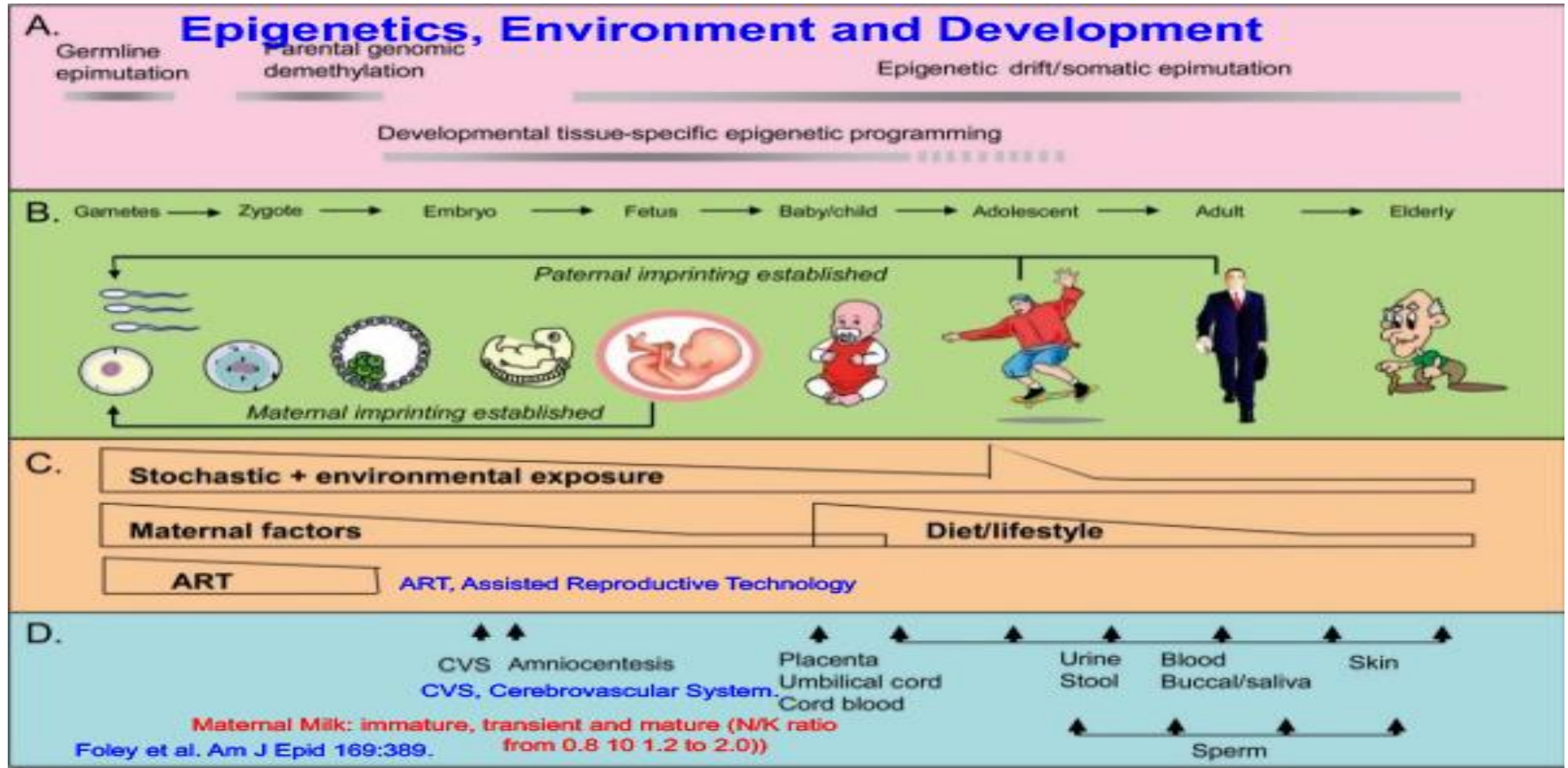
- Molecular basis of disease
- Biomarker identification
- Diagnostics development
- Drug targeting



**You only need to sequence your genome once,
but you need to determine your epigenome
multiple times...**

<https://www.youtube.com/watch?v=JMT6oRYgkTk>

Epigenetics, environment and development



Toxic substances

Key toxic substances affecting the epigenome

Arsenic	Induces <u>genetic</u> and <u>epigenetic</u> changes
Benzene	Benzene and its metabolic product hydroquinone alter <u>methylation</u> profiles and contribute to <u>leukemia</u>
Cadmium	Induces <u>hypermethylation</u> of selected genes in <u>lung cancer</u>
Chromium	Induces <u>hypermethylation</u> in <u>lung cancer</u>
Nickel	Alters <u>chromatin structure</u> and induces <u>histone acetylation</u>
PFOS	Affects <u>prenatal methylation</u> and regulation of <i>GSTP1</i> and <u>LINE/SINE</u> sequences
PAHC	Alters <u>histone H3 acetylation</u> in <u>breast cancer</u> model
Uranium	Contributes to <u>leukemia</u>

PFOS, Perfluorooctane sulfonate

PAHC, Polycyclic aromatic and halogenated compounds

Histone phosphorylation



Aldehyde and nitric oxide, present in cigarette smoke induce phosphorylation of histones resulting in decreased histone deacetylase 2 activity



Endogenous factors

[PloS One](#), 2016 May 12;11(5):e0155554. doi: 10.1371/journal.pone.0155554. eCollection 2016.

Maternal Smoking during Pregnancy and DNA-Methylation in Children at Age 5.5 Years: Epigenome-Wide-Analysis in the European Childhood Obesity Project (CHOP)-Study.

[Ezehak P](#)¹, [Saffery B](#)², [Vandac E](#)³, [Rao E](#)³.

[Author information](#)

Abstract

Mounting evidence of a DNA methylation profile in the blood assessed by Epigenome-wide association studies (EWAS) of DNAm signatures in children at age 5.5 years. This study investigated the biological role by examining the association of maternal smoking during pregnancy with DNA methylation in children at age 5.5 years.

[Transl Psychiatry](#), 2016 Mar 29;6:e765. doi: 10.1038/tp.2016.32.

The effects of maternal anxiety during pregnancy on IGF2/H19 methylation in cord blood.

[Mansell T](#)^{1,2}, [Novakovic B](#)^{1,2}, [Meyer B](#)^{1,2}, [Ezehak P](#)^{1,3}, [Vuillemin P](#)^{1,2,4,5}, [Ponsonby AL](#)^{1,2}, [Collier E](#)^{4,5}, [Burgner D](#)^{1,2}, [Saffery B](#)^{1,2}, [Ryan J](#)^{1,2,6,7}; [EHS investigator team](#).

[Collaborators](#)

[Author information](#)

Abstract

Compelling evidence of a DNA methylation profile in the blood assessed by EWAS of DNAm signatures in children at age 5.5 years. This study investigated the biological role by examining the association of maternal anxiety during pregnancy with DNA methylation in children at age 5.5 years.

Epigenetic Biomarkers

- Environmentally inducible :
- Tissue- and cell-specific
- Factors that may affect the plasticity of human epigenome

Exogenous risk factors

- Lifestyle factors
 - Smoking
 - Alcohol consumption
 - Physical activity
 - Diet
- Environmental Pollutants

Endogenous factors

- Aging
- Oxidative stress
- Inflammation
- Metabolic disorders
- Hormone disorders

Behavior

Epigenetics and behavior (including emotions)



Happiness Genes: Unlock the Positive Potential Hidden in Your DNA by James D. Baird and Laurie Nadel, in which we are told, “**Happiness is at your fingertips, or rather sitting in your DNA, right now! The new science of epigenetics reveals there are reserves of natural happiness within your DNA** that can be controlled by you, by your emotions, beliefs and behavioral choices.”

Epigenetics and behavior

Epigenome-Wide Association Study of Aggressive Behavior

Jeremy van Dongen,^{1,2} Michel G. Nivard,¹ Bart M. L. Smiters,^{1,2} René S. Driks,² Lenneke Ughetti,¹ BOCS Consortium,¹ Bastiaan T. Heijmans,¹ Mieke Bartels,^{1,2} and Dorret I. Boomsma^{1,2}
¹Department of Biological Psychology, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands
²EMGO Institute for Health and Care Research, the University Medical Center, Amsterdam, the Netherlands
³The Erasmus Brain Imagerie Study (EBIS) Consortium is full list of authors is provided in the Supplementary material
⁴Department of Molecular Epigenetics, Radboud University Medical Center, Nijmegen, The Netherlands

Aggressive behavior is highly heritable, while environmental influences, particularly early in life, are also important. Epigenetic mechanisms, such as DNA methylation, regulate gene expression throughout development and adulthood, and may mediate genetic and environmental effects on complex traits. We performed an epigenome-wide association study (EWAS) to identify regions in the genome where DNA methylation levels are associated with aggressive behavior. Significant findings are described in Supplementary material.

Twin Research and Human Genetics, 2018, Mar 29;6:e765. doi: 10.1017/erg.2018.18

The effects of maternal anxiety during pregnancy on IGF2/H19 methylation in cord blood.

Manolis T^{1,2}, Novakovic B^{1,2}, Meyer B^{1,2}, Rzehak P^{1,2}, Willemin P^{1,2,4,5}, Porsiby AL^{1,2}, Collier F^{4,5}, Burdner D^{1,2}, Saffery R^{1,2}, Evans J^{1,2,6,7}, BIS Investigator team

Collaborators (11)

Author information

Abstract

Compelling evidence suggests that maternal mental health genes, insulin-like growth factor 2 (IGF2) and H19, are imprinted. This study aimed to determine the association between differentially methylated regions (DMRs) of IGF2 (DMR0) and H19 (DMR1) in cord blood of offspring. Maternal depression, anxiety and perceived stress were measured during pregnancy. The Australian Twin Infant Study (n=576). DNA methylation was measured in

Chapter 29

Epigenetic Regulation in Biopsychosocial Pathways

Kristin Litzelman and Mukesh Verma

Abstract

Theory and empirical evidence suggest that psychological stress and other adverse psychosocial experiences can contribute to cancer progression. Research has begun to explore the potential role of epigenetic changes in these pathways. In basic, animal and human models, exposure to stressors or to the products of the stress-induced stress response (e.g., cortisol) has been associated with epigenetic changes, such as DNA

Cancer Prevention Fellow

Cross-generational effects

Research Article

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CROSS-GENERATIONAL EFFECTS

Cross-generational effects of alcohol dependence in humans on *HRAS* and *TP53* methylation in offspring

Shirley Y Hill^{1,2}, Gre...

¹Department of Psychiatry, U...

²Center for Neuroscience, U...

³Departments of Anesthesi...

15213, USA

* Author for correspondence

Epigenomics



Toxicoepiggenomics and Cancer: Implications for Screening

Mukesh Verma

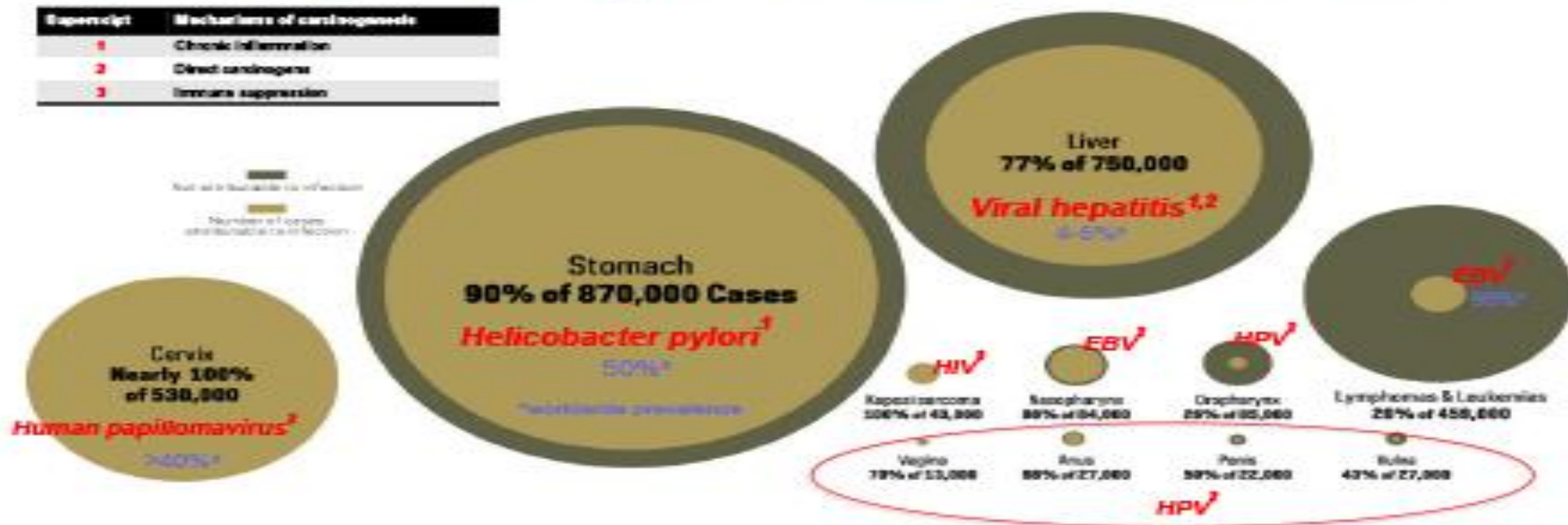
Abstract

Scientists have long considered genetics to be the key mechanism that alters gene expression because of exposure to the environment and toxic substances (toxicants). Recently, epigenetic mechanisms have emerged as an alternative explanation for alterations in gene expression resulting from such exposure. The fact that certain toxic substances that contribute to tumor development do not induce mutations probably results from underlying epigenetic mechanisms. The field of toxicoepiggenomics emerged from the combination of epigenetics and classical toxicology. High-throughput technologies now enable evaluation of altered epigenomic profiling in response to toxins and environmental pollutants. Furthermore, differences in the epigenomic backgrounds of individuals may explain why, although whole populations are exposed to toxicants, only a few people in a population develop cancer. Metals in the environment and toxic substances not only alter DNA methylation patterns and histone modifications but also affect enzymes involved in posttranslational modifications of proteins and epigenetic regulation, and thereby contribute to carcinogenesis. This article describes different toxic substances and environmental pollutants that alter

Infectious agents

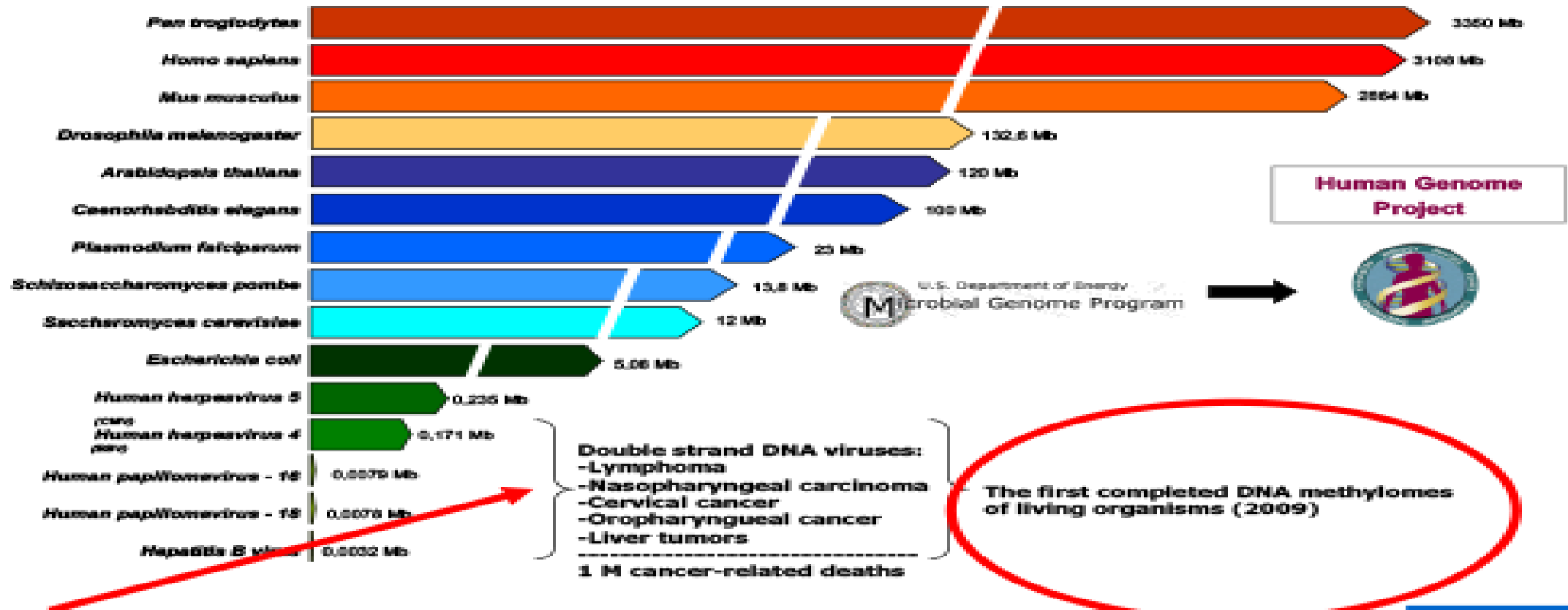
Infectious Agents: Etiologic Role in Cancer and Prevalence

Excerpt	Mechanism of carcinogenesis
1	Chronic inflammation
2	Direct carcinogens
3	Immune suppression



Genomes

Genomes



Oncogenic viruses and bacteria

Oncogenic viruses, bacteria and epigenetics

Viruses:	p16 in HPV16/18 (Cervical Cancer) RASSF1a in SV40 (Mesothelioma) HBV and HCV genes (Liver Cancer) LANA in EBV (Nasopharyngeal Carcinoma)
Bacteria:	COX2 in H.pylori Infected Cells (Gastric Cancer)

Int. J. Cancer: 113, 440–445 (2005)
© 2004 Wiley-Liss, Inc.

Frequent p16INK4a Promoter Hypermethylation in Human Papillomavirus-Infected Female Lung Cancer in Taiwan

Ming-Fang Wu^{1,2}, Ya-Wen Cheng^{2,3}, Ji-Ching Lai⁴, Min-Chih Hsu⁴, Jung-Ta Chen⁵, Wen-Shan Liu⁶, Ming-Chih Chiou^{2,3}, Chih-Yi Chen⁷ and Huei Lee^{3,4*}

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

²Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan

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

LANA

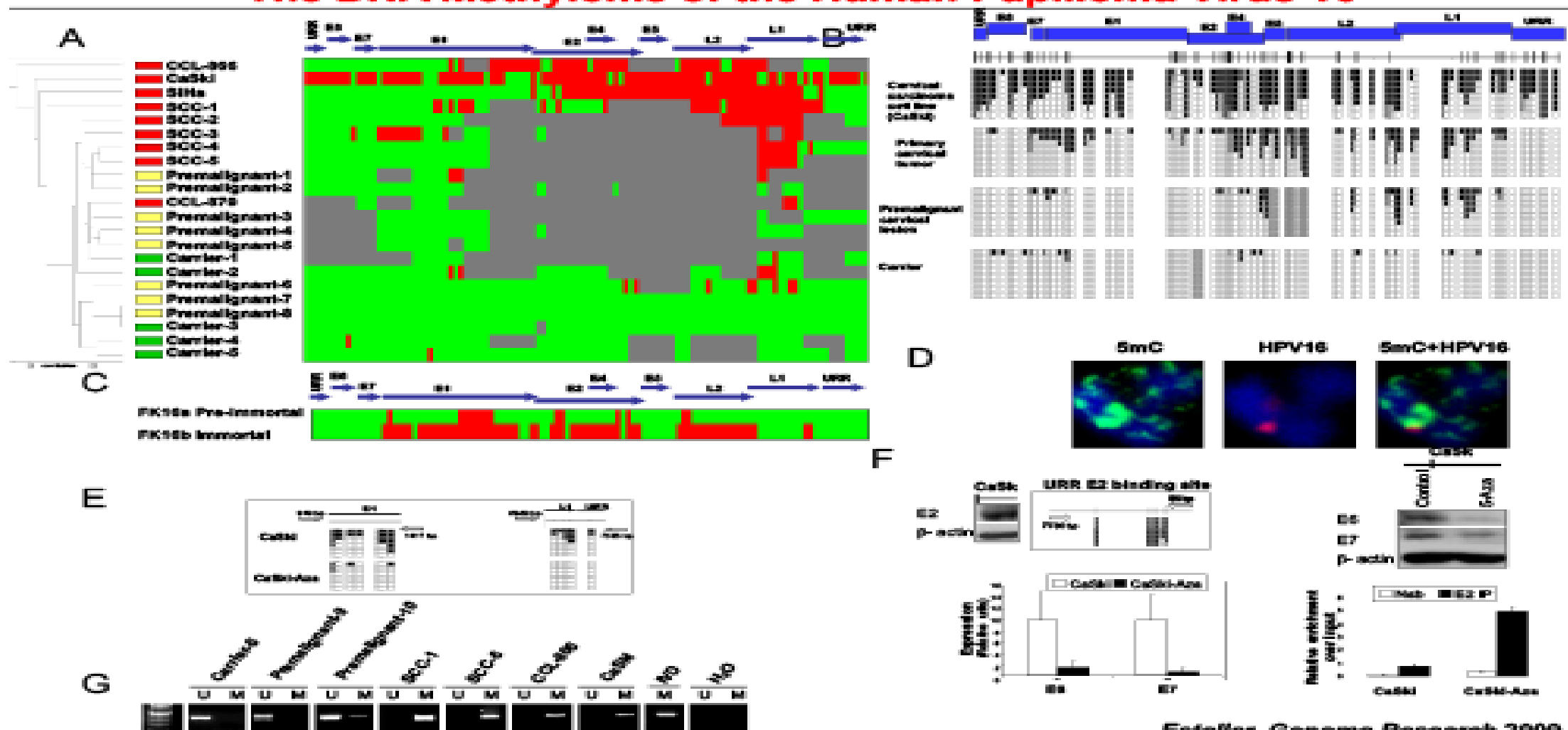
Complete methylome of HPV, EBV,
and HBV.

Esteller M. *Genome Research*. 2009. 19: 438

EBNA

DNA methylome

The DNA Methylome of the Human Papilloma Virus 16



Infection and cancer

Infection and Cancer: New and Emerging Associations

Infectious Agent	Cancer
Merkel cell polyomavirus (MCPV)	Merkel cell carcinoma
Plasmodium falciparum	Endemic Burkitt's lymphoma
Cytomegalovirus	Brain
Salmonella typhi	Gallbladder
Streptococcus bovis	Colorectal
Chlamydia pneumoniae	Lung
Others?	???

> [Nature](#). 2022 Oct;510(7931):381-388. doi: 10.1038/s41586-022-05282-z. Epub 2022 Oct 5.

SARS-CoV-2 disrupts host epigenetic regulation via histone mimicry

John Kee ^{1,2}, Samuel Thudium ^{1,2}, David M Renner ^{1,3,4}, Karl Glastad ^{1,2,5},
Katherine Palozola ^{1,2}, Zhen Zhang ^{2,5}, Yize Li ^{3,4}, Yemin Lan ², Joseph Casare ^{2,6}

FULL TEXT LINKS



ACTIONS

Risk Assessment

Understanding Cancer Etiology and Risk Assessment

Need healthy population (pathologically disease free) (cohort) with information about

- Exposure (Chemicals, Radiations, Infectious Agents, Toxic substance)
- Family History
- Diet and Life Style
- Medication

Need easily collected biospecimens (non-invasive technologies) and analytic tools

Need follow up (for longitudinal studies) for several years

Challenge: Expensive, data sharing

Advantage: Essential to identify risk factors for cancer

Special populations

10/14

Special Populations in EGRP

African-American men & women

South American women

Asian-American & Asian men & women

Latin-American/Hispanics

African men & women

Alaskan & Hawaiian Natives

Middle-Eastern populations

American-Indian, incl. Navajo

Rural South

Chinese

EGRP Studies Are Everywhere

- Senegal
- Malawi
- The Zambia
- China
- Japan
- Egypt
- Israel
- Brazil
- Colombia
- England
- Canada
- Sweden
- Denmark
- France
- Costa Rica
- Singapore
- Poland
- Australia
- U.S., including Alaska & Hawaii

2.3 Million Subjects
Cohorts, CGN and Family Registries

Cohort consortium

The Cohort Consortium (CoCo)



- 73 cohorts, over 4 million individuals
- **Membership:** cohort studies worldwide with >10,000 subjects, blood samples and questionnaire data on important cancer risk factors
- The Cohort Consortium was formed by NCI to address the need for large-scale collaborations for
 - Rapid identification and confirmation of **common polymorphisms** and **cancer susceptibility** (GWAS)
 - Studies of **GxG** and **GxE** interactions in the etiology of cancer.

Loss (or gain) of gene function in cancer



Dr. Shao

Loss (or Gain) of gene function in cancer

Most permanent

Most dynamic



Deletion
Amplification
Point mutations
Chromosomal
Translocation
(Ig rearrangement)

Genetic

Chromatin
Changes
Promoter
Methylation
Silencing

Epigenetic

Transcription
Factor
Changes

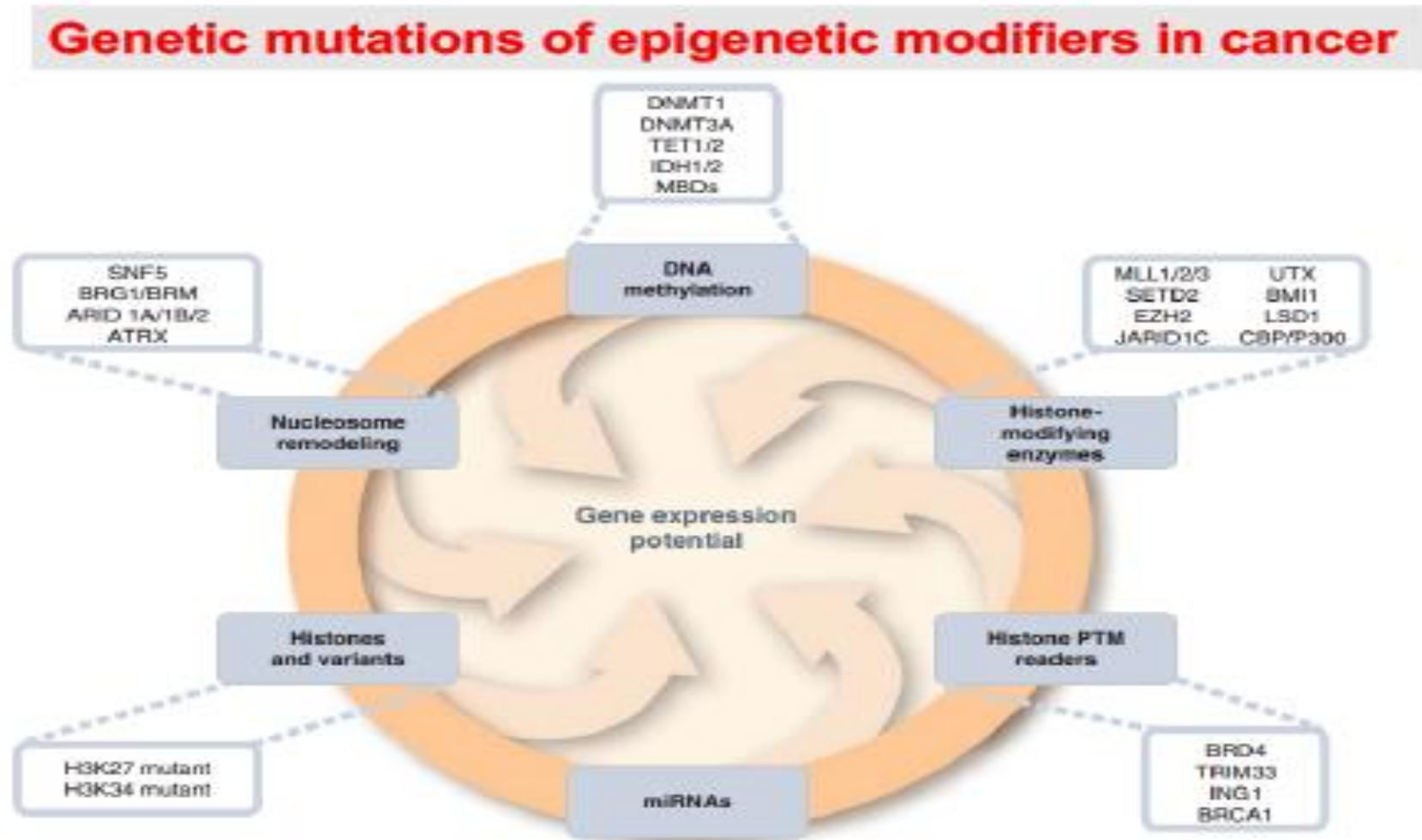
Cell-cycle
Regulated
Changes

Leiman Chew 125:1199 1225



James Leiman

Genetic mutations



Hypomethylation

PubMed
25 National Center for Biotechnology Information

PubMed
Advanced

Format: Abstract
Send to

2014-10-20 20:15:00 (GMT+08:00)

LINE-1 methylation status in prostate cancer and non-neoplastic tissue adjacent to tumor in association with mortality.

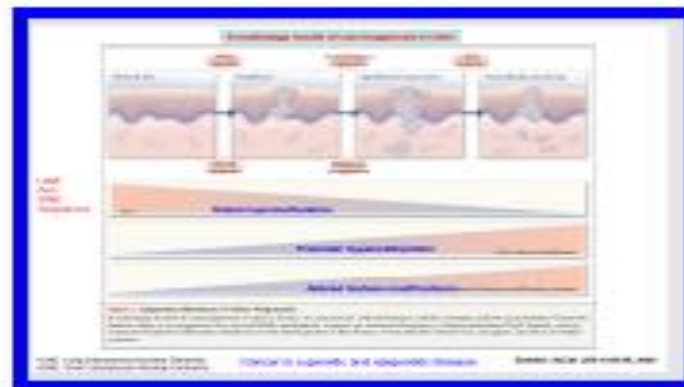
Shankar A, Zou J, Gao J, Devisser L, Srinivasan S, Srinivasan S, Gao J, Shrestha R, Shrestha R.

Author information

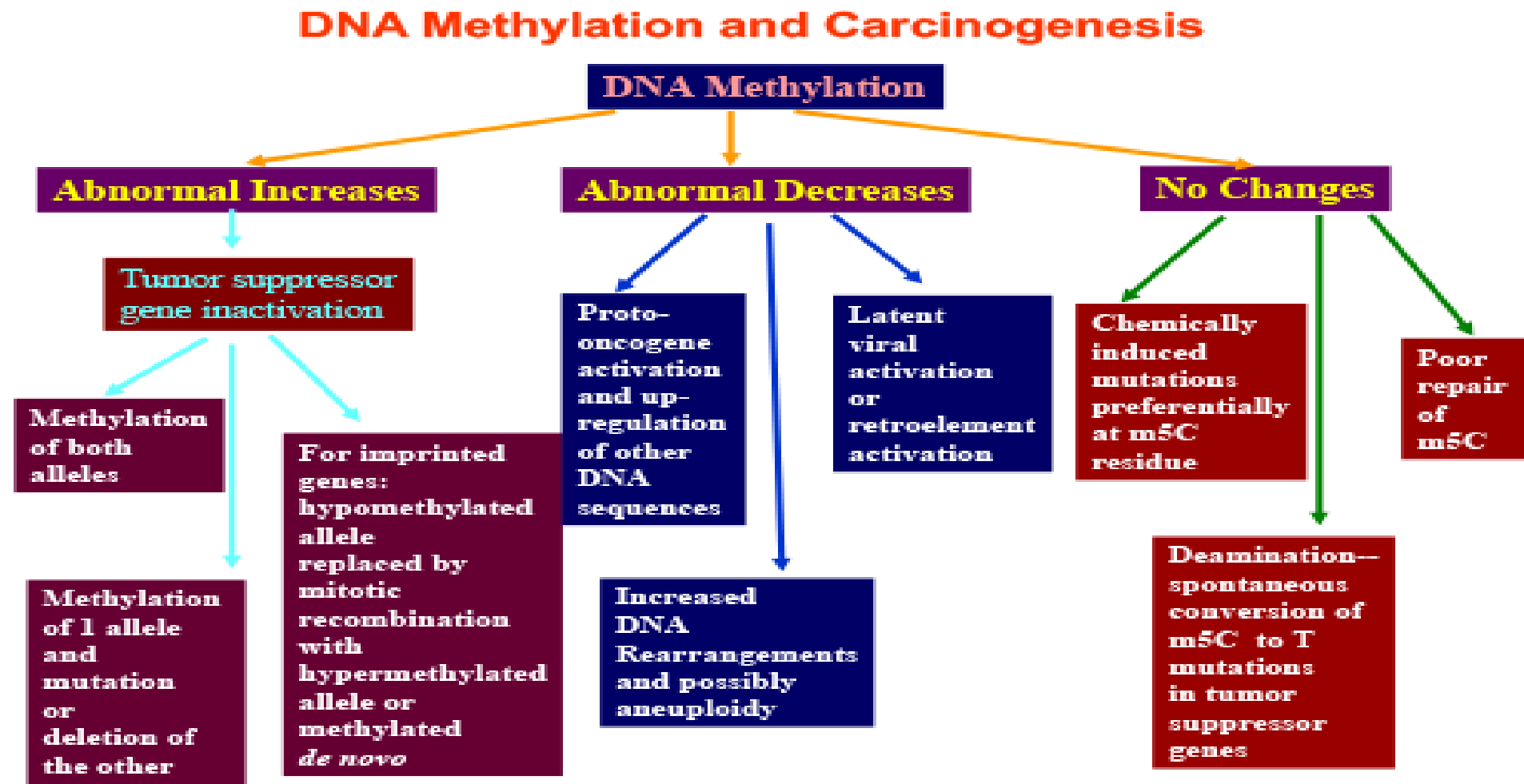
ABSTRACT

Aberrant DNA methylation seems to be associated with prostate cancer behavior. We investigated LINE-1 methylation in prostate cancer and non-neoplastic tissue adjacent to tumor (NTAT) in association with mortality from prostate cancer. We selected 157 prostate cancer patients with available NTAT from two cohorts of patients diagnosed between 1982-1990 and 1993-1996, followed up until 2010. An association between LINE-1 hypomethylation and prostate cancer mortality in tumor was suggested (hazard ratio per 5% decrease in LINE-1 methylation levels: 1.20, 95% confidence interval [CI]: 0.96-3.31). After stratification of the patients for Gleason score, the association was present only for those with a Gleason score of at least 8. Among these, low (<70%) vs. high (>80%) LINE-1 methylation was associated with a hazard ratio of 4.50 (95% CI: 1.03-21.34). LINE-1 methylation in the NTAT was not associated with prostate cancer mortality. Our results are consistent with the hypothesis that tumor tissue global hypomethylation may be a late event in prostate carcinogenesis and is associated with tumor progression.

Tumor tissue global hypomethylation may be a late event in prostate carcinogenesis and is associated with tumor progression.

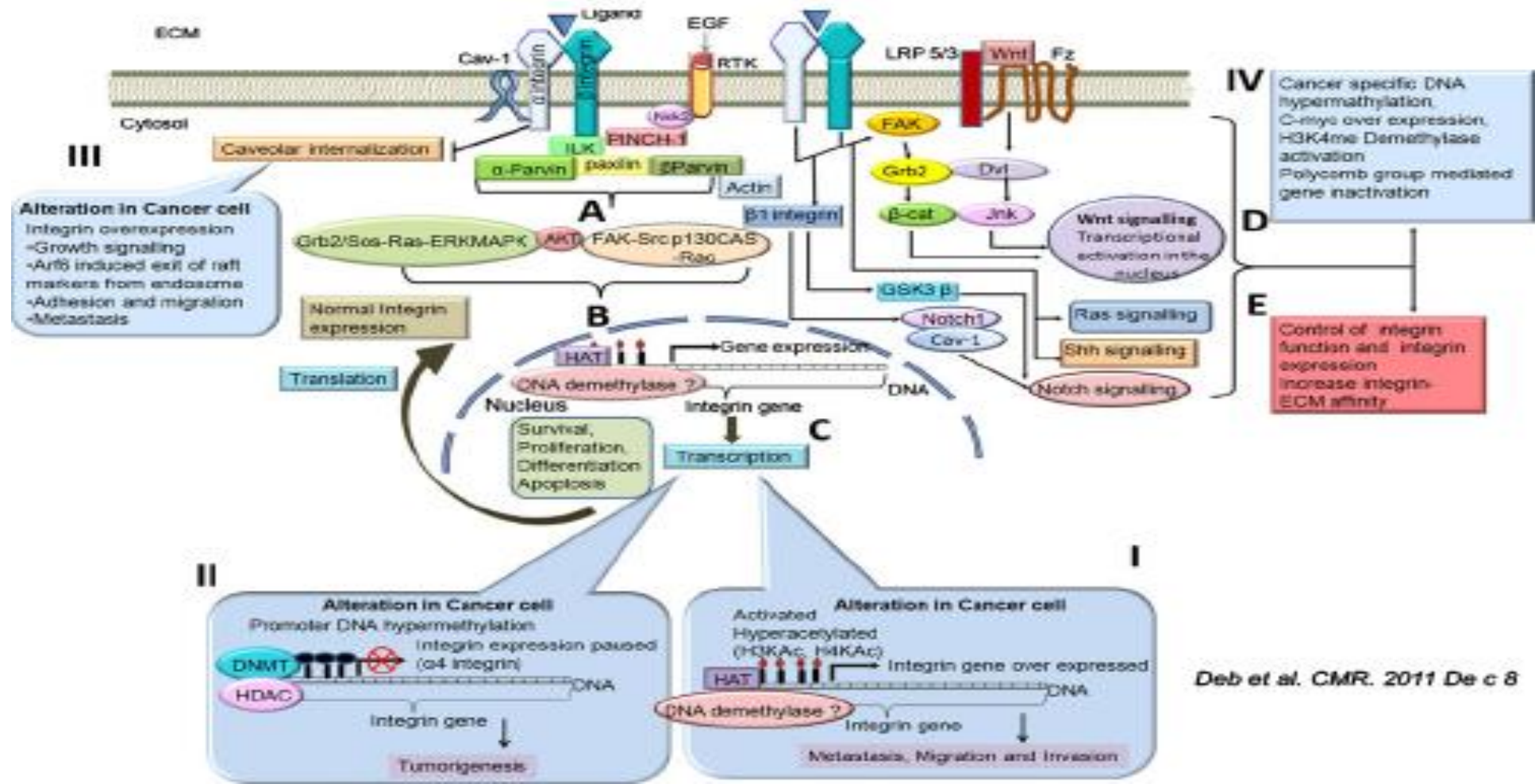


DNA methylation and carcinogenesis



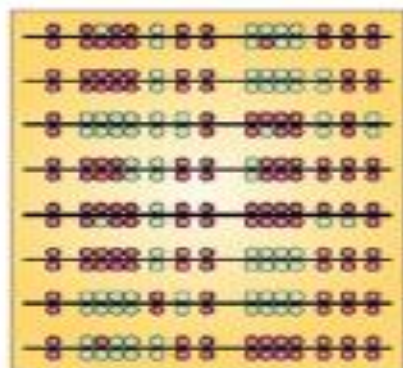
Integrin signaling

Integrin Signaling Network and Epigenetic Regulation

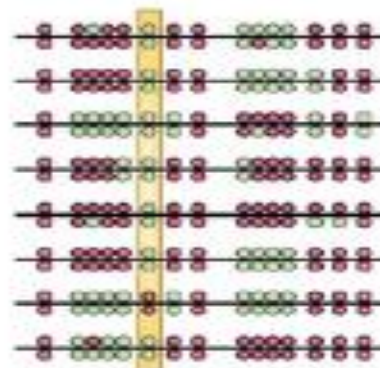


Methylation

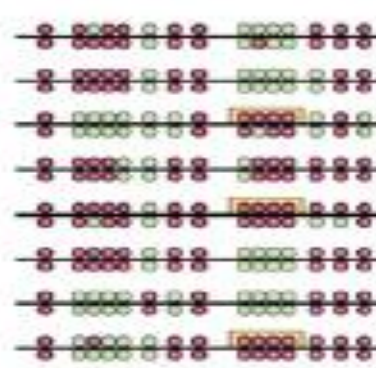
a Methylation content



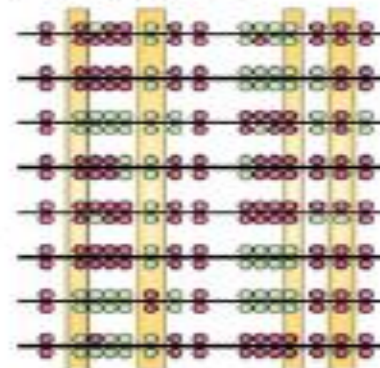
b Methylation level



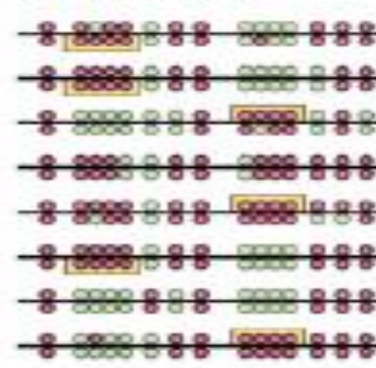
c Methylation pattern



d Level profile



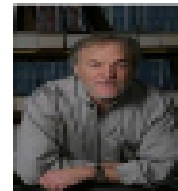
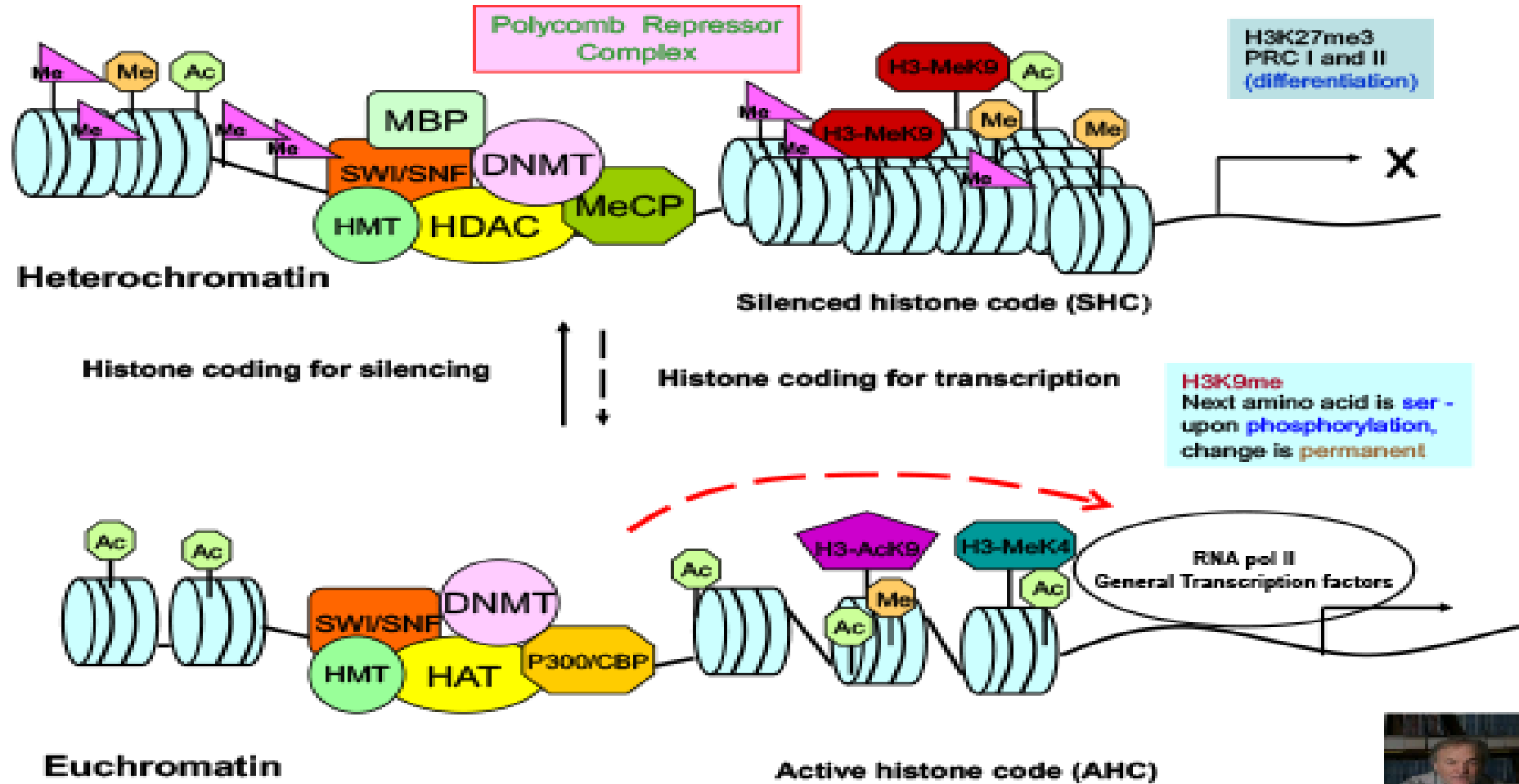
e Pattern profile



- 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

Histone acetylation



Micro RNA signatures

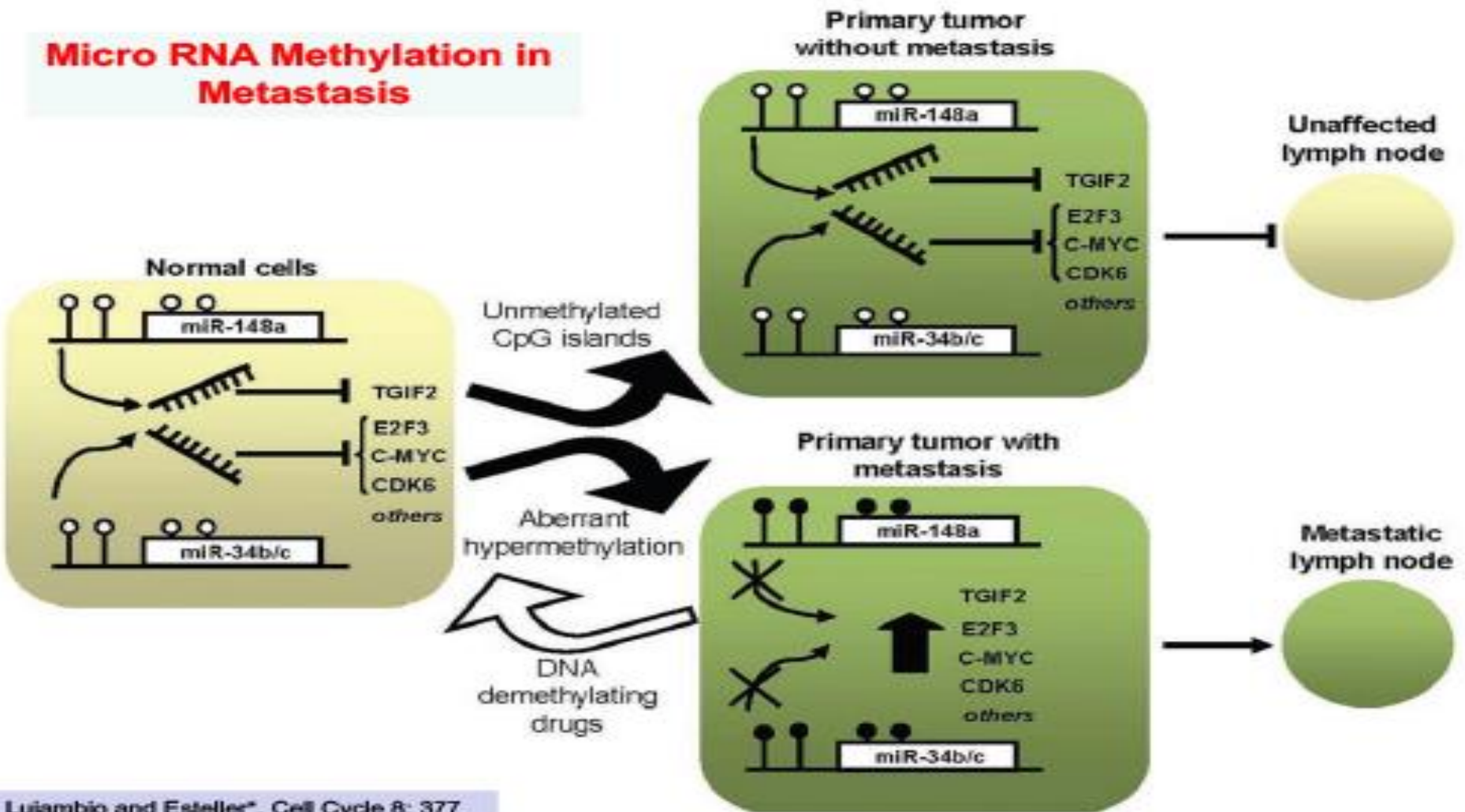
Mirco RNA Signatures in Human Cancers



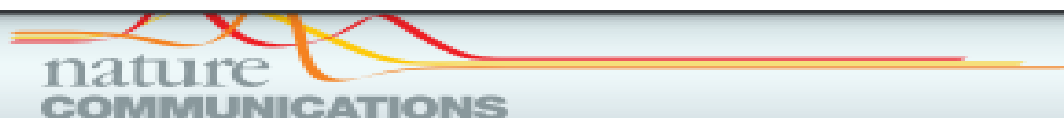
Micro RNA Polymorphism to Identify High Risk Populations

Micro RNA methylation

Micro RNA Methylation in Metastasis



Methylation of microRNAs





ARTICLE

<https://doi.org/10.1038/s41467-019-11826-1>

OPEN

Distinct methylation levels of mature microRNAs in gastrointestinal cancers

Masamitsu Konno ^{1,10}, Jun Koseki^{2,10}, Ayumu Asai^{1,2,10}, Akira Yamagata^{3,10}, Teppei Shimamura⁴, Daisuke Motooka⁵, Daisuke Okuzaki ⁵, Koichi Kawamoto⁶, Tsunekazu Mizushima⁶, Hidetoshi Eguchi⁶, Shuji Takiguchi^{6,7}, Taroh Satoh¹, Koshi Mimori⁸, Takahiro Ochiya⁹, Yuichiro Doki⁶, Ken Ofusa³, Masaki Mori⁶ & Hideshi Ishii²

The biological significance of micro (mi)RNAs has traditionally been evaluated according to their RNA expression levels based on the assumption that miRNAs recognize and regulate their targets in an unwavering fashion. Here we show that a fraction of mature miRNAs including miR-17-5p, -21-5p, and -200c-3p and let-7a-5p harbor methyl marks that potentially alter their stability and target recognition. Importantly, methylation of these miRNAs was significantly increased in cancer tissues as compared to paired normal tissues. Furthermore, miR-17-5p methylation level in serum samples distinguished early pancreatic cancer patients from healthy controls with extremely high sensitivity and specificity. These findings provide a

Extracellular vesicles

Verma et al. *BMC Clinical Pathology* (2015) 15:6
DOI 10.1186/s12907-015-0005-5

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BMC
Clinical Pathology

REVIEW

Open Access

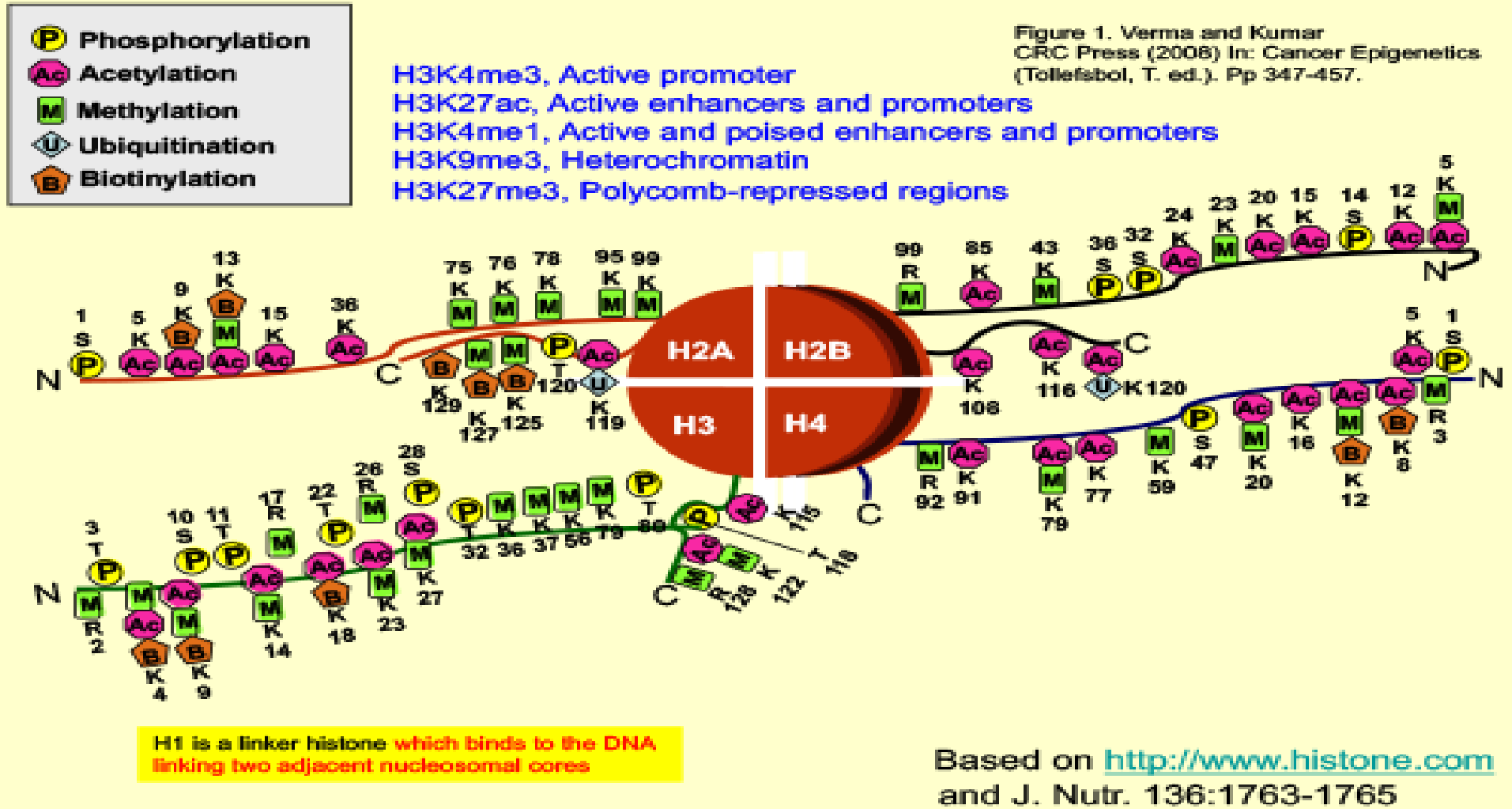
Extracellular vesicles: potential applications in cancer diagnosis, prognosis, and epidemiology

Mukesh Verma^{1*}, Tram Kim Lam, Elizabeth Hebert and Rao L Divi

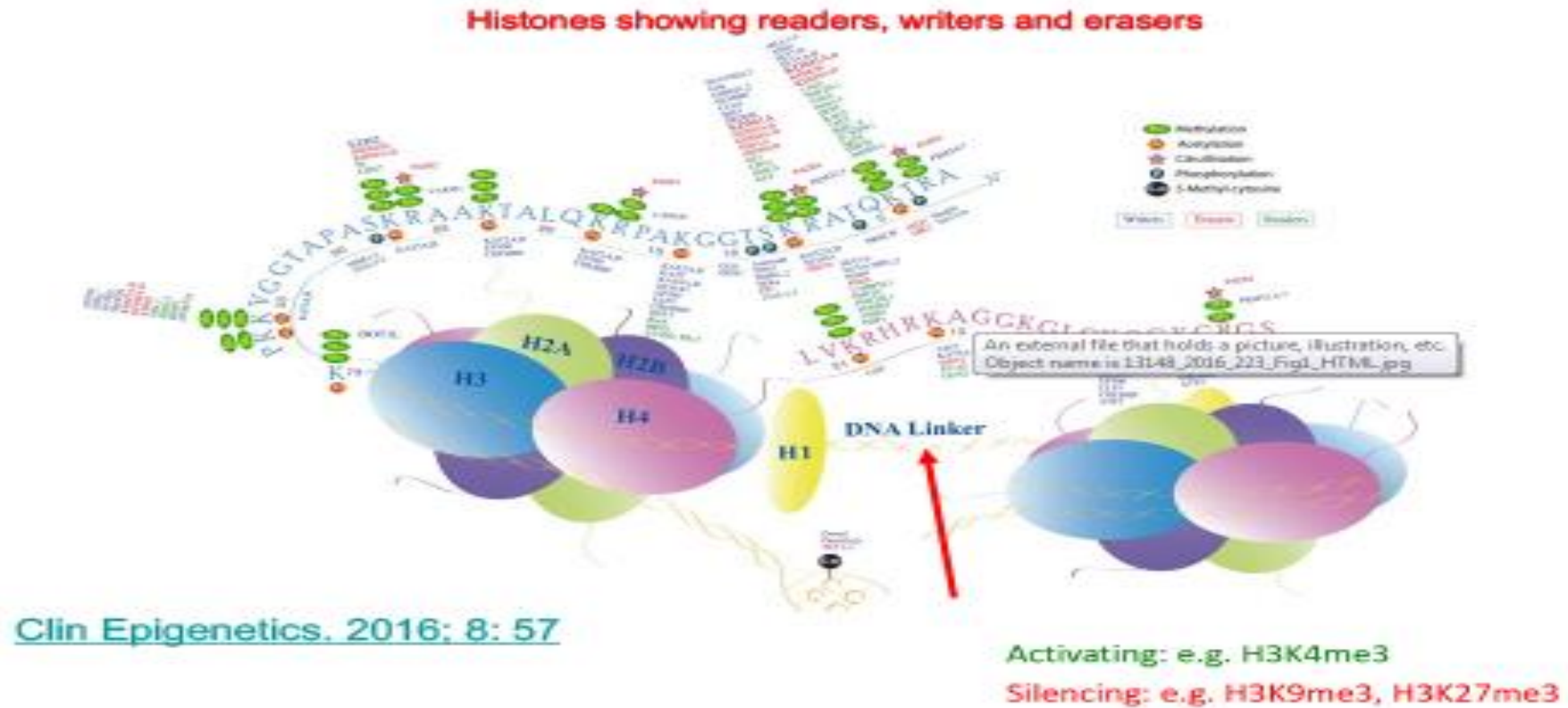
Abstract

Both normal and diseased cells continuously shed extracellular vesicles (EVs) into extracellular space, and the EVs carry molecular signatures and effectors of both health and disease. EVs reflect dynamic changes that are occurring in cells and tissue microenvironment in health and at a different stage of a disease. EVs are capable of altering the function of the recipient cells. Trafficking and reciprocal exchange of molecular information by EVs among different organs and cell types have been shown to contribute to horizontal cellular transformation, cellular reprogramming, functional alterations, and metastasis. EV contents may include tumor suppressors, phosphoproteins, proteases,

Histone modifications



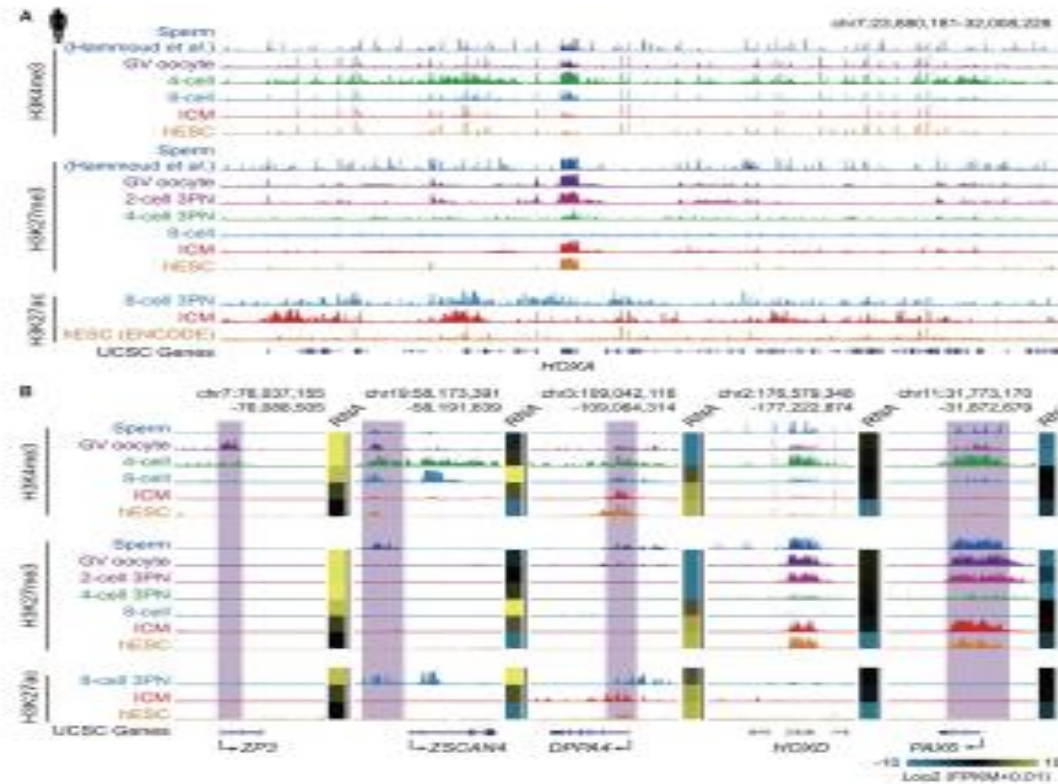
Histones



Histone modifications

Fig. 1 Mapping histone modifications in human gametes and preimplantation embryos.

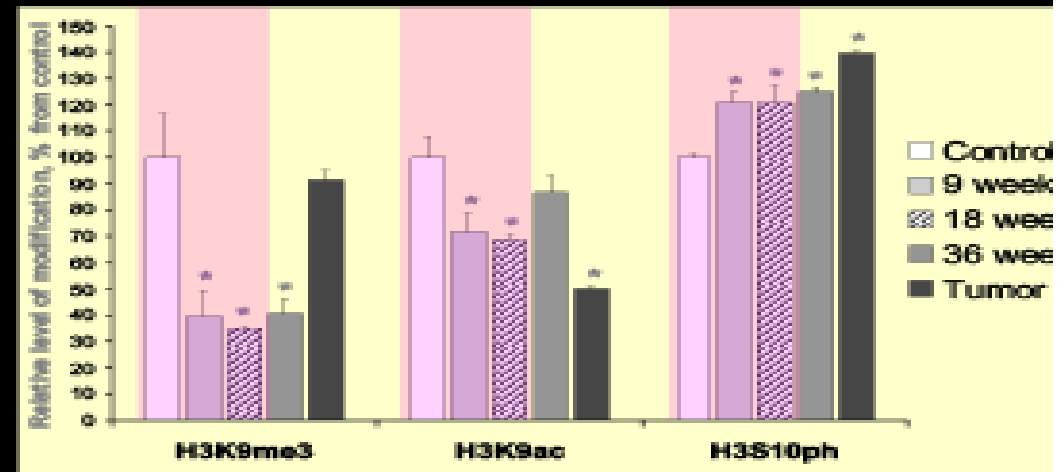
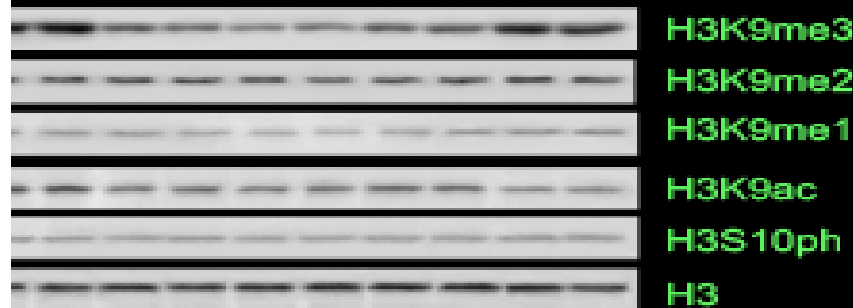
Histone mapping



Wei-kun Xia et al. *Science* 2019;365:353-360

Histone H3 modifications

ALTERATIONS OF HISTONE H3 MODIFICATIONS IN LIVER DURING METHYL DEFICIENCY



Interplay between H3K9me3, H3K9Ac, and H3S10ph

Epigenetic regulation

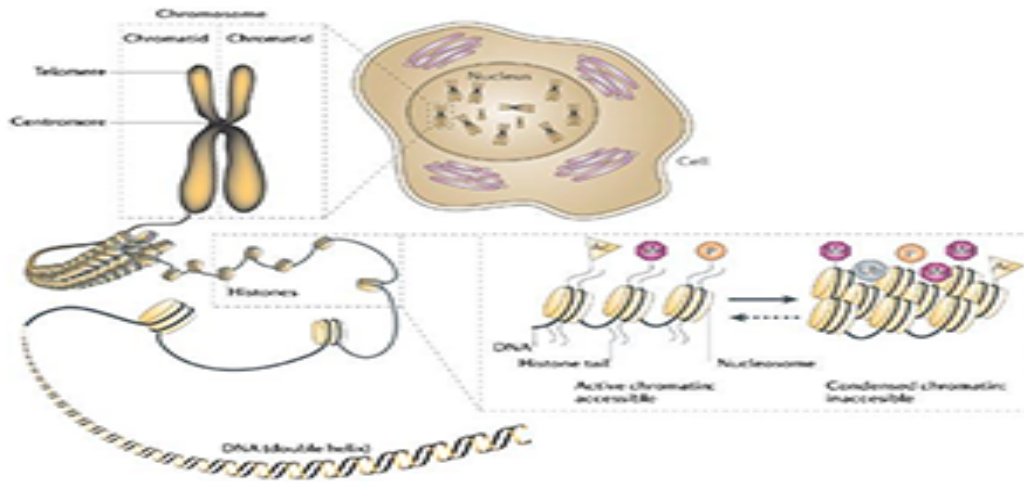
Epigenetic Gene Regulation:

Modification		Methylation			Acetylation
		Mono-methylation	Di-methylation	Tri-methylation	
DNA		Repression	--	--	--
Histone	H3K4	Activation	Activation	Activation	--
	H3K9	Activation	Repression	Repression	Activation
	H3K27	Activation	Repression	Repression	--
	H3K36	--	Repair	Activation	Activation
	H3K79	Activation	Activation	Activation Repression	--
	H3R17	--	Activation	--	--
	H4K5	--	--	--	Activation
	H4K8	--	--	--	Activation
	H4K12	--	--	--	Activation
	H4K16	--	--	--	Activation
	H4K20	Activation	Activation	Repression	--
	H4K16	--	--	--	Activation



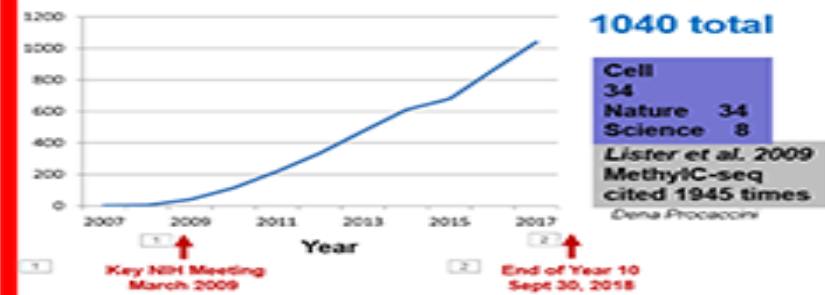
Epigenetics roadmap

Epigenetics Roadmap



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Nature Reviews | Cancer

Cumulative Roadmap Epigenomics Program
Publications as of August 4, 2017



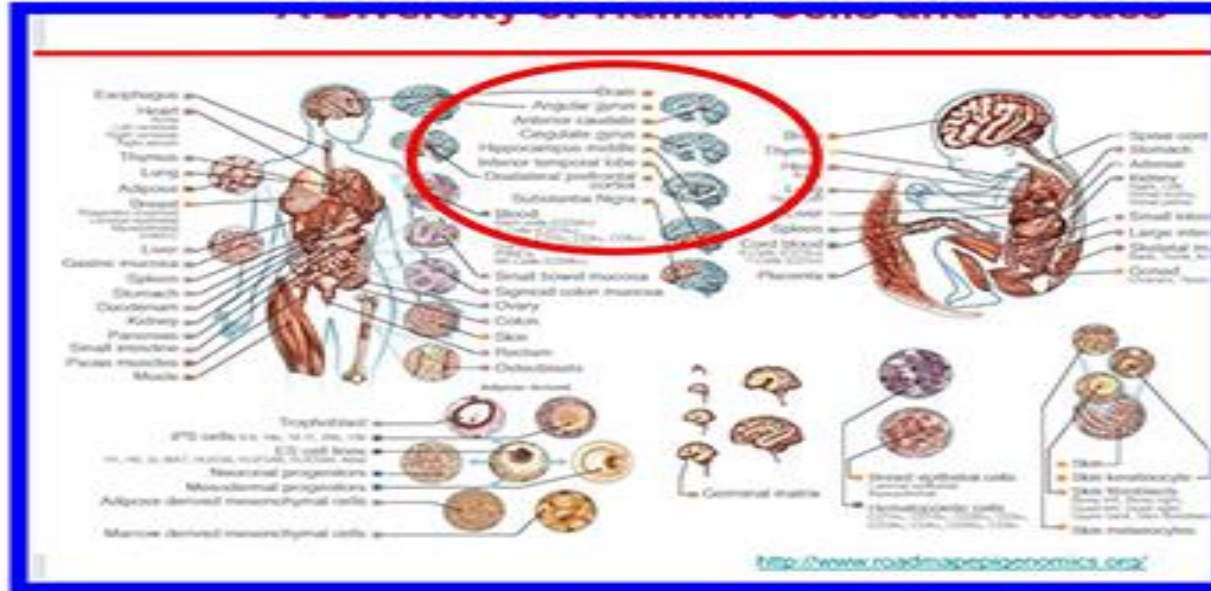
Epigenetically Regulated Diseases:

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

The NIH Roadmap Epigenomics Mapping Consortium was launched with the goal of producing a public resource of human epigenomic data to catalyze basic biology and disease-oriented research.

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

Epigenetics roadmap



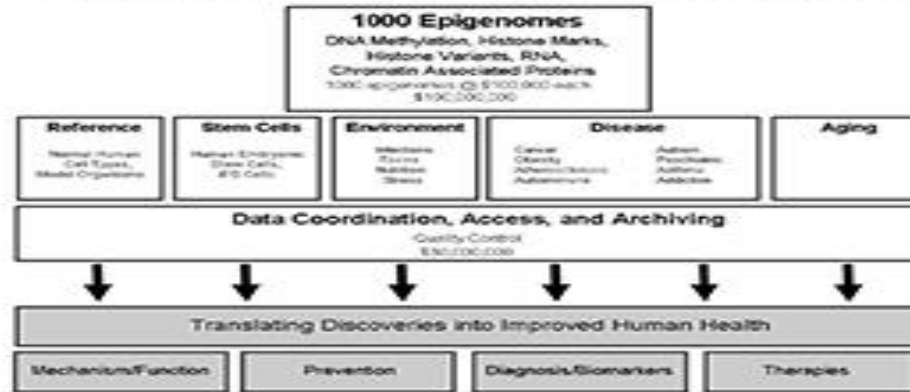
Epigenomics Program Budget
(M funds in millions)

Component	FY08	FY09	FY10	FY11	FY12	FY13	FY14	FY15	Total
RFA 1: Mapping Centers	10	10	10	10	10				50
RFA 2: RM/IC Projects		4	8	12	16	20	16	12	88
RFA 3: Data Analysis/Coord	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	12
RFA 4: Tech Development	3.5	3.5	7	7	7	7	7		42
RFA 5: Discovery of Novel Marks	3.5	3.5	4	2	2				15
NCBI: Public access	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	12
8-Year Total \$219 M (plus \$3M JUMPSTART)									

□ = RFA released

Epigenome consortium

INTERNATIONAL HUMAN EPIGENOME CONSORTIUM



<http://ihec-epigenomes.org/>

Participants in IHEC Meeting: Paris, Jan. 25-26, 2010

Australia
Austria
Canada
China
Euro. Comm. (EU)
France
Germany
Israel
Italy
Japan
Korea
Netherlands
Norway
Poland
Singapore
Spain
Sweden
Switzerland
UK
USA

Funding Agencies

Consiglio Nazionale delle Ricerche, Italy
European Science Foundation
Genome British Columbia, Canada
German Research Foundation, Germany
National Natural Science Foundation, China
Netherlands Genomic Initiative, Netherlands
NIH, USA
Wellcome Trust, UK

Industrial Participants

Affymetrix
Genoscope
Novartis

Other

AACR

Publishers

Nature
Science

Histone modifications

20 Diagnosing Cancer Using Histone Modification Analysis

Mukesh Verma and Deepak Kumar

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> Nature. 2022 Oct;610(7931):381-388. doi: 10.1038/s41586-022-05282-z. Epub 2022 Oct 5.

SARS-CoV-2 disrupts host epigenetic regulation via histone mimicry

John Kee^{1,2}, Samuel Tivudium^{*1,2}, David M Renner^{*3,4}, Karl Glastad^{*2,5},
Katherine Palozola^{1,2}, Zhen Zhang^{2,5}, Yize Li^{3,4}, Yemin Lan², Joseph Cesare^{2,6},

FULL TEXT LINKS



ACTIONS

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20.3.3 Cervical Cancer	350
20.3.4 Colon Cancer	350

ISBN 9781420045796 - CAT# 45792

Single cell epigenomics

SINGLE CELL EPIGENOMICS

Single cells isolated from

- Blood
- Breast milk
- Exfoliated cells
- Hair
- Oral swab
- Pancreatic fluid
- Saliva
- Skin
- Tissue
- Urine

1. Methylation profiling
2. Histone modifications
3. miRNA profiling
4. Chromatin Accessibility

Single Cell
Epigenomics

Identify open and closed chromatin

Identify cell-specific transcription factors

Determine nucleosome position

Identify active and repressive
transcription state

Implications of single cell epigenomics

Risk Assessment to identify high-risk individuals

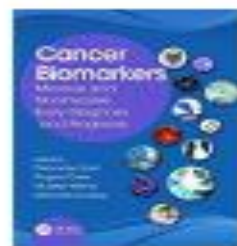
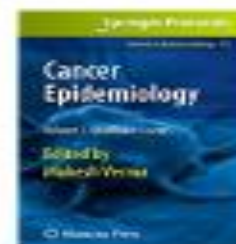
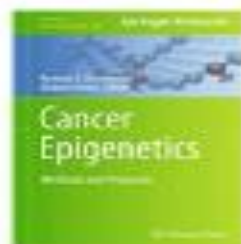
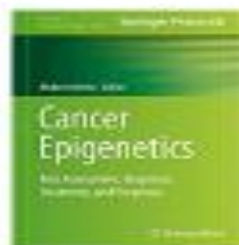
Diagnosis

Prognosis

Screening

Follow up treatment and co-morbidity

Books



Books edited by Mukesh Verma

Epigenetic changes

The image shows a screenshot of a web browser displaying a Nature article. The browser's address bar shows the URL: <http://www.nature.com/news/epigenetics-reversing-the-cancer-code-1.11411>. The article title is "Epigenetics: unravelling the cancer code".

The article text includes the following paragraphs:

DNA with increased methylation and hypothesized that if a tumour suppressor gene was hypermethylated, its activity would decrease or stop entirely — just as if it were a genetic mutation — allowing the tumour to flourish. In other words, Baylín reasoned, this epigenetic change would produce the same result as a genetic mutation.

Firm evidence came in 1994. Baylín and his colleague, oncologist James Herman, were investigating renal cell carcinoma (RCC), the most common type of kidney cancer in adults. Around 60% of RCCs are caused by an inherited mutation in the von-Hippel Lindau tumour-suppressor gene (VHL), which hobbles the gene's ability to express the tumour suppressing protein. Baylín and Herman showed that 20% of the remaining, non-inherited form of RCC did not have a mutation in VHL. Their genes were silenced not by a mutation, but rather by hypermethylation.

The following year, in collaboration with Sidransky's lab at Johns Hopkins, Baylín and his colleagues showed that human cancers commonly arise when a particular tumour suppressor gene, known as p16, is inactivated. Moreover, in many cancers (including RCC), epigenetic and genetic mutations often work together: one copy of a tumour suppressor gene is inactivated by genetic mutation, while the other copy is silenced by hypermethylation. This finding "convinced us that epigenetic abnormalities could play an important driving role in cancer, and many others have been pursuing this possibility ever since," says Baylín.

The move from a purely genetic to an epigenetic model is crucial for prevention strategies. As numerous gene therapy trials have shown, it is very difficult to treat a genetic disease by re-activating the dormant, mutated gene or by replacing it with a non-mutated one. "Epigenetic changes are reversible, and therefore have an edge over genetics," says Mukesh Verma, an epigeneticist at the National Cancer Institute's division of cancer control and population sciences in Bethesda, Maryland. Furthermore, epigenetic changes in cancer occur before genetic mutations. "If you can prevent methylation of those tumour suppressor genes, you might have a valuable prevention strategy," says Baylín.

The environmental link

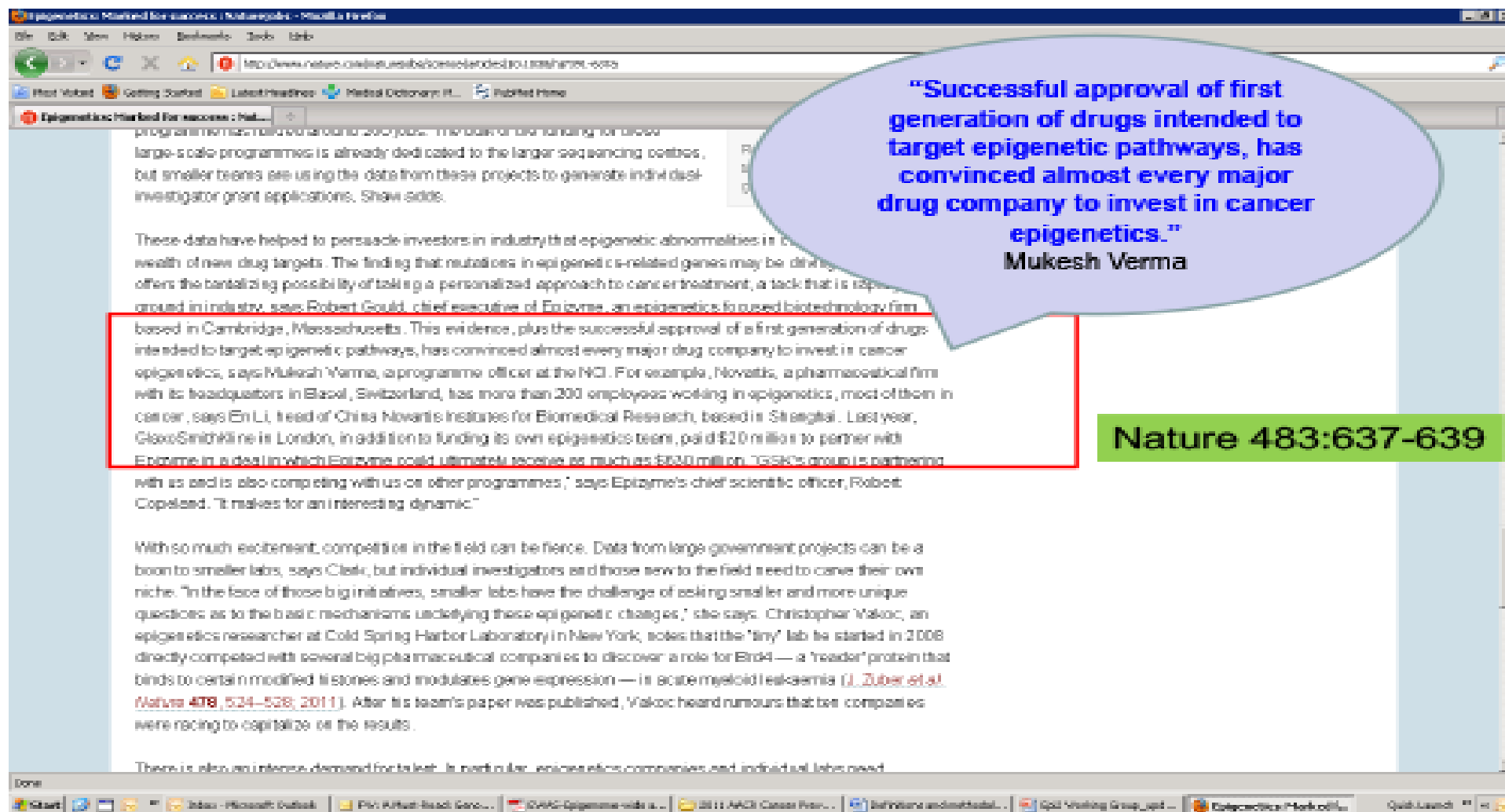
Epigenetics has also provided clues that link environmental factors with cancerous genetic changes. Changes in methylation can be detected in the blood of cancer-free individuals who smoke and eat high-fat diets, and these

Annotations:

- A yellow box highlights the text: "Nature 471: s12-s13".
- A blue speech bubble contains the text: "Epigenetic changes are reversible, and therefore have an edge over genetics" Mukesh Verma Nature 471: s12-s13".
- A red box highlights the text: "Epigenetic changes are reversible, and therefore have an edge over genetics," says Mukesh Verma, an epigeneticist at the National Cancer Institute's division of cancer control and population sciences in Bethesda, Maryland. Furthermore, epigenetic changes in cancer occur before genetic mutations.

The browser's taskbar at the bottom shows several open windows, including "Start", "Internet Explorer", "DNA - Epigenetics", "2011 NCI Cancer R...", "Definitions and...", "Bioscience - Process...", "Epigenetics: unrav...", and "Quick Launch".

Epigenetic drugs



The image shows a screenshot of a web browser displaying an article about epigenetic drugs. A callout box contains a quote from Mukesh Verma, and a red box highlights a specific paragraph of text. A green box at the bottom right contains a reference to Nature 483:637-639.

**“Successful approval of first generation of drugs intended to target epigenetic pathways, has convinced almost every major drug company to invest in cancer epigenetics.”
Mukesh Verma**

Epigenetics: Marked for success: Nature 483:637-639

programmers involved in large-scale sequencing projects. The role of bioinformatics in large-scale programmes is already dedicated to the larger sequencing centres, but smaller teams see using the data from these projects to generate individual investigator grant applications. Show slide.

These data have helped to persuade investors in industry that epigenetic abnormalities in cancer offers the tantalizing possibility of taking a personalized approach to cancer treatment, a tack that is top priority in industry, says Robert Gould, chief executive of Epizyme, an epigenetics-focused biotechnology firm based in Cambridge, Massachusetts. This evidence, plus the successful approval of a first generation of drugs intended to target epigenetic pathways, has convinced almost every major drug company to invest in cancer epigenetics, says Mukesh Verma, a programme officer at the NCI. For example, Novartis, a pharmaceutical firm with its headquarters in Basel, Switzerland, has more than 200 employees working in epigenetics, most of them in cancer, says Eli Li, head of Chiesi Novartis Institute for Biomedical Research, based in Singapore. Last year, GlaxoSmithKline in London, in addition to funding its own epigenetics team, paid \$20 million to partner with Epizyme in a deal in which Epizyme could ultimately receive as much as \$630 million. Glaxo's group is partnering with us and is also competing with us on other programmes," says Epizyme's chief scientific officer, Robert Copeland. "It makes for an interesting dynamic."

With so much excitement, competition in the field can be fierce. Data from large government projects can be a boon to smaller labs, says Clark, but individual investigators and those new to the field need to carve their own niche. "In the face of those big initiatives, smaller labs have the challenge of asking smaller and more unique questions as to the basic mechanisms underlying these epigenetic changes," she says. Christopher Yakov, an epigenetics researcher at Cold Spring Harbor Laboratory in New York, notes that the "tiny" lab he started in 2008 directly competed with several big pharmaceutical companies to discover a role for Bdnf—a "reader" protein that binds to certain modified histones and modulates gene expression—in acute myeloid leukaemia ([J. Zuber et al. Nature 478, 524–528, 2011](#)). After his team's paper was published, Yakov heard rumours that big companies were racing to capitalize on the results.

There is also an intense demand for talent in particular epigenetics companies and individual labs need

Nature 483:637-639

Tumors and epigenetics

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} , CREP1, MGMT, hMLH1, DAPK, TIMP-3, APC
Endometrium	hMLH1
Esophagus	p16, p14 ^{ARF} , GSTP1, CDH1/APC
Head and Neck	p16, MGMT, DAPK
Kidney	p16, p14 ^{ARF} , MGMT, GSTP1, TIMP-3, APC
Leukemia	p15, MGMT, DAPK1, CDH1, p73
Liver	p16, CREP1, GSTP1, APC
Lymphoma	p16, p15, CREP1, MGMT, DAPK, p73
Lung	p16, p14 ^{ARF} , CREP1, MGMT, GSTP1, DAPK, FHIT, TIMP-3, RARB α , 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

SMUNs are a group of proteins with histone deacetylase inhibiting and apoptosis inhibitor properties

Venkov and Simeonov (2002) *Leuk. Oncol.* 3: 755-763
 Venkov et al (2004) *Crit. Rev. Clin. Sc.* 41: 555-607
 Venkov and Mladenov (2006) *Crit. Rev. Hematol. Oncol.* 60:9-18
 Venkov et al (2006) *Mol. Diag. Therapy* 10:1-15

Histone enzymes

Category	Gene		Category	Gene			
HDACs	HDAC1		DNMTs	DNMT1			
	HDAC2			DNMT3A			
	HDAC8			DNMT3B			
Sirtuins	SIRT1		(2OG)-Fe(II)-dependent oxygenases	TET1			
	SIRT2			TET2			
	SIRT3			MBD1			
	SIRT7			MBD2			
HMTs	KDM1A		Methyl-CpG binding proteins	MBD3			
	KDM2B			MBD4			
	KDM4C			MECP2			
	KDM5A						
	KDM5B						
	KDM5C						
	KDM5A						
	KDM5B						
HATs	CREBBP		Histone variants	H2AFZ			
	EP300			ARID1A			
	MYST3			CHD5			
	MYST4			CHD7			
	HMTs			KAT5		Chromatin remodeling factors	MTA1
				MLL			MTA2
				EZH2			MTA3
				NSD1			SMARCA2
				PRDM9			SMARCA4
	HMTs			SMYD3		Histone modification readers	SNF5
WHSC1							

Sirtuins are a group of proteins with histone deacetylase inhibiting and anti apoptosis inhibition properties

Verma and Srivastava (2002). *Lancet Oncol.* 3: 355-363;
 Verma et al (2004). *Crit. Rev. Clin. Sc.* 41: 585-607;
 Verma and Menne (2005). *Crit. Rev. Hematol. Oncol.* 60: 9-18;
 Verma et al (2005). *Mol. Diag. Therapy.* 10: 1-15.

Epigenetic drugs

Target	Drug	Clinical Trial	
DNA Methylation	5-Azacytidine	Phase I/II/III	
	5-Aza-2'deoxyctidine	Phase I/II/III	
	FCD R		
	Zebularine		
	Procainamide		
	EGCG	Phase I	
Histone deacetylase	Psamaplin A		
	Antisense Oligomers	Phase I	
	Phenylbutyric acid	Phase I/II	Vannasa, Phil (G) et al
	SAHA (Suberoylanilide hydroxamic acid) or Vorinostat	Phase I/II	
	Depsipeptide	Phase I/II	
	Valproic Acid	Phase I/II	Jean-Pierre Issa Temple Univ.

Adverse Experiences
SAHA
Cuneo et al (2007).
Ann Oncol 18:109-111.

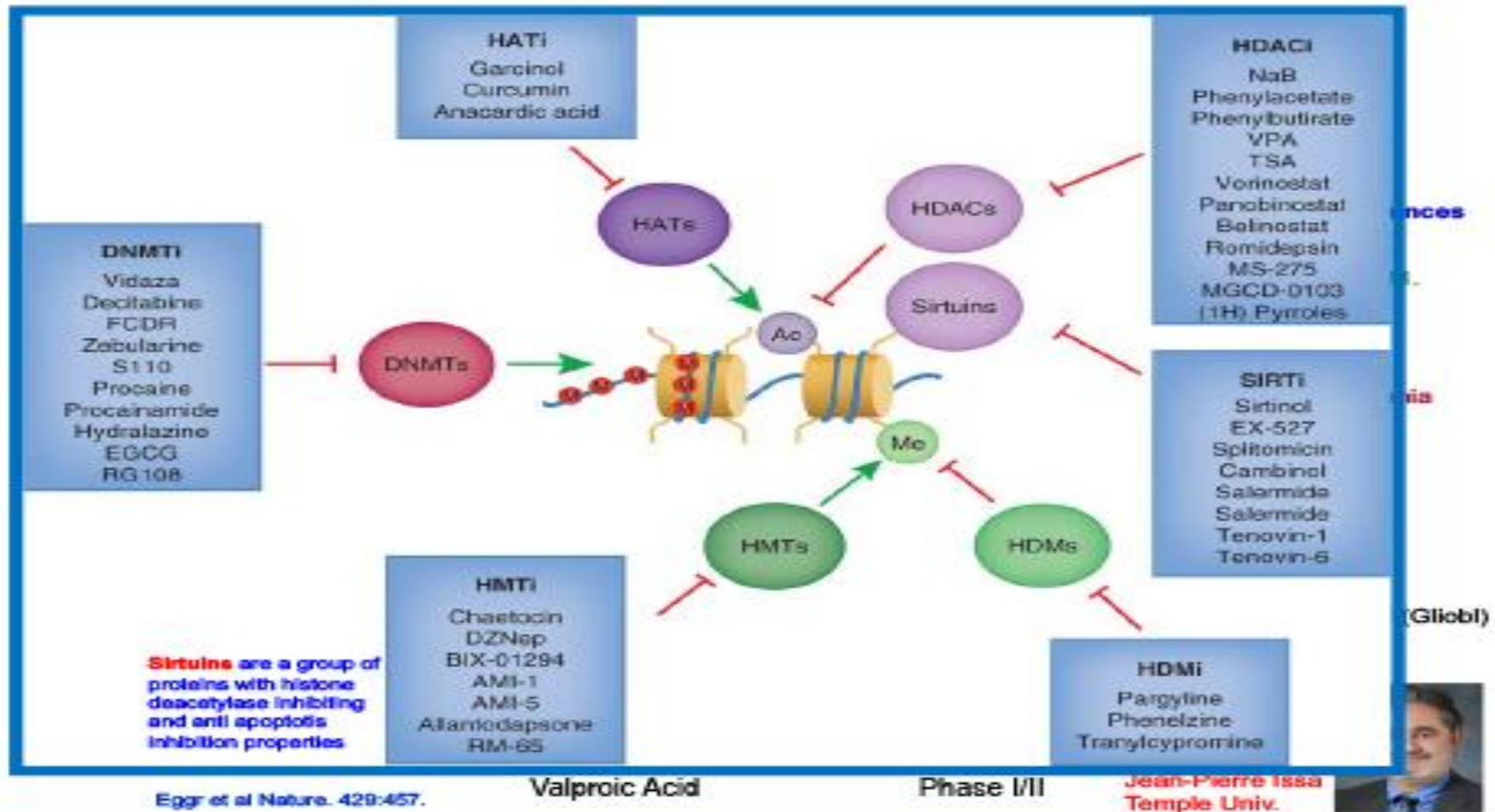
- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting

SAHAs are a group of proteins with histone deacetylase inhibiting and anti-apoptosis inhibitor properties

Epigenetics Nature 429:457



Methylation and acetylation enzymes



Histone deacetylase inhibitors

Table 4. Classification of Histone Deacetylase Inhibitors

Class	Compounds	Concentration needed for inhibition of histone deacetylase	Clinical trials	Notes
Short chain fatty acids	Phenylbutyrate	Milli-mole	Yes	Not ideal drug because of high dose requirement
Aliphatic compounds with hydroxamic acid	Trichostatin A, Suberoylanilide hydroxamic acid	Nano-mole Micro-mole	No Yes	Chelate Zn ion at catalytic site of HDAC.
Cyclic tetrapeptides	Trapoxin B, FK 228	Nano-molar Nano-molar	No Yes	FK228, a natural prodrug
Benzamides	MS-27-275	Micro-mole	Yes	Strong anti-tumor activity

Current Medicinal Chemistry, 2006, 13, 2909-2919

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

Phase I study

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

Braitheh F, Soriano AO, Garcia-Manero G, Hong D, Johnson MM, Silva Lde P, Yang H, Alexander S, Wolff J, Kurzrock R. Clin Cancer Res. 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



Peripheral blood
• Pyrosequencing
• Chip

Analysis
Day 1, 10 and 28

28 Days Cycle

55 people with
Advanced cancer
Median age 60

• 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.

5-azacytidine, valproic acid and ATRA

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*. 110(7):2302-8.

- Combination of 5-azacytidine (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

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)
Recruiting	Phase II Study of Valproic Acid With FEC100 for Patients With Locally Advanced Breast Cancer

Total : 84 studies

Methylation inhibitors

Methylation Inhibitors in Clinical Trials (Clinicaltrials.gov)

STATUS	STUDY
Completed	A Phase II Study of Epigenetic Therapy: Quercetin Chemotherapy Resistance in Refractory Solid Tumors
Active Not Recruiting	Azacitidine and Valproic Acid in Patients With Advanced Cancer
Recruiting	Azacitidine With or Without M-CSF-273 in Treating Patients With Myelodysplastic Syndromes, Chronic Myelomonocytic Leukemia, or Acute Myeloid Leukemia
Active Not Recruiting	PHIL-001: Azacitidine Plus Valproic Acid and Etoposide/Vincristine in Intermediate- and High-Risk MDS
Recruiting	Dacitabine With or Without Interferon Alfa-2 b in Treating Patients With Unresectable or Metastatic Solid Tumor
Recruiting	Hydroxyurea, Valproic Acid, or Gemtuzumab for Cervical Cancer
Recruiting	Hydroxyurea, Valproic Acid, or Gemtuzumab for Cervical Cancer
Recruiting	Dacitabine in Treating Patients With Previously Untreated Acute Myeloid Leukemia
Recruiting	Chronic Hepatitis C Non-Responder Study With Aso Met and Pegging
Recruiting	Azacitidine, Docetaxel, and Prednisone in Treating Patients With Metastatic Prostate Cancer That Did Not Respond to Hormone Therapy
Recruiting	Low-Dose Dacitabine + Interferon Alfa-2 b in Advanced Renal Cell Carcinoma

Total : 51 studies

<http://clinicaltrials.gov/ct2/results?term=methylation+inhibitors>

Schering-Plough (Dacitabine (5-aza-Deoxycytidine) Trial for melanoma) (8 hrs to inactivate DNMT1)
 Bristol-Myers Squibb (other compounds)

Three-drug combination



Novel drug combination shows promise in advanced HER2-negative breast cancer treatment

A novel three-drug combination achieved notable responses in patients with advanced HER2-negative breast cancer, according to new research directed by investigators from the Johns Hopkins Kimmel Cancer Center.

The treatment included a histone deacetylase inhibitor, a drug that causes a chemical change to stop tumor cells from dividing, with two types of immunotherapy known as checkpoint inhibitors, which unharness the power of the immune response against cancer.

The multicenter phase IB study, which aimed to improve response to checkpoint inhibitors by sensitizing the tumor microenvironment, found that the combination therapy resulted in a 25%

HER2-NEGATIVE BREAST CANCER

Three-drug combination achieved notable responses in patients with advanced HER2-negative breast cancer

HDAC (**entinostat**)

Drug causing chemical change to stop tumor cell from dividing

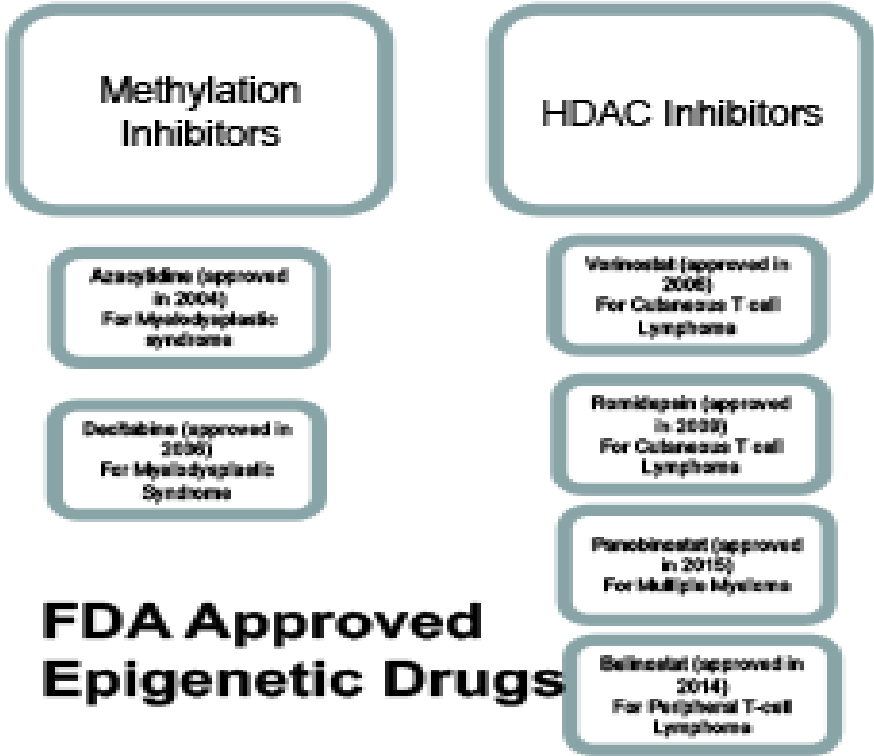
Checkpoint inhibitors

PD-1/PD-L1 inhibitor **nivolumab**

CTLA-4 inhibitor **ipilimumab**

25% reduction in response rate in advanced breast cancer patients for 6 months (tumor either destroyed or reduced)

Approved epigenetic drugs



Childhood cancer data initiative



The screenshot shows the NIH National Cancer Institute website. The header includes the NIH logo and the text "NATIONAL CANCER INSTITUTE". Below the header is a navigation menu with options: "About Cancer", "Cancer Types", "Research", "Grants & Training", "News & Events", and "About NCI". A search bar is located on the right side of the header. The main content area is titled "Childhood Cancer Data Initiative (CCDI)". On the left, there is a sidebar with a "Childhood Cancer Data Initiative" section containing links: "About CCDI", "CCDI Progress", "Community Stories", "Data Ecosystem", "CCDI Programs", and "Events & Webinars". The main content area has a heading "Childhood Cancer Data Initiative (CCDI)" followed by a paragraph: "NCI's Childhood Cancer Data Initiative (CCDI) is building a community centered around childhood cancer care and research data. Through better data sharing, we can advance our understanding of cancer biology and improve childhood cancer diagnosis, treatment, quality of life, and survivorship." Below this is a section titled "CCDI goals" with the text: "CCDI has three foundational goals:". To the right of the main content is a box titled "Engage with CCDI" containing two bullet points: "Sign up for our 9/10 webinar on the impact of a CCDI program on CNS tumors and our 10/8 webinar comparing proton and photon therapy" and "Download and visualize CCDI".

CCDI's Key Goals

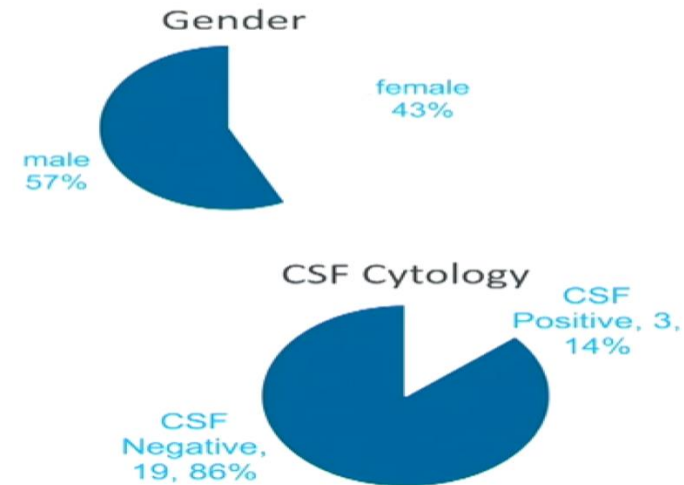
- Gather data from every child, adolescent, and young adult with childhood cancer
- Create a national strategy of molecular characterization to inform diagnosis and treatment
- Develop a platform and tools for clinical and research data to improve prevention, treatment, quality of life, and survivorship
- Engaging the entire childhood cancer care and research community



CNS Diagnosis

MCI CNS Diagnosis: Diffuse Midline Glioma, H3K27 altered (n=86)

- Median age: 9 years (range 3-20)
- Gender: 57% male:43% female
- Stage: 3/22 (14%) positive CSF cytology (most not tested)
- One Year Follow-Up available for first 14 patients: 43% survival
- All tumors with alterations in addition to H3K27



Epigenetic age

- Selecte
- *FGF*
- *PDG*
- *PI3K*
(10 M
- 7 Fu
(4 M
- Gerr
(CH

Epigenetic Age and Chronological AGE

Table 1. Published associations between epigenetic age acceleration and all-cause mortality in non-cancer cohorts⁴⁵⁻⁴⁸

Epigenetic clocks	Hazard Ratio*	P-value
Hannum (2013)	1.21 (1.16–1.25)	45e-15
Horvath (2013)	1.09 (1.05–1.13)	17e-21
Horvath (2018)	1.15 (1.10–1.20)	96e-08
PhenoAge (2018)	1.23 (1.20–1.26)	79e-47
GrimAge (2019)	1.50 (1.45–1.60)	20e-75

*Associations scaled to represent association per 5-year difference between biological age and chronological age

Clinical trials

nature
REVIEWS **CANCER**

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nature.com | journal home | archive | issue | perspectives | opinion | full text | table 1

Table 1: Summary of clinical trials of epigenetic therapies in solid tumours as drug resistance modulators

From
Poised epigenetic states and acquired drug resistance in cancer
 Robert Brown, Edward Curry, Luca Magnani, Charlotte S. Wilhelm-Benartzi & Jane Borley
Nature Reviews Cancer **14**, 747–753 (2014) | doi:10.1038/nrc3819

[← back to article](#)

Table 1: Summary of clinical trials of epigenetic therapies in solid tumours as drug resistance modulators

Cancer type	Epigenetic therapy	Drug combination	Patient selection	Response	Pharmacodynamic target validation?	Refs ¹
Gastrointestinal stromal tumours	Pancobinostat (pan-deacetylase inhibitor)	Pancobinostat and imatinib	Patients with metastatic gastrointestinal stromal tumours refractory to imatinib and sunitinib	1 of 11 partial response; 7 of 11 stable disease; 3 of 11 progressive disease	Yes	87
Wild-type KRAS metastatic colorectal cancer	Decitabine (demethylating agent)	Decitabine and panitumumab (monoclonal antibody against EGFR)	Patients with progressive disease on standard therapy and previously treated with cetuximab	2 of 20 partial response; 11 of 20 stable disease; 7 of 20 progressive disease	No	88
Advanced solid tumours	Azacytidine (demethylating agent); Valproic acid (pan-deacetylase inhibitor)	Azacytidine, valproic acid and carboplatin	Advanced cancer and progression following standard therapy (platinum-based) or no standard effective therapy available	6 of 32 stable disease; 26 of 32 progressive disease	Yes	89
			Initial response by	1 of 15 CA125		

Combination therapy

AML subtypes and combination therapy

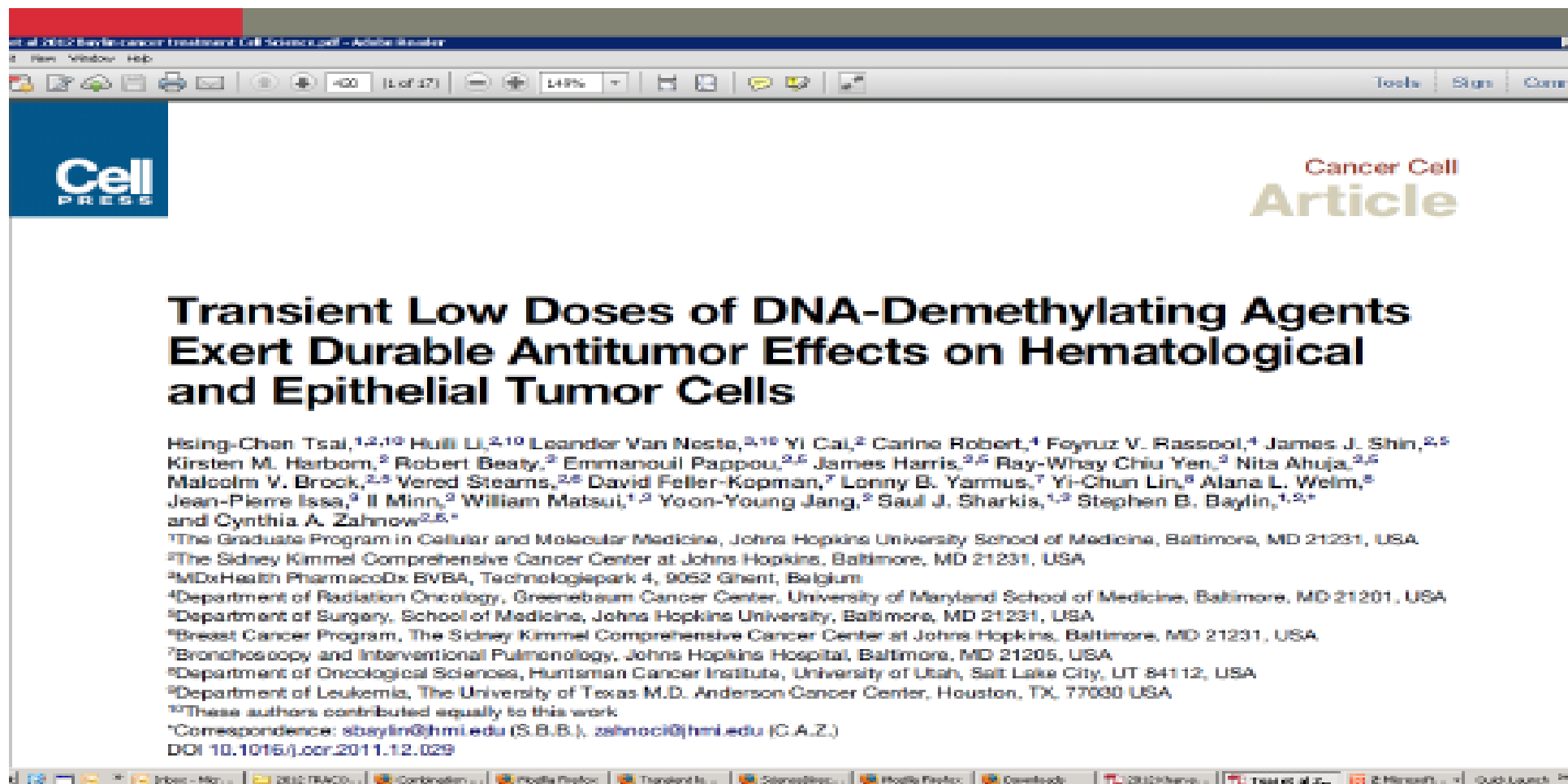


Pharmaceutical Participation

AML Subtype	Drug	Company
Tet2/WT1	CD33 + Aza	BI
IDH2 Mutation	Enasidenib	Celgene
MLL	Entospletinib (Syk inhibitor)	Gilead
CBF	Samalizumab (CD200 Ab) + induction	Alexion
P53 mutation	Entospletinib (Syk inhibitor) + Decitabine	Gilead
Complex Karotype	Entospletinib (Syk inhibitor) + Decitabine	Gilead
P53 mutation	Pevonedistat (Nedd8 inhibitor) + Aza	Takeda
Marker Negative	CD33 + Aza	BI
NPM1 w FLT3 WT	Entospletinib (Syk inhibitor)	Gilead
FLT3 mutation	Gilteritinib	Astellas
IDH1 Mutation	Ivosidenib + Aza	Agios

Source: Leukemia & Lymphoma Society

Low doses of DNA-demethylating agents



et al. 2012 Baylin cancer treatment Cell Science.pdf - Adobe Reader

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Cancer Cell
Article

Transient Low Doses of DNA-Demethylating Agents Exert Durable Antitumor Effects on Hematological and Epithelial Tumor Cells

Hsing-Chen Tsai,^{1,2,10} Huili Li,^{2,10} Leander Van Neste,^{2,10} Yi Cai,² Carine Robert,⁴ Foyruz V. Rassool,⁴ James J. Shin,^{2,5} Kirsten M. Harbom,² Robert Beaty,² Emmanouil Pappou,^{2,6} James Harris,^{2,6} Ray-Whay Chiu Yen,² Nita Ahuja,^{2,6} Malcolm V. Brook,^{2,3} Vered Stearns,^{2,6} David Feller-Kopman,⁷ Lonny B. Yarnus,⁷ Yi-Chun Lin,⁸ Alana L. Weim,⁸ Jean-Pierre Issa,⁹ Il Minn,² William Matsui,^{1,2} Yoon-Young Jang,² Saul J. Sharkis,^{1,2} Stephen B. Baylin,^{1,2,*} and Cynthia A. Zahnow^{2,6,*}

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²The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231, USA
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⁶Breast Cancer Program, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231, USA
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⁸Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA
⁹Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX, 77030 USA
¹⁰These authors contributed equally to this work
*Correspondence: sbaylin@jhmi.edu (S.B.B.), zahnowc@jhmi.edu (C.A.Z.)
DOI 10.1016/j.ccr.2011.12.029

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Reprogramming and editing

Epigenome Reprogramming and Epigenetic Editing

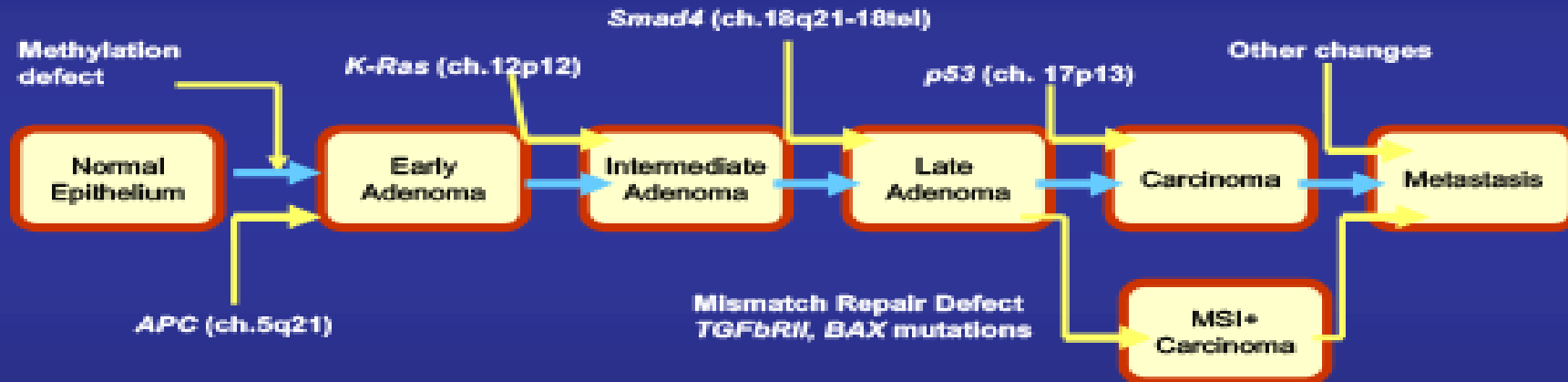
Table. Selected epigenomic editing companies		
Company, Year Founded	Recent Funding Events	Selected ASGCT 2023 Presentations ^a
Chroma Medicine, 2021	\$125M Series A (November 2021) \$135M Series B (March 2023)	Development of a Human PCSK9-Targeting Epigenetic Editor with Durable, Near-Complete In Vivo Silencing Efficiency Multiplexed Editing without Chromosomal Rearrangements Using Epigenetic Editors
Epic Bio, 2022	\$55M Series A (July 2022)	EPI-321: A Potential Cure for FSHD
Modalis Therapeutics, 2016 ^b		Advancing Epigenetic Editing with CRISPR-GNDM: Novel Muscle-Tropic AAV Vectors Deliver Promising Single-Dose Treatment for LAMA2-CMD
Navega Therapeutics, 2018	\$3.8M SBIR/NCI (September 2019) \$2M NINDS (June 2021)	N/A
Tune Therapeutics, 2020	\$40M Series A (December 2021)	Transient Delivery of Epigenome Editors Stably Represses PCSK9 and Lowers LDL Cholesterol in Nonhuman Primates
Rejuvenate Bio, 2017	\$10M Series A (April 2021)	Relevance of Animal Data for Human Health Programs

^aNamed EdGene when founded in 2016 and renamed Modalis Therapeutics in 2019.

^bOral abstract session or scientific symposium.

Intervention

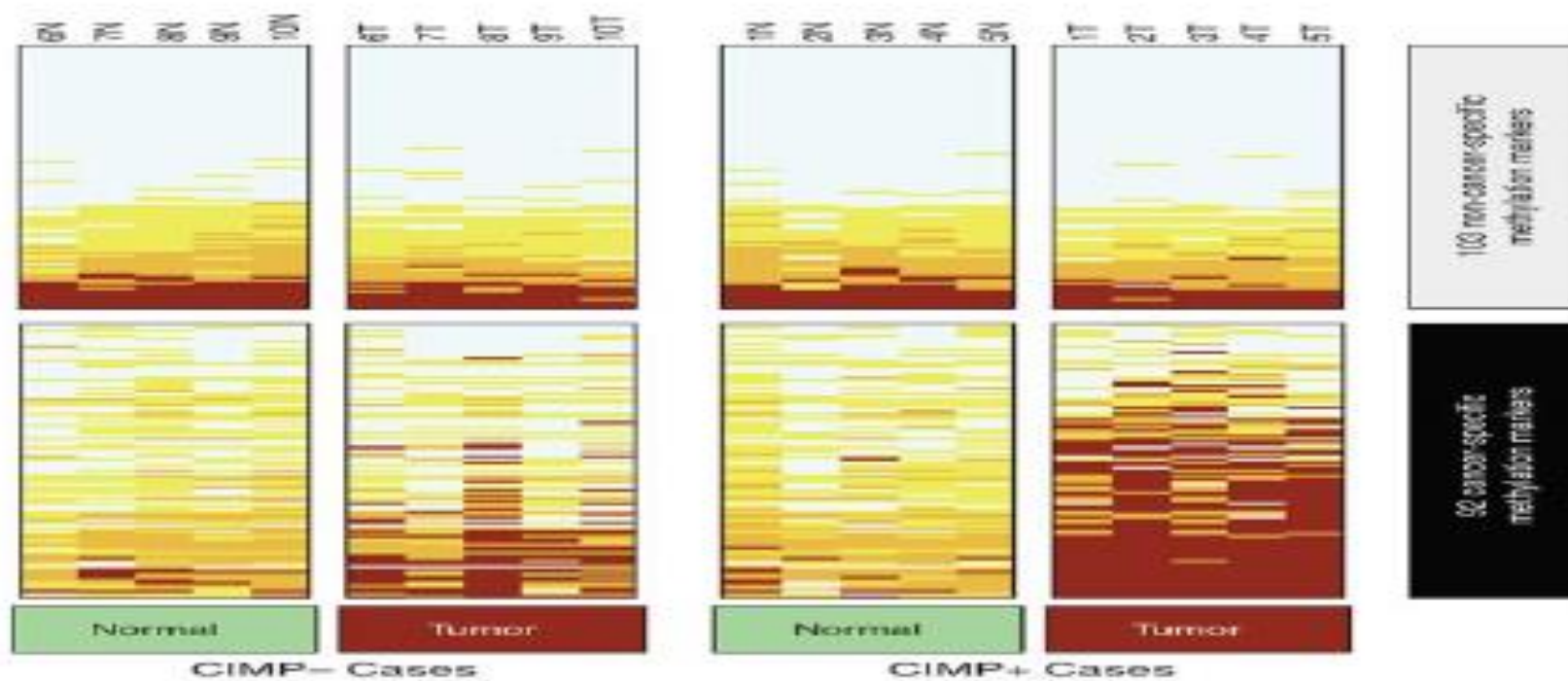
Potential Steps for Intervention



A Model for Colorectal Tumorigenesis

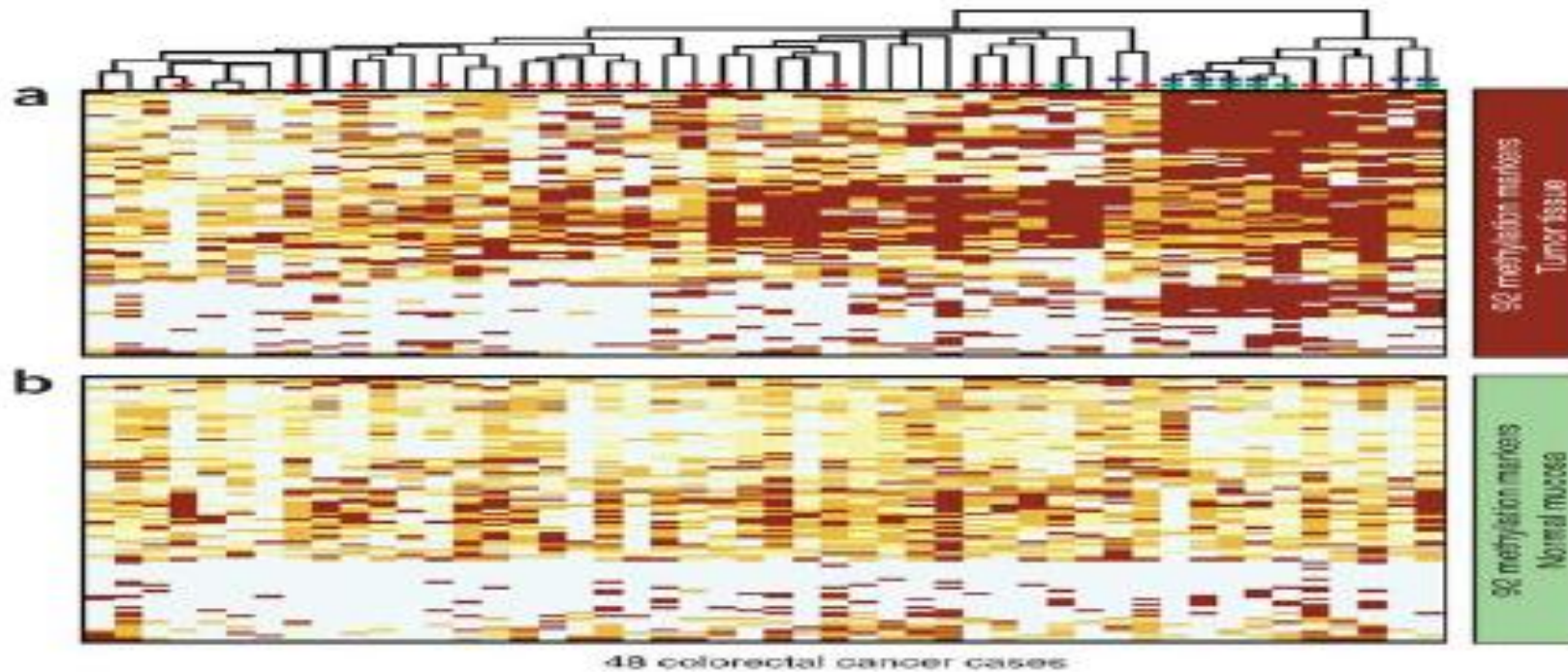
Microsatellite instability

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



Tumor clusters

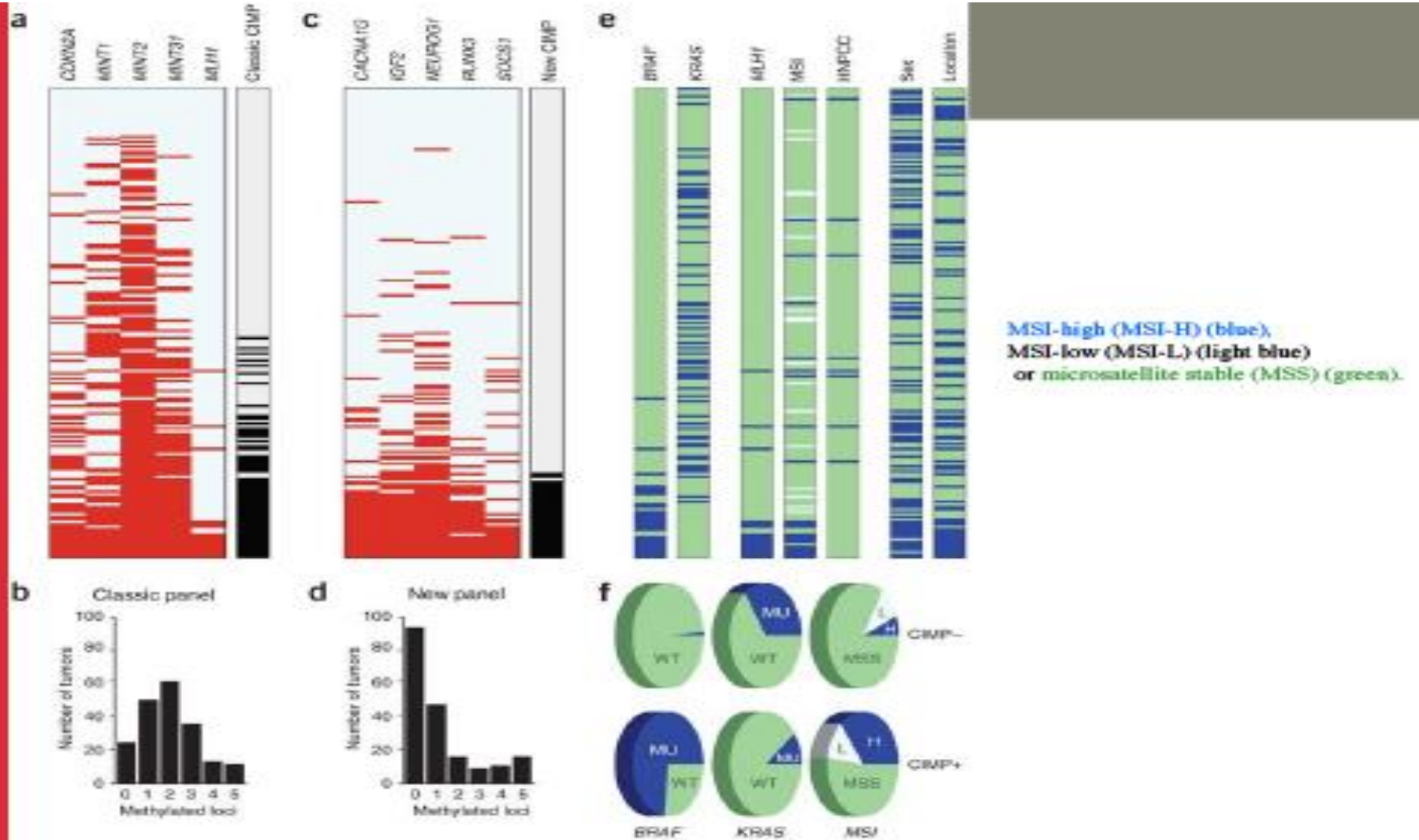
Identification of tumor clusters.



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

Genetic analysis



CRC markers

Validated CRC Markers in Clinical Trials

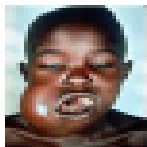
Table 1:

Validated DNA-methylation biomarkers for colorectal cancer

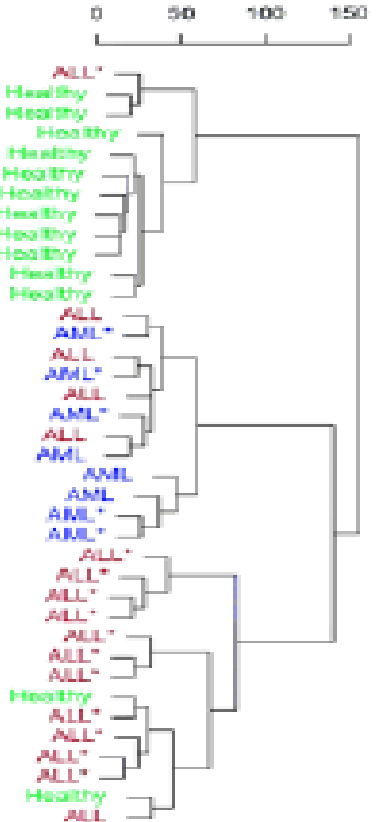
Clinical Use	Biomarkers	Available Commercial Assays	Study Design of Major Trials with Assay	Ref.
Stool-based CRC screening	mTME	ColoSure™	Case (N=42) control (N=241) study	109
	mBMP3 and mMDR4	ColoGuard® (detects mutant KRAS and includes a FIT test)	Prospective cohort based clinical trial in screening population (N=9939)	110
blood-based diagnostic marker	mSEPT9	EpigenColo® 1.0, ColoVantage®, RealTime m99	Multiple trials: 1) Prospective cohort based clinical trial in screening population (N=7941) (Church); 2) Case-Control study (N=289) (deVos); 3) Case-Control study (N=312) (Lifton-Bay 2008)	125 135,136,139
	mSCAT3 mMZFF	Colvera	Cross-sectional study (N=220)	138
Tissue-based prognostic markers	CGMP panel	NA	Multiple trials: 1. Case-Control study from 2 phase 1/II clinical trials (N=31)(Ogino, 2007); 2. Case-Control study from phase 3 clinical trial (N=818) (Shiovitz, 2014); 3) Observational cohort study (N=2050)(Pappa, 2015)	140, 142, 159, 160
Diagnostic test to screen for Lynch syndrome	mMLM	MUS1: Hypermethylation analysis	1. Cross sectional study (N=1066)	67

Methylation analysis

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

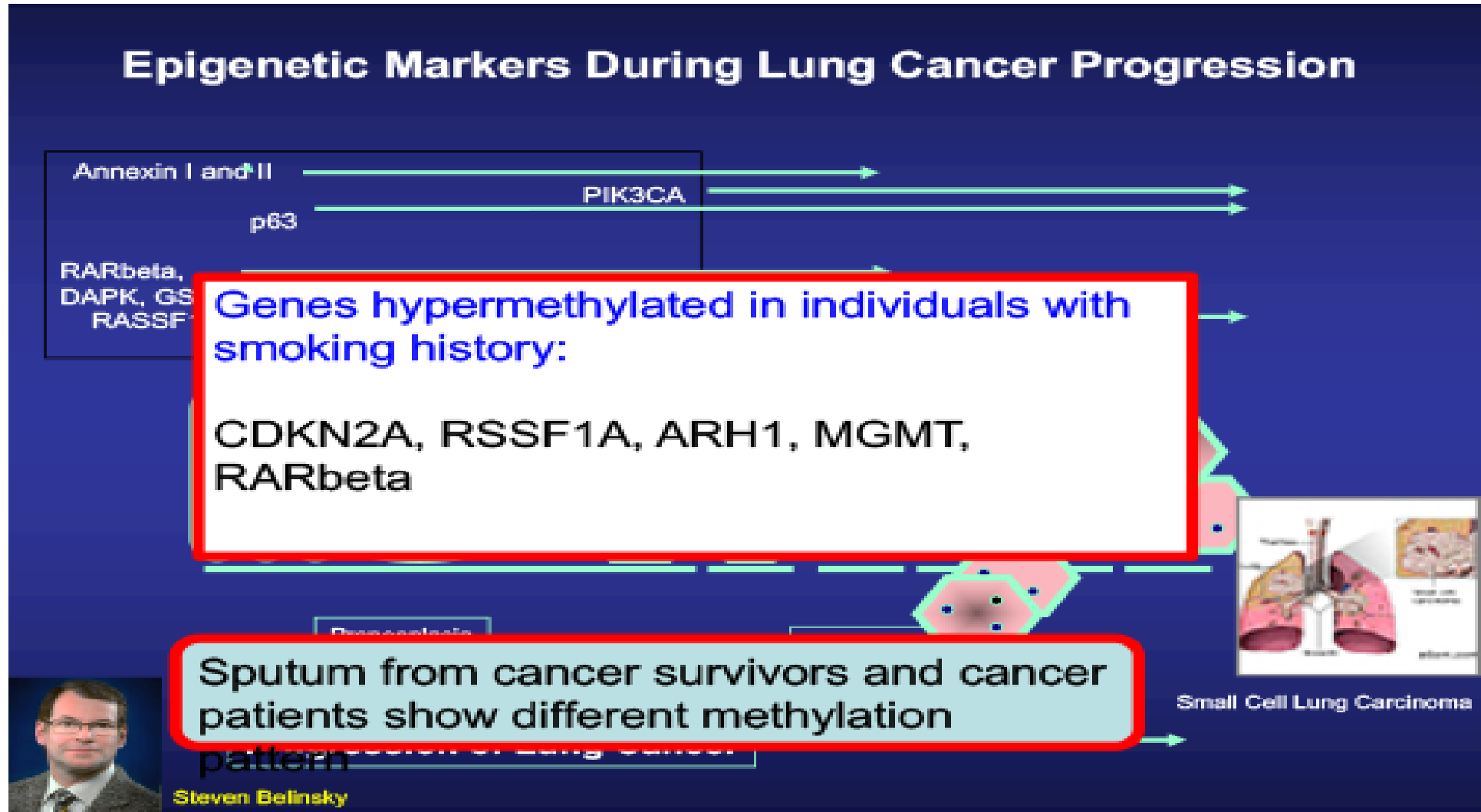


Lymphoma



AML: Acute Myeloid Leukemia
 ALL: Acute Lymphoblastic Leukemia

Epigenetic markers



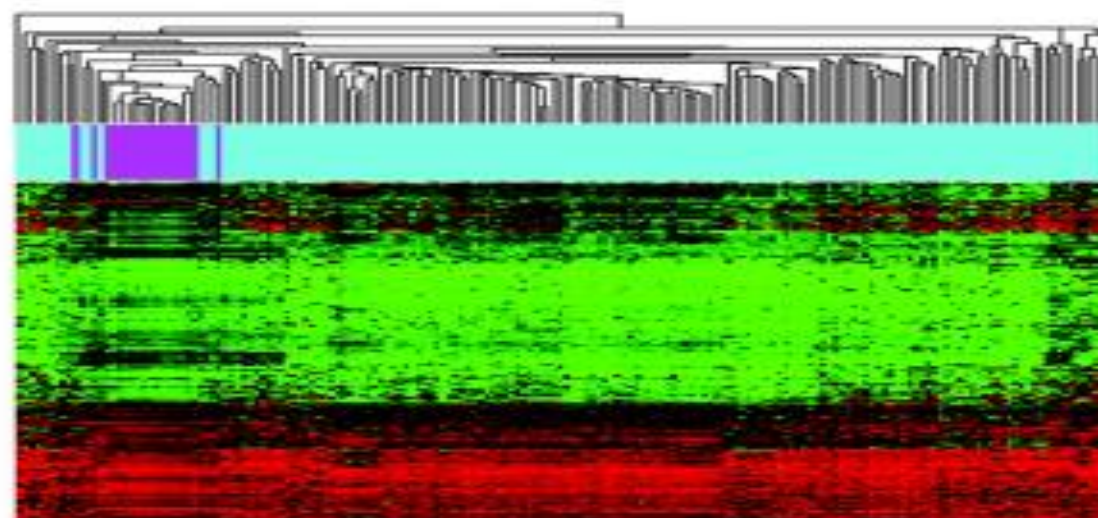
Mesothelioma

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

ASBESTOS

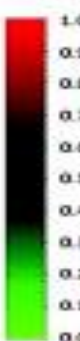
MESOTHELIOMA

Non-Mutagenic carcinogen



Tumor
Nontumor

Average β



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

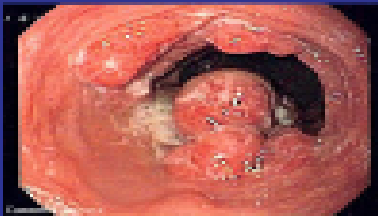
Cancer Research

Epigenetic pattern

Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

Cancer Research

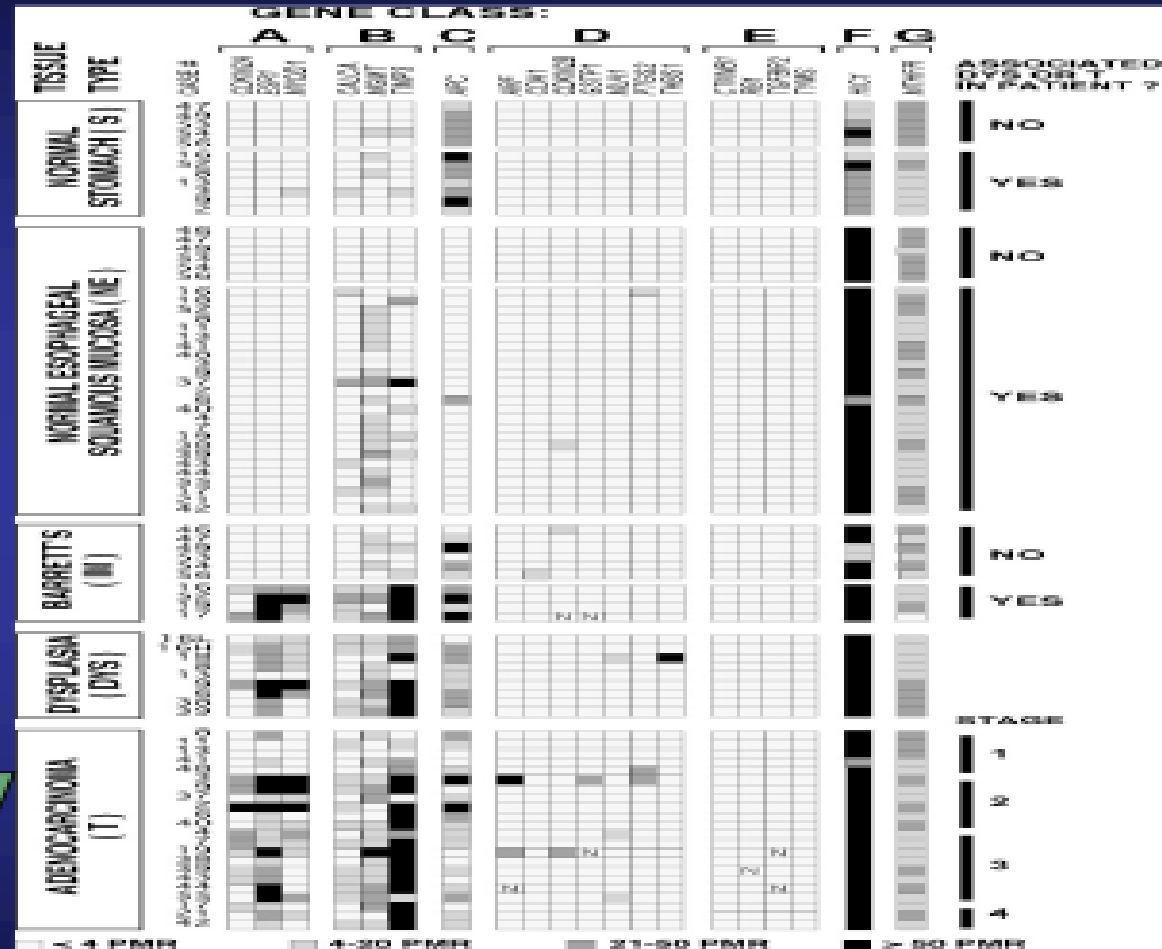
61:3410



Cancer Progression

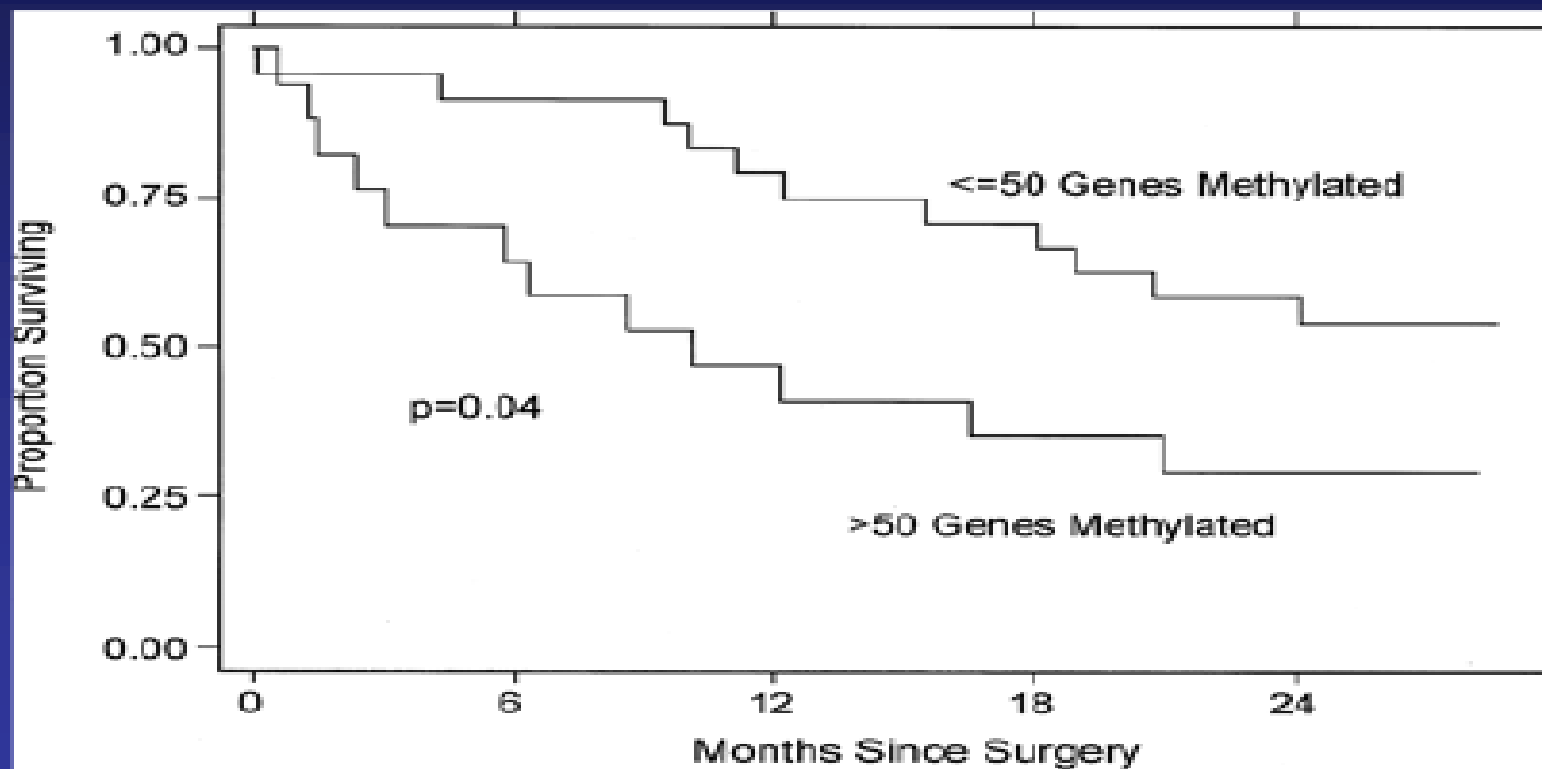
Risk factors

- Gastroesophageal Reflux Disease (GERD)
- Smoking
- Higher Body Mass Index (BMI) or obesity



Esophageal cancer

Esophageal Cancer: Probability of Survival



Pancreatic cancer

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

Pancreatic Carcinoma (PCA) : 39

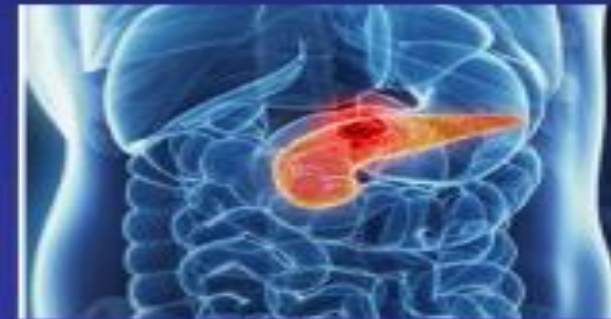
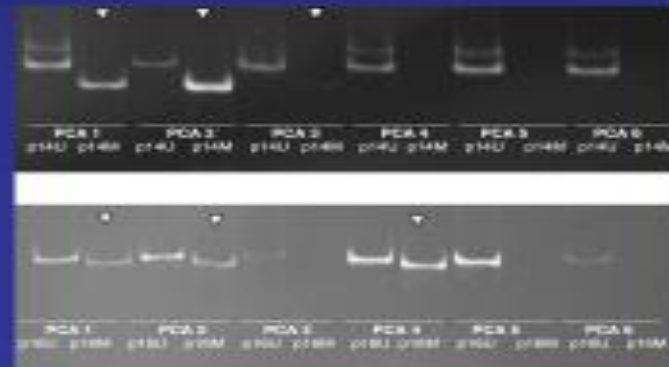
19/39 p16INK4a

Chronic Pancreatitis (CP) : 16

0/16 p16INK4a

Normal Pancreatogram (NAD) : 6

0/6 p16INK4a



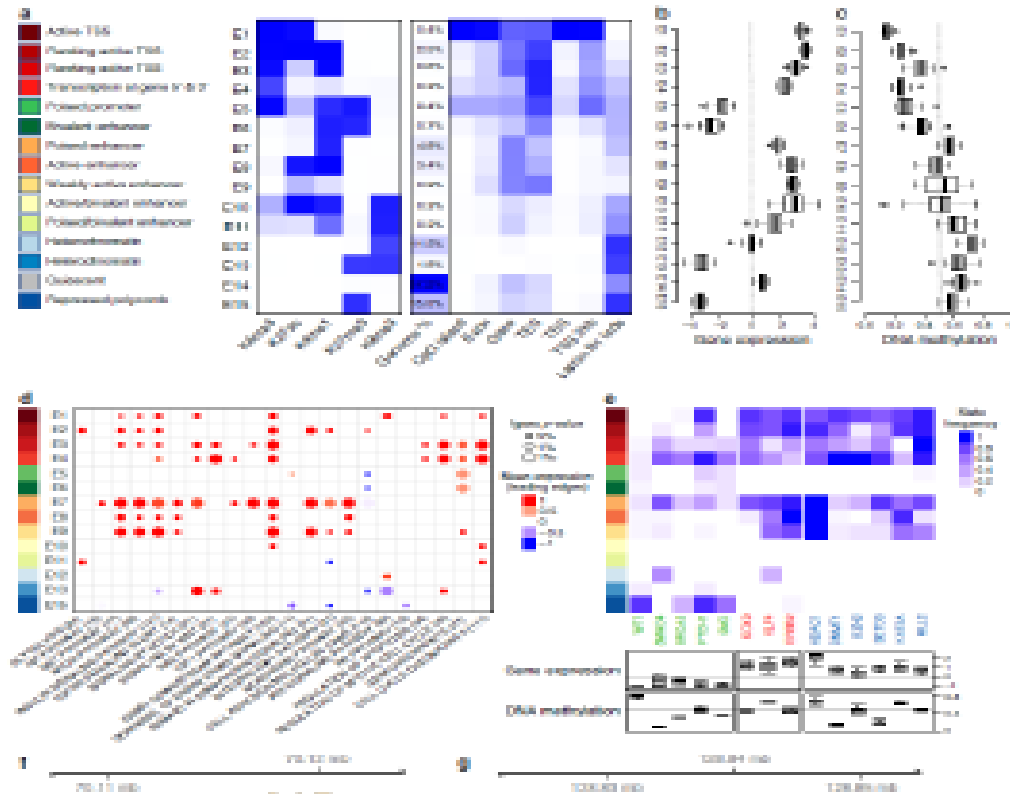
Sample: Pancreatic Fluid

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

Chromatin states

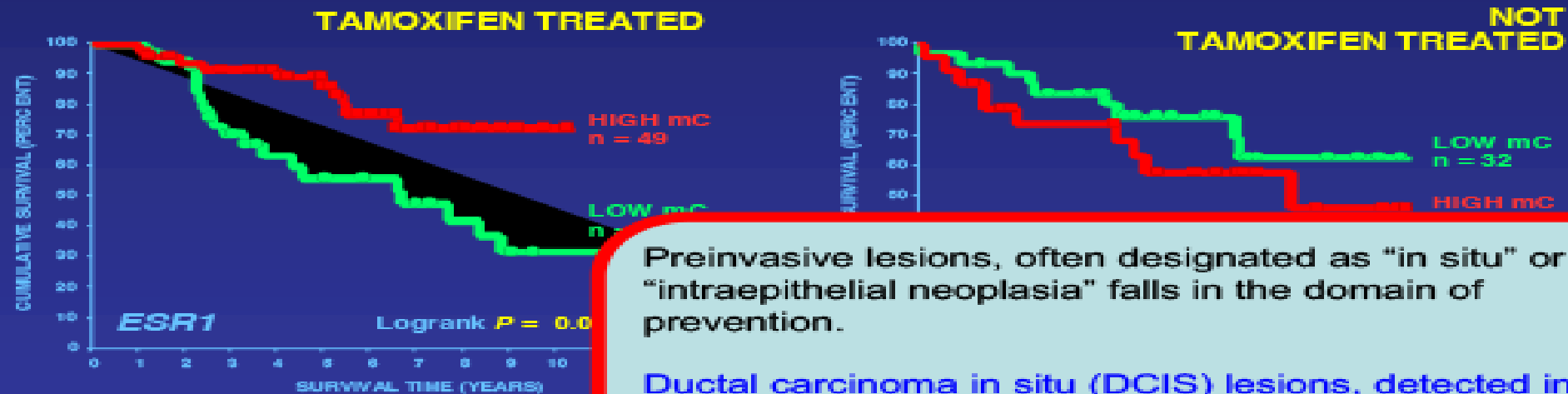
Distinct chromatin states of human PDAC

NATURE COMMUNICATIONS | (2018) 9:1978



Breast cancer

Breast Cancer Response to Tamoxifen Treatment by ESR1 Methylation

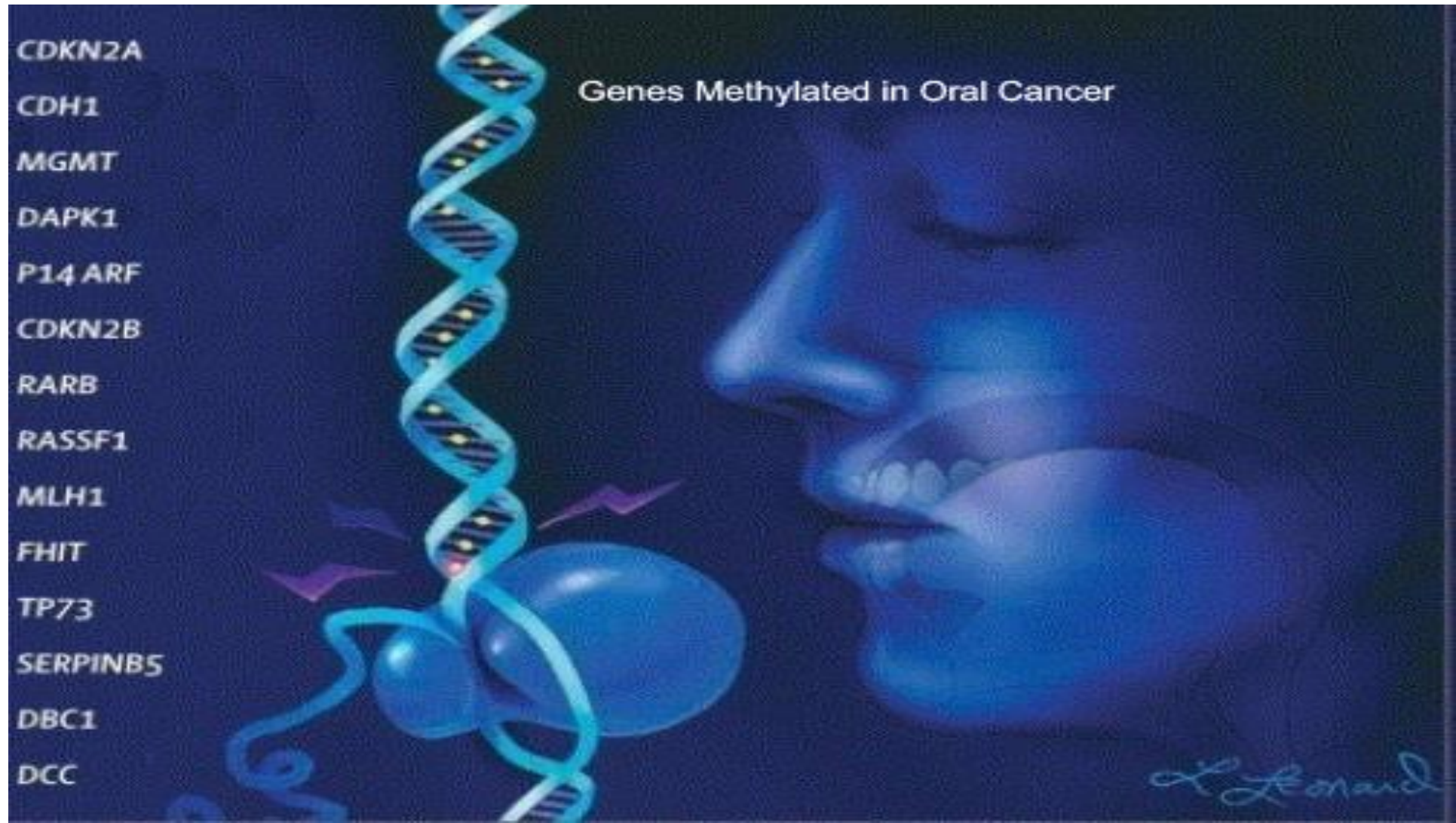


Preinvasive lesions, often designated as “in situ” or “intraepithelial neoplasia” falls in the domain of prevention.

Ductal carcinoma in situ (DCIS) lesions, detected in screening are generally treated aggressively, although all DCIS do not lead to breast cancer (over treatment).

Methylation profiling of DCIS lesions can distinguish aggressive from indolent DCIS.

Methylated genes



Biomarkers

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 Incorporates
leading provider of diagnostic
services.

...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

...marker

Methyl-Profiler™ DNA Methylation PCR ARRAYS

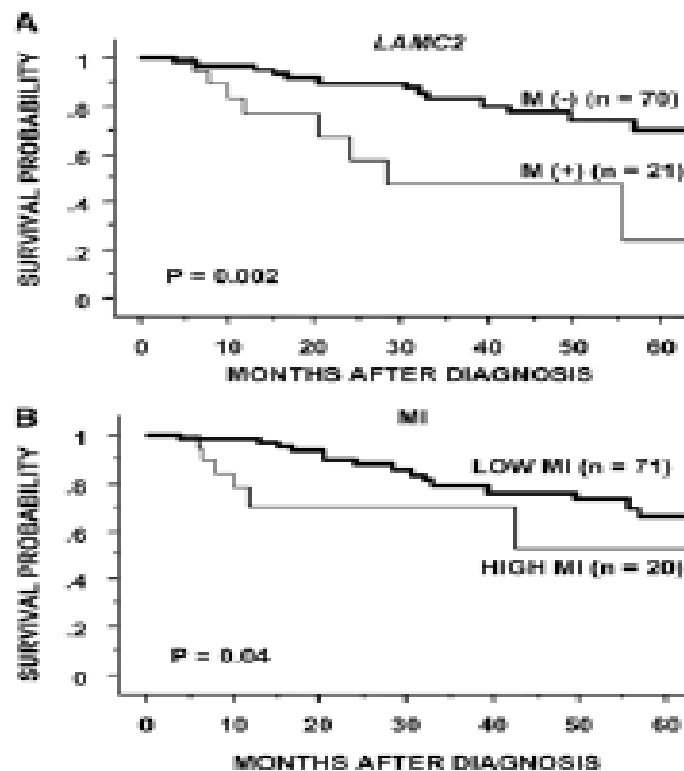
Product*	Catalog #	Price*
Human Breast Cancer - Signature Panel	MeAH-011	\$ 499
Human Gastric Cancer - Signature Panel	MeAH-021	\$ 499
Human Liver Cancer - Signature Panel	MeAH-031	\$ 499
Human Lung Cancer - Signature Panel	MeAH-041	\$ 499
Human Prostate Cancer - Signature Panel	MeAH-051	\$ 499
Human 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 (56 genes).

Bladder cancer methylation



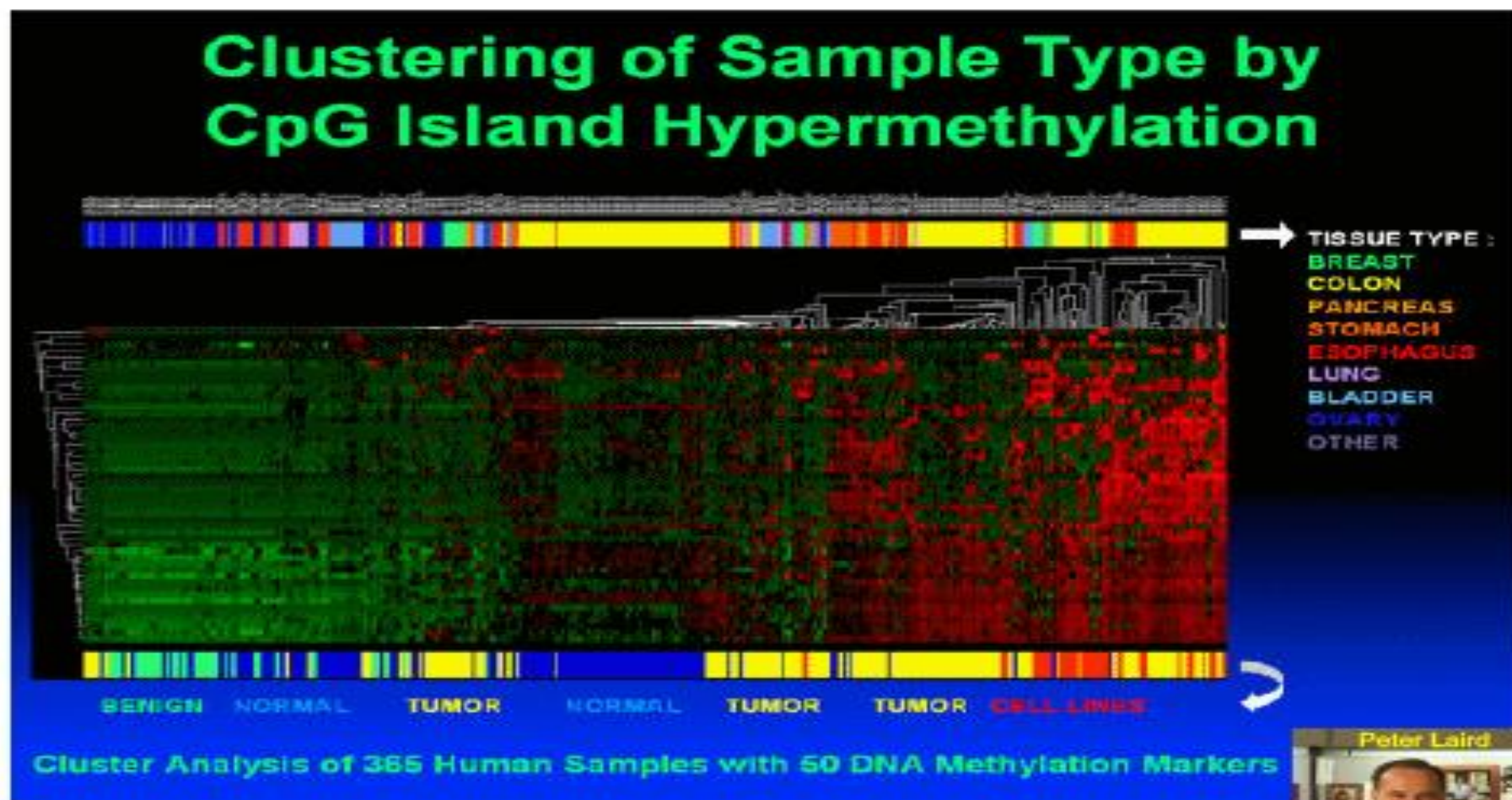
Bladder Cancer Methylation of LAMC2 in Exfoliated Cells Isolated from Urine



Another Study:
Schistosomes and Bladder Cancer

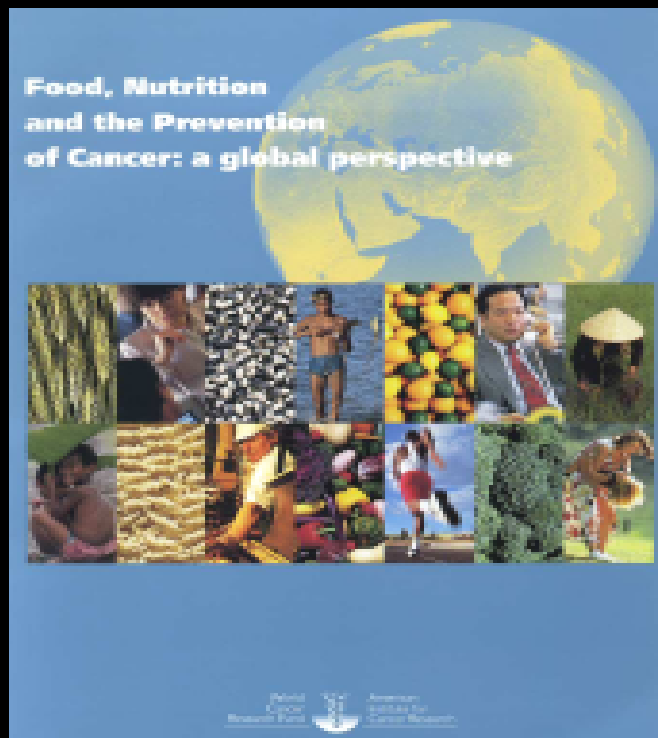
MI, Methylation Index

CpG island hypermethylation



Diet and cancer

DIET AND CANCER: FOCUS ON PREVENTION



Food, Nutrition
and the Prevention
of Cancer: a global perspective

Cancer is principally caused by environmental factors, of which the most important are tobacco, diet and factors related to diet, including body mass and physical activity, and exposures in the workplace and elsewhere.




Between 30% and 40% of cancer cases throughout the world are preventable by feasible dietary means.

- Understanding the determinants of the earliest detectable phenotypes in initiated cells
- Uncovering the molecular mechanisms of action of dietary nutrients leading to cancer formation and prevention
- Defining effects of dietary compounds not only on cancer cells but on normal and preneoplastic cells
- Determining factors that can modulate effect of diet

Methyl deficiency

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 ⁺ -foci	>54 weeks, GST ⁺ -tumor	Liver tumor
			

(part of a JHEP journal communication)

Sci. Data. 2014;3:1242 | doi: 10.1038/sdata.1242 | Epub 09 June 2014

Nutritional Epigenetics and the Prevention of Hepatocellular Carcinoma with Bioactive Food Constituents.

Shen, D¹, Wang, J¹, Chen, J²

Author information

Abstract
Hepatocellular carcinoma (HCC) is an aggressive and life-threatening disease often diagnosed at intermediate to advanced stages, which substantially limits therapeutic approaches to successful treatment. This indicates that the prevention of HCC may be the most promising strategy to reducing its incidence and mortality. Emerging evidence indicates that sufficient nutrients and consistent dietary bioactive components can reduce the occurrence and/or delay the development of HCC through modifications of deregulated epigenetic mechanisms. This review examines the

Keywords
epigenetic potential of bioactive food components, including dietary methyl group donors, epigenetic DNA methylase, sodium butyrate, resveratrol, curcumin, and sulforaphane, on liver carcinogenesis. Future direction and potential

References
in the effective use of bioactive food constituents in the prevention of HCC are highlighted and discussed.

Association of TNRSP12A Methylation With Prognosis in Hepatocellular Carcinoma With History of Alcohol Consumption.

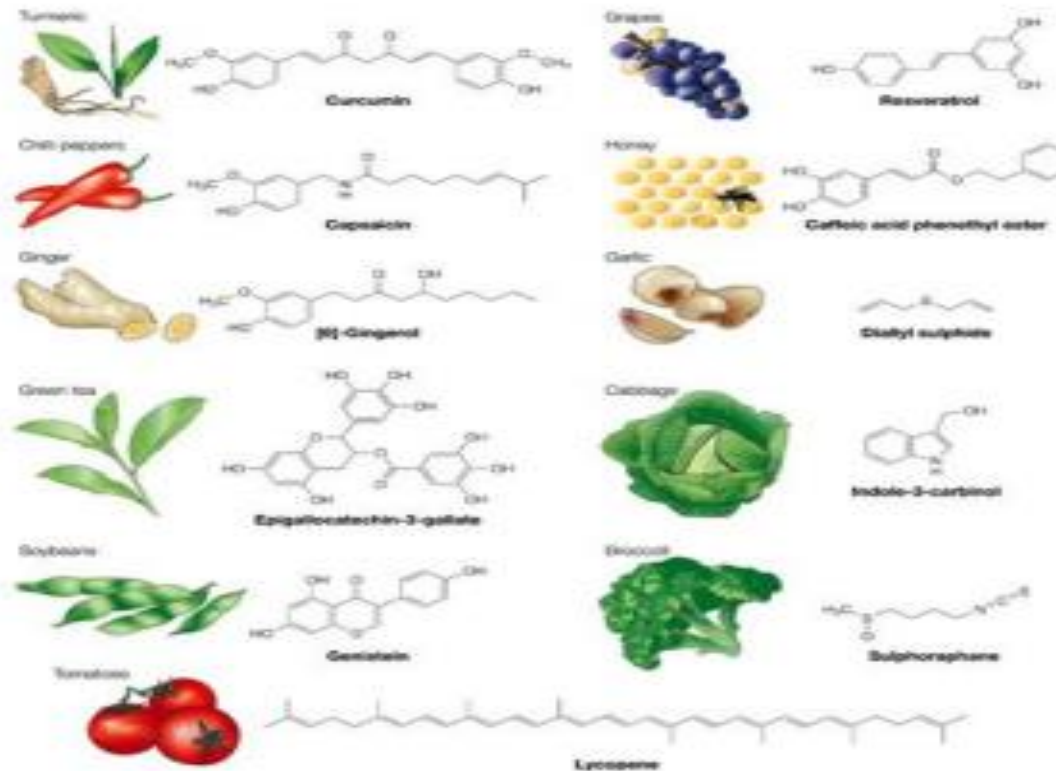
Wang, J¹, Shen, D¹, Wang, J², Wang, J³, Wang, J⁴, Wang, J⁵, Wang, J⁶, Wang, J⁷, Wang, J⁸, Wang, J⁹

Author information

Abstract
Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide with a poor prognosis. Alcohol liver disease accounts for approximately one-third of all HCC cases. Current evidence proved that aberrant over-expression of TNRSP12A correlates with the severity of disease, making it a likely marker of disease a poor prognosis and worse prognosis outcome. Emerging studies have confirmed that epigenetic changes are critical events in the development and progression of liver cancer. The study to investigate the mechanisms by which alcohol abuse mediated changes in the methylation level of TNRSP12A affect the occurrence, development and prognosis of HCC were under way. Thus, in this study we used two publicly available datasets to detect the association between DNA methylation level of CpG sites in gene TNRSP12A and the development of HCC in those with alcohol abuse history. Finally, we discovered that the hypermethylation of two CpG sites (cg0010441 and cg00000000) could identify HCC from other non-HCC liver diseases. Also, hypermethylation of these two sites could identify alcohol abusers from other non-hepatocellular carcinoma liver diseases. Most important, the prognostic analysis revealed that the hypermethylation of cg0010441 and cg00000000 in HCC patients with alcohol abuse history could predict poor prognosis. Further stratified analyses by gender discovered that in male HCC patients with alcohol abuse history, hypermethylation of cg00000000 signified poor prognosis. The tumor mechanism analysis revealed that the DNA methyltransferase DNMT3L might regulate TNRSP12A methylation and affect the occurrence, development and prognosis of HCC, especially in patients with a history of alcohol abuse. These findings provide new insights into the role of epigenetic mechanisms in the transformation of alcohol liver disease into HCC.

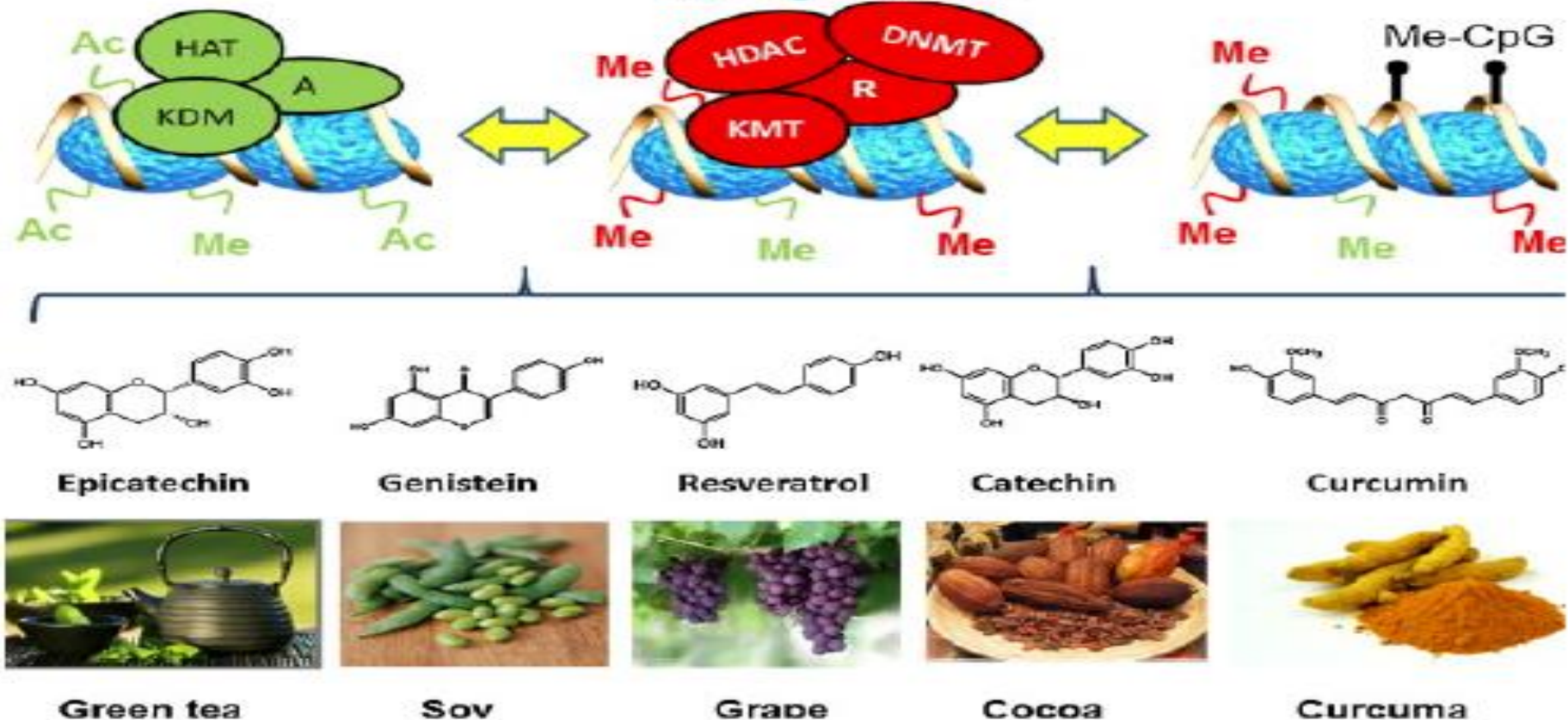
Anticancer phytochemicals

ANTICANCER PHYTOCHEMICALS (Representative chemopreventive phytochemicals and their dietary sources)

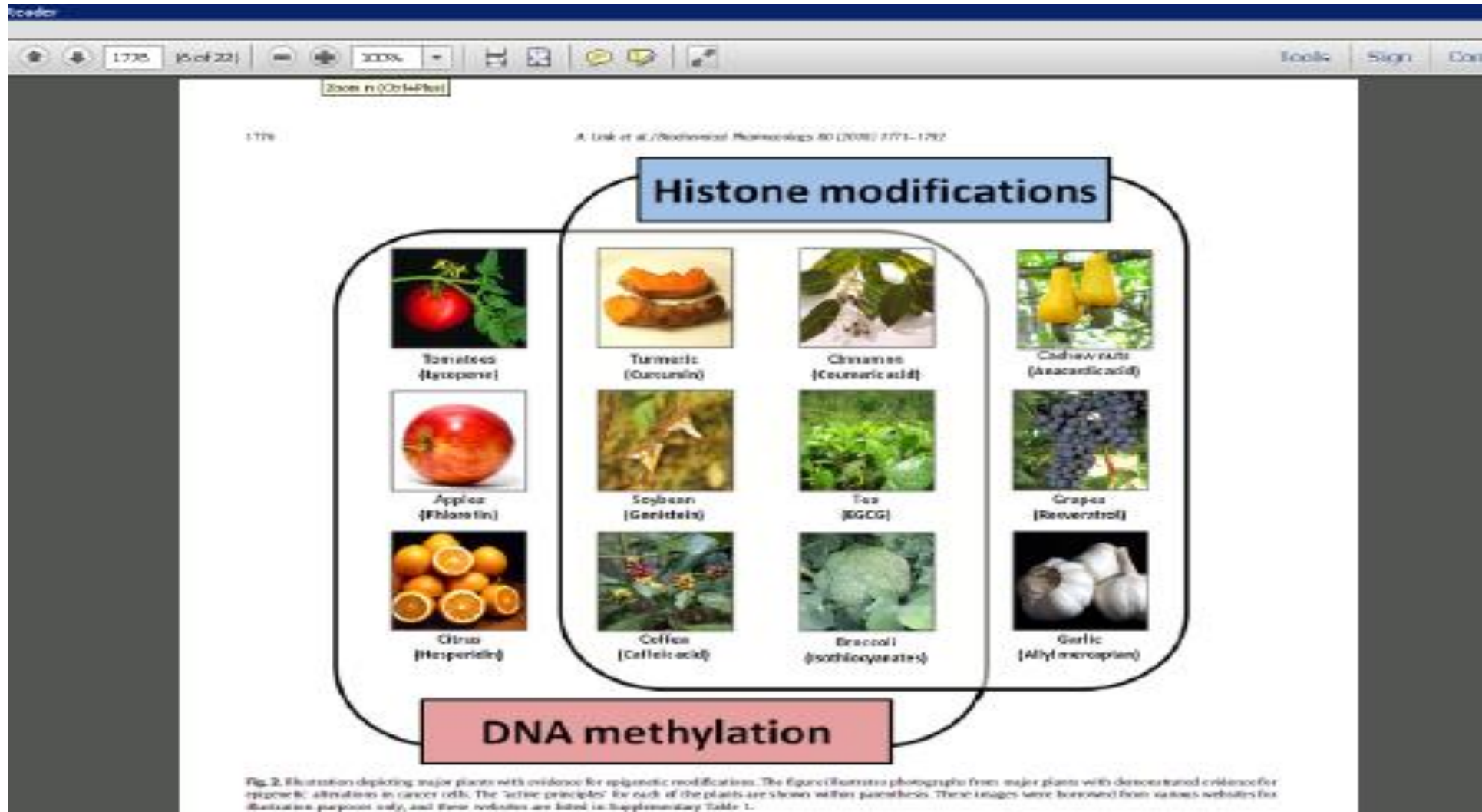


Dietary supplements

Development of functional foods or dietary supplements as nutrition based epigenetic modulators of chromatin writers, readers and erasers in cancer chemoprevention



Epigenetic foods



Myplate



Myplate.gov
Explore the MyPlate Food Groups

 <p>Fruits Fruits and whole grains Lentils, beans</p>	 <p>Vegetables Many more varieties Salads, soups</p>	 <p>Grains Whole, half whole grains, whole grains Cereals, breads</p>	 <p>Protein Foods Many, many protein choices Beans, lentils</p>	 <p>Dairy Milk, yogurt, cheese or calcium-rich foods Milk, yogurt, cheese</p>
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MyPlate Kitchen
Find recipes and resources in our very own kitchen. Sort by food group to find recipes based on what you already have or to get started on a grocery list. Save your favorites, make personalized cookbooks, and more!

[Go to MyPlate Kitchen](#)

 <p>Shop Simple with MyPlate Find savings in your area and discover new ways to prepare budget-friendly foods. Learn more</p>	 <p>MyPlate on Alexa Get MyPlate nutrition tips on Amazon Alexa devices or the free Alexa app. Learn more</p>	 <p>Start Simple with MyPlate App Build healthy eating habits one goal at a time! Download the Start Simple with MyPlate app today. Learn more</p>
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<https://www.myplate.gov/>

Mobile food record

Mobile food record



Research opportunities

Research Opportunities and Challenges

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?

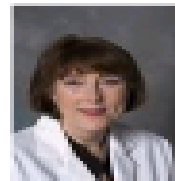
Are there race/ethnicity specific miRNAs and noncoding RNAs?

How can we use this information for better define cancer subcategories?

How can we overcome EWAS technical challenges?



Christopher Plass (Heidelberg)



Nancy Khvil (Seattle)

Christine Amberson
(Roswell Park, Buffalo)



Research challenges

Research Opportunities and Challenges

Can we predict cancer recurrence or secondary cancer development based on epigenetics marks (or in combination with other omics marks)?

Why is it difficult to harmonize epigenetic data with other omics data sets?

Is there a window of susceptibility of exposure? How can we develop epigenetic approaches to intervene?

How to avoid activity of DNMT and HDAC inhibitors on normal cell functions?

What is the role of non-histone proteins in gene regulation?

How to target cancer stem cells using epigenetic approaches?

How much microbiome-specific metabolites can affect epigenetic regulation? How effective are probiotics in cancer prevention?

Conclusions

Conclusions

- **Epigenetic regulation is needed for normal development.**
- **External and internal environment contribute to alterations in epigenetic components and gene expression resulting in disease initiation and development.**
- **Epigenetic changes are reversible.**
- **Epigenetic inhibitors have been used successfully in combination therapy.**

