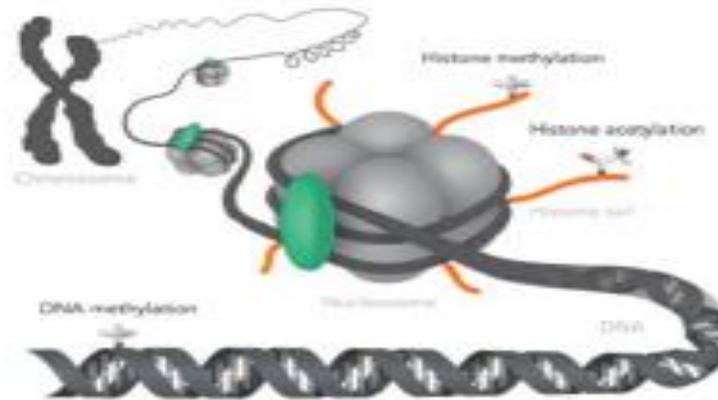


Epigenetics and cancer

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

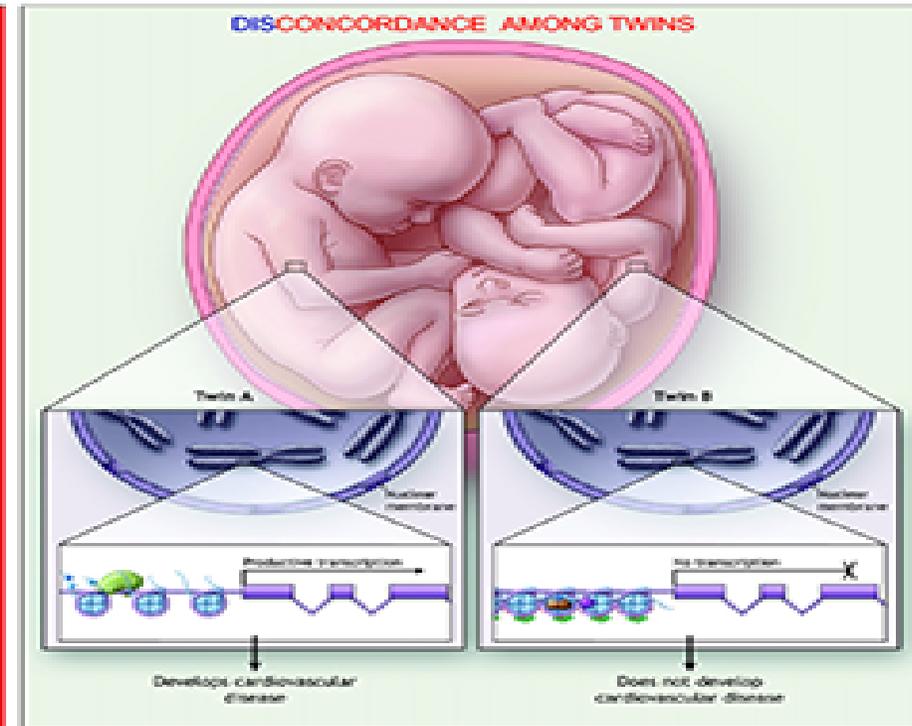
The genetic information provides the blue print for the manufacture of all the proteins necessary to create a living organism, whereas the epigenetic information provides the instructions on how, where and when the genetic information will be used.



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

Adapted from Matouk and Marsden *Cir Res* 102:873

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.

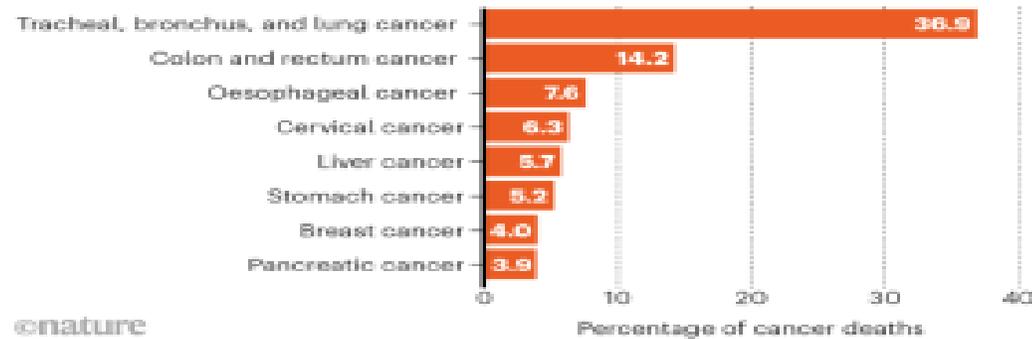
<https://www.nature.com/articles/d41586-022-02355-x>

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 Coufides

nature briefing

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Email address

e.g. j.smith@university.ac.uk

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Cancer continuum

DCCPS covers cancer continuum



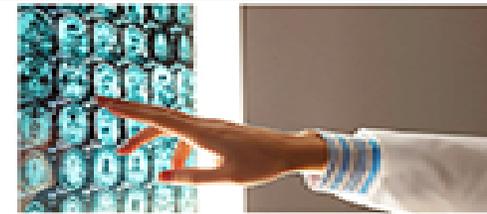
Prevention

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

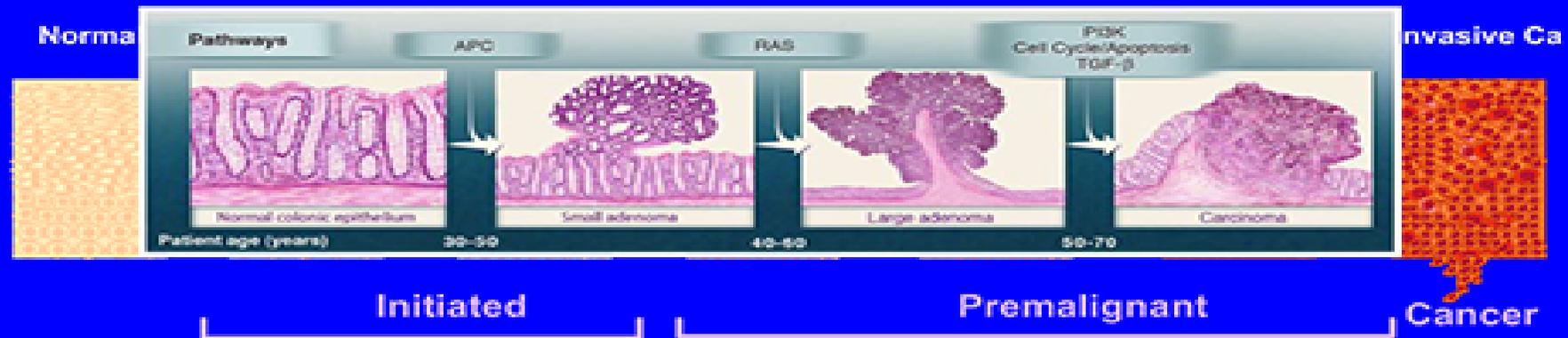


Cancer recurrence
Secondary cancer

Prevention: restoring transcription, halting progression, or stopping metastasis

Cancer development

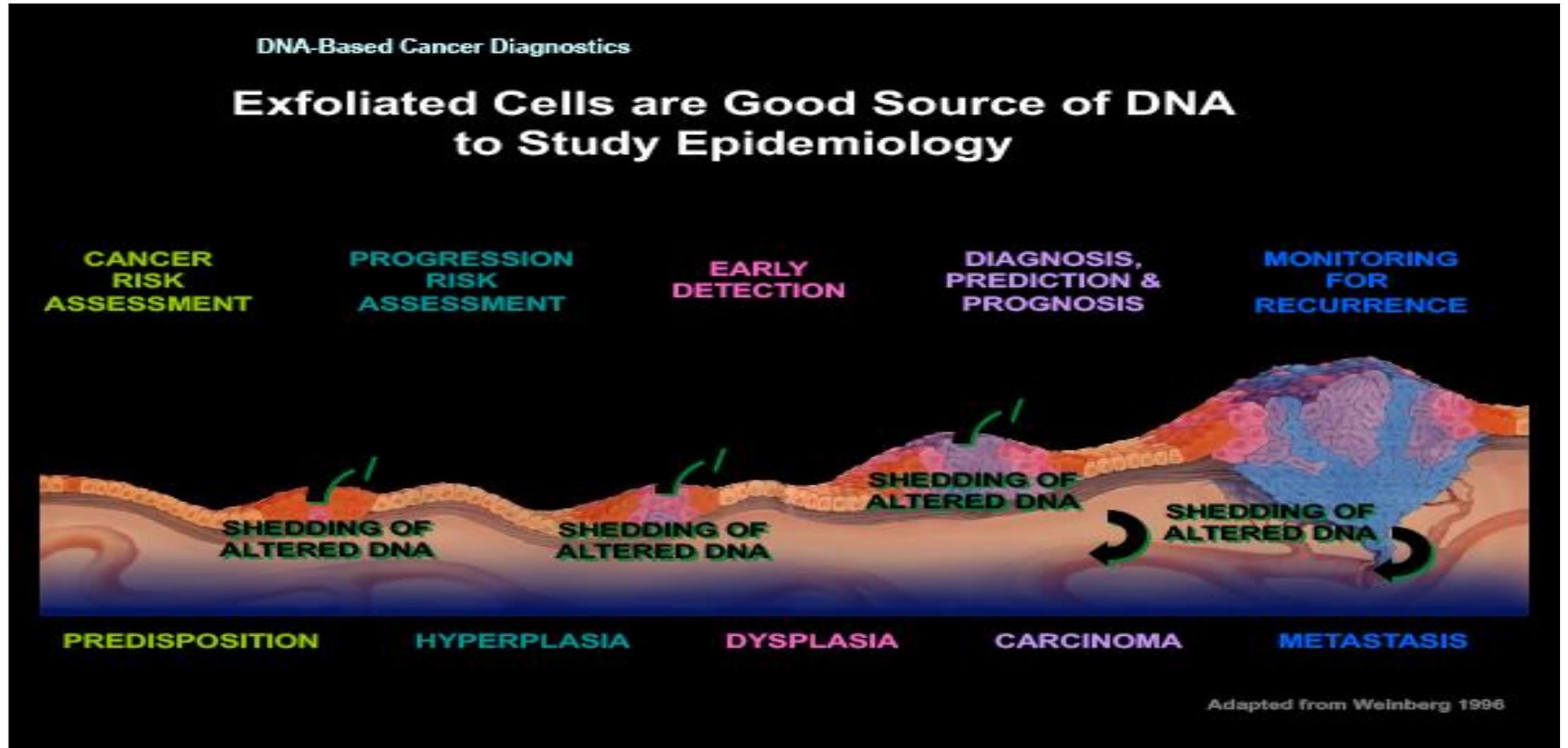
Cancer Development is a Multi-step Process



Genetic alterations and the progression of colorectal cancer

The major signaling pathways that drive tumorigenesis are shown at the transitions between each tumor stage. One of several driver genes that encode components of these pathways can be altered in any individual tumor. Patient age indicates the time intervals during which the driver genes are usually mutated. Note that this model may not apply to all tumor types. TGF- β , transforming growth factor- β .

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*

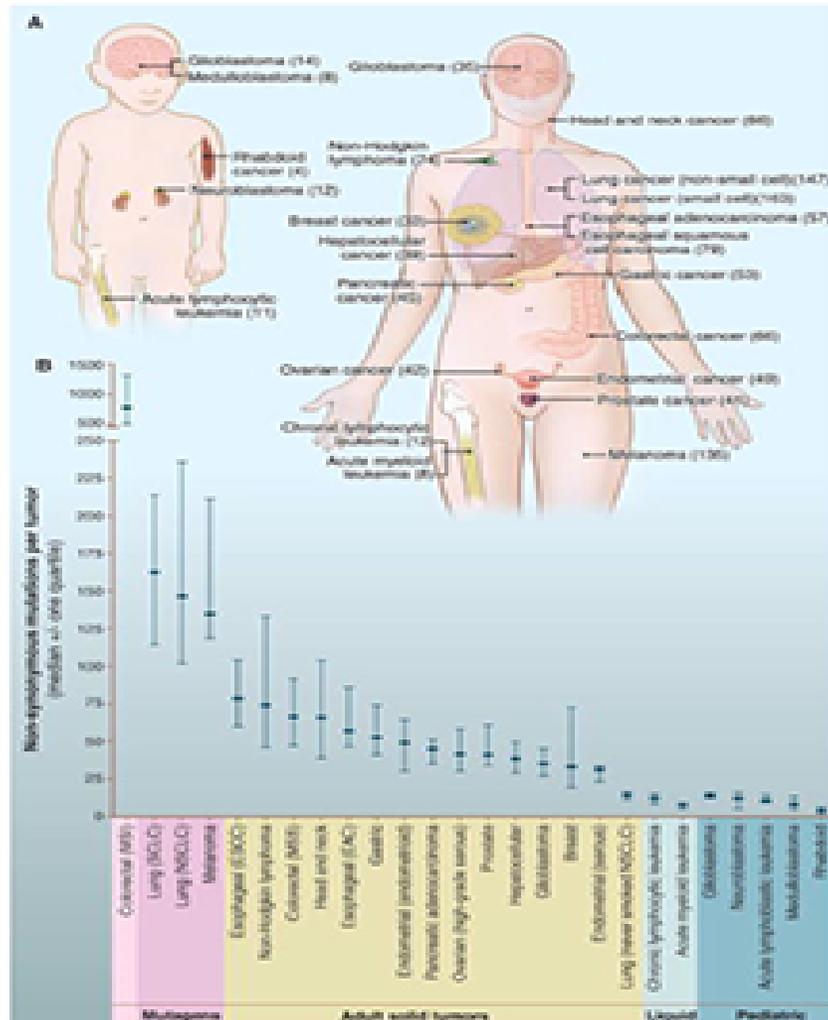
1950-2000 : Age of DNA

*structure, genetic code,
genome sequence*

2000 - : Age of epigenetics

*epigenetic code, epigenome,
epigenetic medicine*

Genome landscape



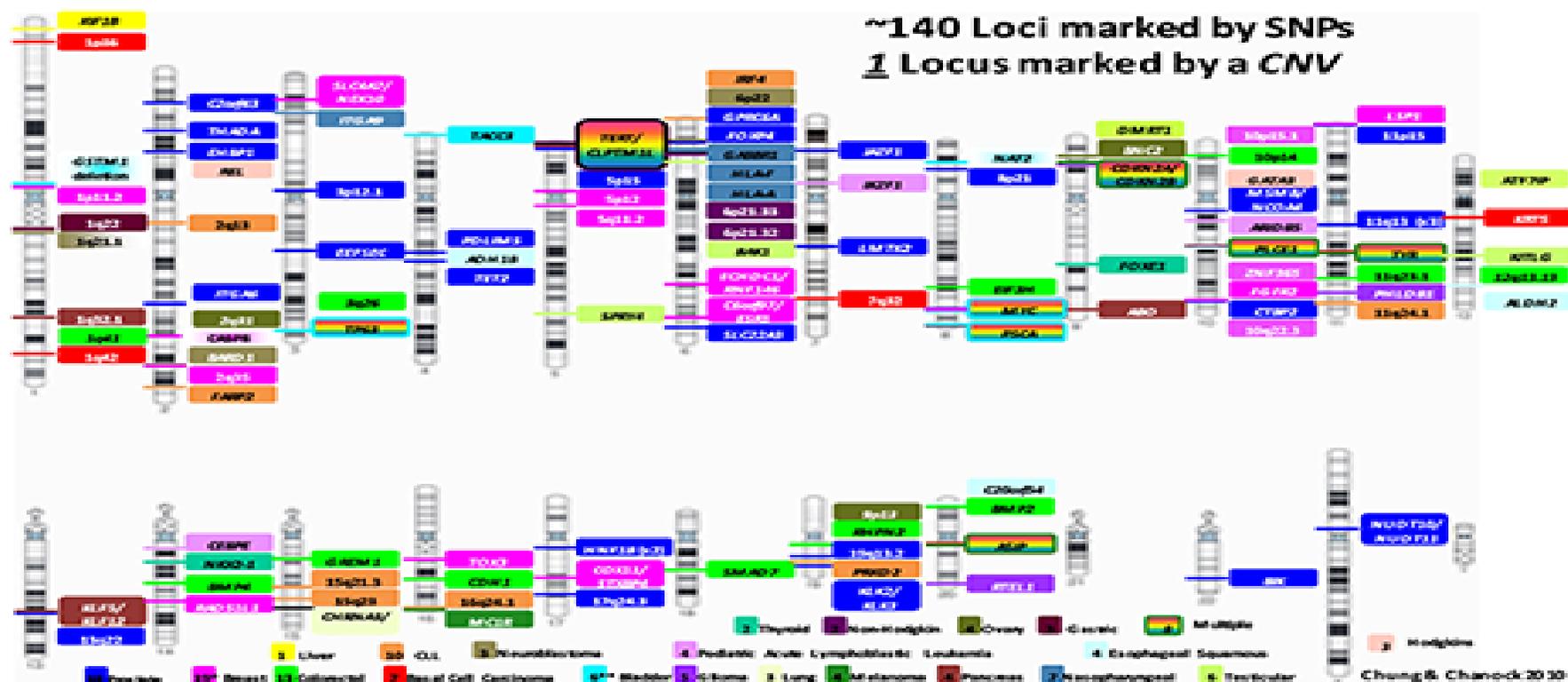
CANCER GENOME LANDSCAPE
 Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies



Adapted from Vogelstein and Kinzler (Science 2013)

GWAS hits

Published GWAS Etiology Hits (2010)

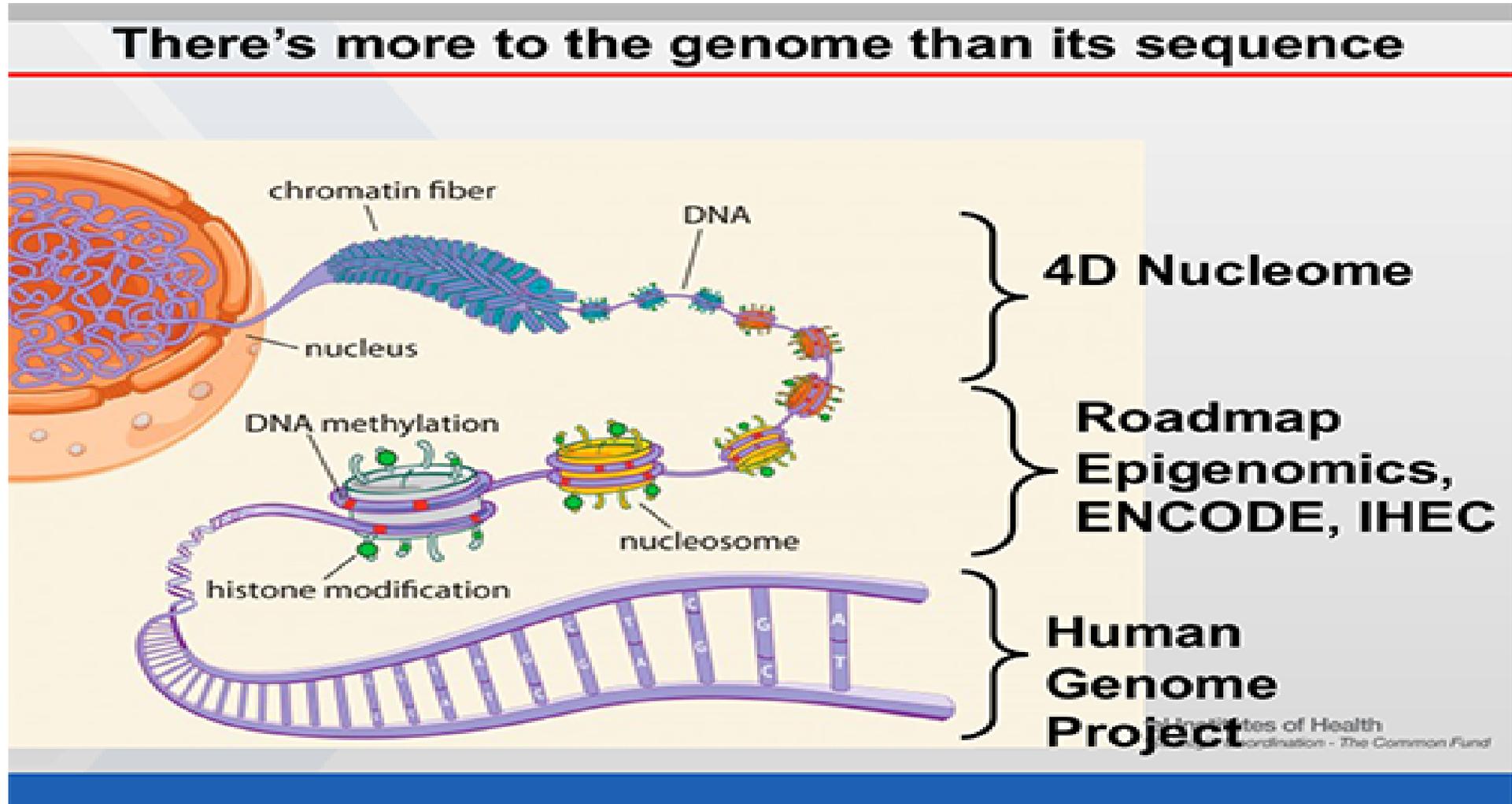


Genome associations

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



Genome sequence



Kornberg and nucleosome

Nucleosomes (Units of Chromatin)

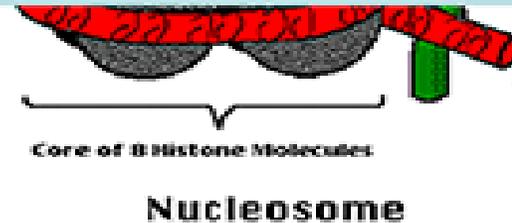
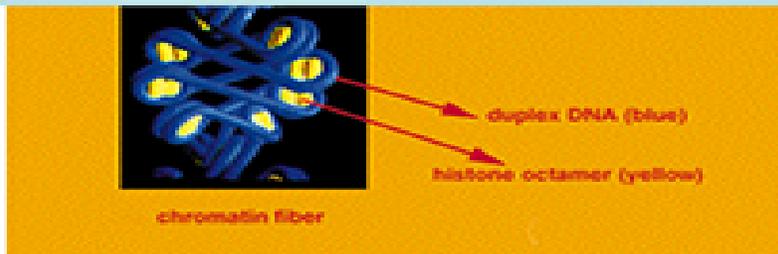
DNA
Histones H2a, H2b, H3, H4

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

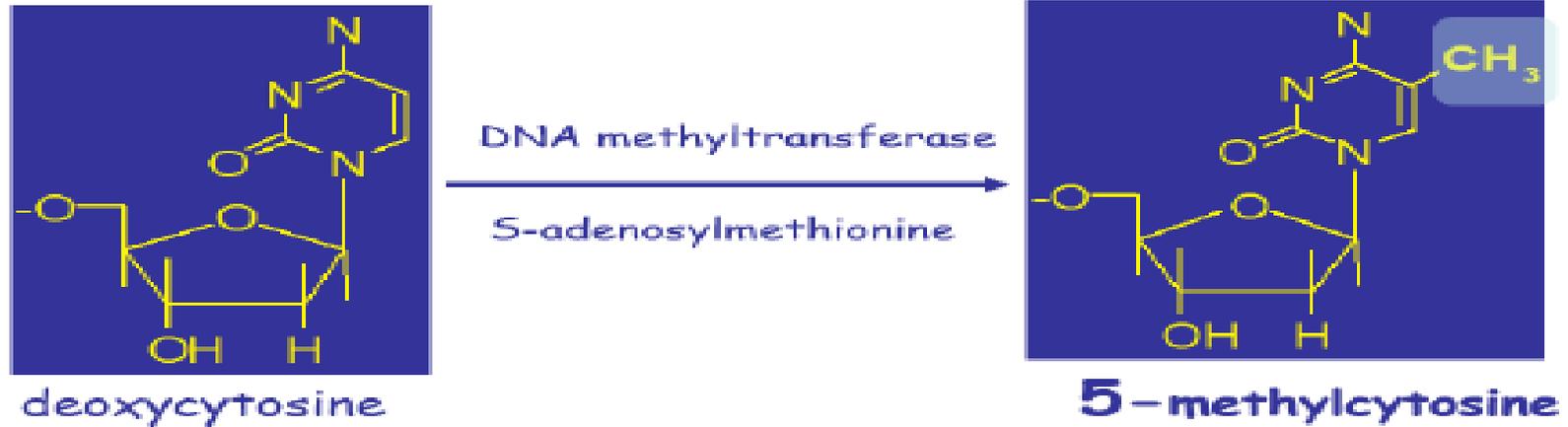
1974: Roger Kornberg discovers nucleosome who won Nobel Prize in 2006.



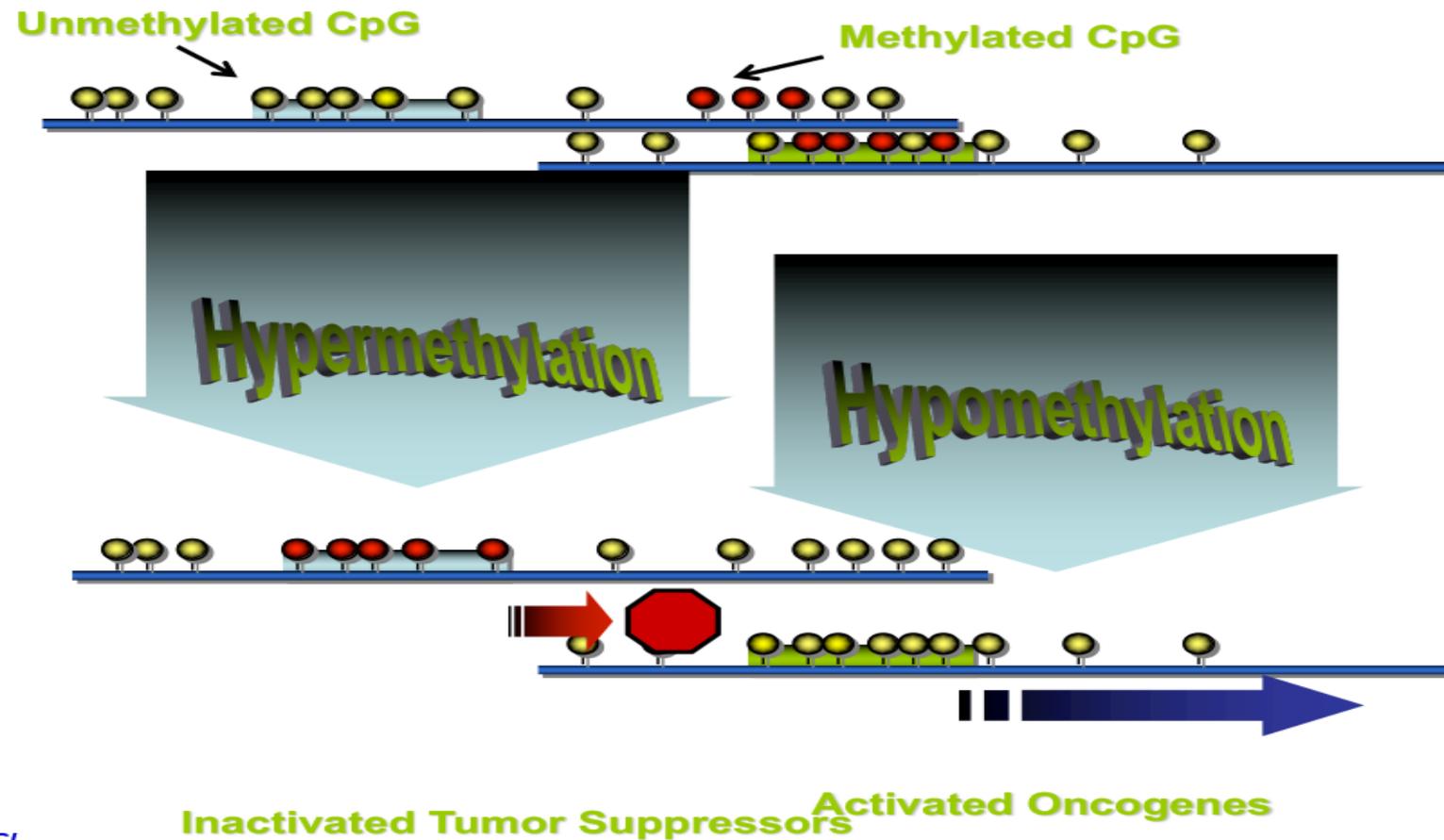
Shores are 0-2kb from islands
Shelves are 2-4 kb and enhancers are beyond shelves

DNA methylation

DNA Methylation



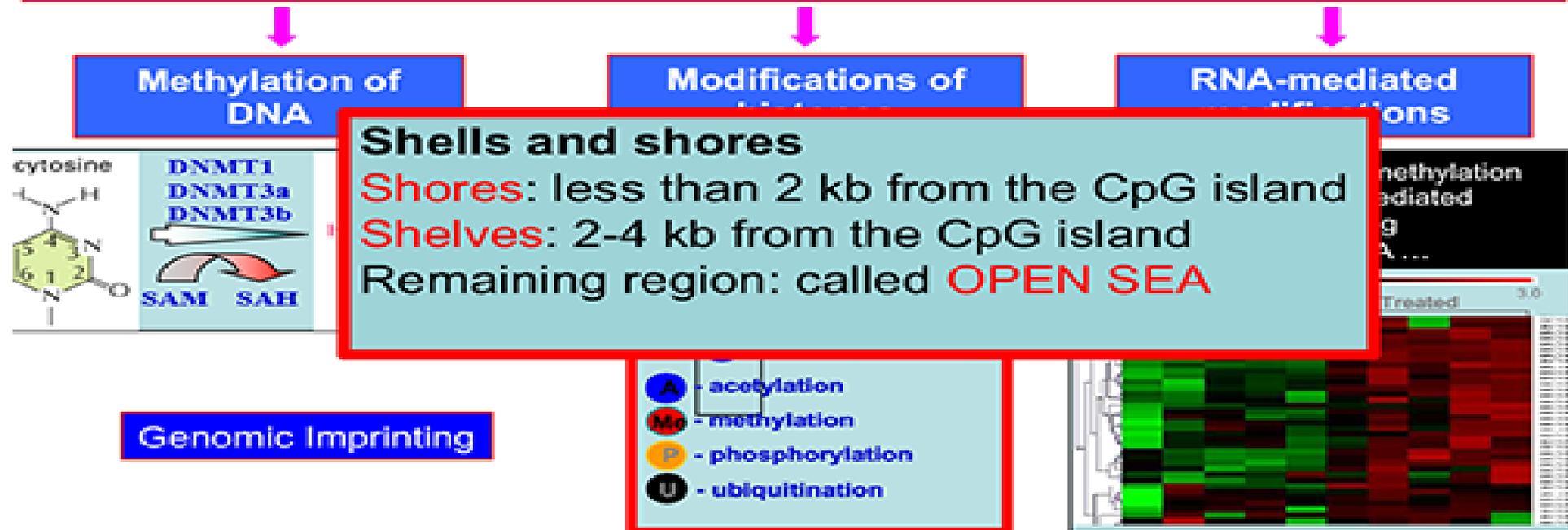
DNA methylation



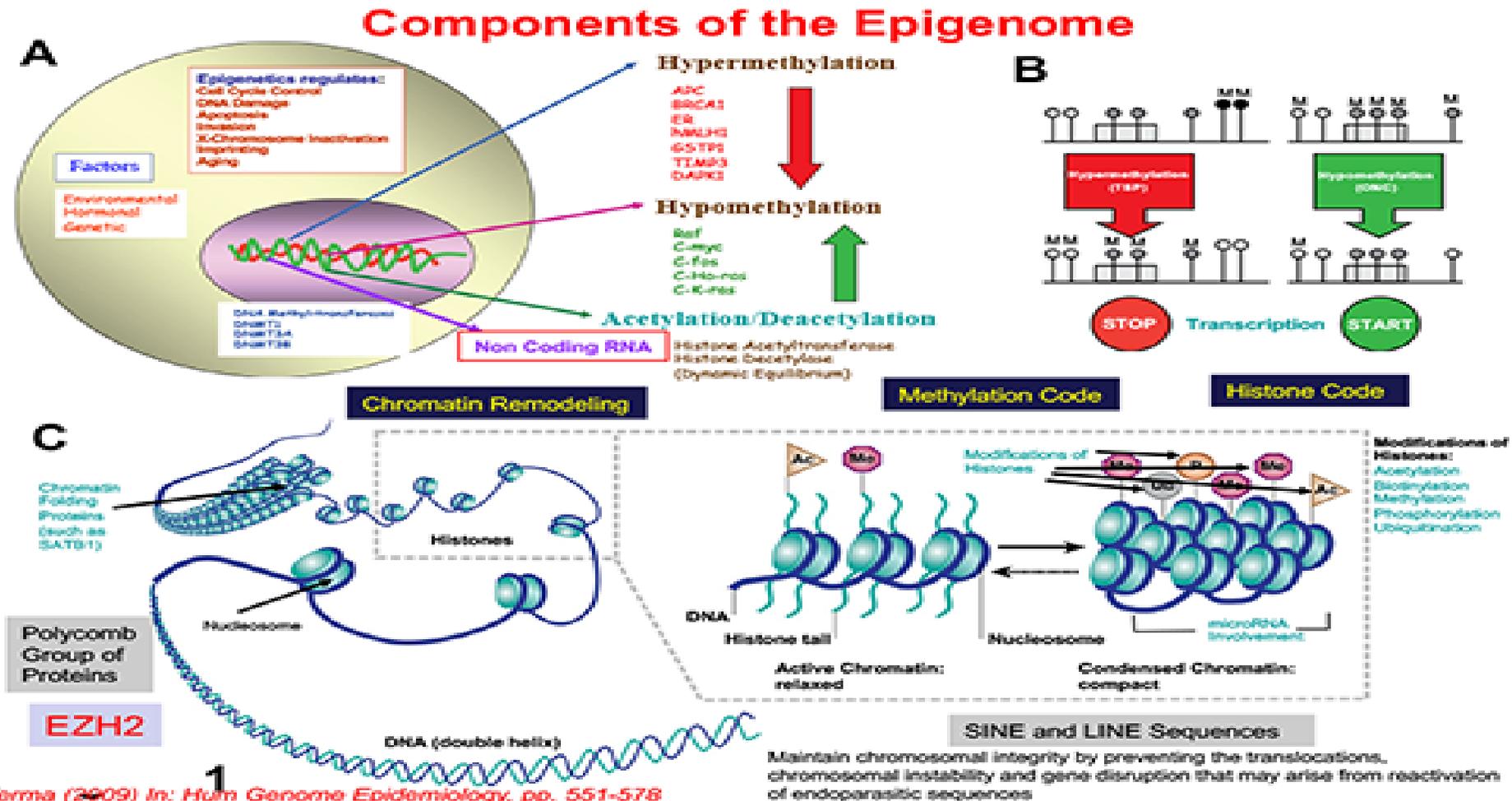
Epigenetics

EPIGENETICS

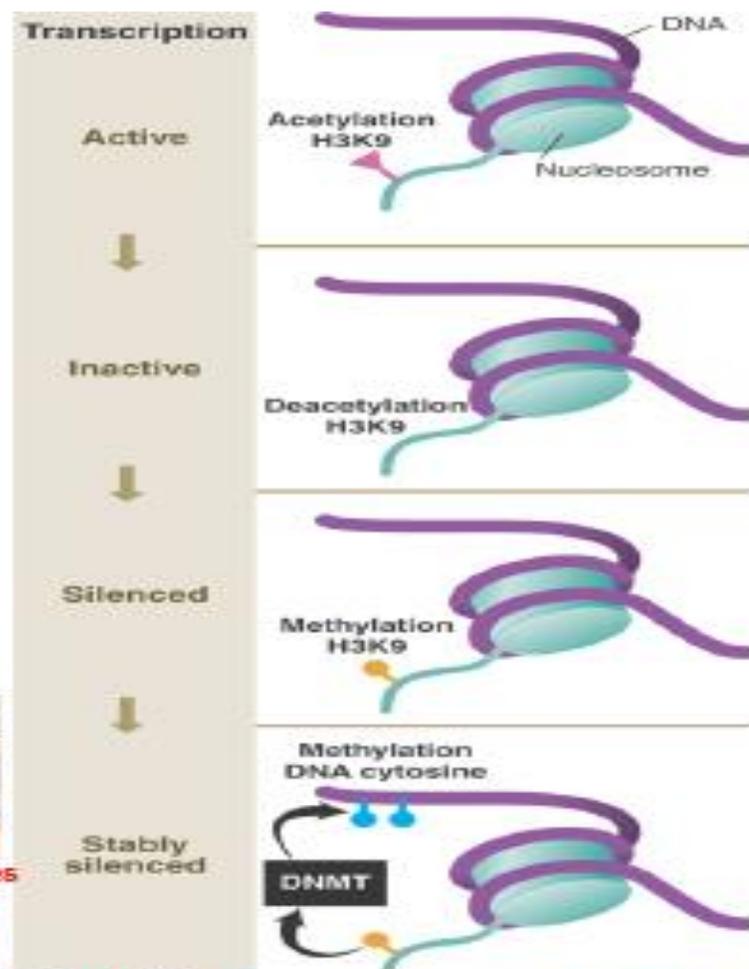
Epigenetic alterations – changes induced in cells that alter expression of the information on transcriptional, translational, or post-translational levels without change in DNA sequence



Epigenome components



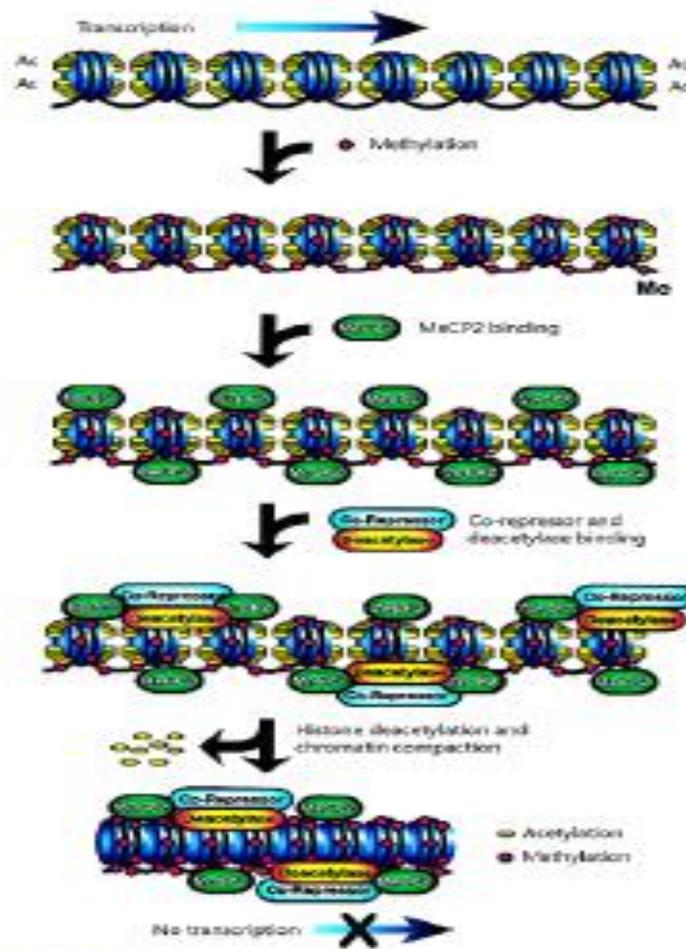
Methylation



Peter Jones



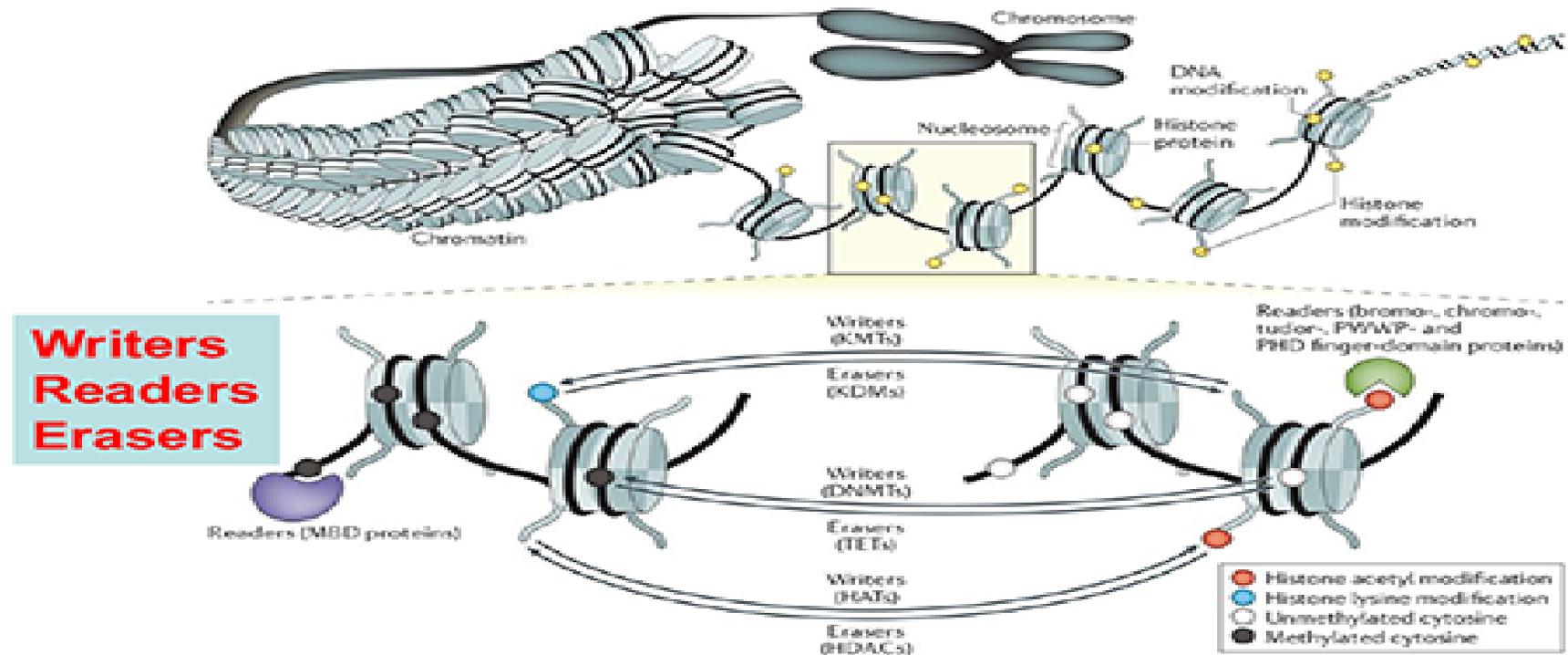
Andrew Feinberg



Chromatin modifications

Figure 1 : Modulation of covalent modifications on chromatin.

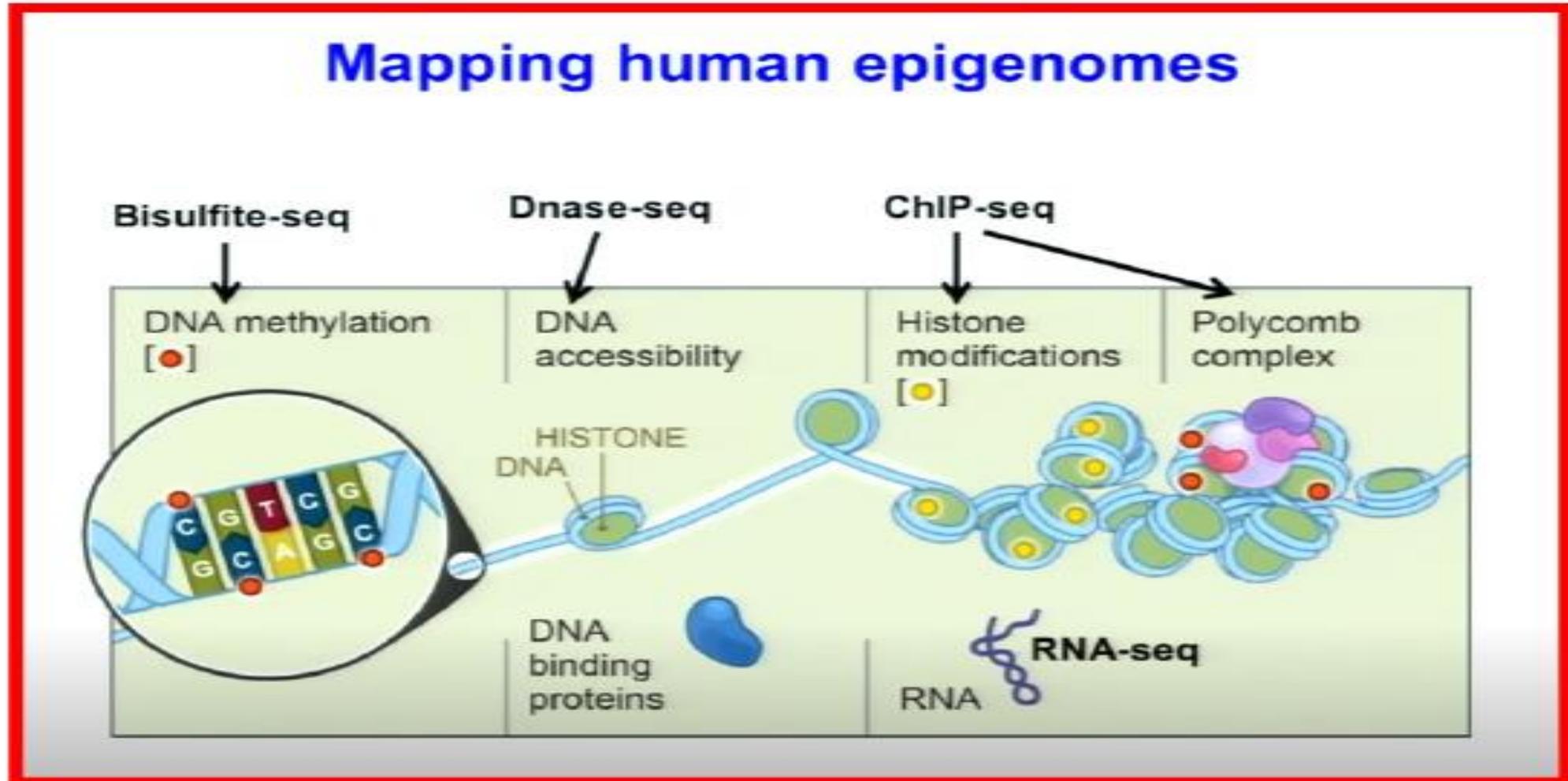
From Targeting the cancer epigenome for therapy



Writers
Readers
Erasers

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

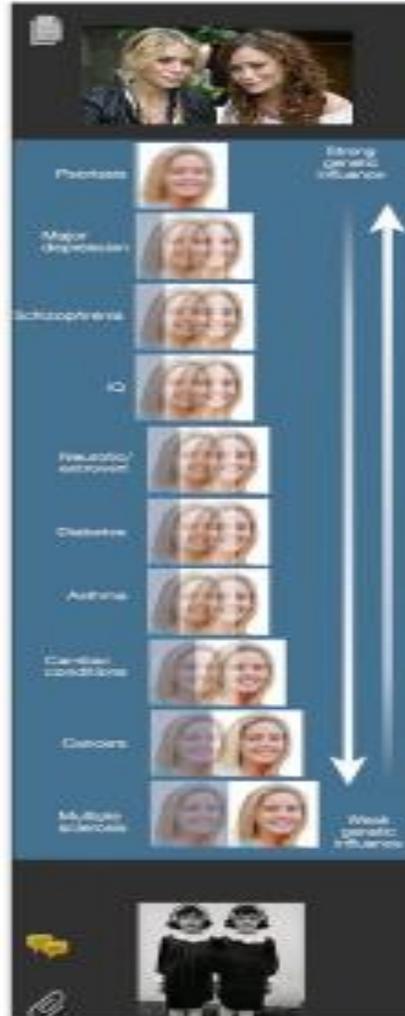
Epigenomes



Genome versus epigenome



Genome vs. epigenome – why is it important?



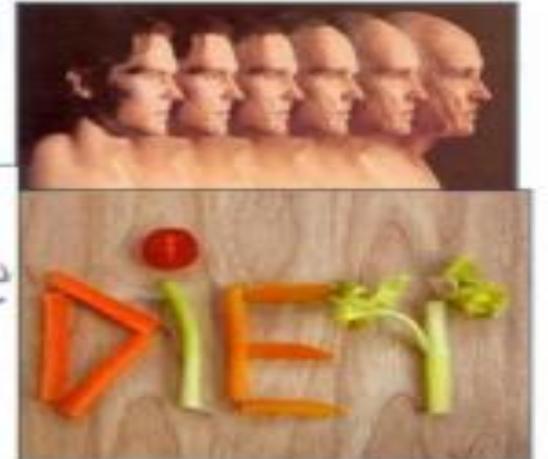
• Genome is generally constant; epigenome changes

- Age
- Diet
- Disease
- Lifestyle
- Environment



• Areas of interest:

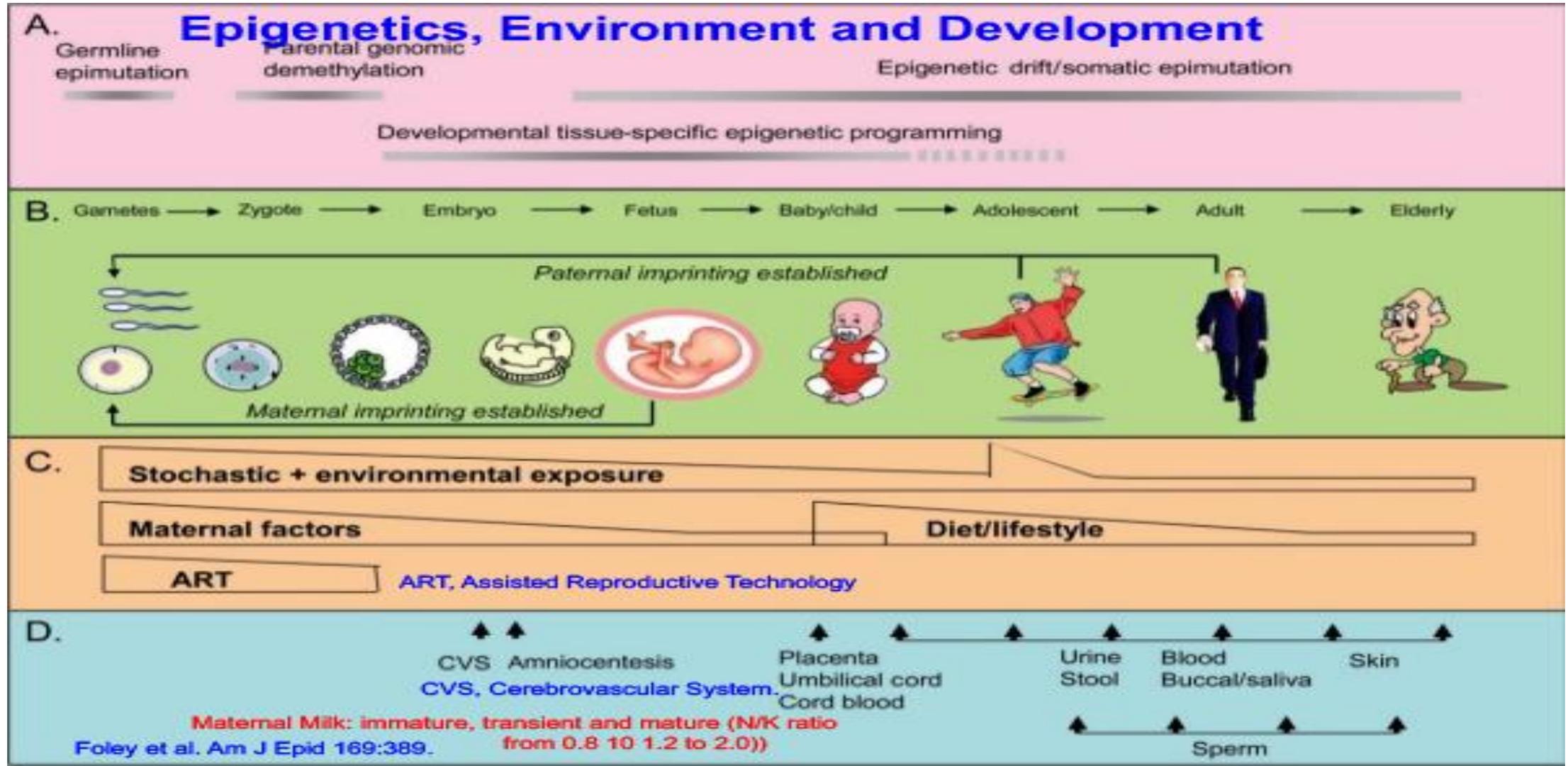
- Molecular basis of disease
- Biomarker identification
- Diagnostics development
- Drug targeting



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

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 Ughetto,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}, Porsatny AL^{1,2}, Collier F^{4,5}, Burger 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 and differentially methylated regions (DMRs) of IGF2 (DMR0) are differentially methylated in cord blood of offspring. Maternal depression, anxiety and perceived stress during pregnancy are associated with IGF2/H19 methylation in the Infant Study (n=576). DNA methylation was measured in

Chapter 29

Epigenetic Regulation in Biopsychosocial Pathways

Kristin Litzelman and Mukesh Verma

Abstract

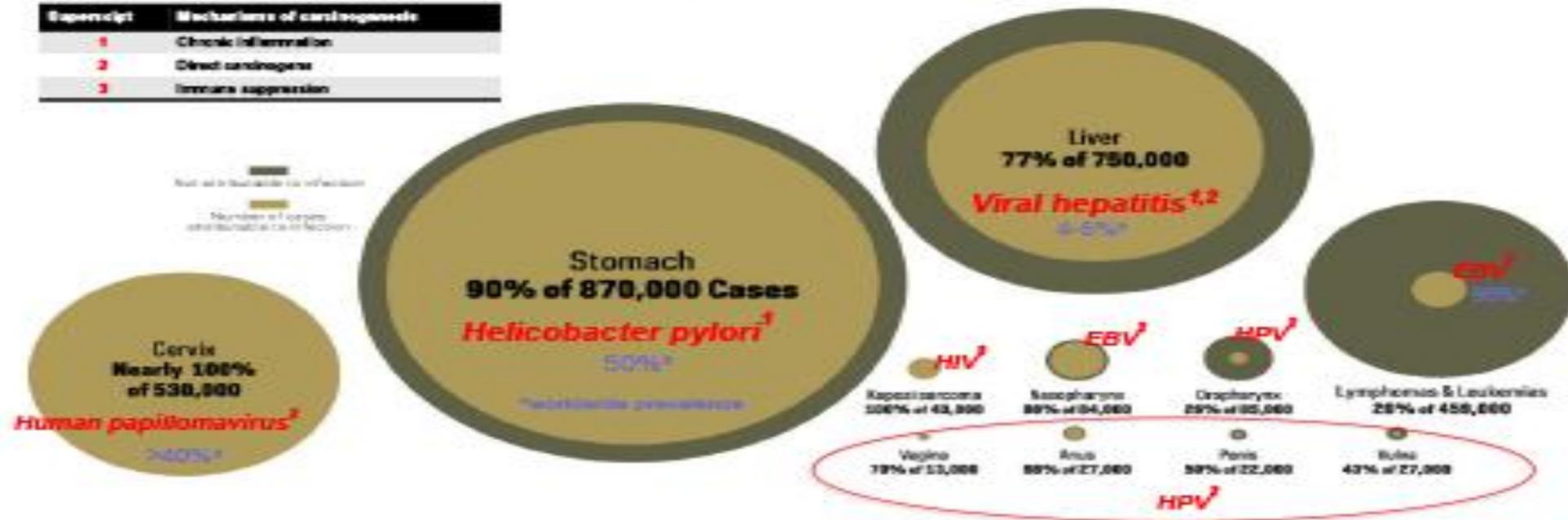
Theory and empirical evidence suggest that psychological stress and other adverse psychosocial experiences can contribute to cancer progression. Research has begun to explore the potential role of epigenetic changes in these pathways. In basic, animal and human models, exposure to stressors or to the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

Cancer Prevention Fellow

Infectious agents

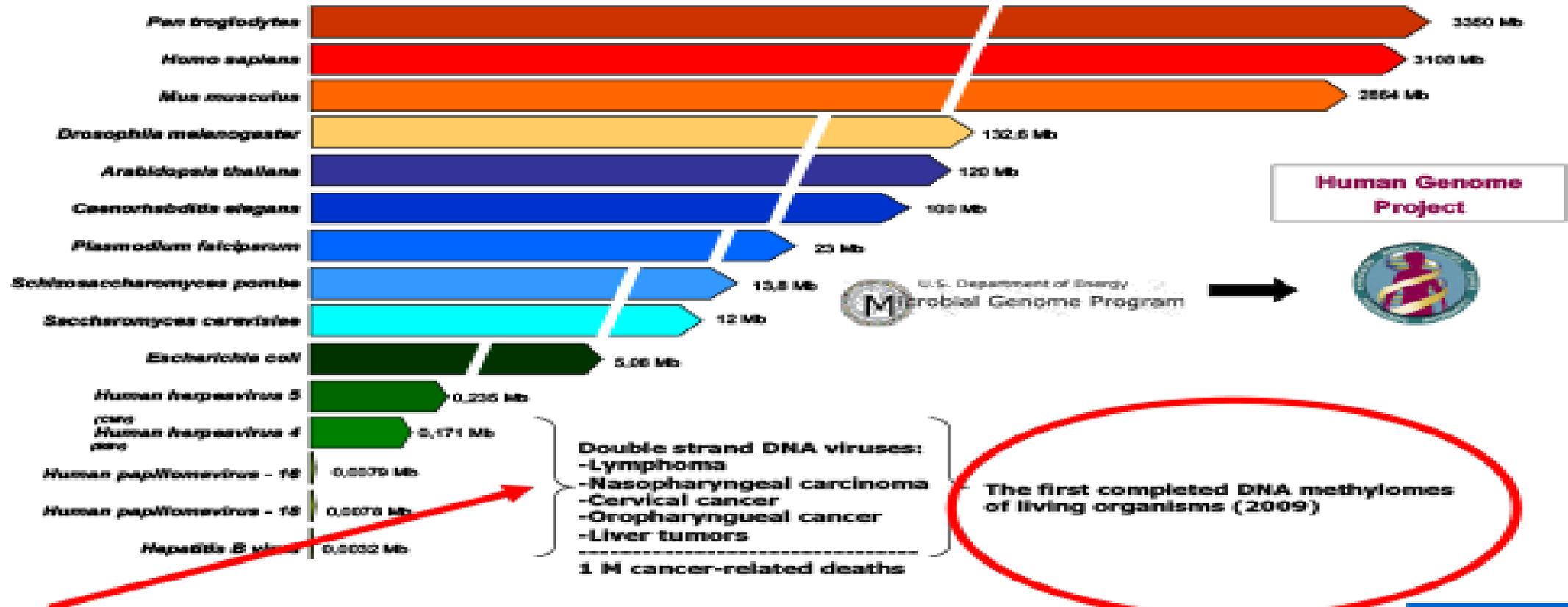
Infectious Agents: Etiologic Role in Cancer and Prevalence

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

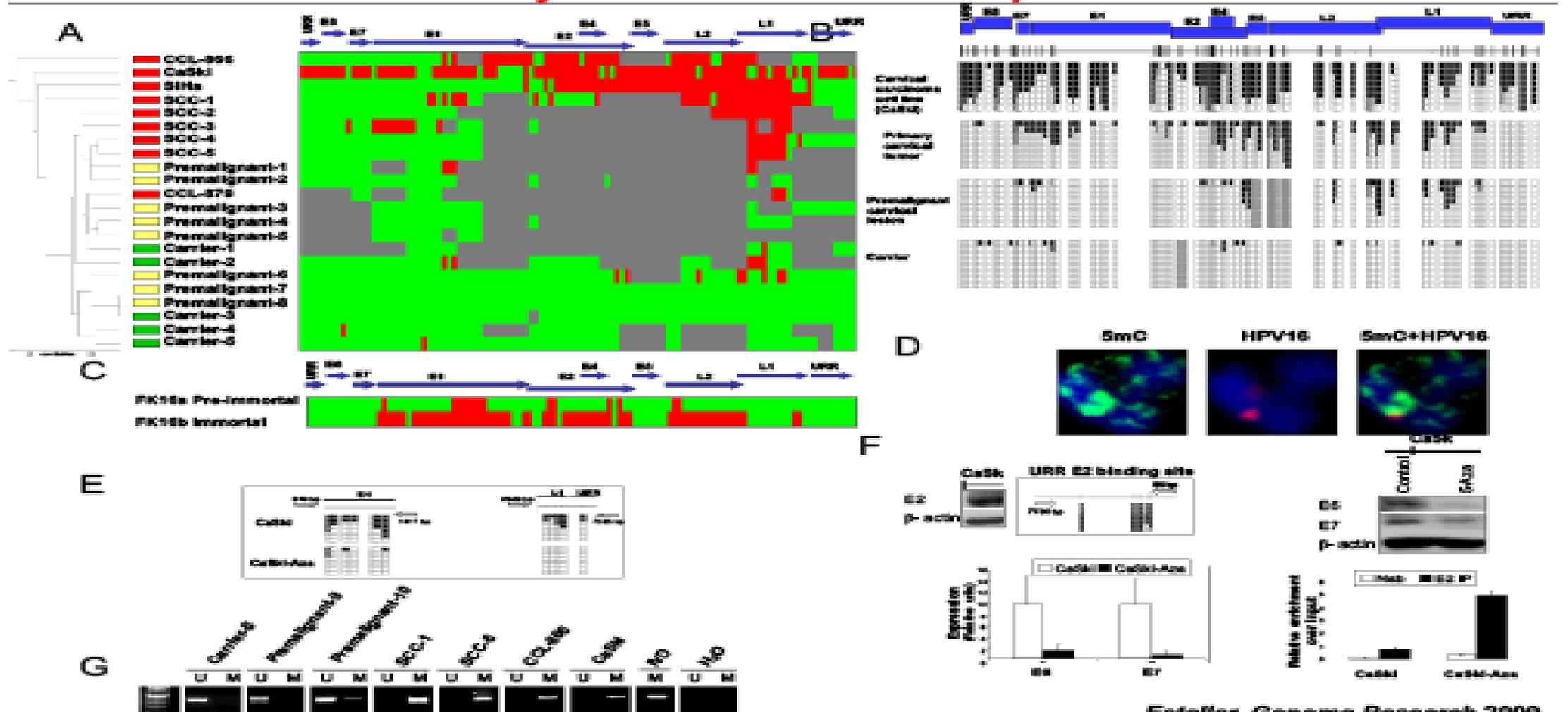
Complete methylome of HPV, EBV,
and HBV.

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;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 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 Lan², 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



Prof. Sherr

Loss (or Gain) of gene function in cancer

Most permanent

Most dynamic



Deletion
Point mutations
Amplification
Chromosomal
Translocation
(Ig rearrangement)

Genetic

Chromatin
Changes
Promoter
Methylation
Silencing

Epigenetic

Transcription
Factor
Changes

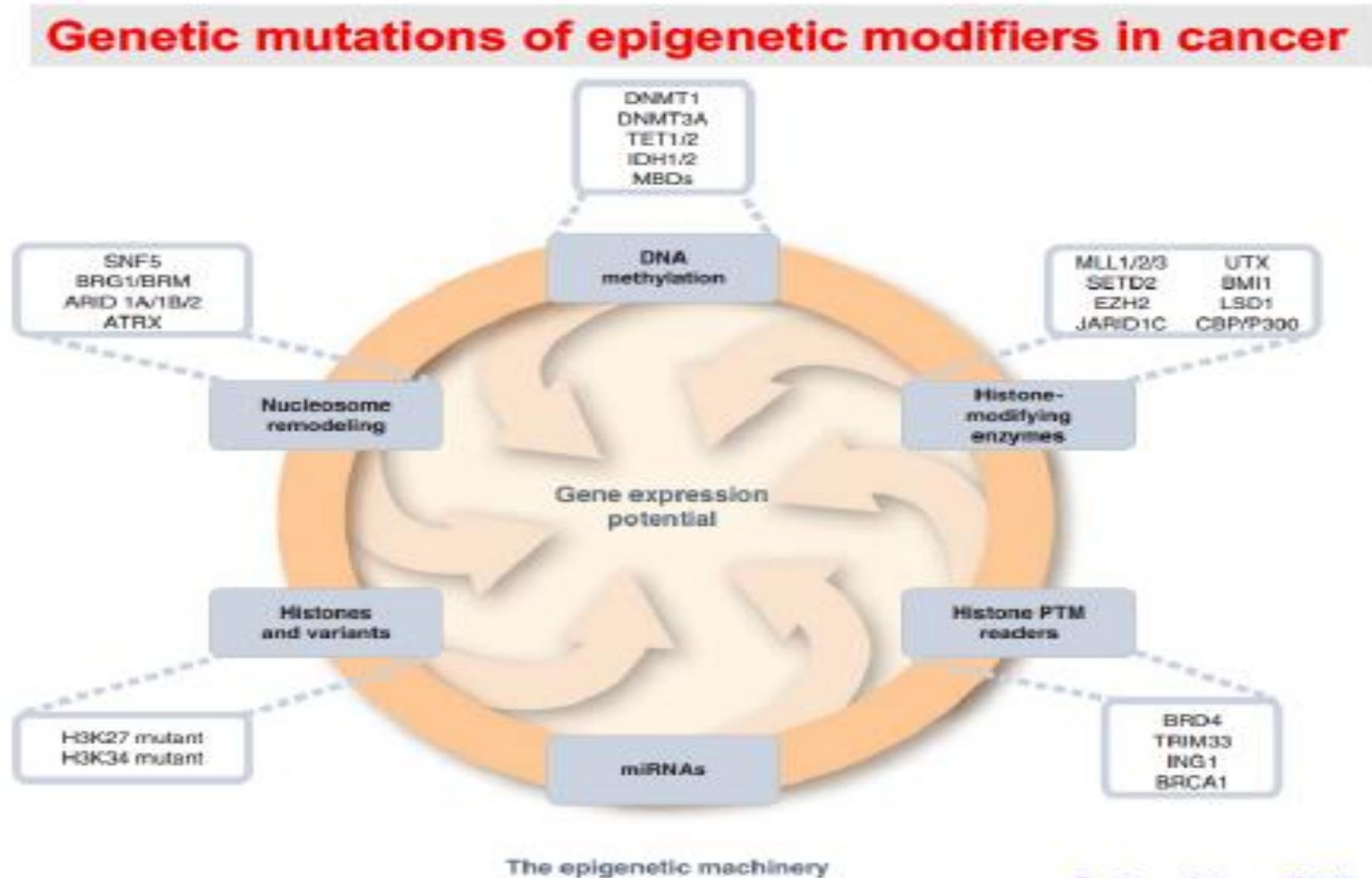
Cell-cycle
Regulated
Changes

Lerman Chew 125:1199-1225



Lerman Lerman

Genetic mutations



Hypomethylation

PubMed
25 National Center for Biotechnology Information

Advanced

Format: Abstract

Send to

December 2014; Volume 20(12); Pages 1940-1946 (17 pages)

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

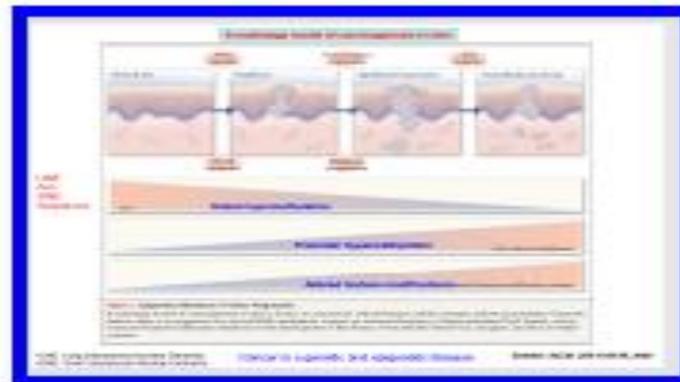
Shankar A, Zou J, Gao S, Devisser L, Srinivasan S, Srinivasan S, Gao S, Gao S, Shankar A, Shankar A.

Author information

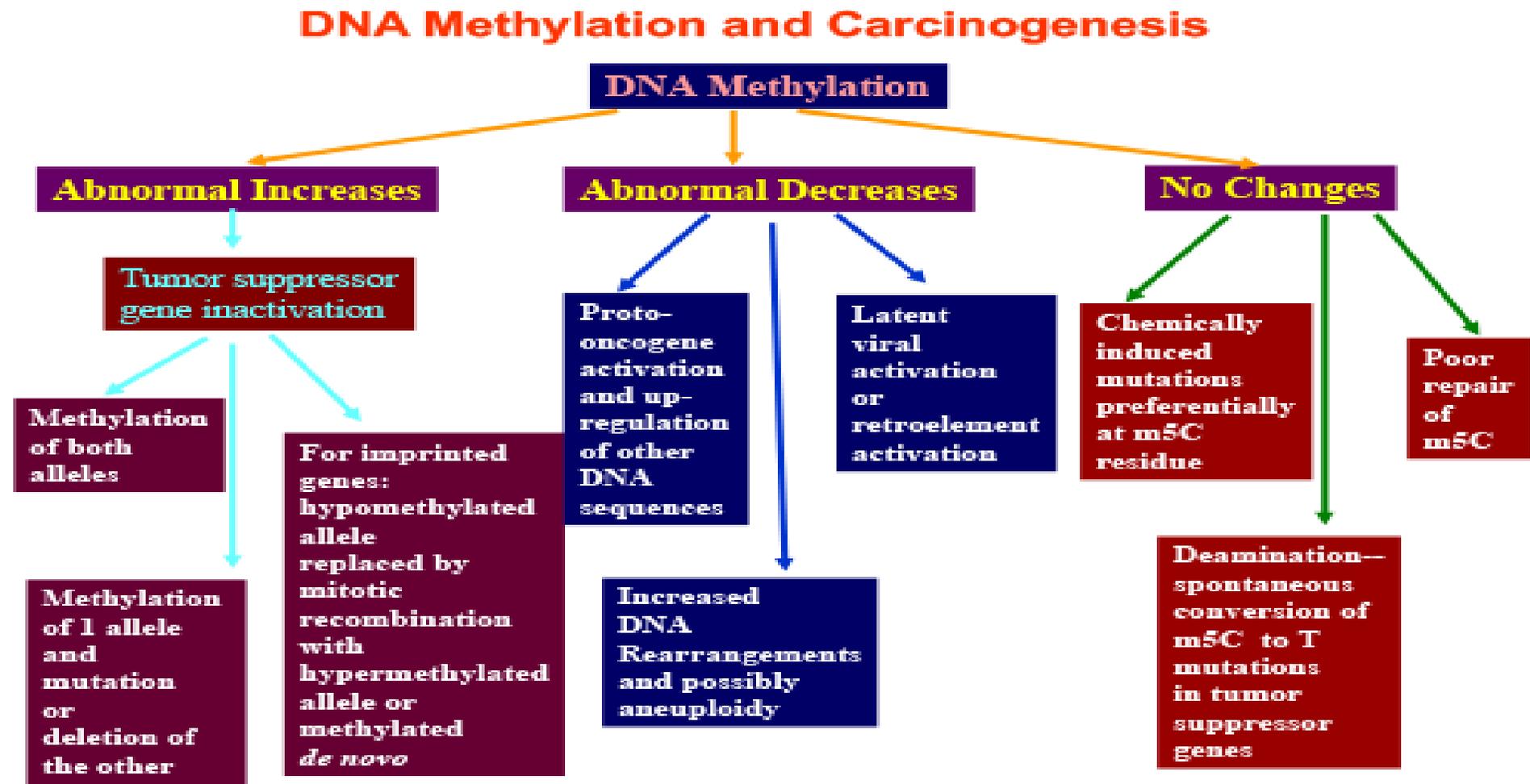
Abstract

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.

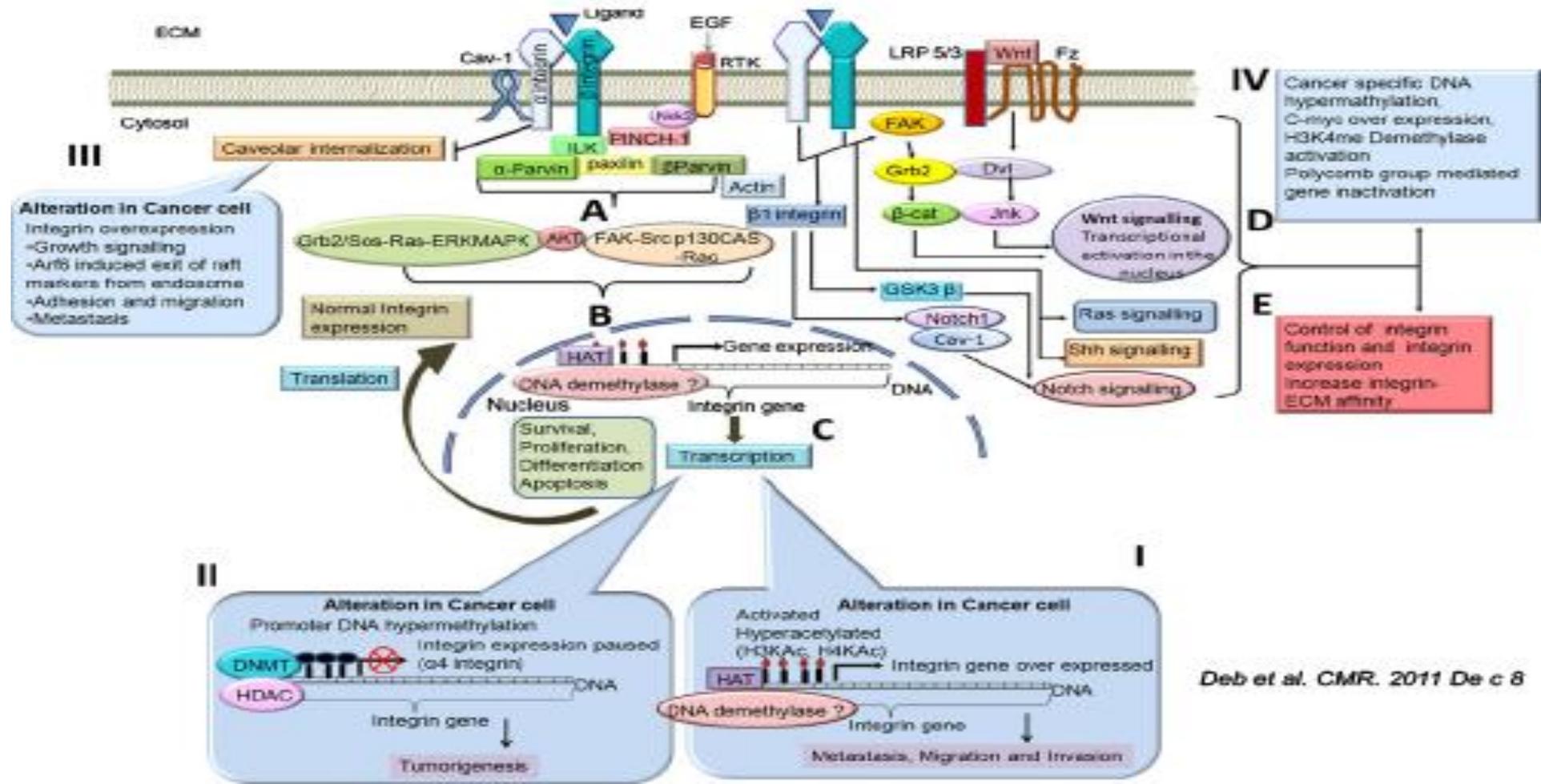


DNA methylation and carcinogenesis



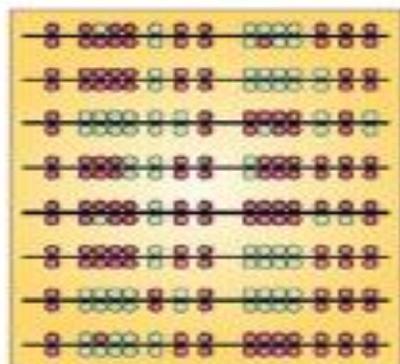
Integrin signaling

Integrin Signaling Network and Epigenetic Regulation

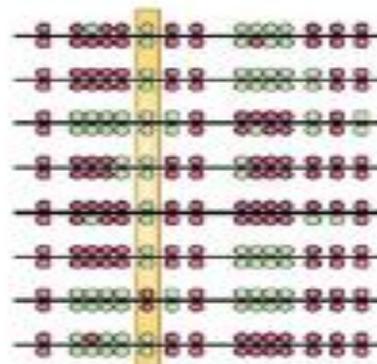


Methylation

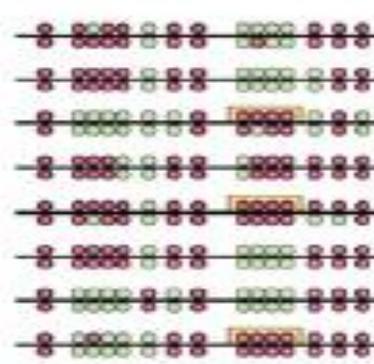
a Methylation content



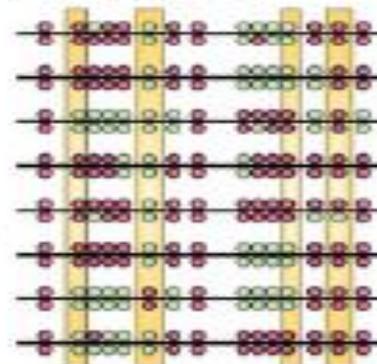
b Methylation level



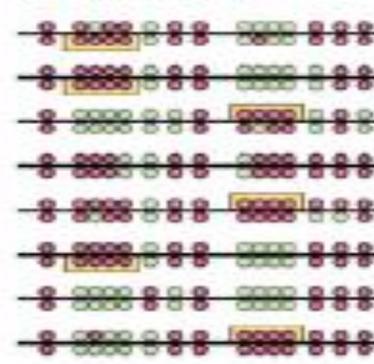
c Methylation pattern



d Level profile



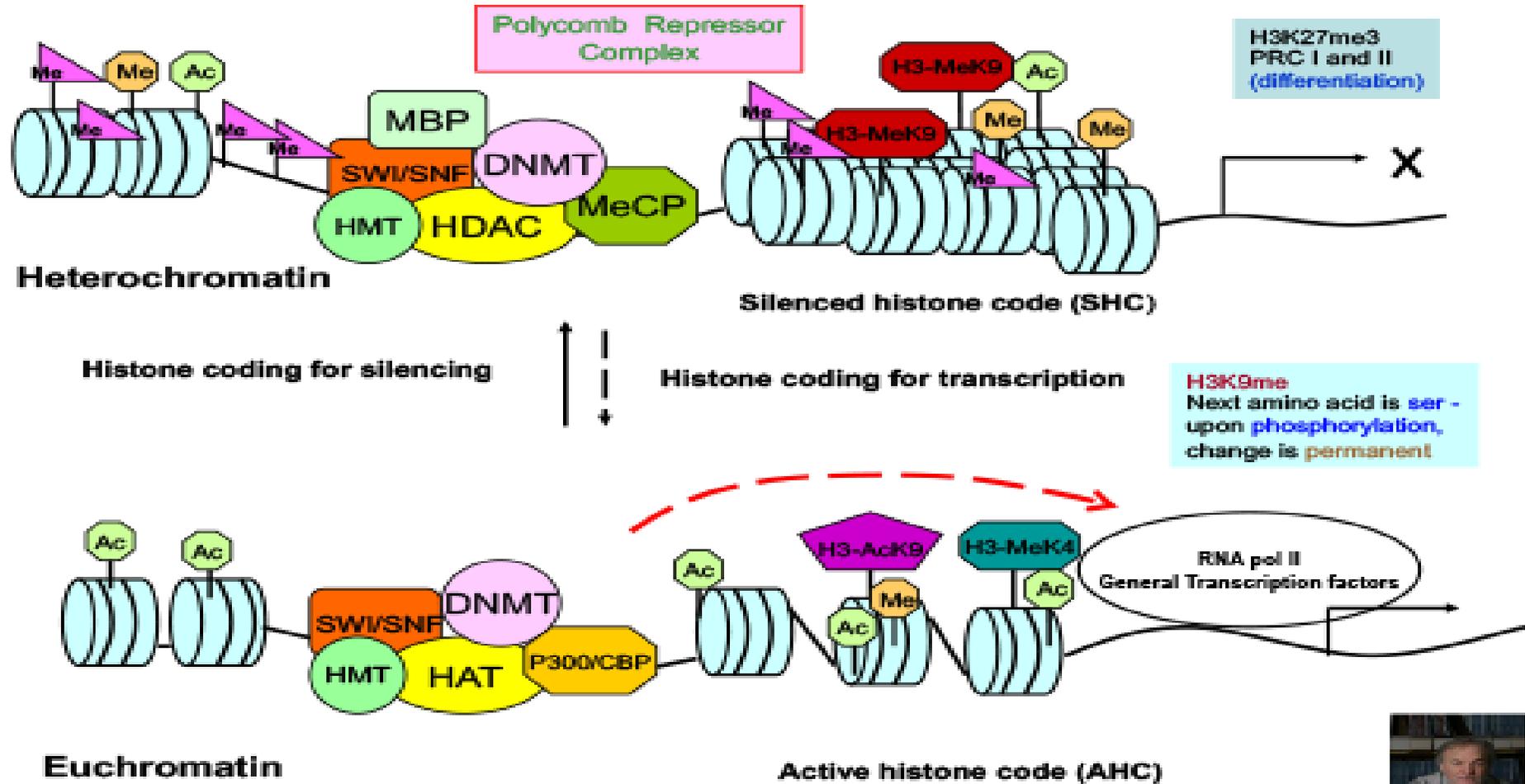
e Pattern profile



- 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

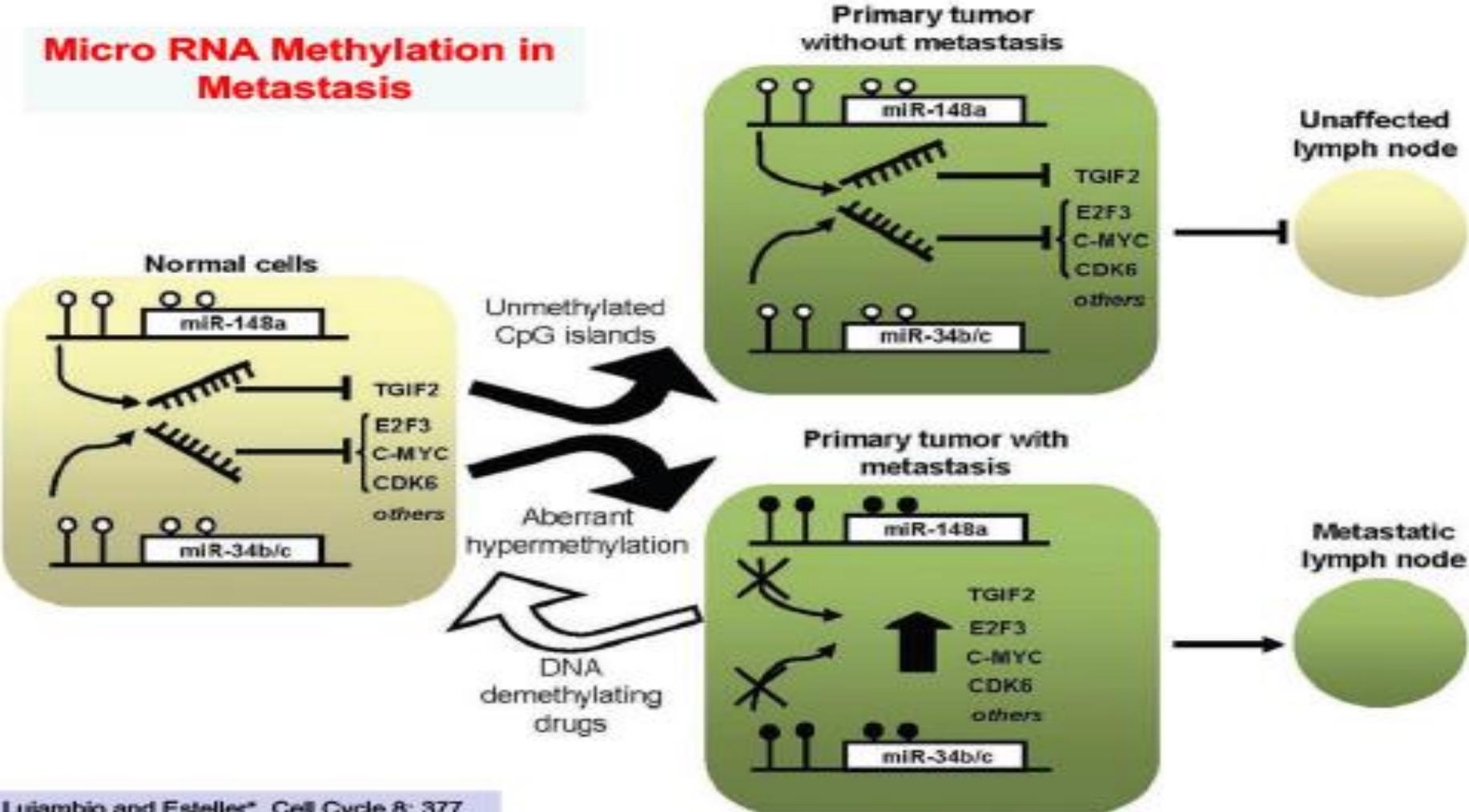
Mirco RNA Signatures in Human Cancers



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

RNA epigenetics

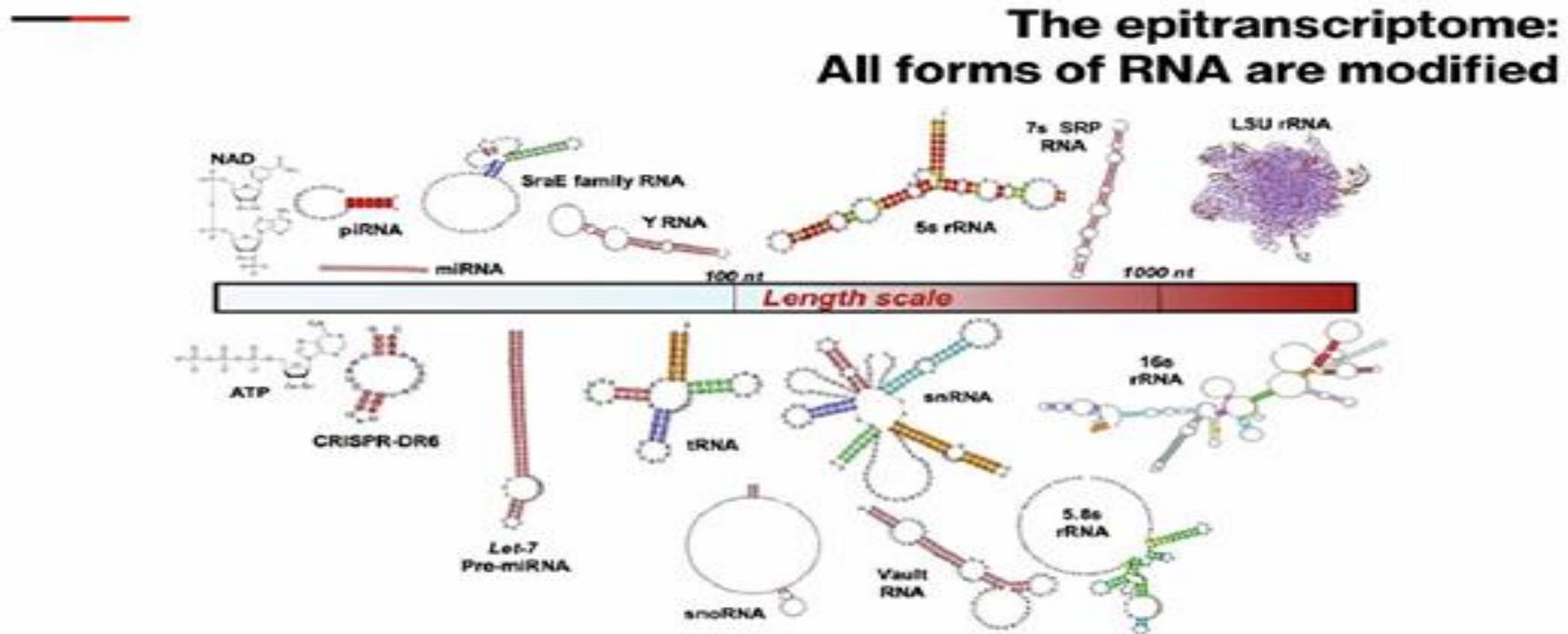
Epitranscriptomics or RNA Epigenetics

- Epitranscriptomics, also known as RNA epigenetics, is the study of chemical modifications to RNA molecules that occur after transcription.
- Can affect the structure of RNA, its stability, and how it's translated.
- Can also impact gene expression and regulation.

Some examples of RNA modifications include:

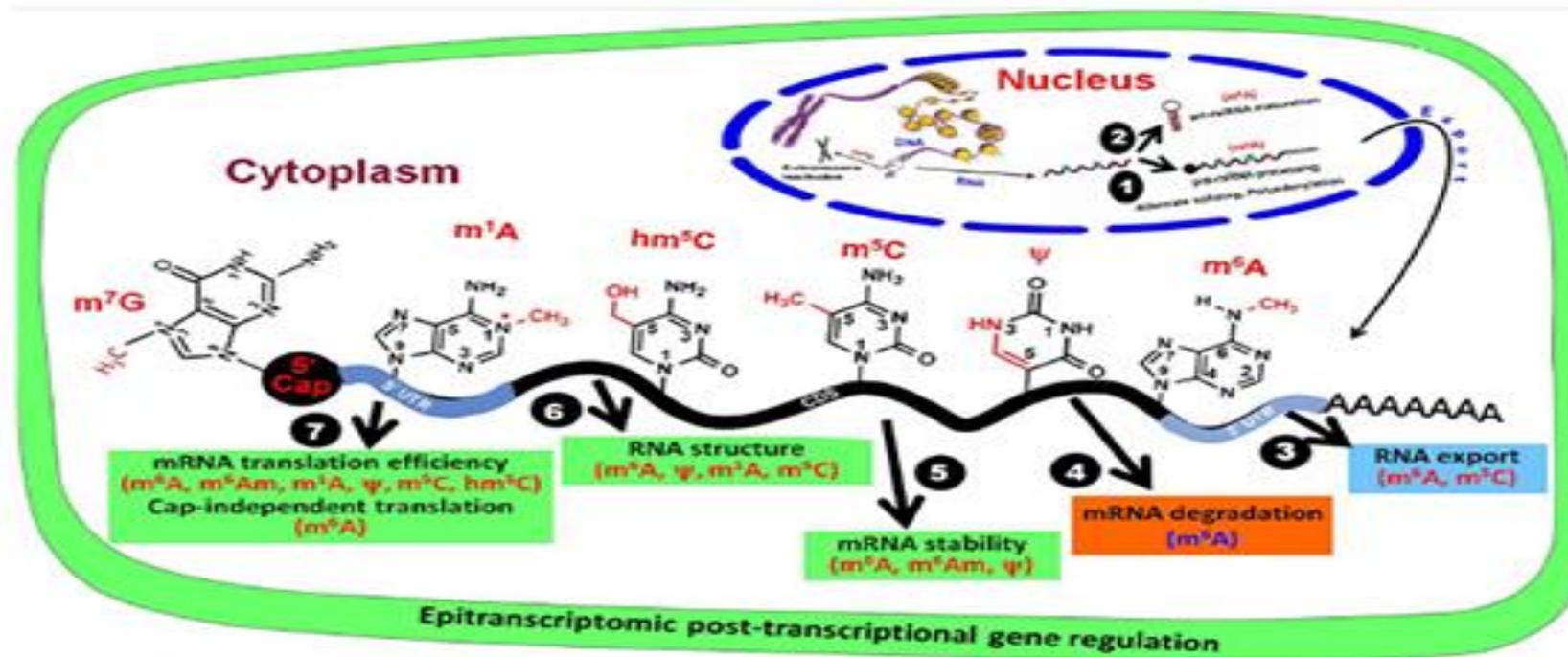
- N6-methyladenosine (m6A),
- N1-methyladenosine (m1A),
- 7-methylguanosine (m7G), Pseudouridine (Ψ),
- 5-methylcytidine (m5C).

Epitranscriptome

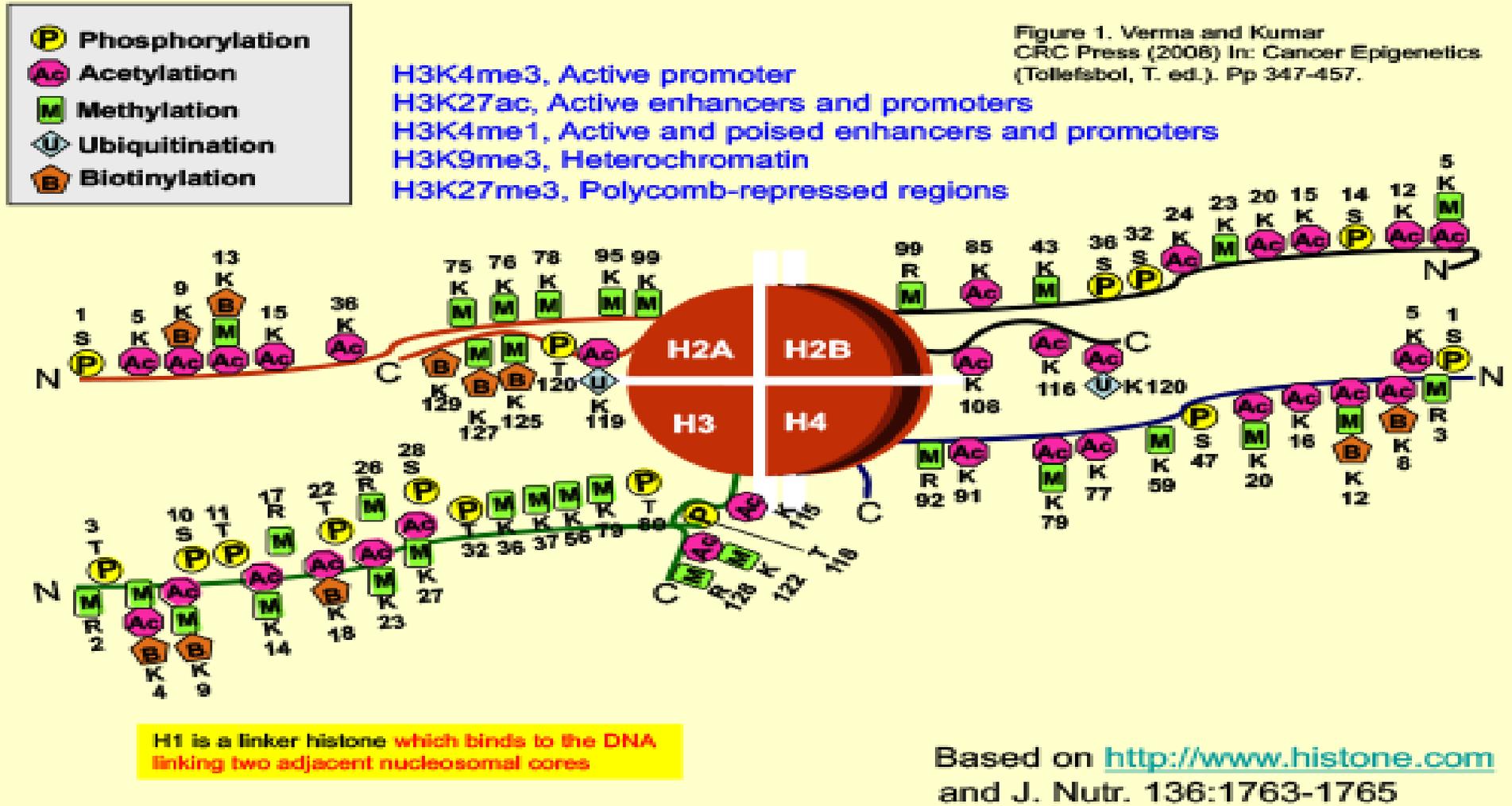


RNA epigenetics

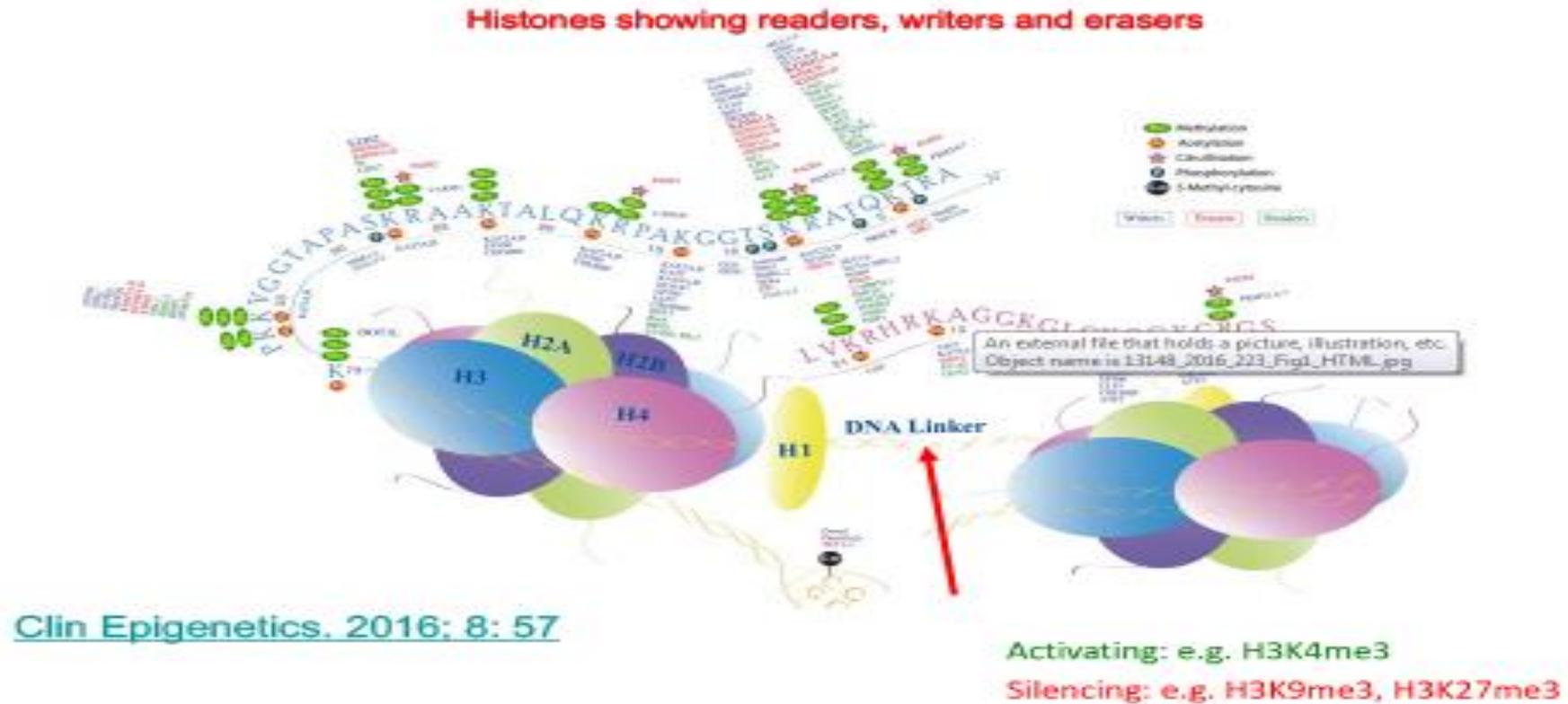
Gene Regulation by RNA Epigenetics (Epitranscriptomics)



Histone modifications



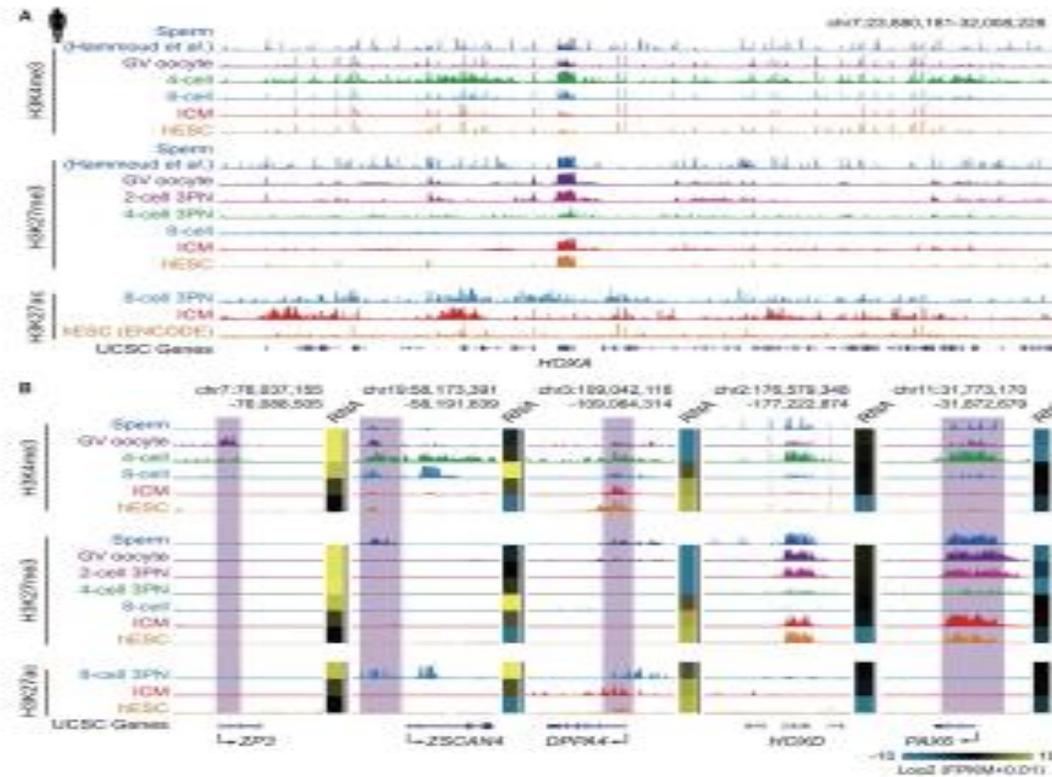
Histones



Histone modifications

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

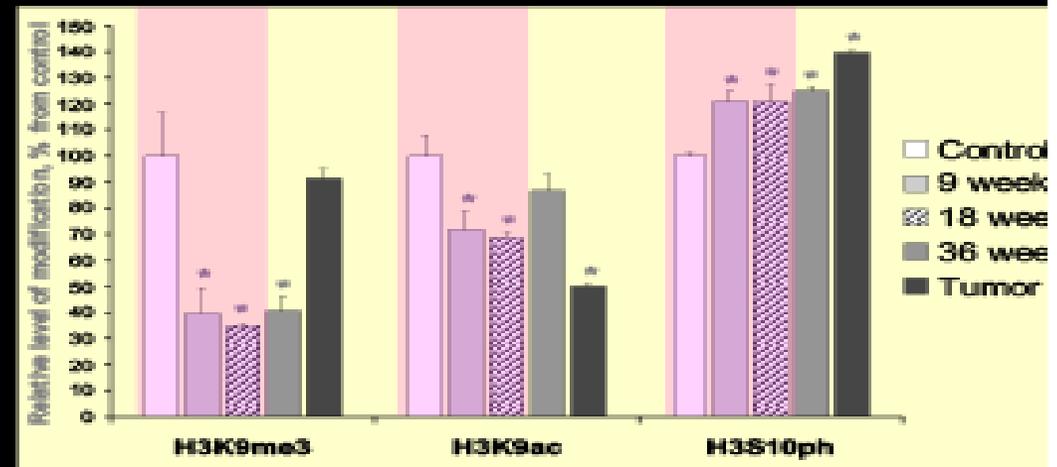
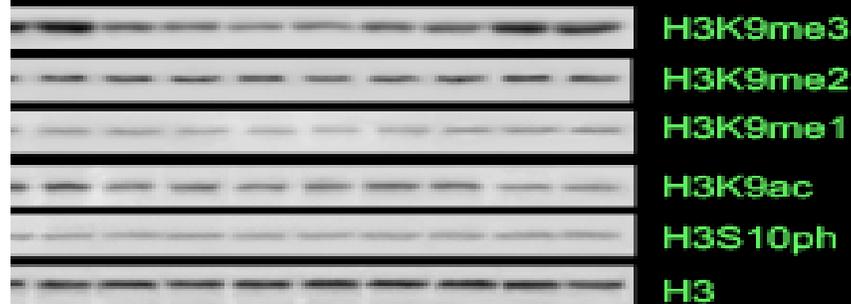
Histone mapping



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

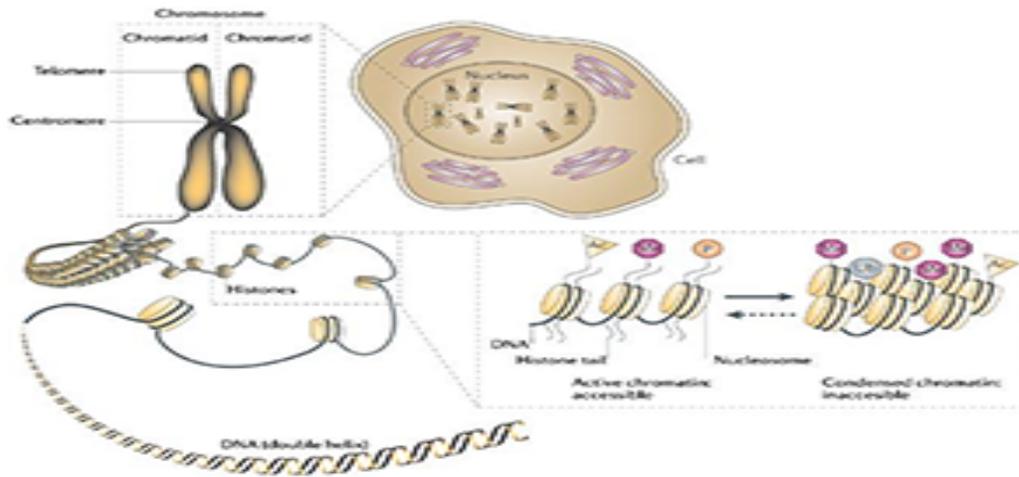
Epigenetic Gene Regulation:

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

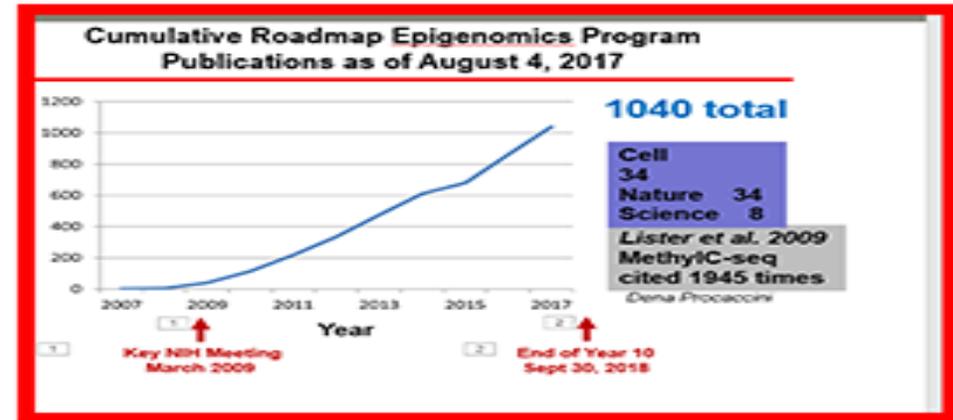


Epigenetics roadmap

Epigenetics Roadmap



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Nature Reviews | Cancer



Epigenetically Regulated Diseases:

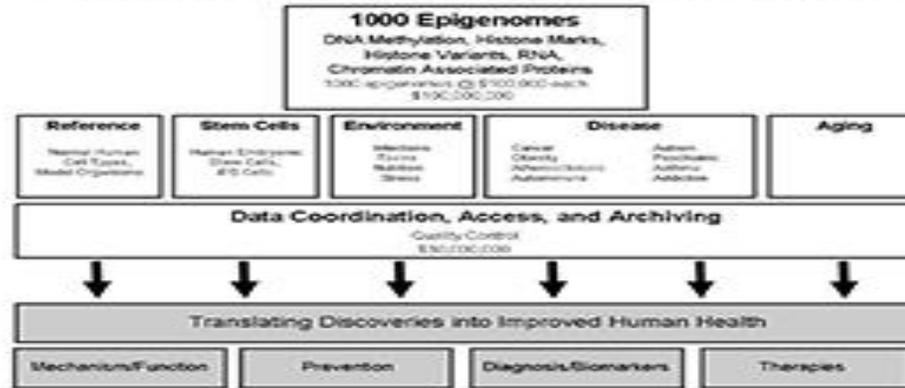
Several cancers, autoimmune disorders, reproductive disorders, and neurobehavioral and cognitive dysfunctions

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/>

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

CONTENTS

20.1	Background	347
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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

20.3.2	Breast Cancer	350
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

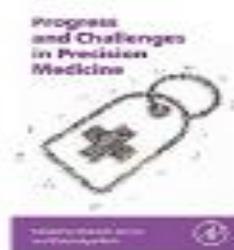
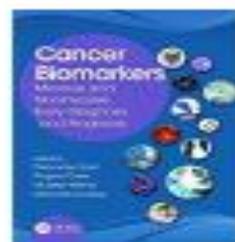
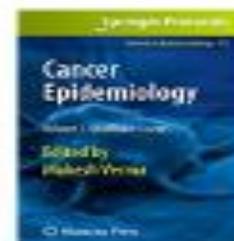
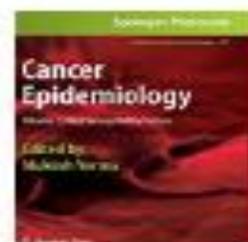
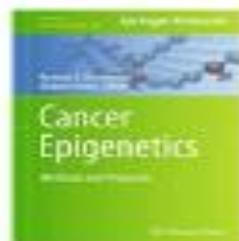
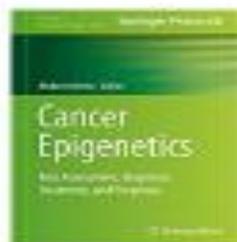
Diagnosis

Prognosis

Screening

Follow up treatment and co-morbidity

Books



Books edited by Mukesh Verma

Epigenetic changes

The image is 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 hypermethylation².

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".

Tumors and epigenetics

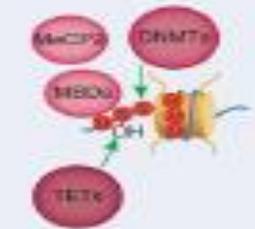
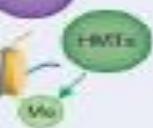
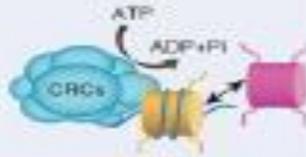
Tumor Types and Genes Regulated by Epigenetic Mechanism

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: 333-343
 Venkov et al (2004) *Crit. Rev. Clin. Sc.* 41: 355-607
 Venkov and Mladenov (2006) *Crit. Rev. Hematol. Oncol.* 60:9-18
 Venkov et al (2006) *Mol. Diag. Therapy* 10:1-13

Histone enzymes

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

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 Manne (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	

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

- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting

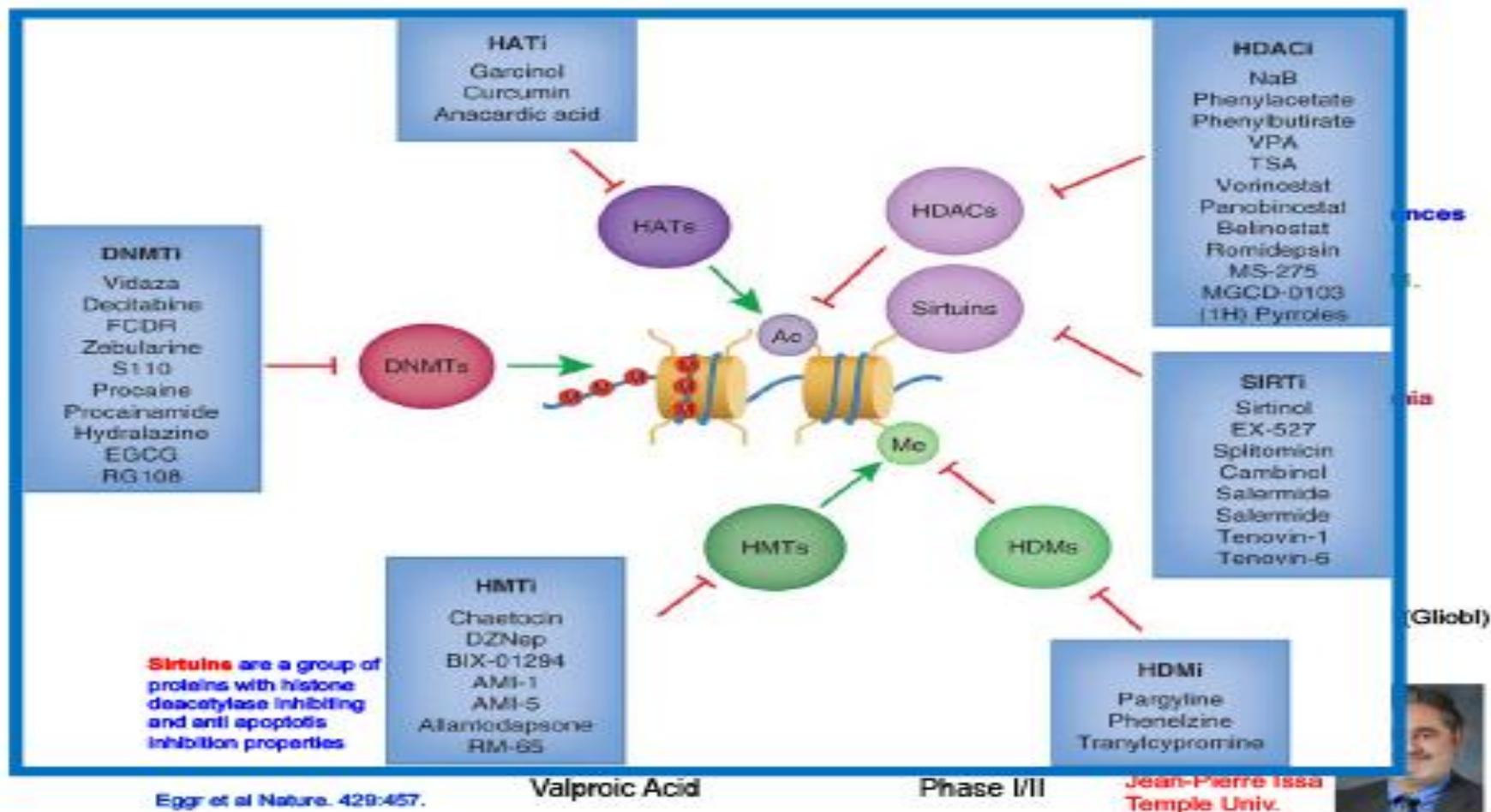
SAHAs are a group of proteins with histone deacetylase inhibiting and anti-apoptosis inhibitor properties

Epigenetics Nature 429:457

Jean-Pierre Issa
Temple Univ.



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-23 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%

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

HDAC (**entinostat**)

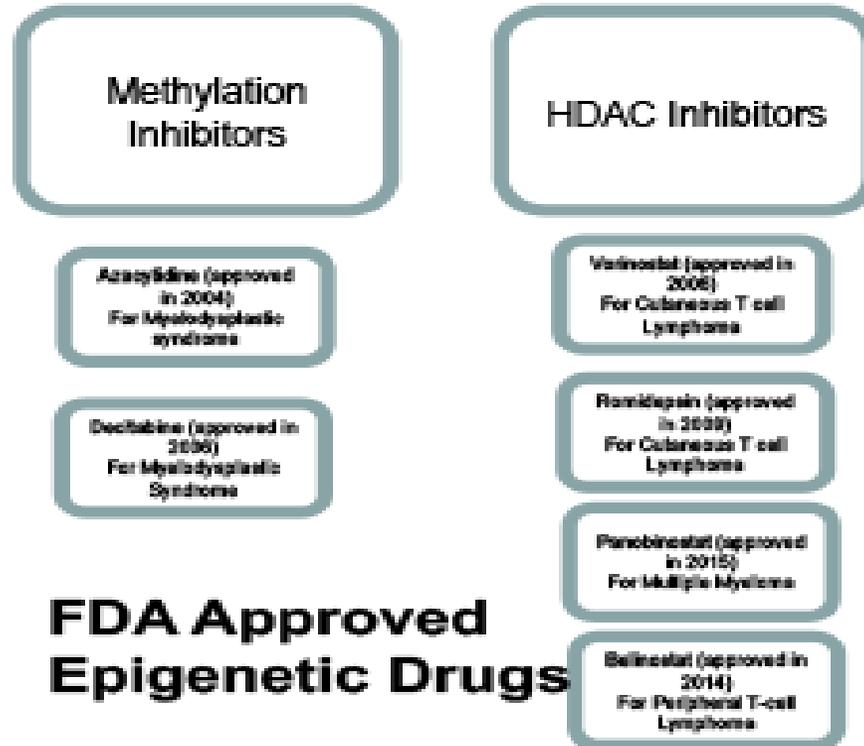
Drug causing chemical change to stop tumor cell from dividing
Checkpoint inhibitors

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

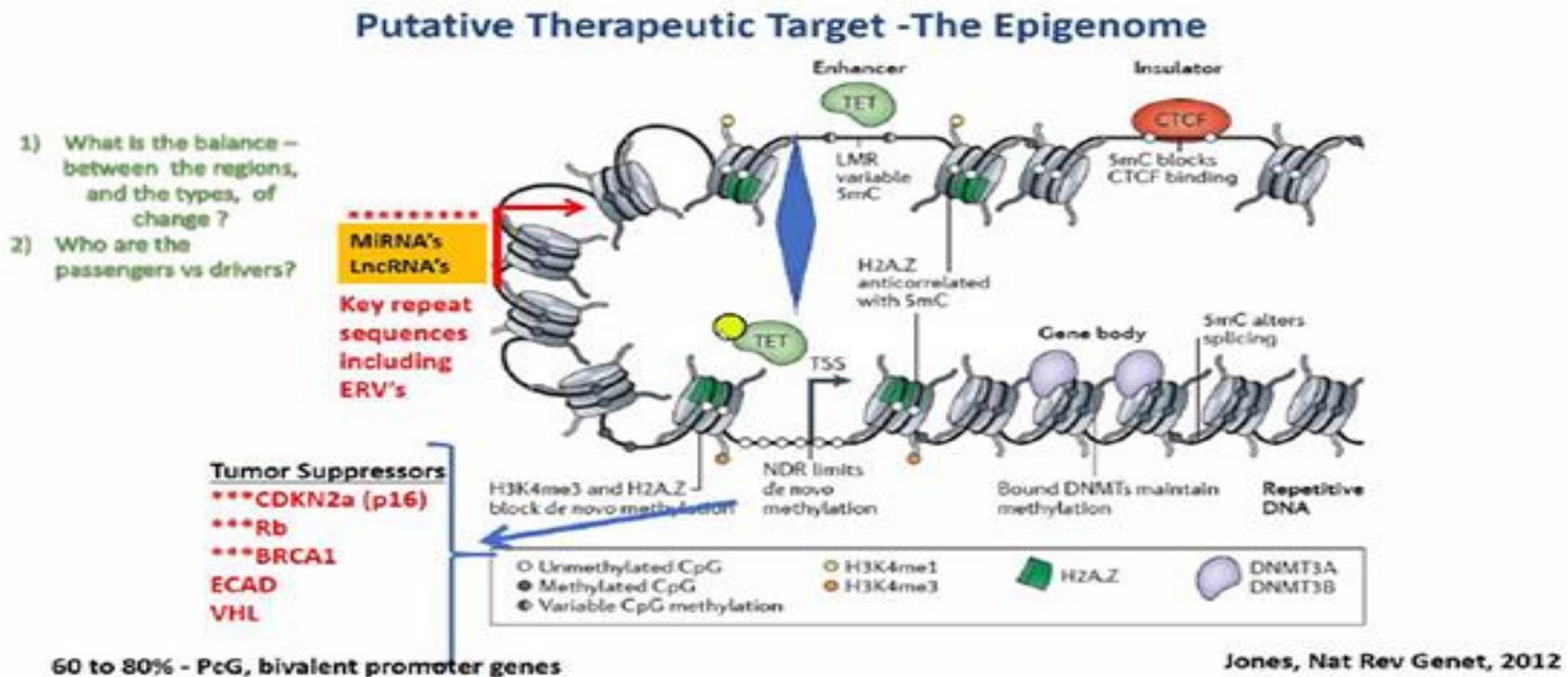
CTLA-4 inhibitor **ipilimumab**

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

Approved epigenetic drugs



Therapeutic target



Epigenetics of cancer type

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, 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, CRBP1, GSTP1, APC
Lymphoma	p16, p15, CRBP1, MGMT, DAPK, p73
Lung	p16, p14 ^{ARF} , CRBP1, MGMT, GSTP1, DAPK, FHIT, TIMP-3, RARBeta, RASSF1A
Ovary	p16, BRCA1, DAPK
Pancreas	p16, MGMT, APC
Prostate	GSTP1, p27(kip1)
Stomach	p14 ^{ARF} , P16, APC, hMLH1, MGMT
Uterus	p16, p14 ^{ARF} , MGMT

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

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

AML subtypes and 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

Cancer letters 17 July 2018

Combination epigenetic therapy



The screenshot shows a PubMed article page. The title is "Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer." The authors listed are Juergens RA, Wrange J, Venzetti FP, Murphy SC, Zhou N, Coleman R, Sebree R, Rodgers K, Hooker CM, Franco N, Lee R, Tsai S, Delgado E, Rudek M, Belinsky SA, Herman JS, Baylin SB, Brock MV, Rudin CM. The abstract discusses a phase III trial of combined epigenetic therapy with azacitidine and entinostat in patients with refractory advanced non-small cell lung cancer. It reports that the therapy is well tolerated and shows objective responses, including a complete response and a partial response in a patient who remains alive without disease progression approximately 2 years after completing protocol therapy. Median survival in the entire cohort was 6.4 months (95% CI 3.8-9.2), comparing favorably with existing therapeutic options. Demethylation of a set of 4 epigenetically silenced genes known to be associated with lung cancer was detectable in serial blood samples in these patients and was associated with improved progression-free (P = 0.034) and overall survival (P = 0.035). Four of 19 patients had major objective responses to subsequent anticancer therapies given immediately after epigenetic therapy. Significance: This study demonstrates that combined epigenetic therapy with low-dose azacitidine and entinostat results in objective, durable responses in patients with solid tumors and defines a blood-based biomarker that correlates with clinical benefit.

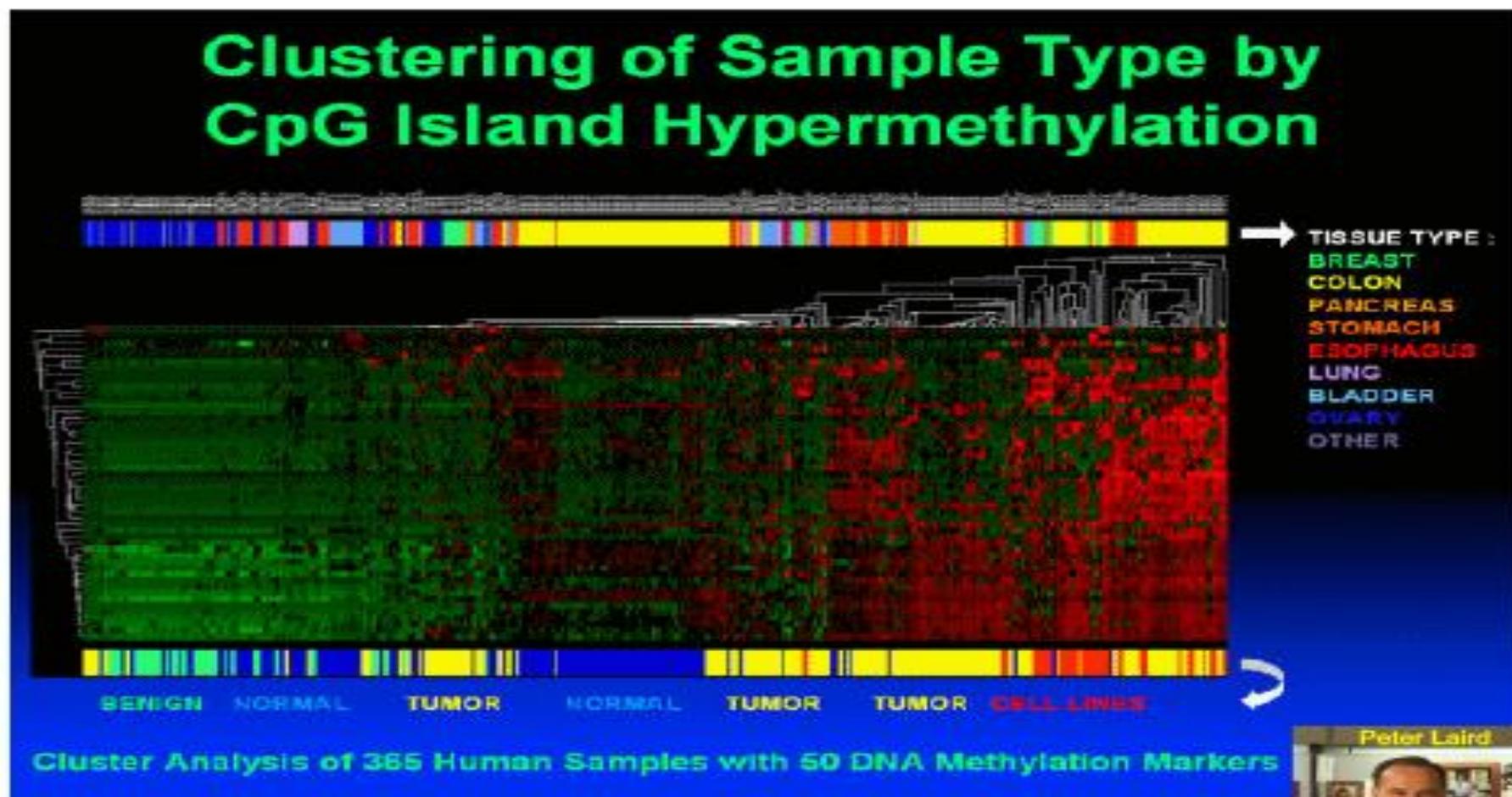
Epigenetic Therapy for Colorectal Cancer

Vivek Vaish, Tripti Khare, Mukesh Verma, and Sharad Khare

Methods Mol Biol. 2015;1238:771-82. doi: 10.1007/978-1-4939-1804-1_40

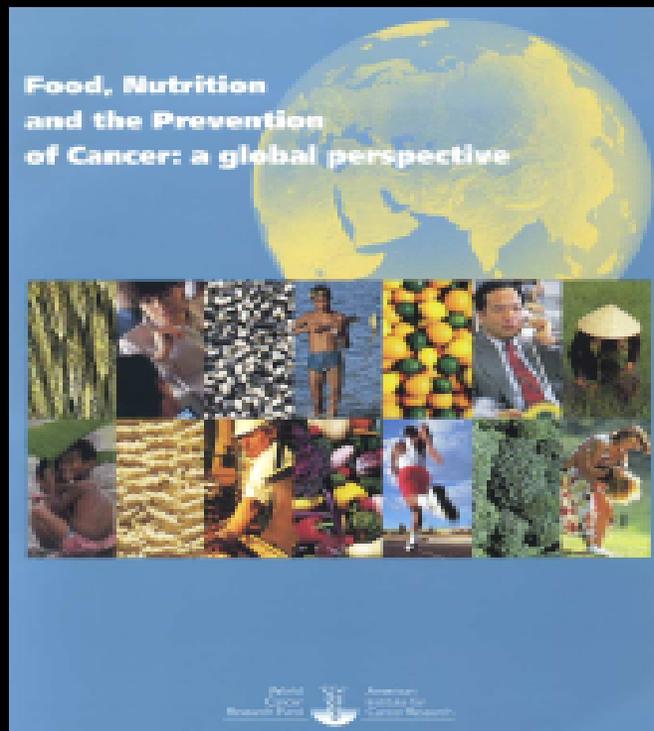
Aberrations in epigenome that include alterations in DNA methylation, histone acetylation, and miRNA (small RNA) expression may occur in the progression of colorectal cancer (CRC). These epigenetic changes

CpG island hypermethylation



Diet and cancer

DIET AND CANCER: FOCUS ON PREVENTION



Gary Pagan

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

(part of a JHEP journal communication)

Sci. Data. 2014; 3:1242 | doi: 10.1038/sdata.2014.1242 | Epub 09 June 2014

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

Shen, D¹, Wang, D¹, Chen, D²

1) Author Information

Abstract
 Hepatocellular carcinoma (HCC) is an aggressive and life-threatening disease often diagnosed at intermediate or 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 numerous 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 in vivo and in vitro **chemopreventive potential of epigenetic food components, including dietary methyl-group donors, epigenetic 3-oxo-alkane, sodium butyrate, resveratrol, curcumin, and sulforaphane, on liver carcinogenesis. Future direction and potential challenges 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.

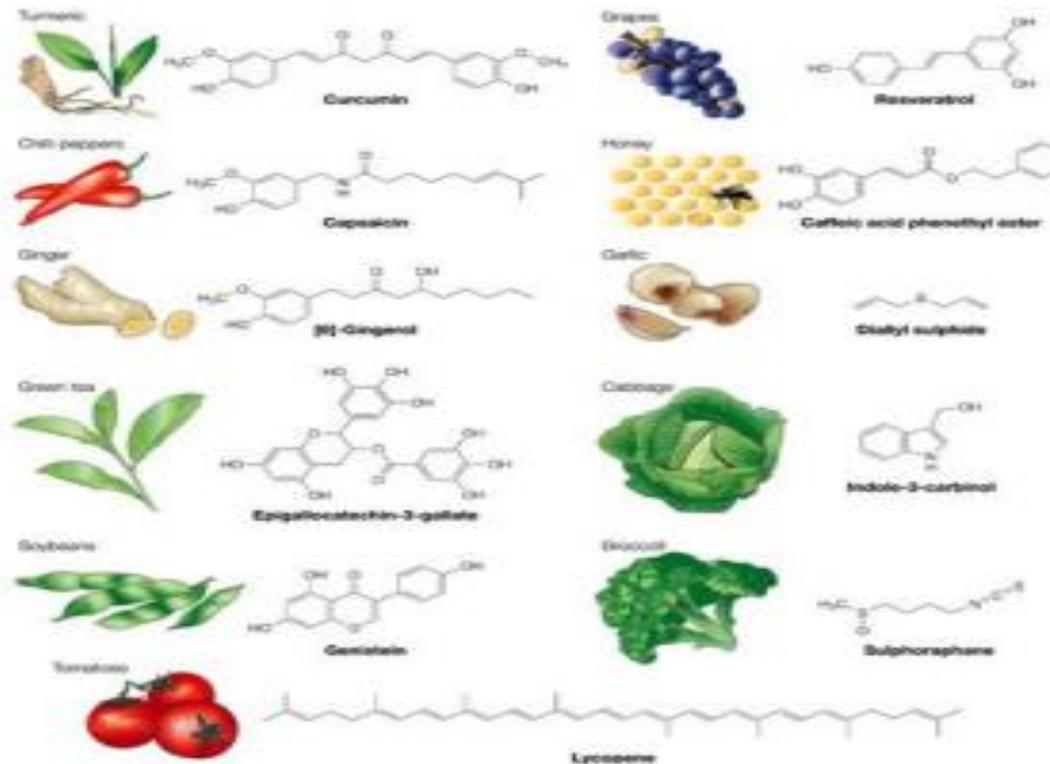
Shen, D¹, Wang, D¹, Chen, D², Wang, D¹, Wang, D¹, Wang, D¹, Wang, D¹, Wang, D¹, Wang, D¹, Wang, D¹

1) 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 predictor of disease's tumor aggressiveness 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. Firstly, we discovered that the hypermethylation of two CpG sites, cg00010441 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 cg00010441 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.

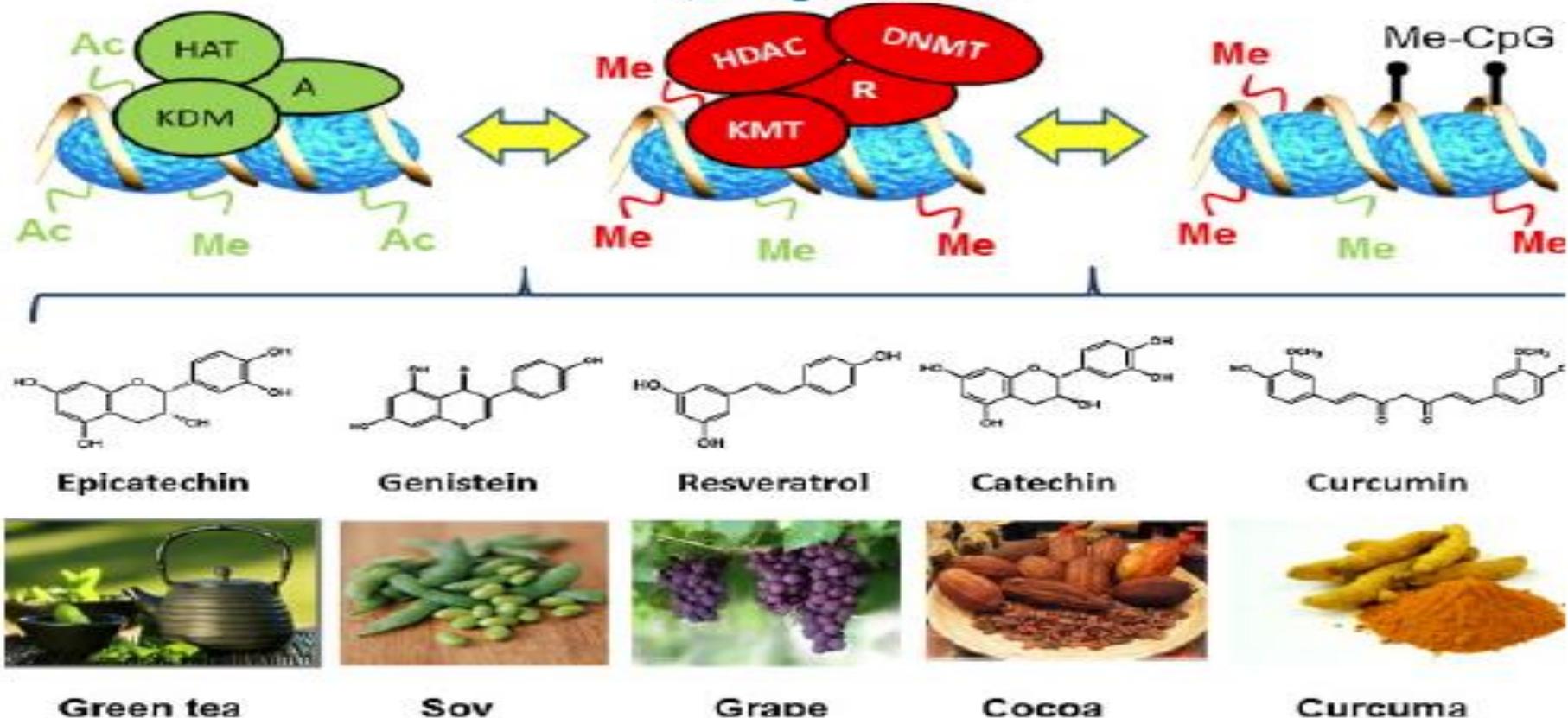
Anticancer phytochemicals

ANTICANCER PHYTOCHEMICALS (Representative chemopreventive phytochemicals and their dietary sources)

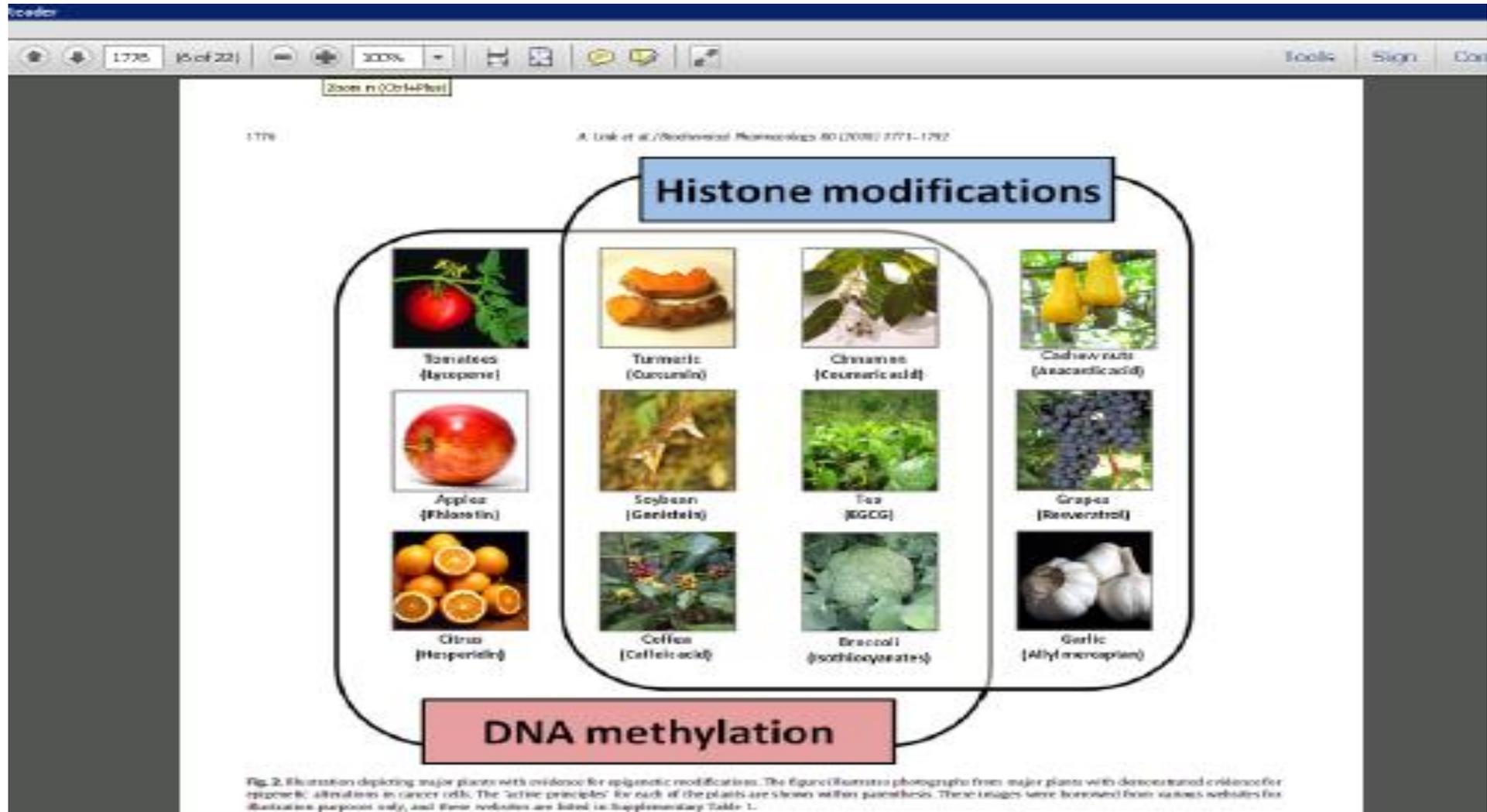


Dietary supplements

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 Pears, pineapple, kiwi, kiwifruit, kiwifruit</p> <p>Learn more</p>	 <p>Vegetables Mint, green beans, lettuce, spinach</p> <p>Learn more</p>	 <p>Grains Whole, half, whole grain, whole grain, whole grain</p> <p>Learn more</p>	 <p>Protein Foods Wild, trout, protein, trout, trout</p> <p>Learn more</p>	 <p>Dairy Milk, low-fat, low-fat, low-fat, low-fat</p> <p>Learn more</p>
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MyPlate Kitchen

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<https://www.myplate.gov/>

Mobile food record

Mobile food record



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.**

