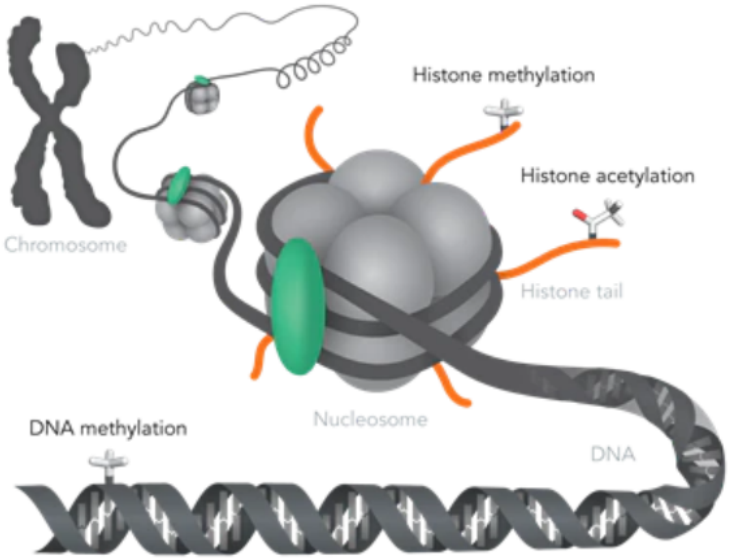
National Cancer Institute

Epigenetics and Cancer



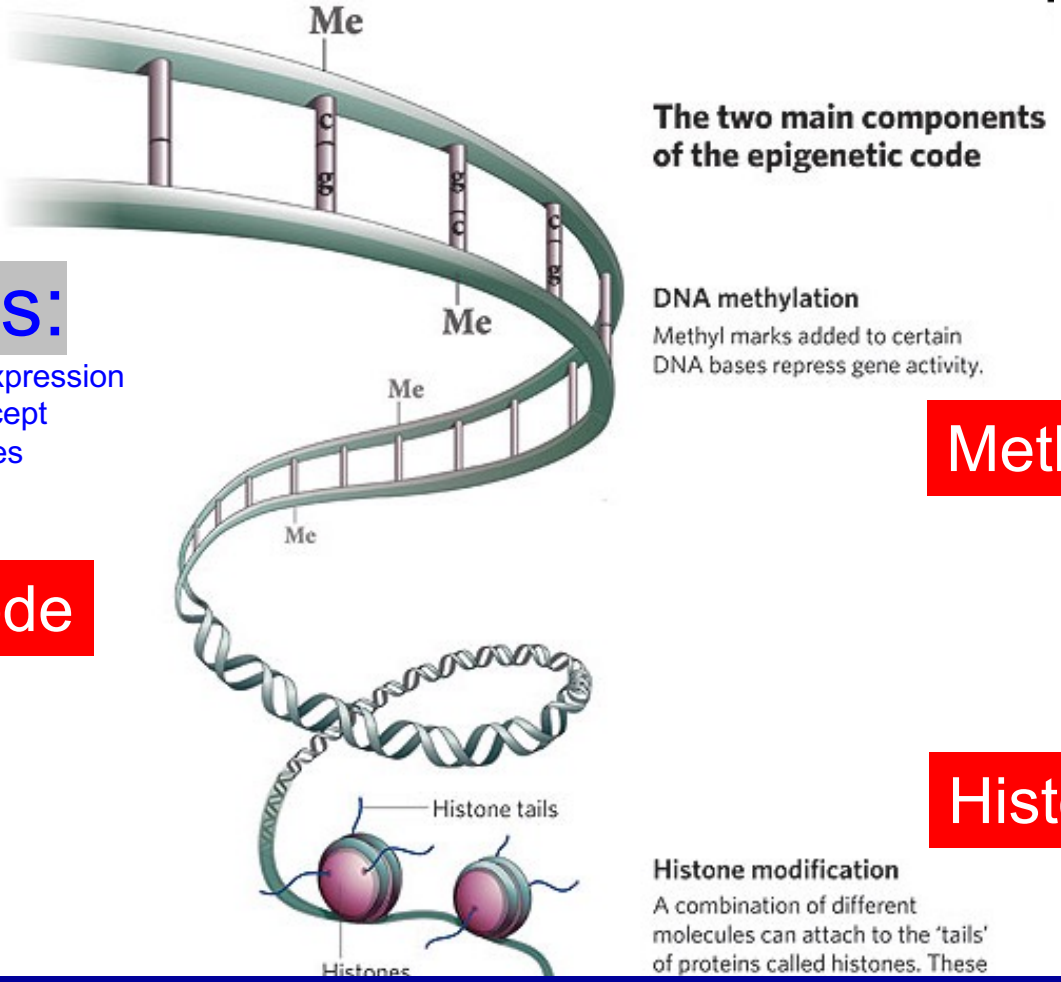
Mukesh Verma, Ph.D.
Chief, Methods and Technologies Branch
Program Director,
Epidemiology and Genomics Research Program
DCCPS, NCI, NIH

Fig. Credit: https://horizondiscovery.com/en/applications/cell-line/epigenetics


NESSA CAREY
THE EPIGENETICS REVOLUTION
How Modern Biology Is Rewriting Our Understanding of Genetics, Disease, and Inheritance
TIME
WHY YOUR DNA ISN'T YOUR DESTINY
Science
AAAS
GENOTYPE TO PHENOTYPE
NATIONAL
TWINS
ALIKE BUT NOT ALIKE

Epigenetics:

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



The two main components of the epigenetic code

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

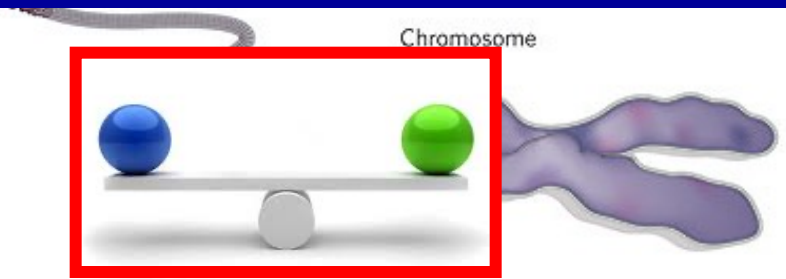
Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These

Genetic Code

Methylation Code

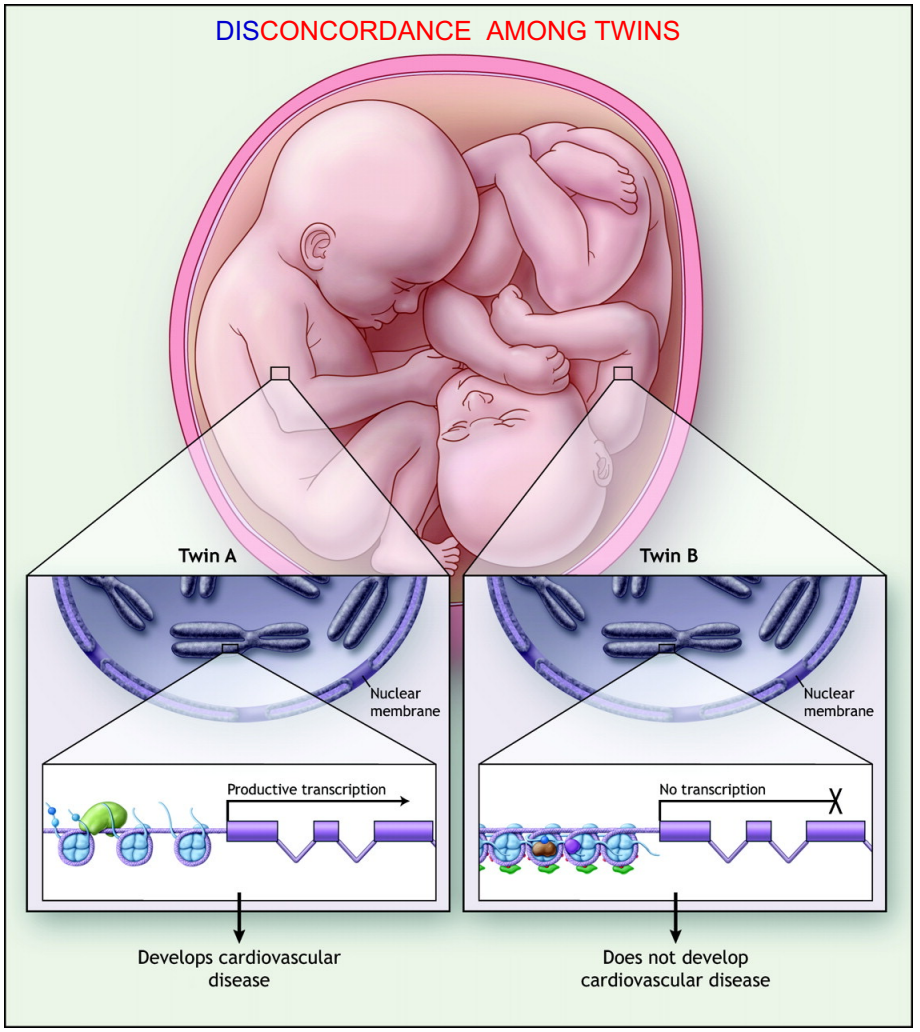
Histone Code

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



Qiu NATURE 441: 143

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



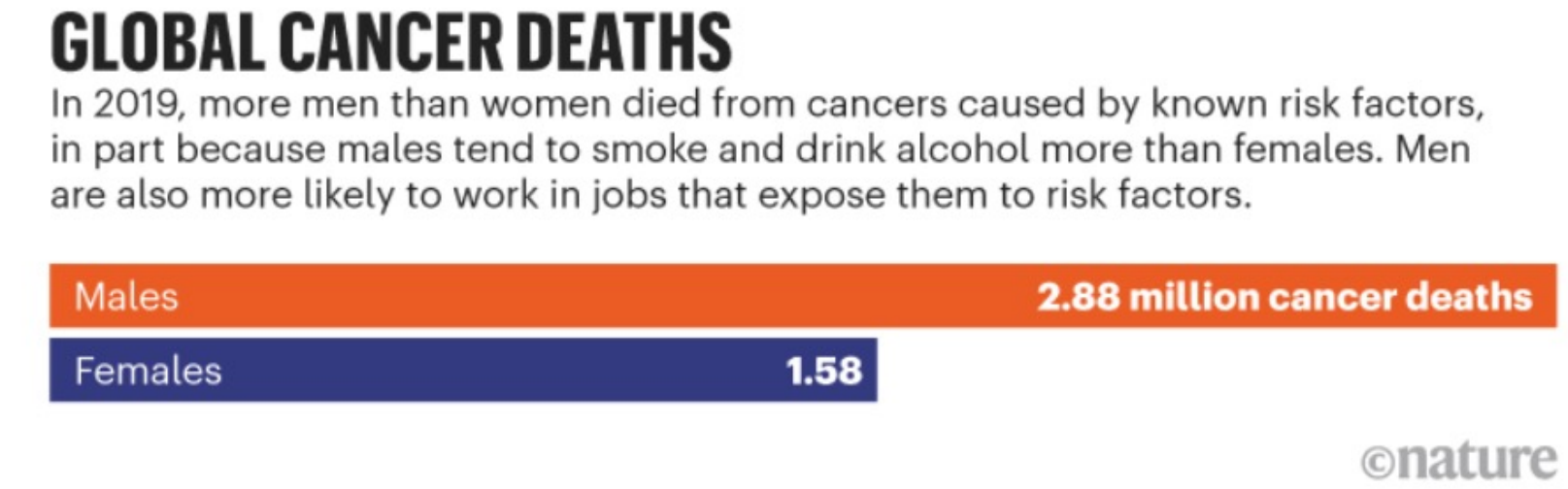
Epigenetic predisposition to angiogenesneis? Individual? Populations?

Pharmacogenomics and pharmacoepigenomics (personalized medicine)

Microenvironment, microbiome, and gene expression

GWAS and EWAS

Adapted from Matouk and Marsden Cir Res 102:873



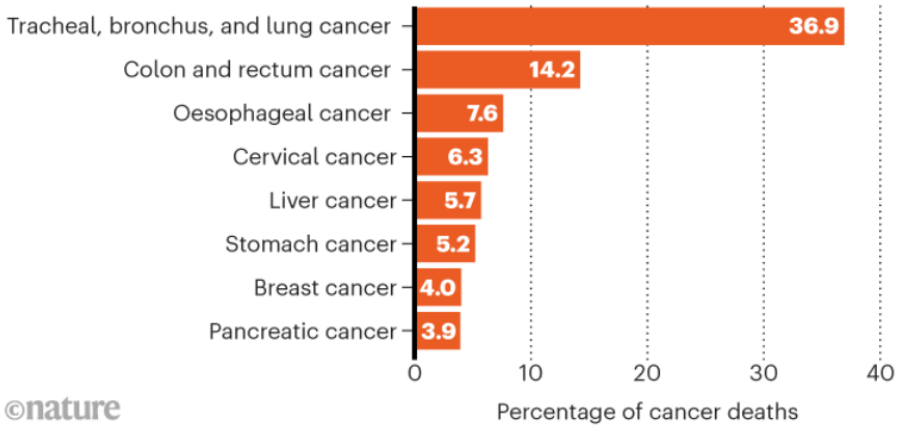
Source: Ref 1.

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

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

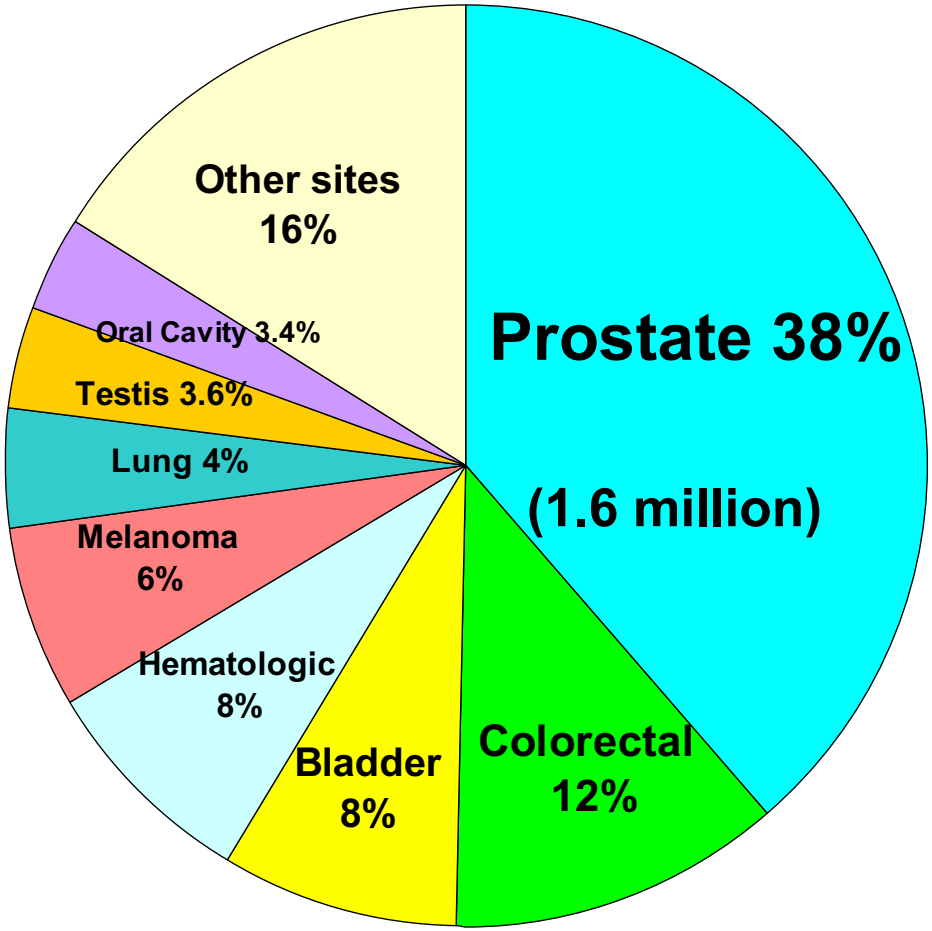
CANCER DEATHS BY TUMOUR TYPE

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.

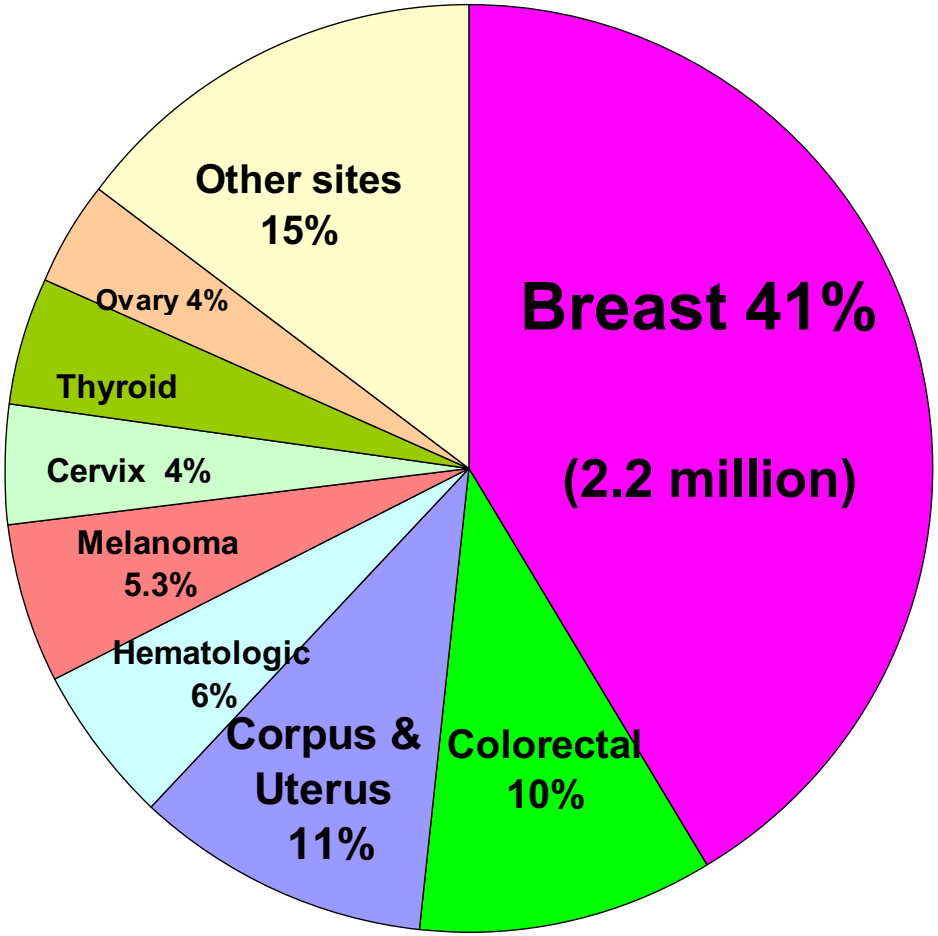


doi: https://doi.org/10.1038/d41586-022-02355-

Estimated Number of Persons Alive in the U.S. Diagnosed with Cancer by Site



Men (4.24 million)



Women (5.31 million)

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

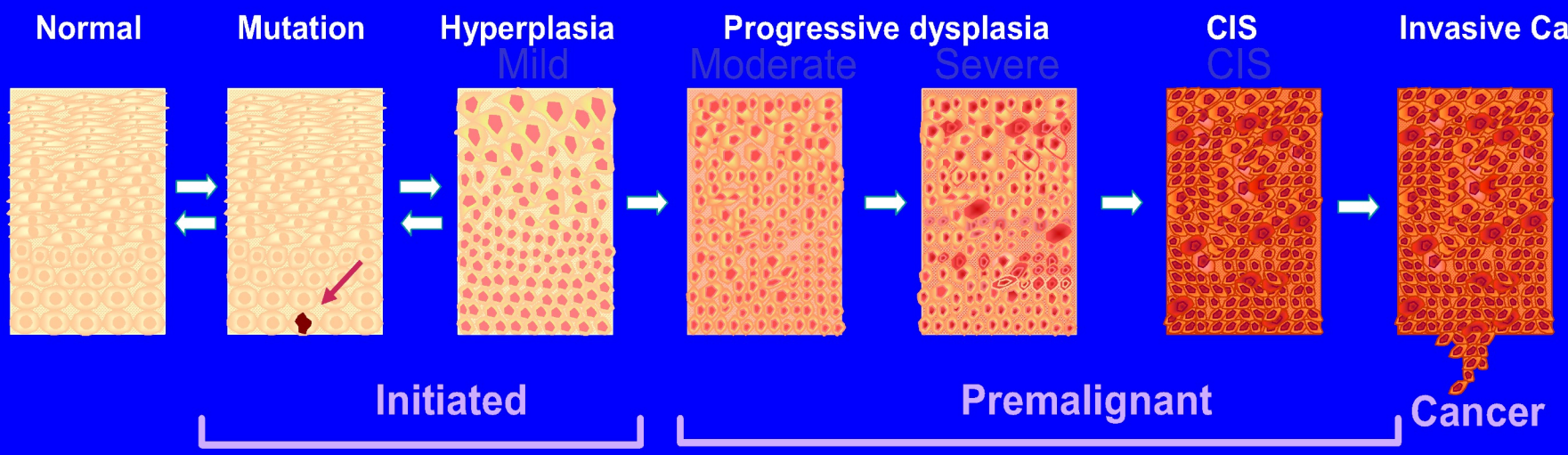
End of Life

Mortality, Person – years of life lost

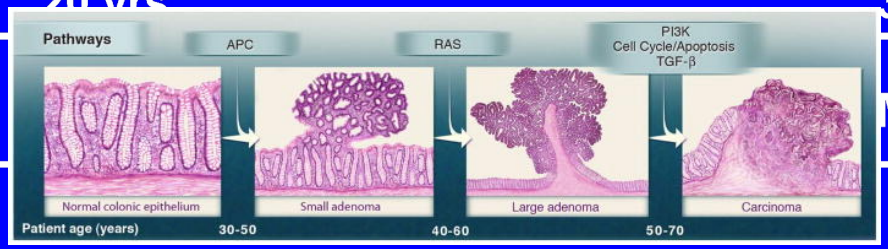
Prevention → Cancer recurrence
Secondary cancer

Prevention: restoring transcription, halting progression, or stopping metastasis

Cancer Development is a Multi-step Process



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



Adapted from Vogelstein et al 2013

DNA-Based Cancer Diagnostics

Exfoliated Cells are Good Source of DNA to Study Epidemiology

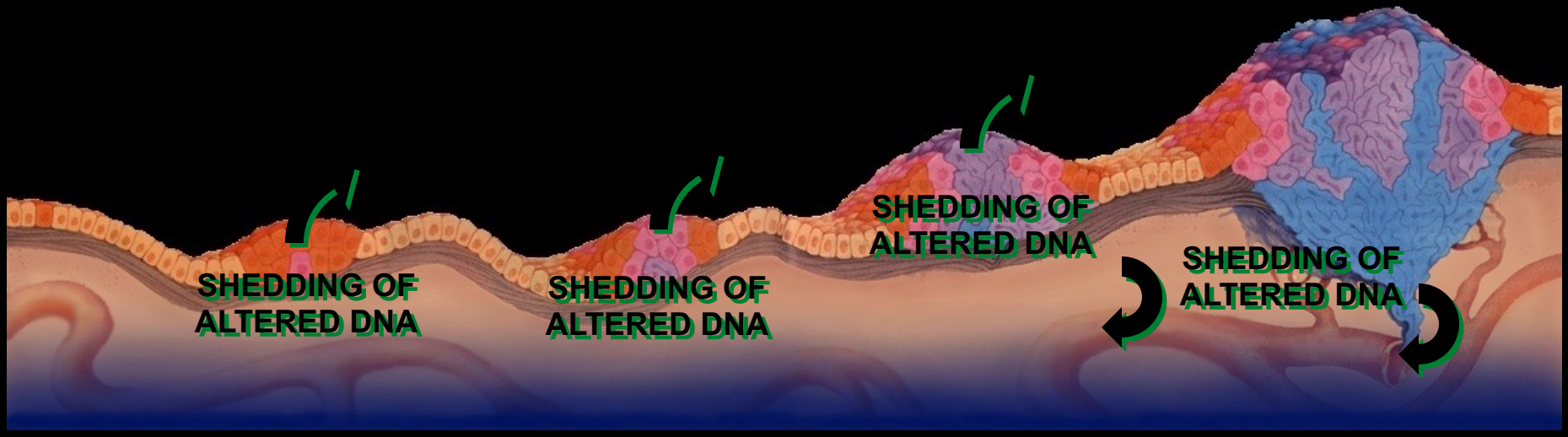
CANCER RISK ASSESSMENT

PROGRESSION RISK ASSESSMENT

EARLY DETECTION

DIAGNOSIS, PREDICTION & PROGNOSIS

MONITORING FOR RECURRENCE



PREDISPOSITION

HYPERPLASIA

DYSPLASIA

CARCINOMA

METASTASIS

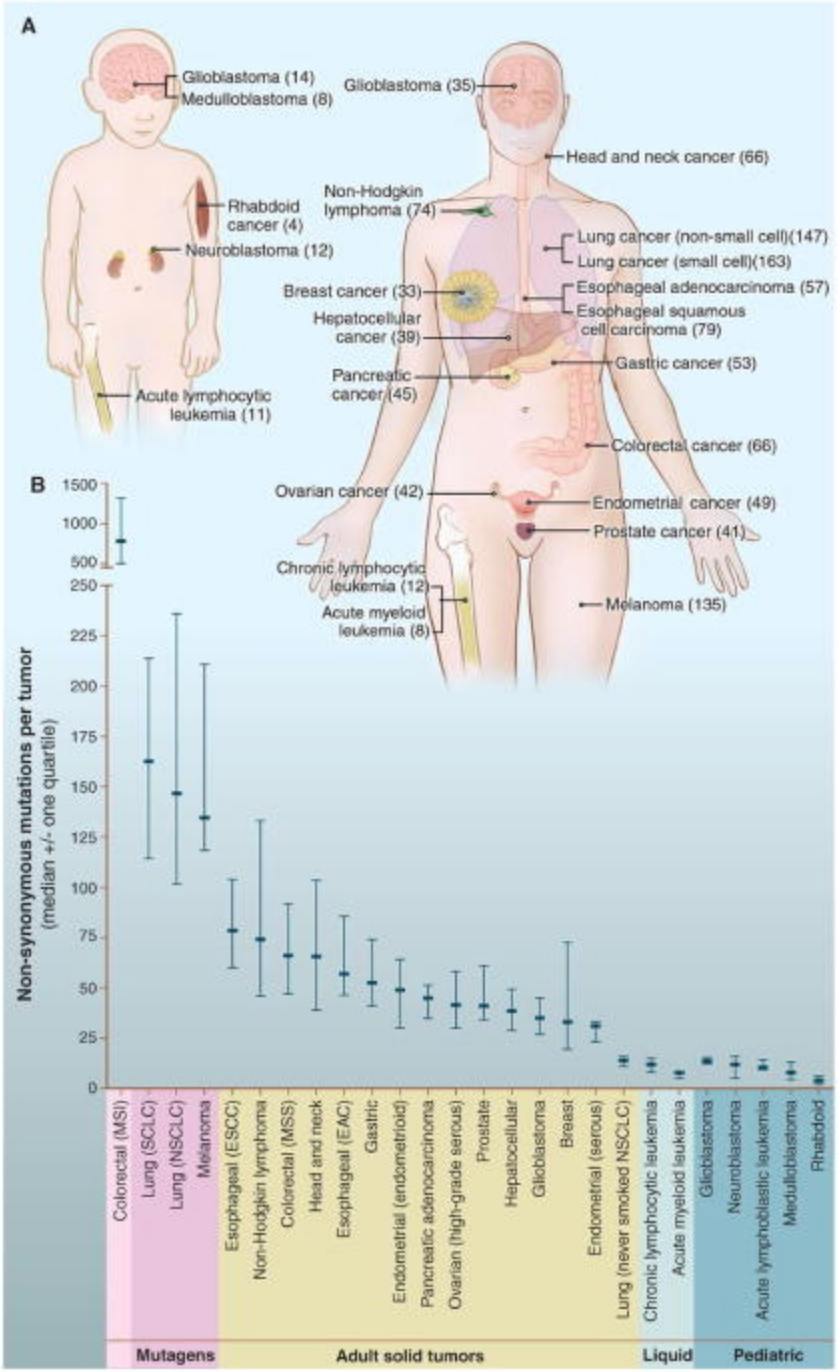
Adapted from Weinberg 1996

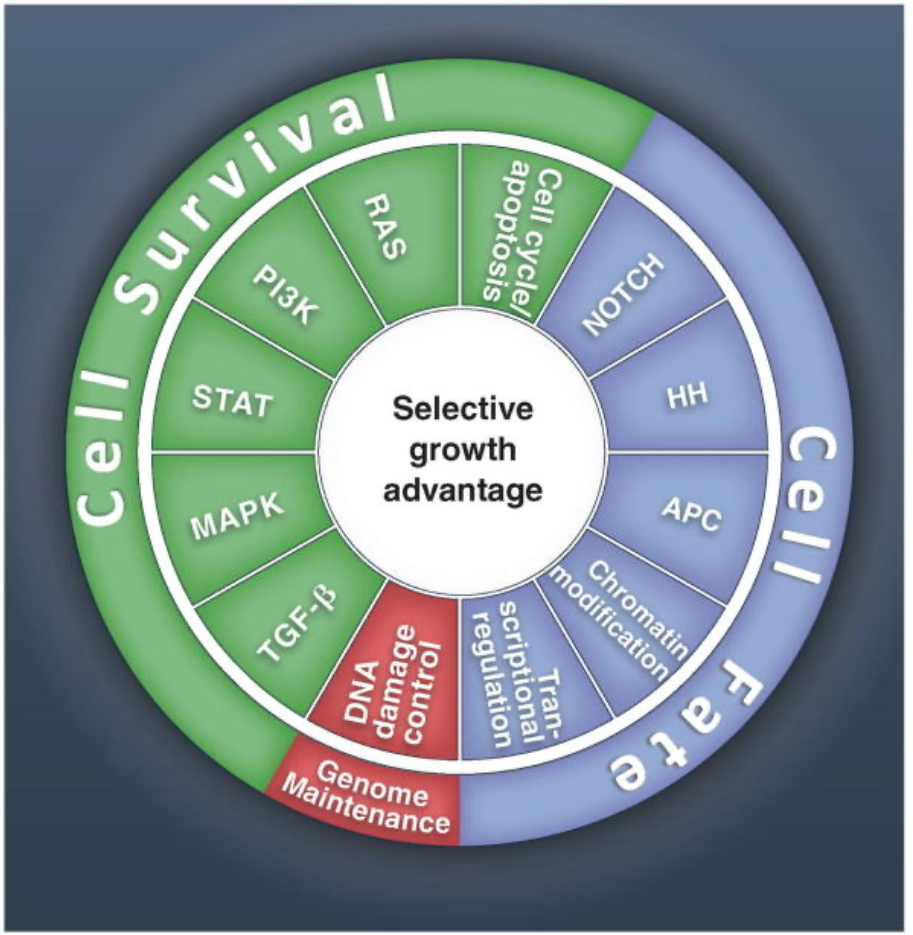
Paradigm shifts in genetics

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*

CANCER GENOME LANDSCAPE

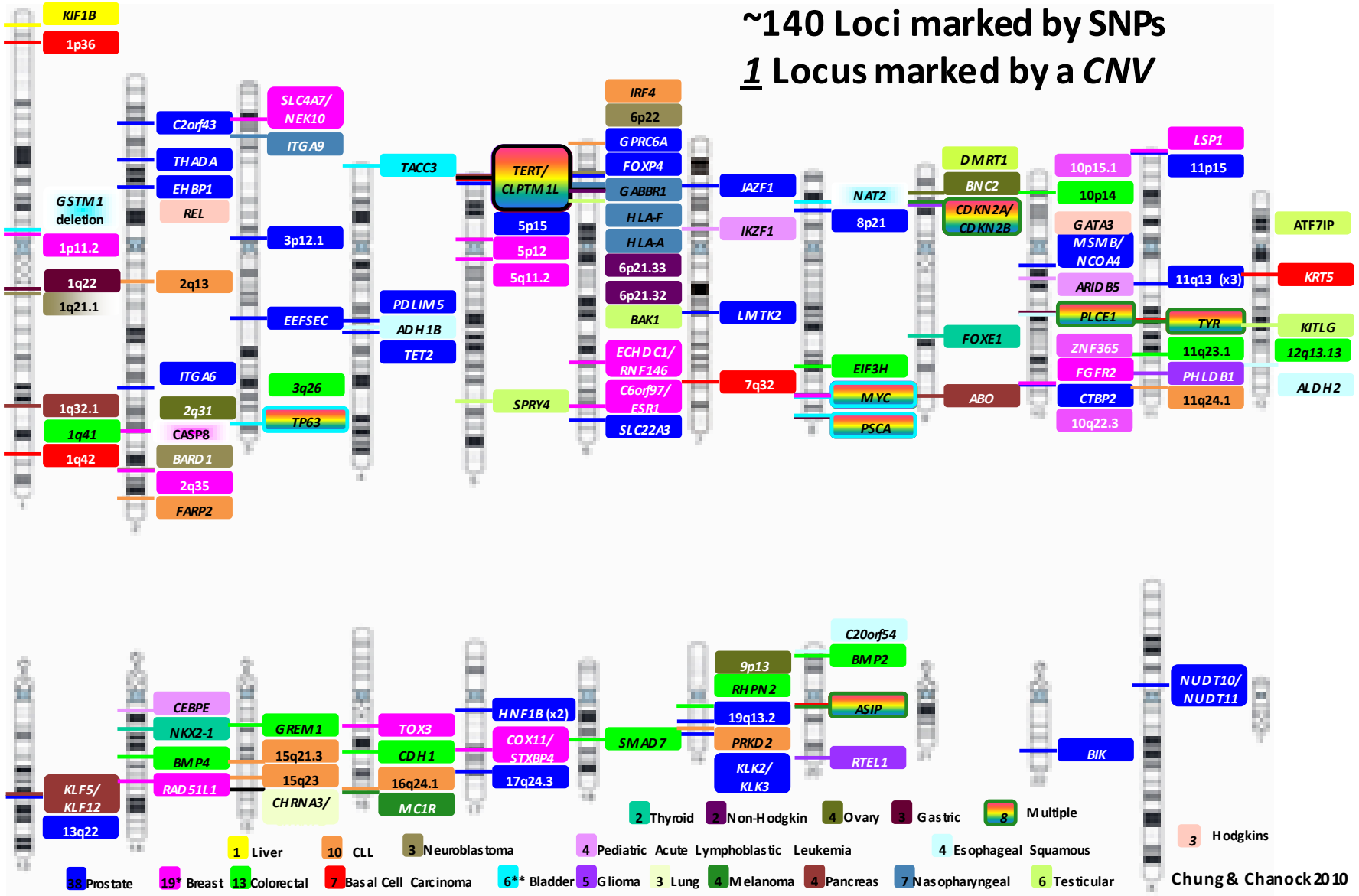
Number of somatic mutations in representative human cancers, detected by genome-wide sequencing studies





Adapted from Vogelstein and Kinzler (Science 2013)

Published GWAS Etiology Hits (2010)

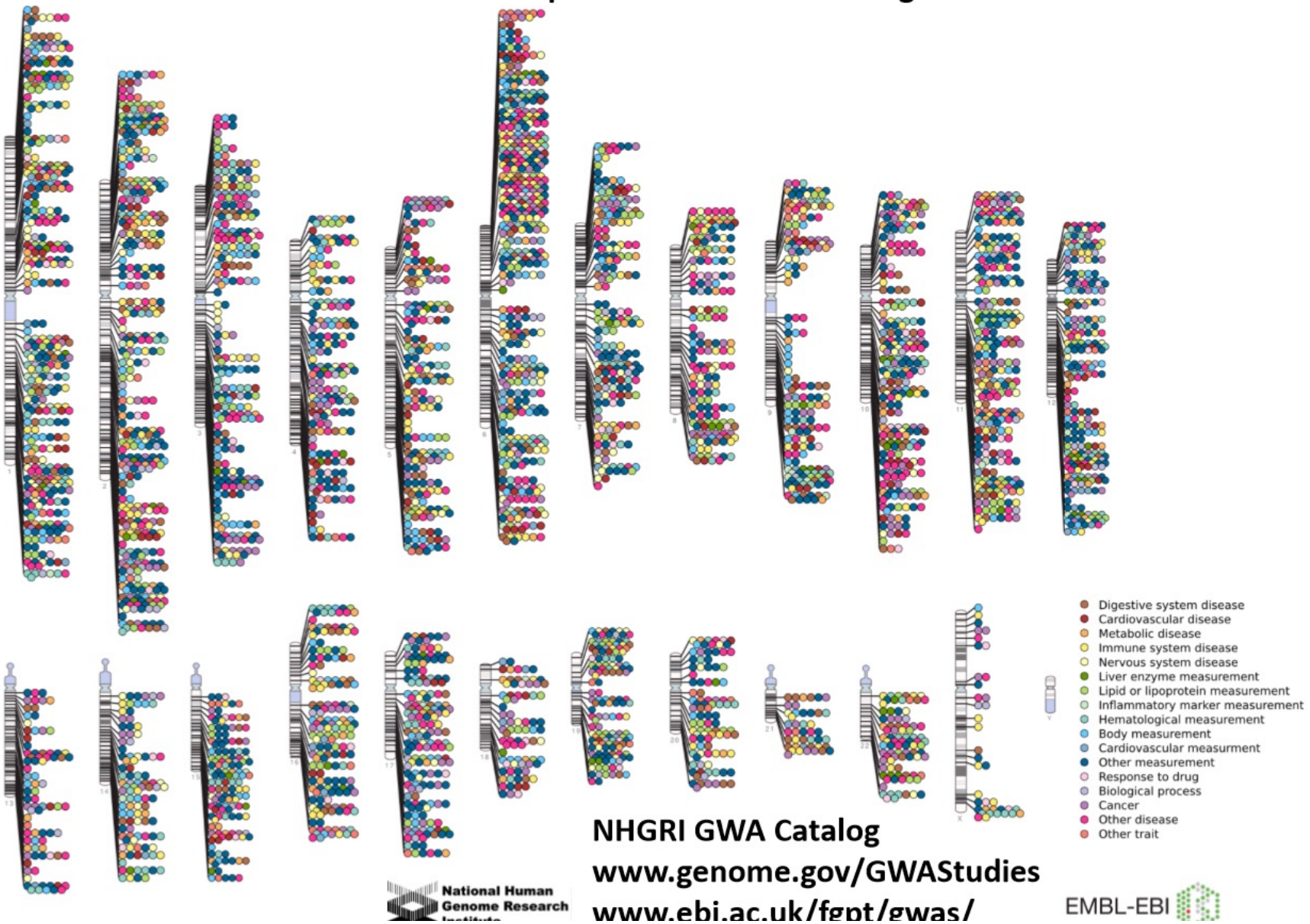


Hindorff L, Gillanders EM, Manolio T.

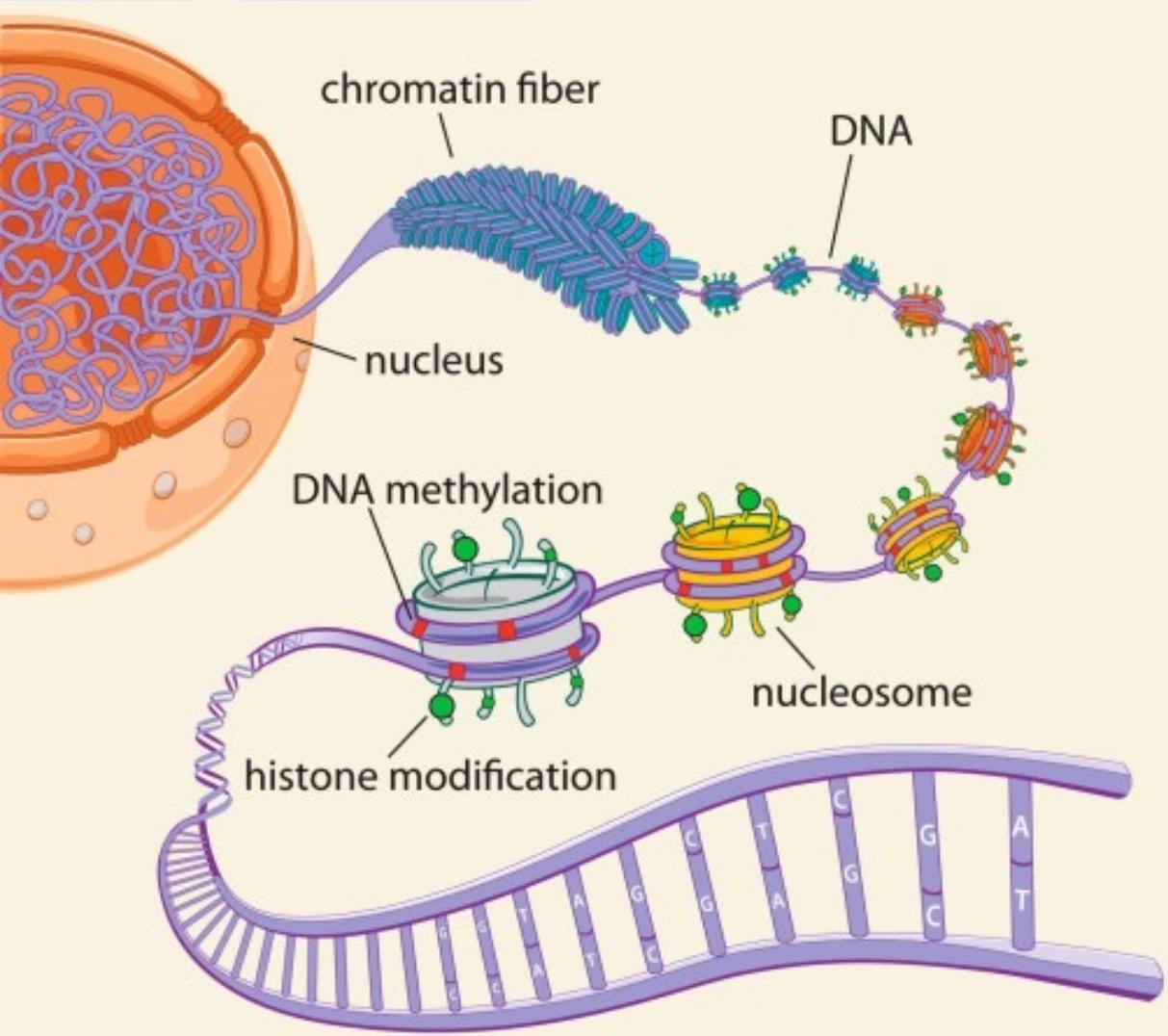
Adapted from T. Manolio

Published Genome-Wide Associations through 12/2013

Published GWA at $p \leq 5X10^{-8}$ for 17 trait categories



There's more to the genome than its sequence



4D Nucleome

Roadmap Epigenomics, ENCODE, IHEC

Human Genome Project

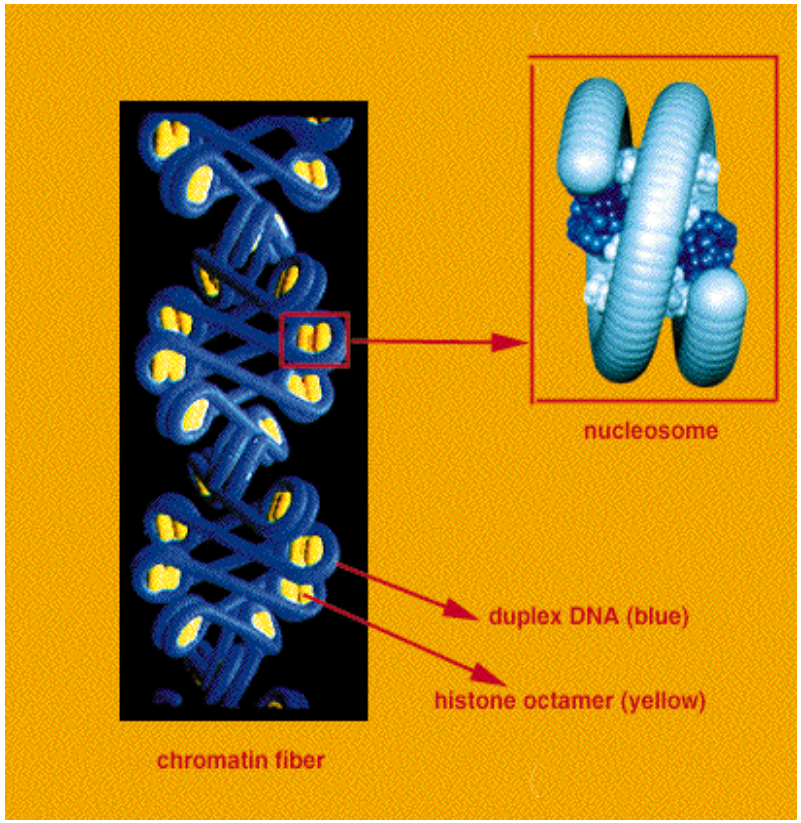
Nucleosomes (Units of Chromatin)

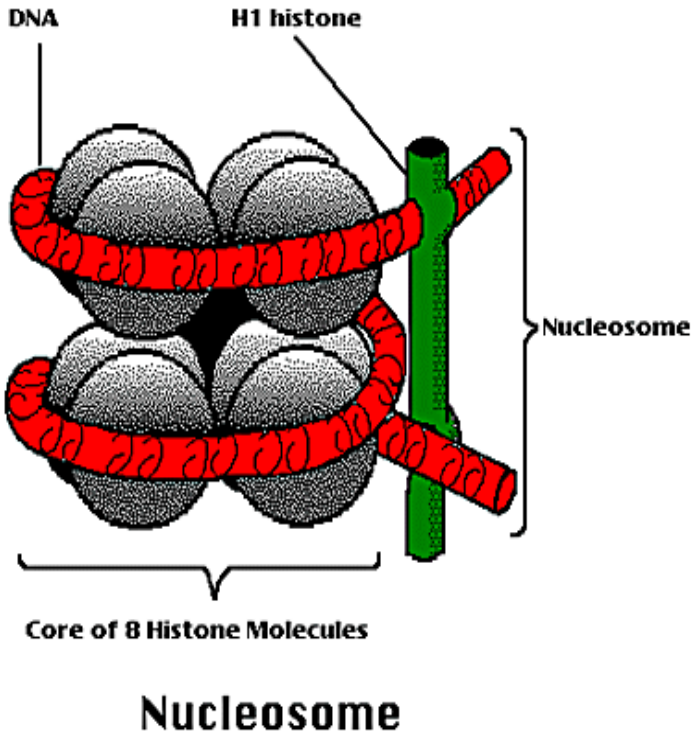
DNA
Histones H2a, H2b, H3, H4

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 octomeric complex of two of each of histone types



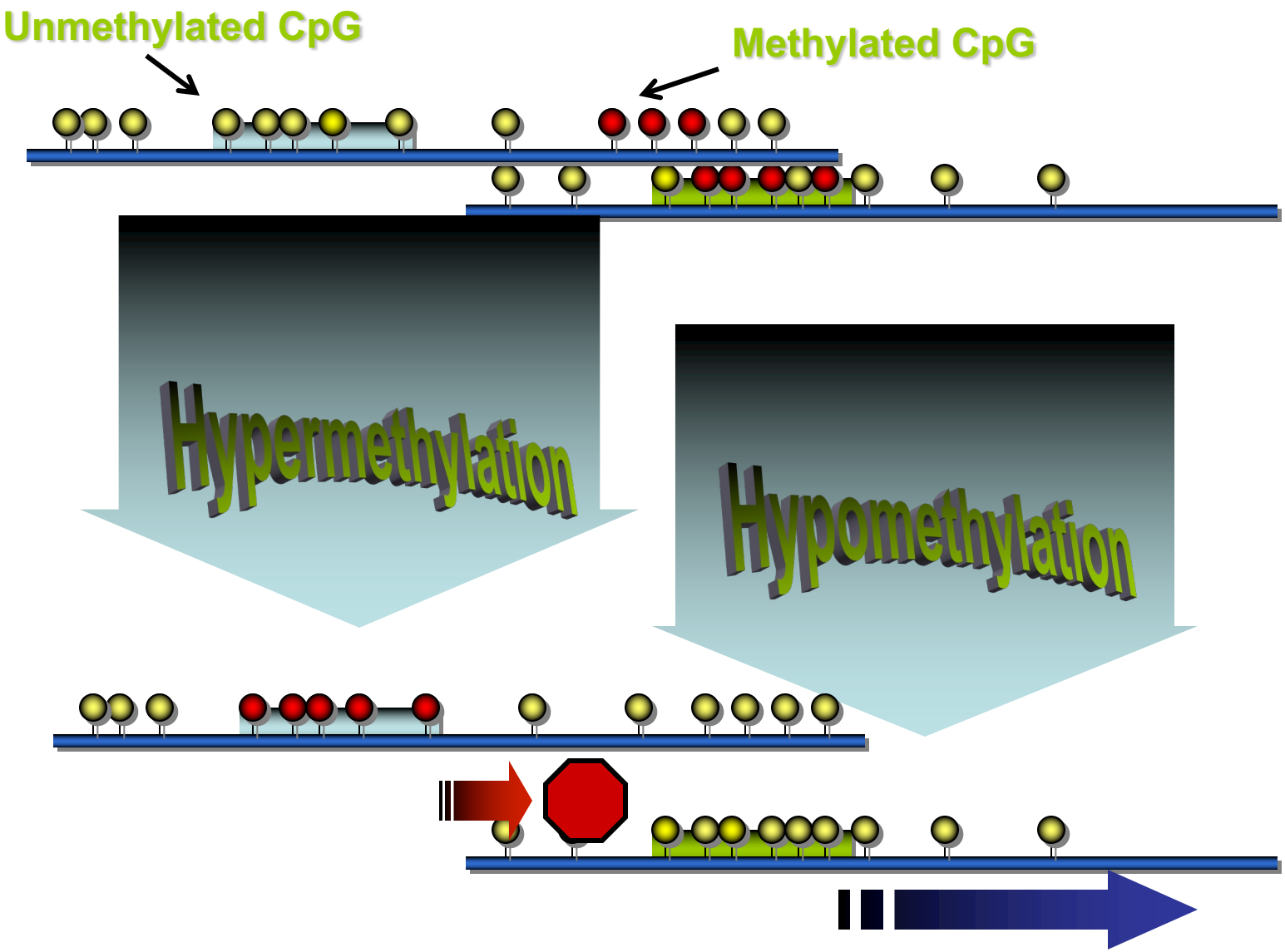


Shores are 0-2kb from islands
Shelves are 2-4 kb and enhancers are beyond shelves

DNA Methylation

deoxycytosine

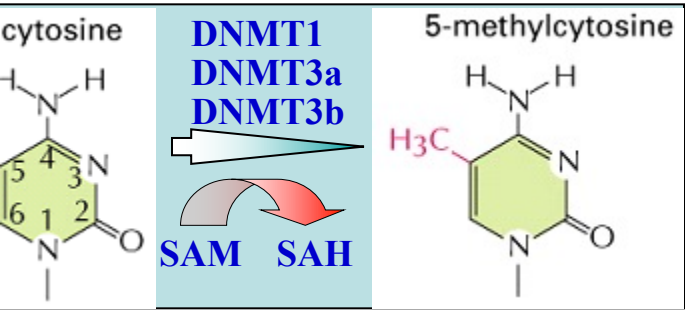
5-methylcytosine


Unmethylated CpG
Methylated CpG
Hypermethylation
Hypomethylation
Inactivated Tumor Suppressors
Activated Oncogenes

EPIGENETICS

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

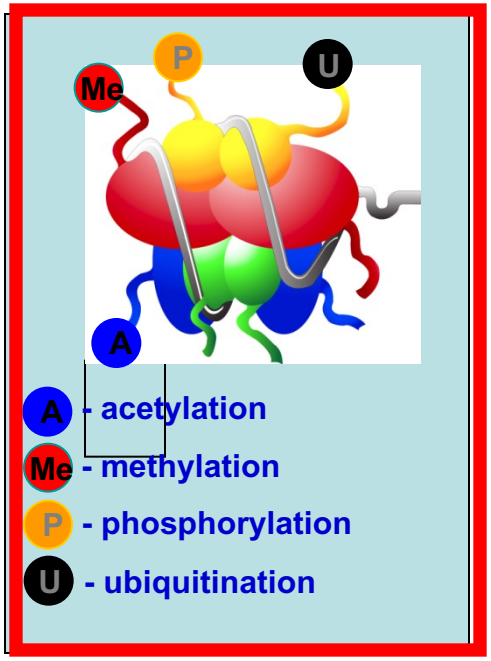
Methylation of DNA



Genomic Imprinting

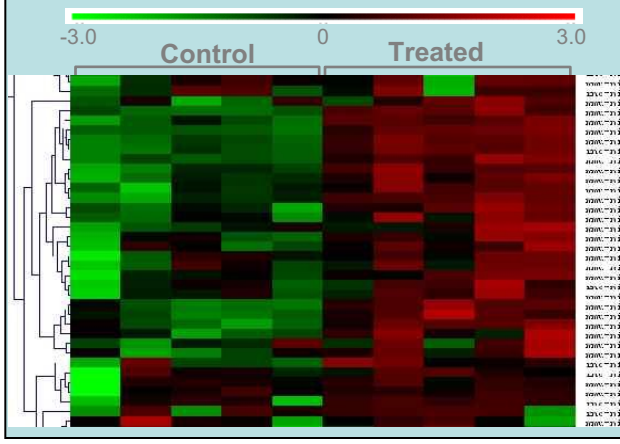
Shores: less than 2 kb from the CpG island
Shelves: 2-4 kb from the CpG island
Remaining region: called OPEN SEA

Modifications of histones

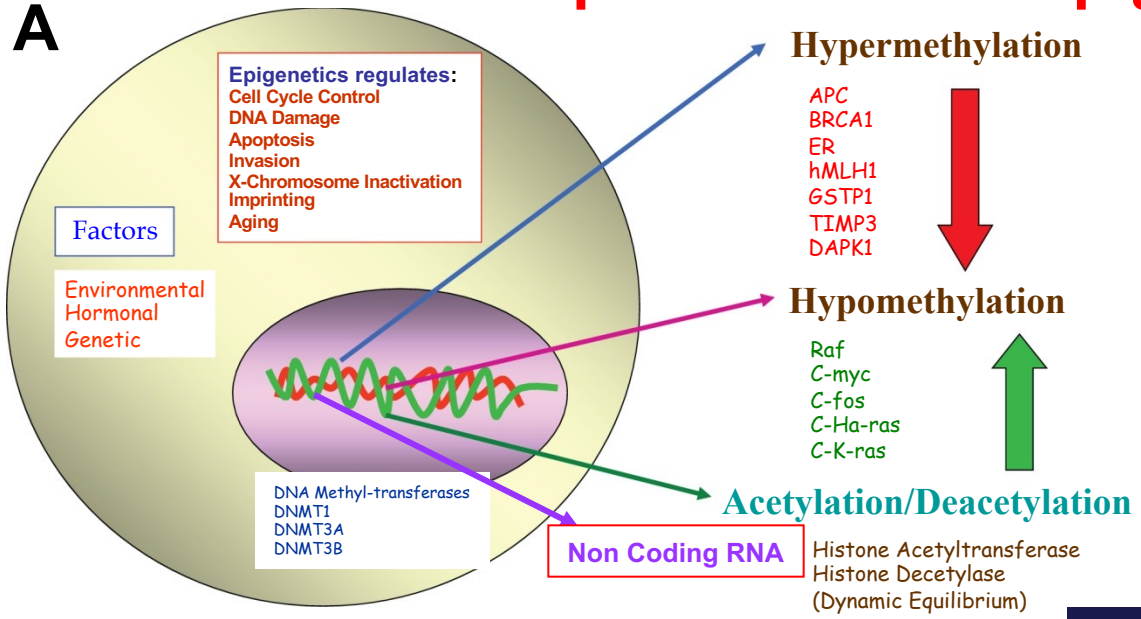


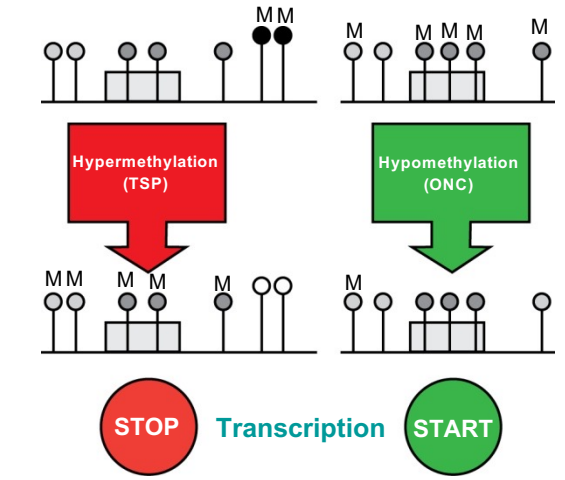
RNA-mediated modifications

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



Components of the Epigenome

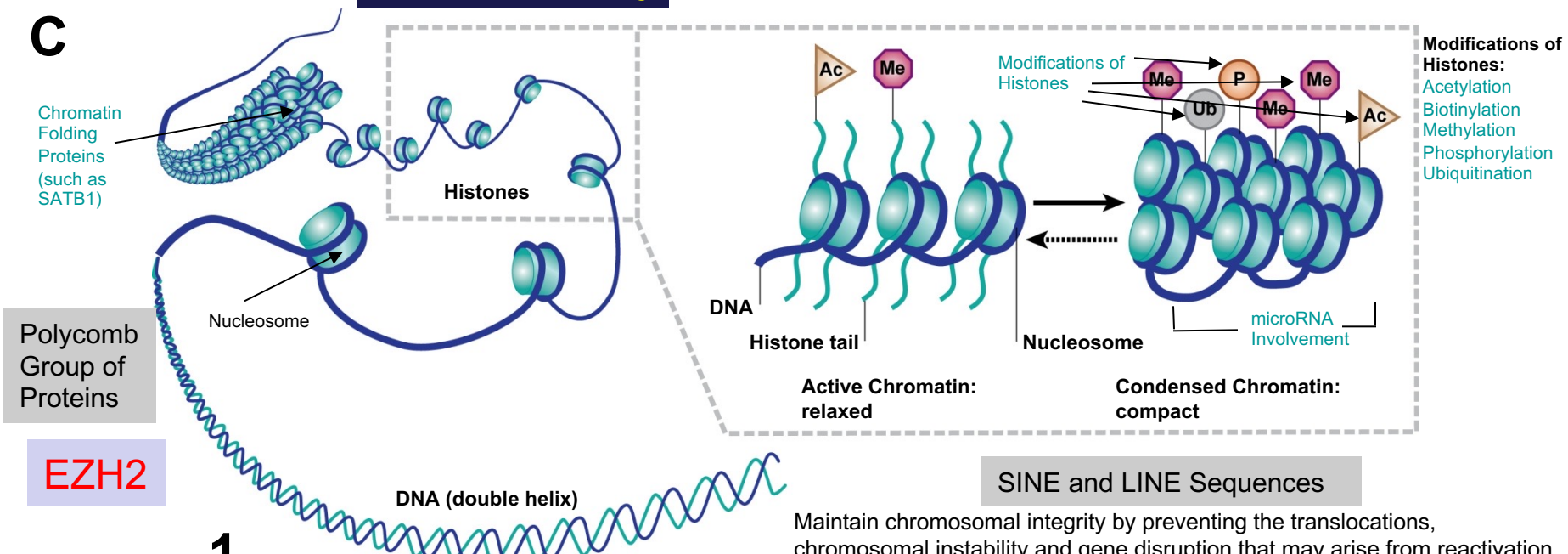




Chromatin Remodeling

Methylation Code

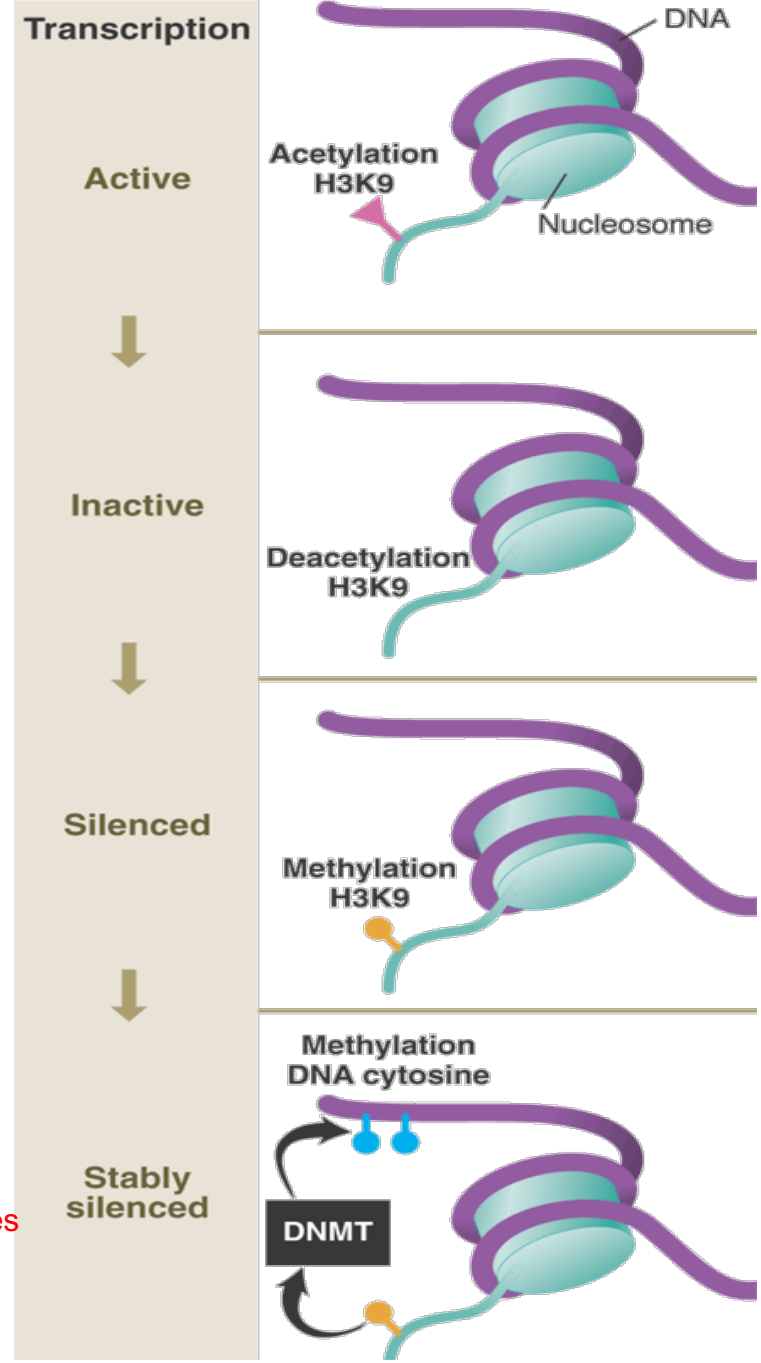
Histone Code



SINE and LINE Sequences

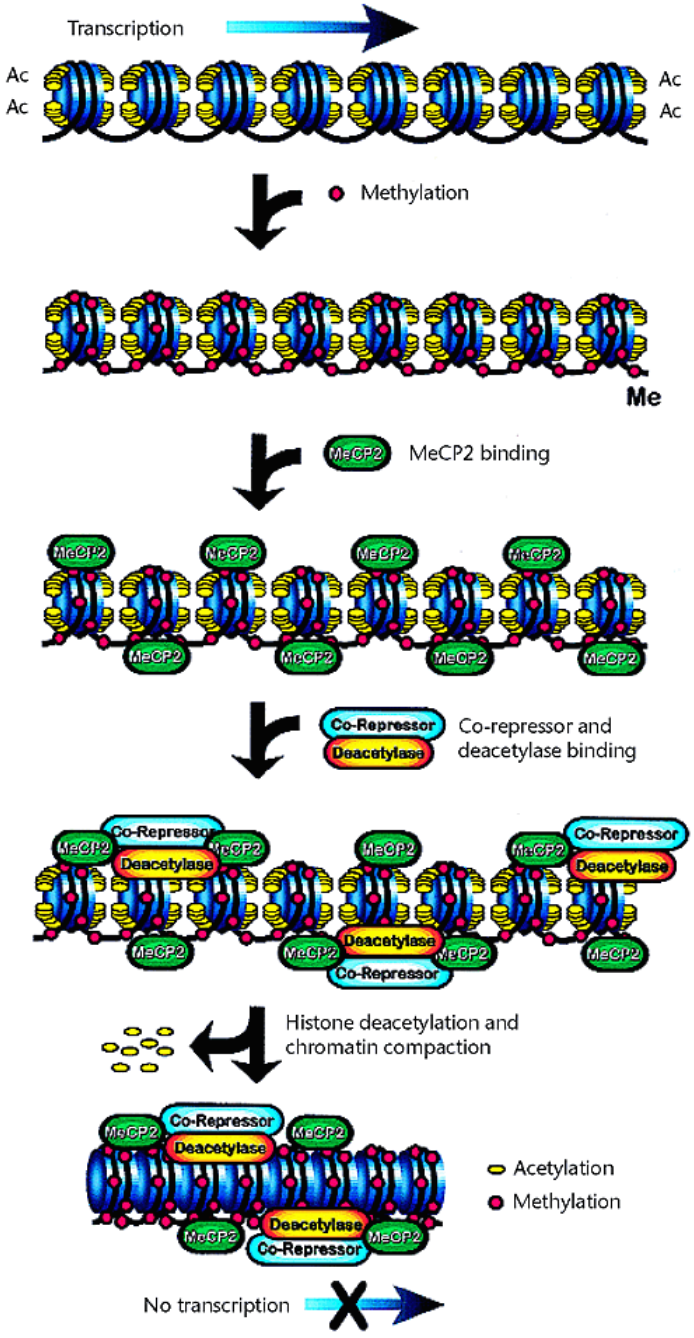
Maintain chromosomal integrity by preventing the translocations, chromosomal instability and gene disruption that may arise from reactivation of endoparasitic sequences

Verma (2009) In: Hum Genome Epidemiology, pp. 551-578



Peter Jones

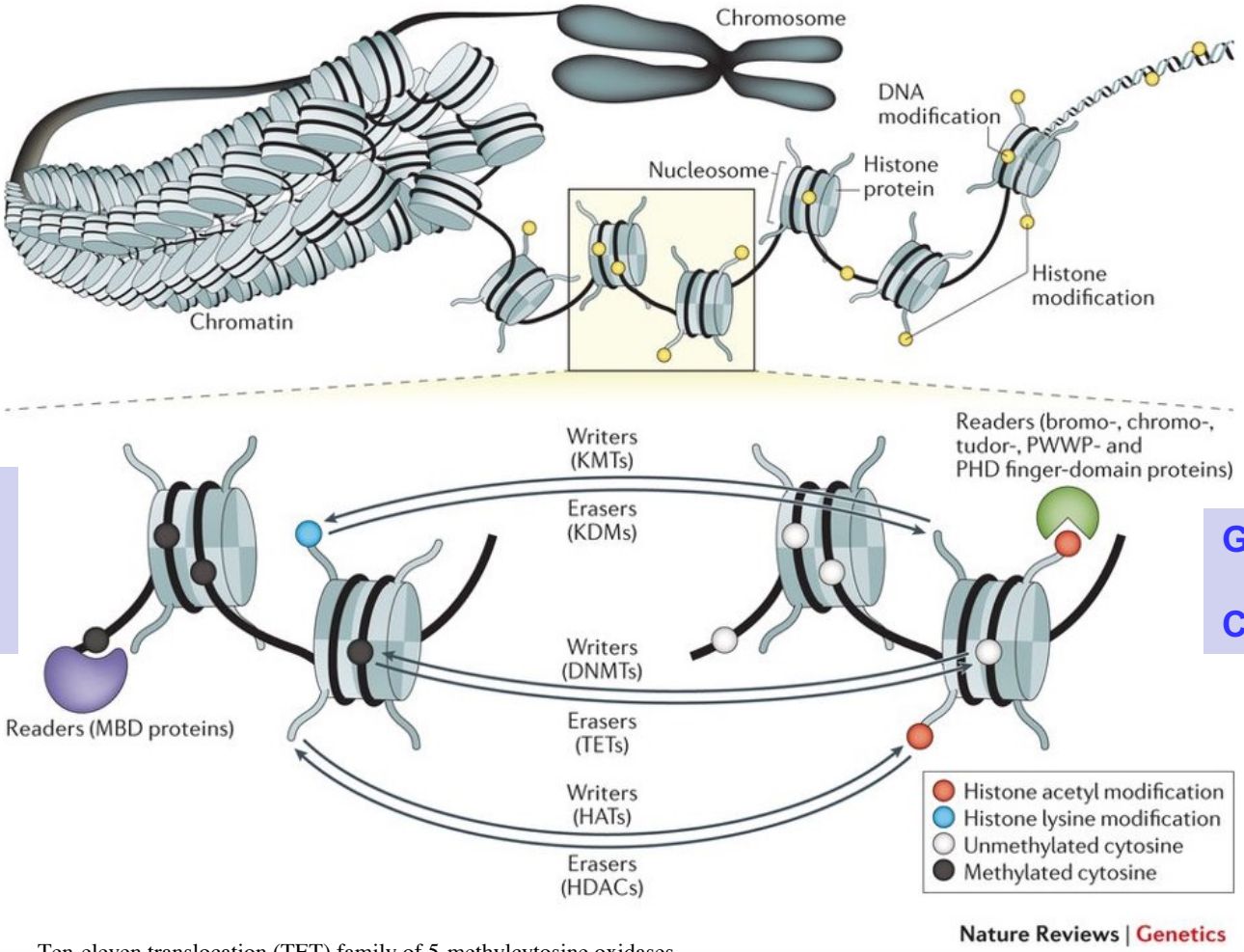
Andrew Feinberg



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

Figure 1 : Modulation of covalent modifications on chromatin.

From: Targeting the cancer epigenome for therapy



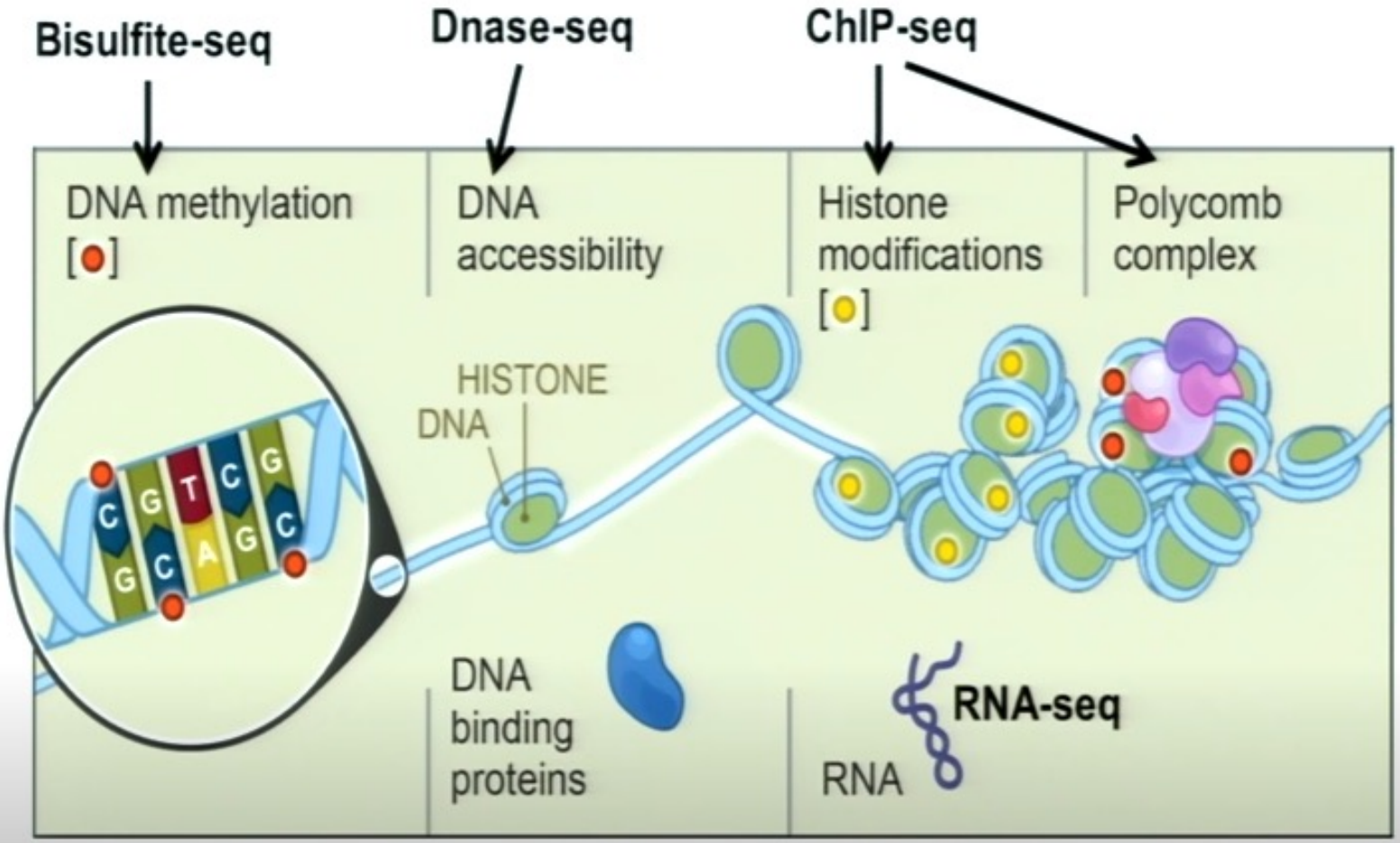
Writers
Readers
Erasers

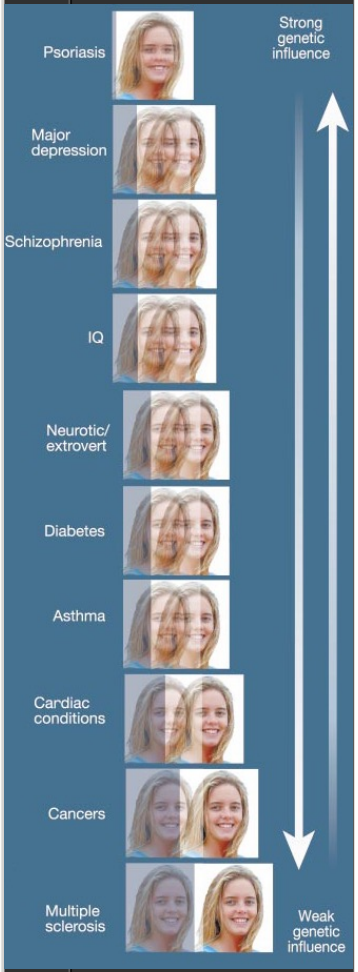
Gatekeeper genes

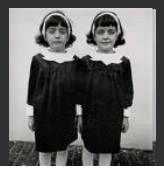
Caretaker genes

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

Mapping human epigenomes



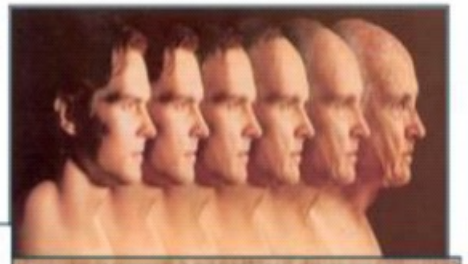


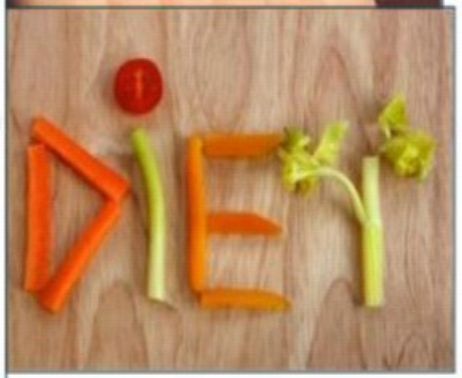


Genome vs. epigenome – why is it important?

- Genome is generally constant; epigenome changes
  - Age
  - Diet
  - Disease
  - Lifestyle
  - Environment

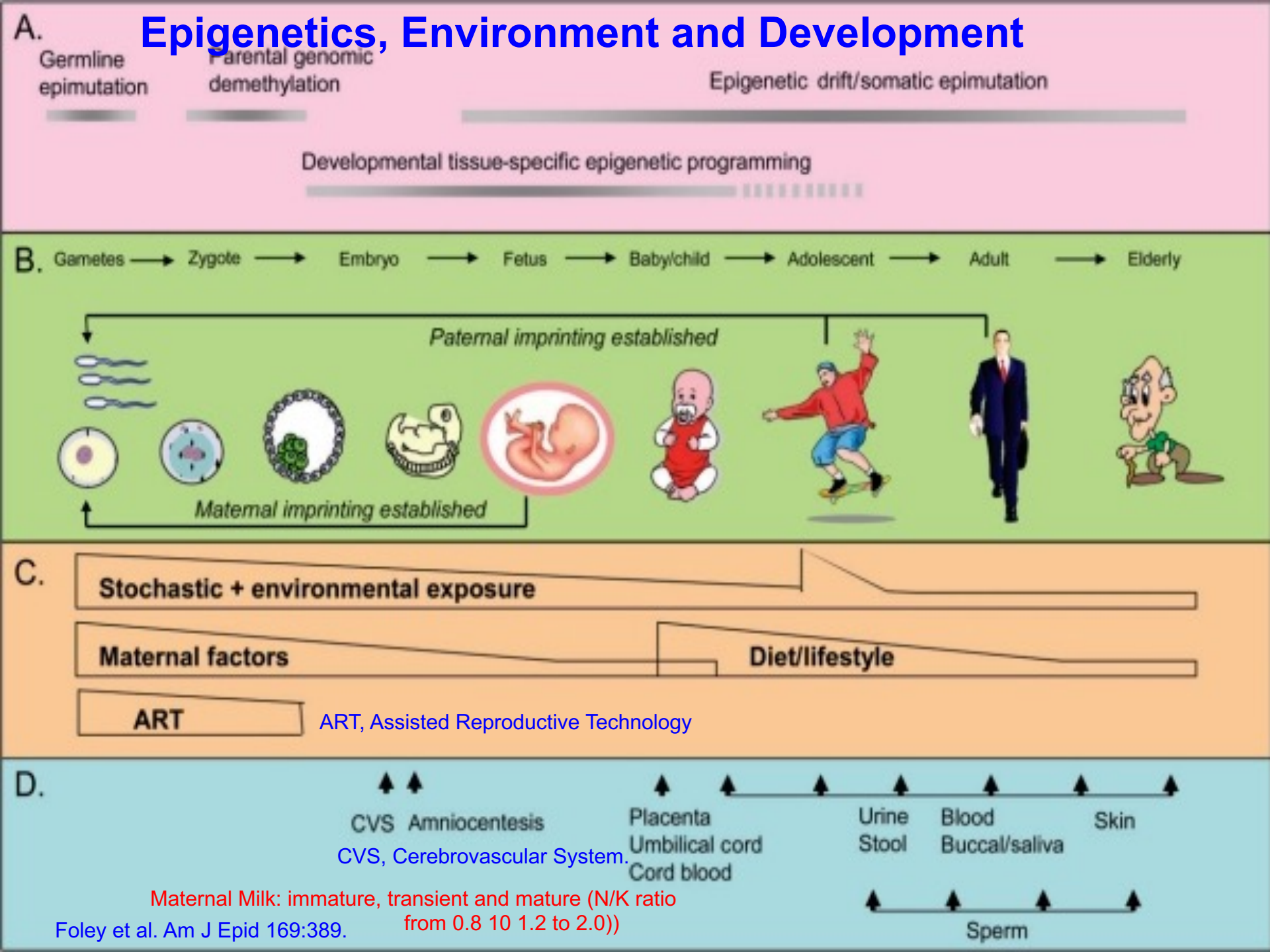
- Areas of interest:
  - Molecular basis of disease
  - Biomarker identification
  - Diagnostics development
  - Drug targeting





You only need to sequence your genome once, but you need to determine your epigenome multiple times... https://www.youtube.com/watch?v=JMT6oRYgkTk

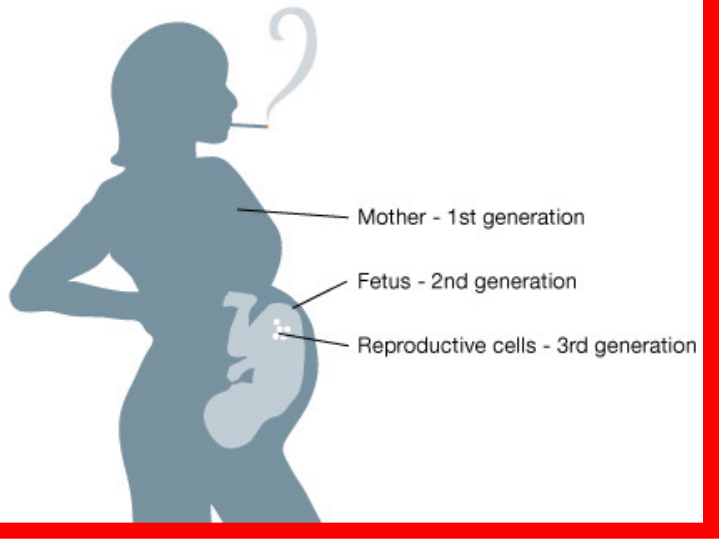
Epigenetics, Environment and Development



Key toxic substances affecting the epigenome

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

PFOS, Perfluorooctane sulfonate
PAHC, Polycyclic aromatic and halogenated compounds



Vaping can damage vital immune system

OPEN ACCESS

ORIGINAL ARTICLE

Pro-inflammatory effects of e-cigarette vapour condensate on human alveolar macrophages

Aaron Scott,[1] Sebastian T Lugg,[1] Kerrie Aldridge,[1] Keir E Lewis,[2] Allen Bowden,[3] Rahul Y Mahida,[1] Frances Susanna Grudzinska,[1] Davinder Dosanjh,[1] Dhruv Parekh,[1] Robert Foronjy,[4] Elizabeth Sapey,[1] Babu Naidu,[1] David R Thickett[1]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2018-211663).

[1]Birmingham Acute Care Research Group Institute of Inflammation and Ageing (IIA), University of Birmingham, Birmingham, UK
[2]College of Medicine, Swansea

ABSTRACT

Objective Vaping may increase the cytotoxic effects of e-cigarette liquid (ECL). We compared the effect of unvaped ECL to e-cigarette vapour condensate (ECVC) on alveolar macrophage (AM) function.

Methods AMs were treated with ECVC and nicotine-free ECVC (nfECVC). AM viability, apoptosis, necrosis, cytokine, chemokine and protease release, reactive oxygen species (ROS) release and bacterial phagocytosis were assessed.

Key messages

What is the key question?
► Do e-cigarettes have a negative impact on alveolar macrophage viability and function?

What is the bottom line?
► Vapourised e-cigarette fluid is cytotoxic, pro-inflammatory and inhibits phagocytosis in alveolar macrophages.

Thorax: first published as 10.1136/thoraxjnl-2018-211663 on

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

Exercise is important for everyone

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.

Rzehak P[1], Saffery R
Verduci E[6], Riva E[6],

Author informa

Abstract

Mounting evidence
profile in the blood
assessed by Epige
DNAm signatures
of children at age 5
biological role by e
children of the mul

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], Rzehak P[1,3], Vuillermin P[1,2,4,5], Ponsonby AL[1,2], Collier F[4,5], Burgner D[1,2], Saffery R[1,2], Ryan J[1,2,6,7]; BIS investigator team.

Collaborators

Author inform

Abstract

Compelling evider
genes, insulin-like
methylation. This

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**

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."**

Twin Research and Human Genetics
page 1 of 13 ▌© The Author(s) 2015 ▌doi:10.1017/thg.2015.74

Epigenome-Wide Association Study of Aggressive Behavior

Jenny van Dongen,[1,2] Michel G. Nivard,[1] Bart M. L. Baselmans,[1,2] Nuno R. Zilhão,[1] Lannie Ligthart,[1] BIOS Consortium,[3] Bastiaan T. Heijmans,[4] Meike Bartels,[1,2] and Dorret I. Boomsma[1,2]
[1] Department of Biological Psychology, VU Amsterdam, Amsterdam, The Netherlands
[2] EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands
[3] The Biobank-Based Integrative Omics Study (BIOS) Consortium (a full list of authors is provided in the Supplementary Material)
[4] Department of Molecular Epidemiology, Leiden University Medical Center, Leiden, 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 level is associated with aggressive behavior. Subjects took part in longitudinal survey studies

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], Rzehak P[1,3], Vuillermin P[1,2,4,5], Ponsonby AL[1,2], Collier F[4,5], Burgner D[1,2], Saffery R[1,2], Ryan J[1,2,6,7]; BIS investigator team.

⊕ Collaborators (11)

⊕ Author information

Abstract

Compelling evidence suggests that maternal mental hea
genes, insulin-like growth factor 2 (IGF2) and H19, are i
methylation. This study aimed to determine the associati
differentially methylated regions (DMRs) of IGF2 (DMR0
offspring. Maternal depression, anxiety and perceived st
Infant Study (n=576). DNA methylation was measured i

Chapter 29

Epigenetic Regulation in Biopsychosocial Pathways

Kristin Litzelman and Mukesh Verma

Cancer Prevention Fellow

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 physiological stress response (e.g., cortisol) has been associated with epigenetic changes, such as DNA

Research Article

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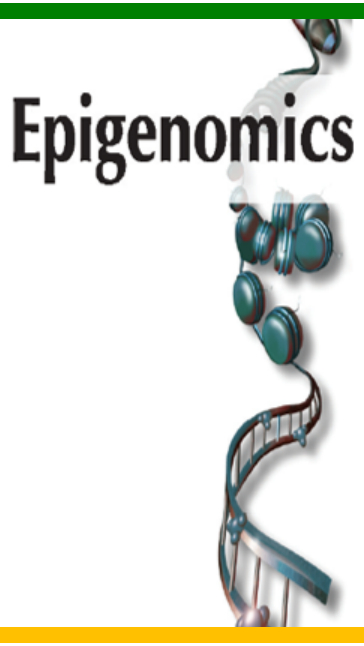


CROSS-GENERATIONAL EFFECTS

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

Shirley Y Hill*,[1], Gre
[1]Department of Psychiatry, U
[2]Center for Neuroscience, U
[3]Departments of Anesthesic
15213, USA
* Author for correspondenc

Toxicoepigenomics 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 toxicoepigenomics 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

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 in EGRP

African-American men & women

South American women

Asian-American & Asian men & women

Latin-American/Hispanics

African men & women

Alaskan & Hawaiian Natives

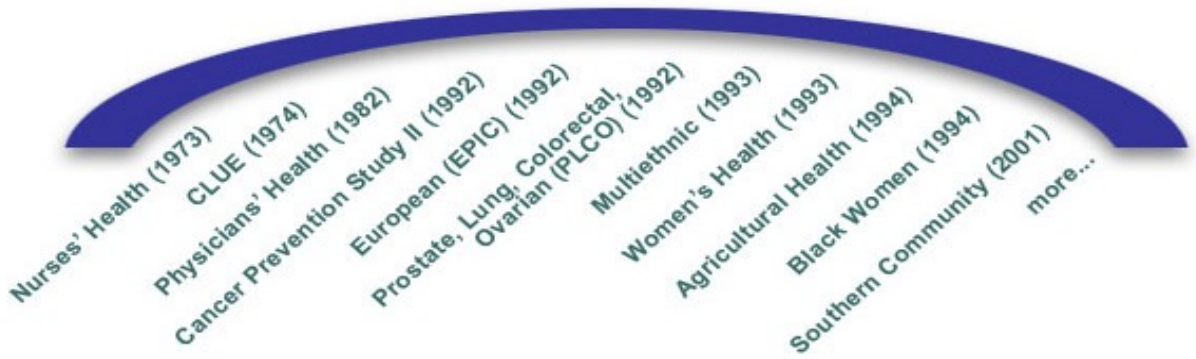
Middle-Eastern populations

American-Indian, incl. Navajo

Rural South

Chinese

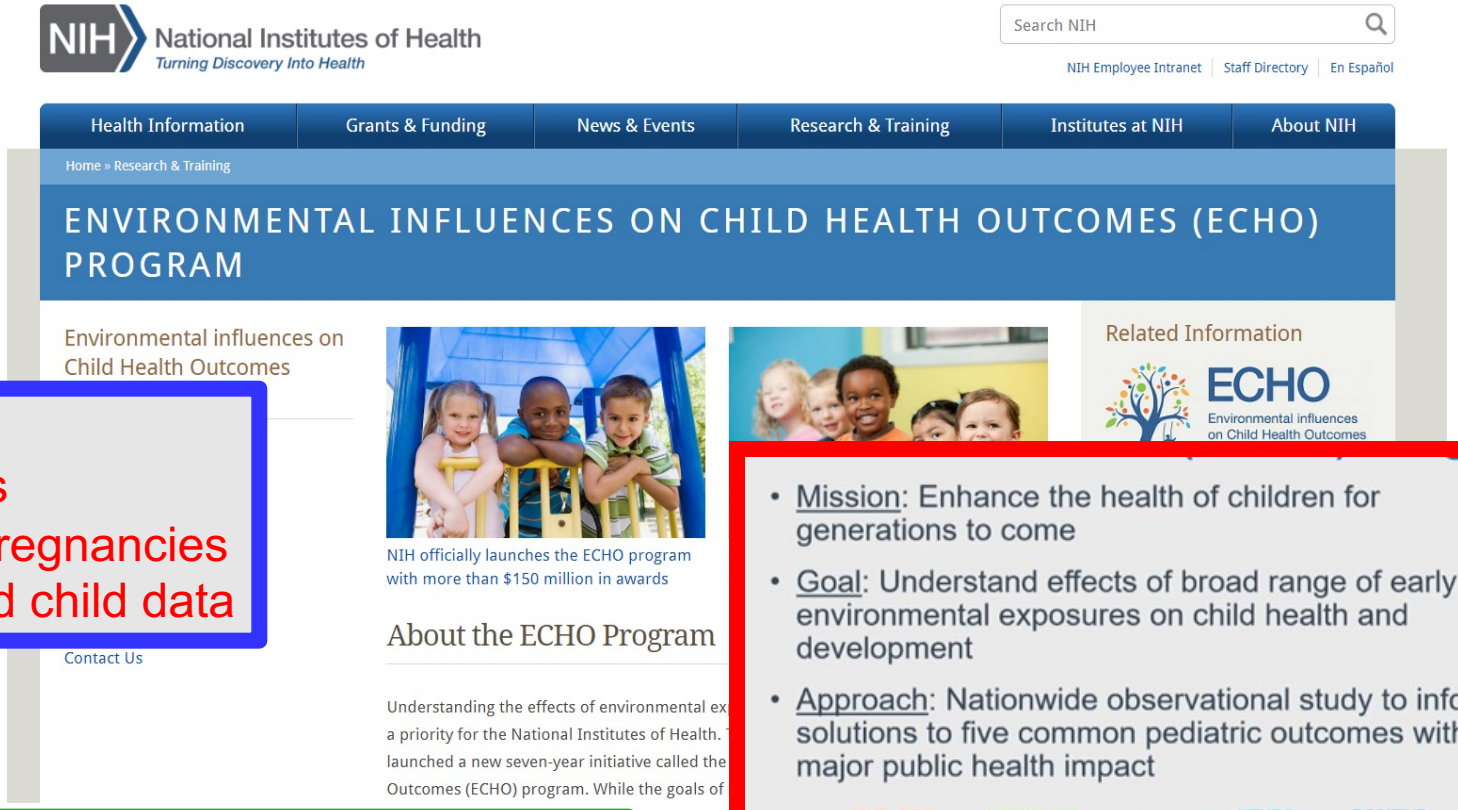
The Cohort Consortium (CoCo)



- 62 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.

ECHO

To evaluate how exposure to a range of environmental factors in early development influences health of children and adolescents



34 PIs
79 Cohorts
>50,000 Pregnancies
Mother and child data

- Allergy and upper airways diseases
- Pre-, Peri-, Post-natal
- Neurological disorders
- Obesity

- Mission: Enhance the health of children for generations to come
- Goal: Understand effects of broad range of early environmental exposures on child health and development
- Approach: Nationwide observational study to inform solutions to five common pediatric outcomes with major public health impact

Developmental Life Stages

Preconception/Prenatal	Anything prior to labor
Perinatal	Labor through discharge (or < 1 month?)
Infancy	1 month through 11 months, 30 days
Early Childhood	12 months through 59 months
Middle Childhood	60 months through 11 years, 11 months
Adolescence	12 years through 18 (or 21?) years

ılk

[RFP FY17]

Serial samples of the same individuals

√

Placenta, cord blood, nail, hair, saliva, urine
Maternal blood, milk before and after pregnancy

ECHO-wide Cohort Protocol

16S amplicons,
Metagenomic and Metatranscriptomic
Cytokine profiling
Metabolomics
Proteomics
Genomics
Exposure data integration
Phenotypic data integration

Advantages of ECHO Research Design

- Longitudinal cohorts – opportunity to examine repeated measures
  - -in utero
  - - early in life
  - - other transition periods
- Look across multiple tissues in same person
- Unifying/harmonizing epigenetic data with other data (including other omics data)
- Potential for single cell analysis
- Across generation

Phil Sharp

Loss (or Gain) of gene function in cancer

Most permanent → Most dynamic

Deletion
Point mutations
Amplification
Chromosomal Translocation (Ig rearrangement)

Chromatin Changes
Promoter Methylation Silencing

Transcription Factor Changes

Cell-cycle Regulated Changes

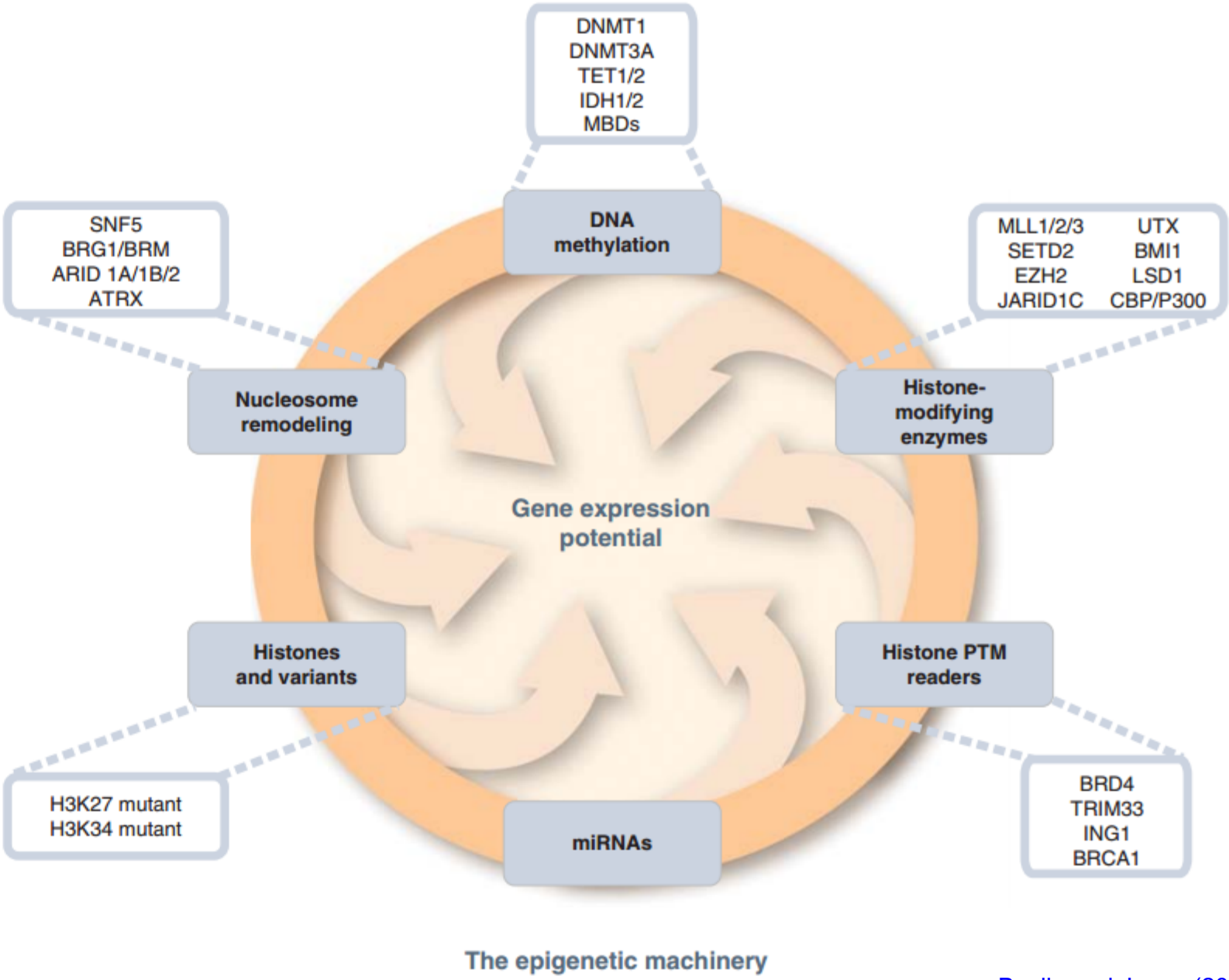
Genetic

Epigenetic

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

James Herman

Genetic mutations of epigenetic modifiers in cancer



Baylin and Jones (2016)

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Epigenetics. 2016 Nov 28:0. [Epub ahead of print]

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

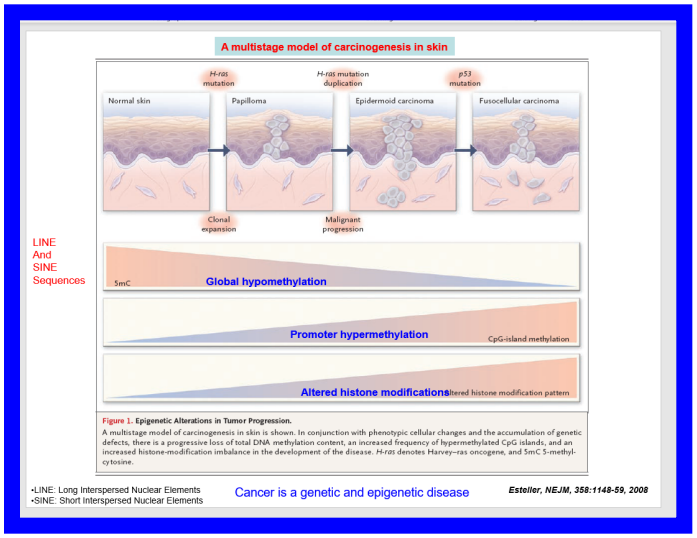
Fiano V[1], Zugna D[1], Grasso C[1], Trevisan M[1], Delsedime L[2], Molinaro L[2], Gillio-Tos A[1], Merletti F[1], Richiardi L[1].

Author information

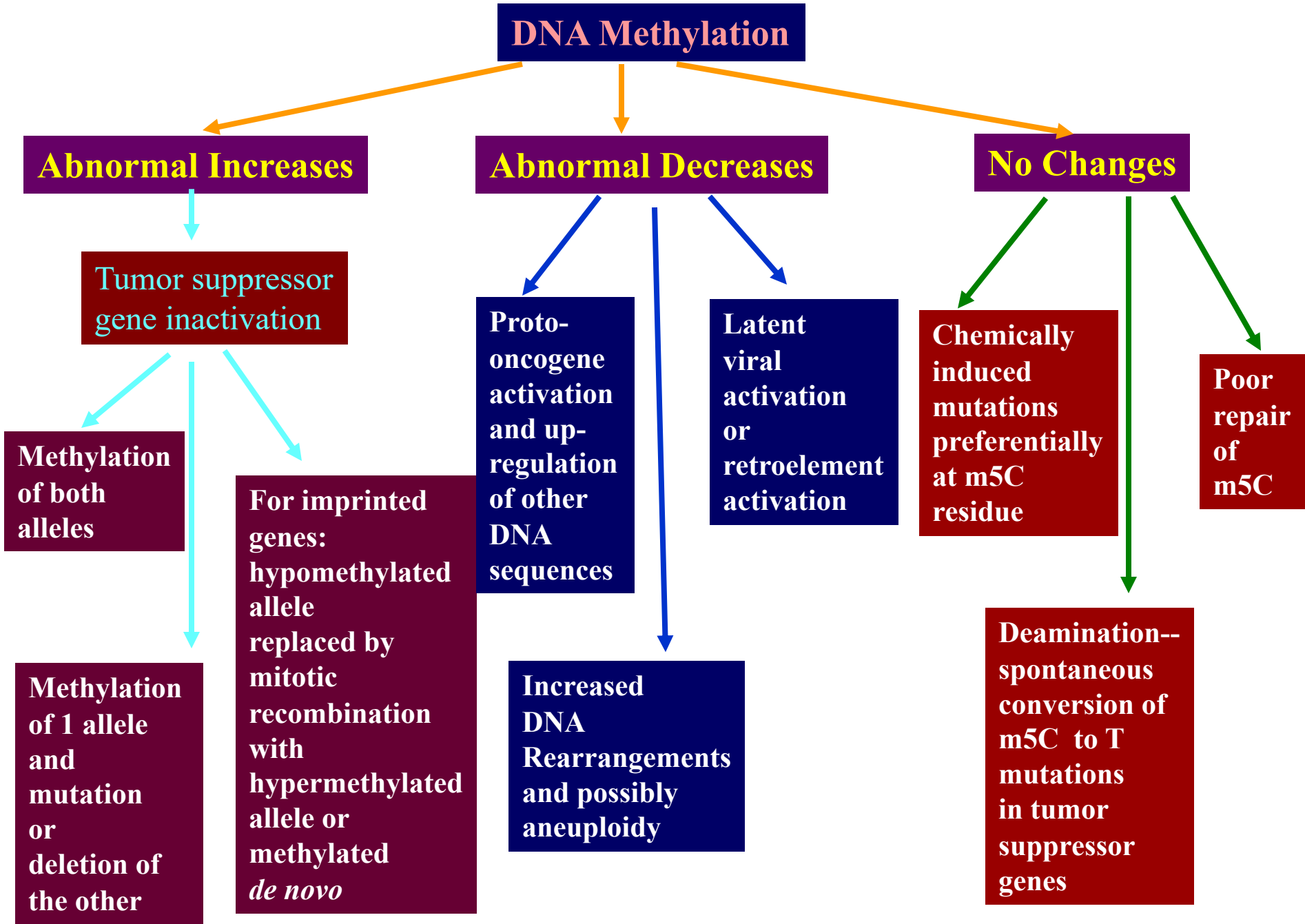
Abstract

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 1982-1988 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: 1.40, 95% confidence interval (CI): 0.95-2.01]. 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 (<75%) vs. high (>80%) LINE-1 methylation was associated with a hazard ratio of 4.68 (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 cancerogenesis and is associated with tumor progression.

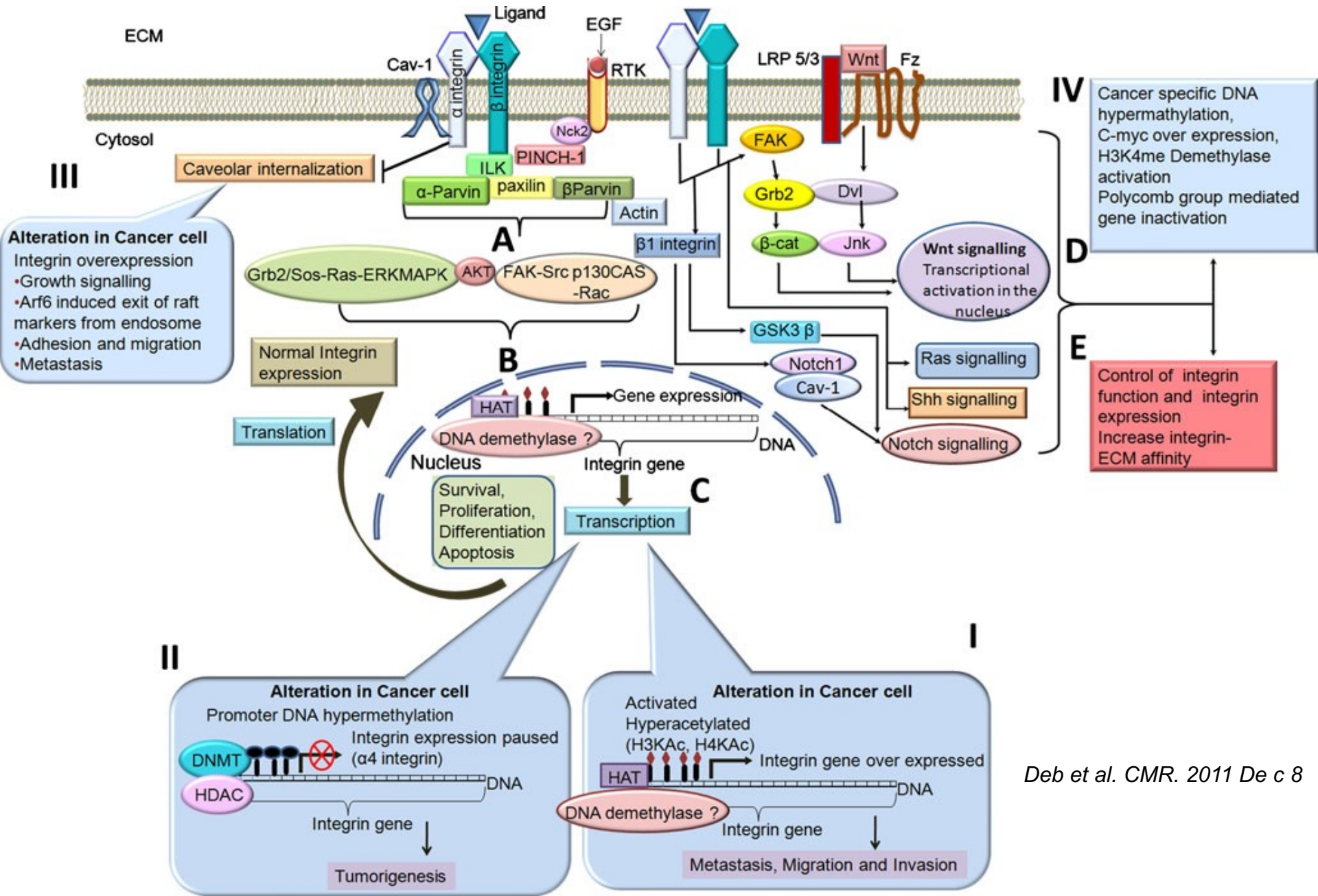
Tumor tissue global hypomethylation may be a late event
in prostate cancerogenesis and is associated with tumor progression.



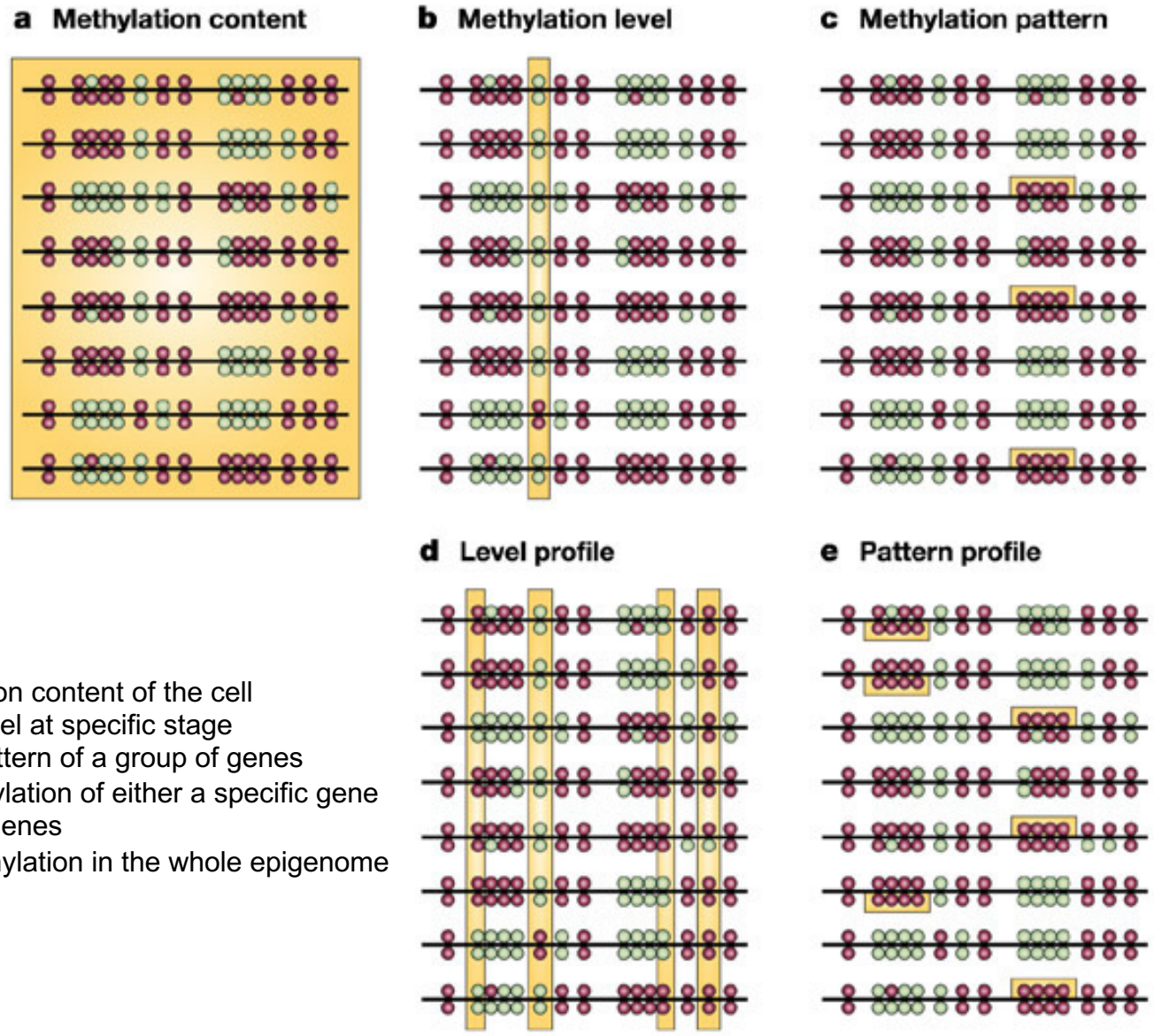
DNA Methylation and Carcinogenesis



Integrin Signaling Network and Epigenetic Regulation



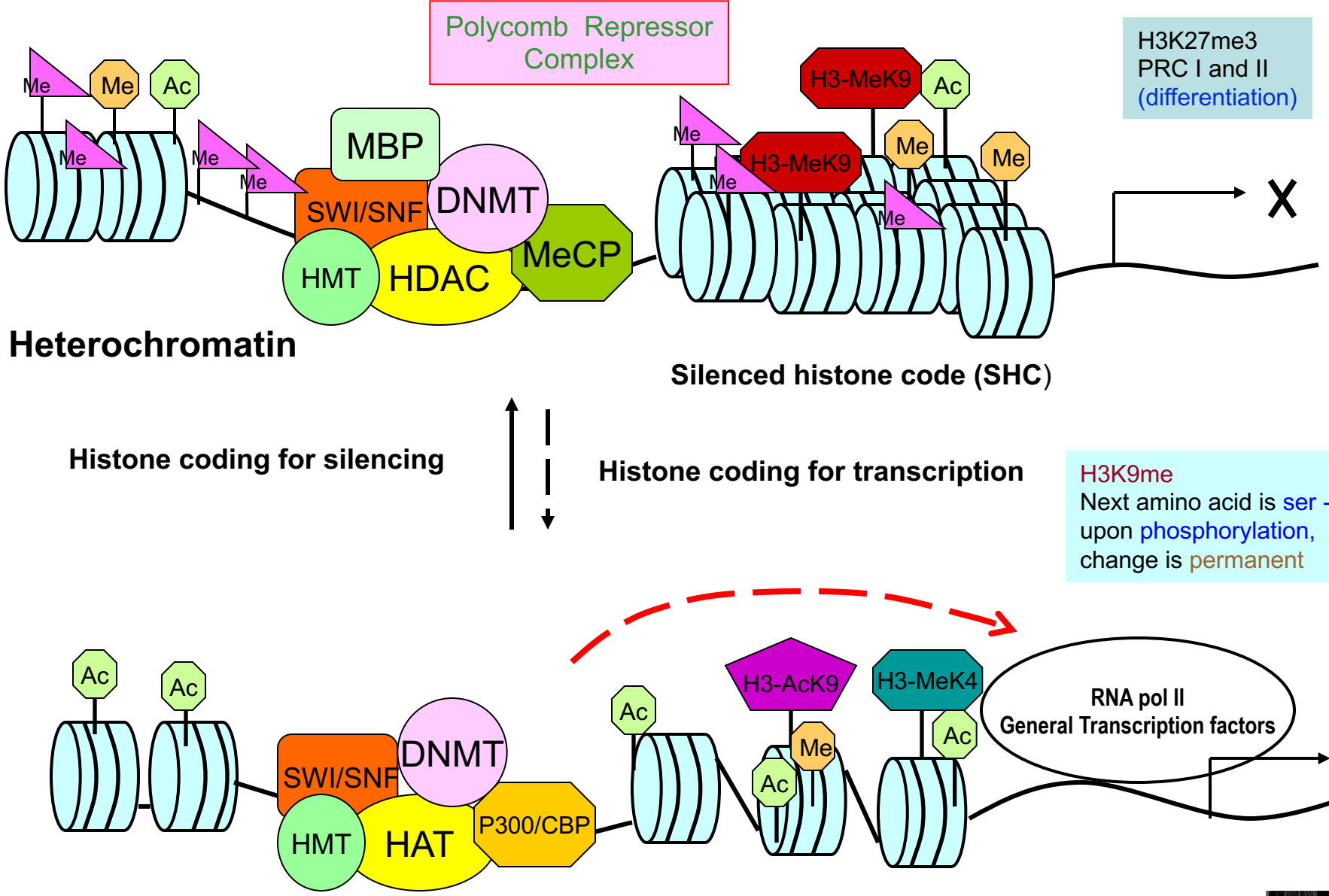
Deb et al. CMR. 2011 De c 8



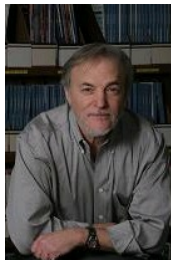
- Total methylation content of the cell
- methylation level at specific stage
- methylation pattern of a group of genes
- profile of methylation of either a specific gene or a number of genes
- pattern of methylation in the whole epigenome

To reduce
- false negative
- false positives

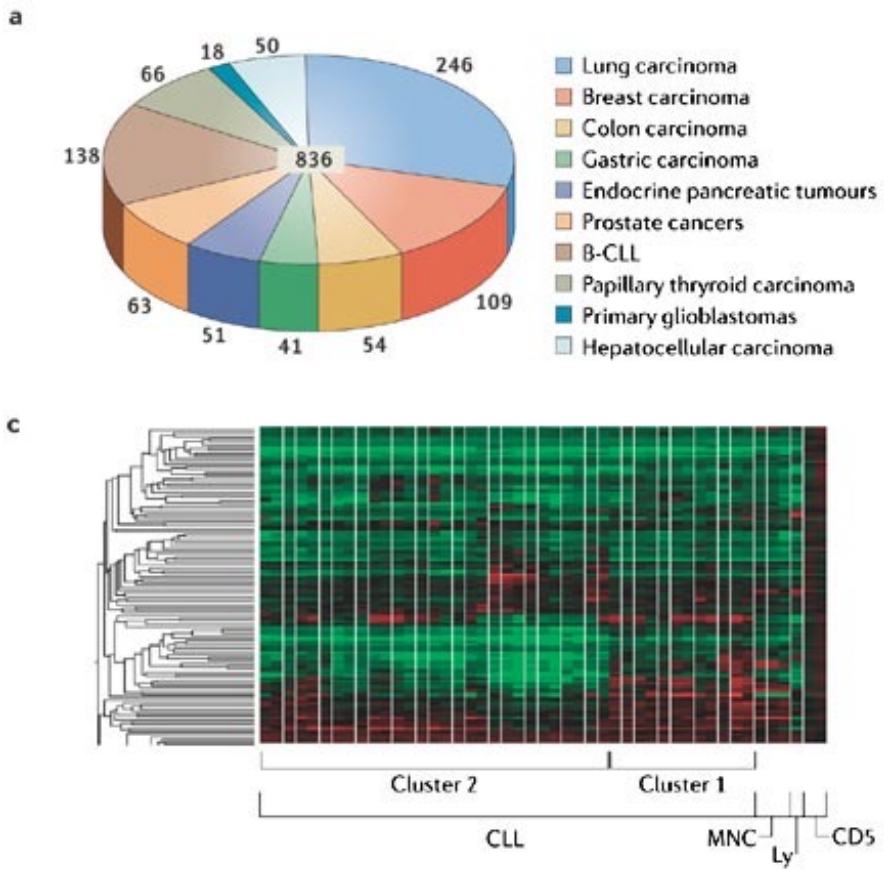
Laird *Nat Rev Cancer* 3:253



Steve Baylin



Mirco RNA Signatures in Human Cancers



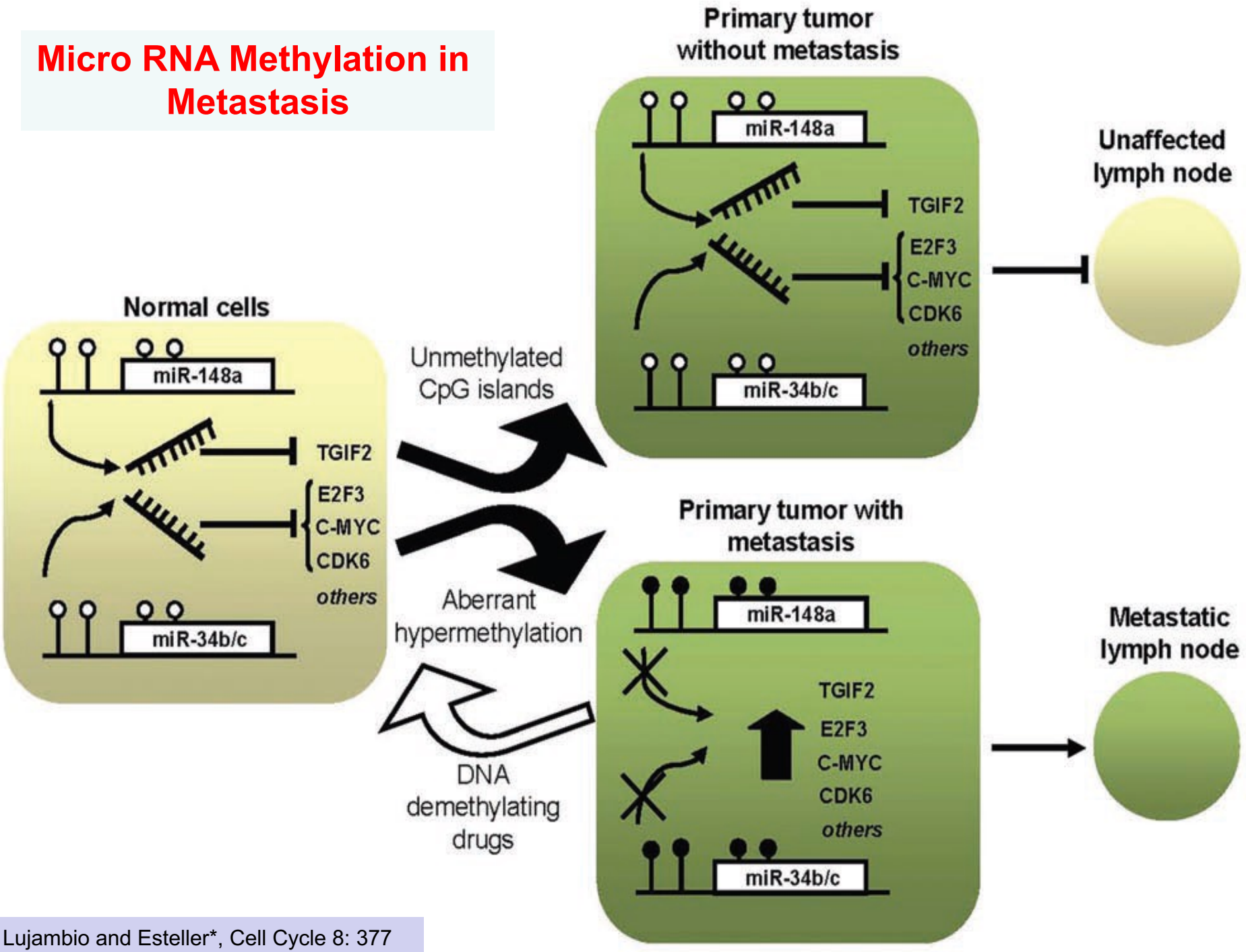
Micro RNA Polymorphism to Identify High Risk Populations

357–866 (November 2006) | doi:10.1038/nrc1997

Mir-31 inhibits metastasis in breast cancer

nature REVIEWS CANCER

Micro RNA Methylation in Metastasis



Lujambio and Esteller*, Cell Cycle 8: 377

nature COMMUNICATIONS

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 Shimamura[4], Daisuke Motooka[5], Daisuke Okuzaki[5], Koichi Kawamoto[6], Tsunekazu Mizushima[6], Hidetoshi Eguchi[6], Shuji Takiguchi[6,7], Taroh Satoh[1], Koshi Mimori[8], Takahiro Ochiya[9], Yuichiro Doki[6], Ken Ofusa[3], Masaki Mori[6] & Hideshi Ishii[2]

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 unvarying 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

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

BMC
Clinical Pathology

REVIEW Open Access

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

Mukesh Verma*, 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,

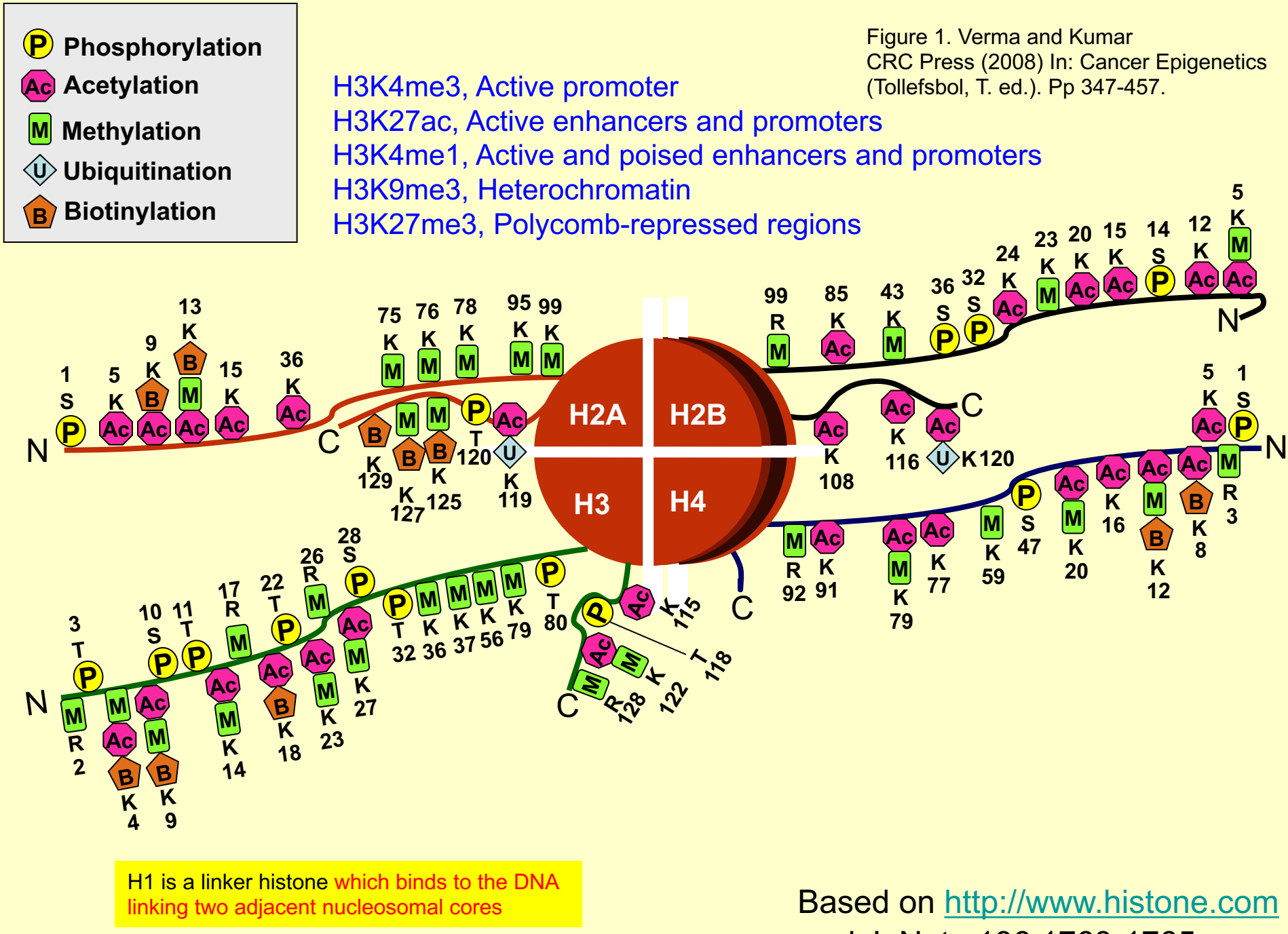
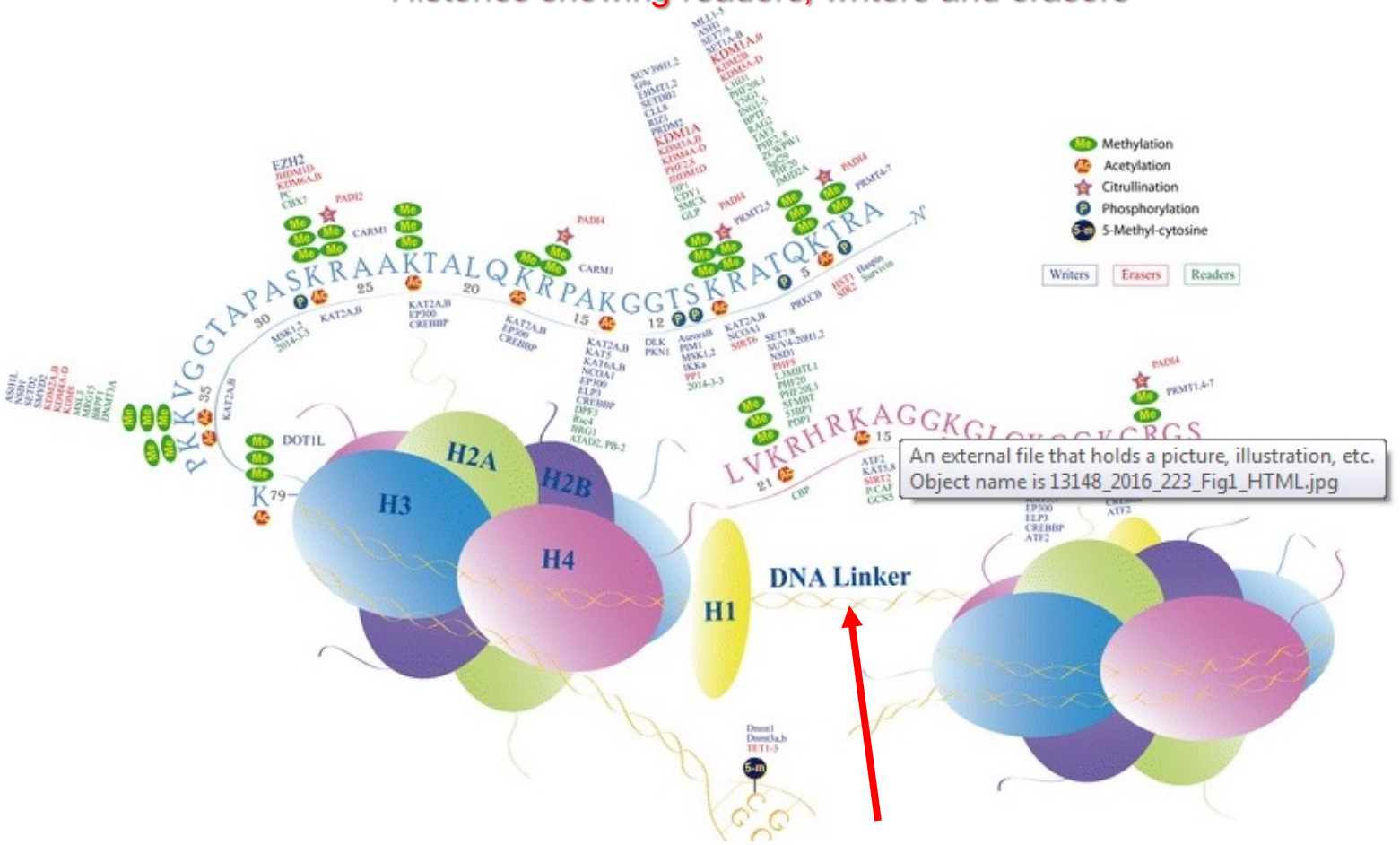


Figure 1. Verma and Kumar
CRC Press (2008) In: Cancer Epigenetics
(Tollefsbol, T. ed.). Pp 347-457.

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

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

Histones showing readers, writers and erasers



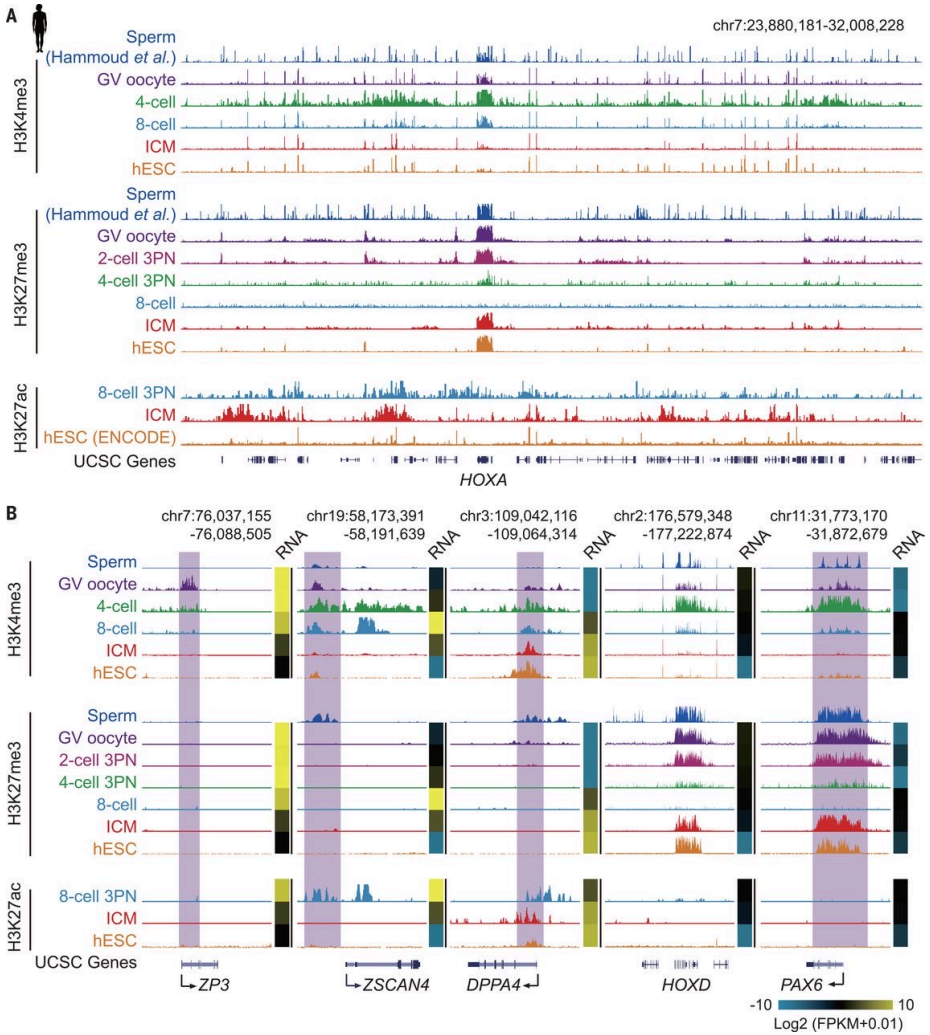
Clin Epigenetics. 2016; 8: 57

Activating: e.g. H3K4me3

Silencing: e.g. H3K9me3, H3K27me3

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

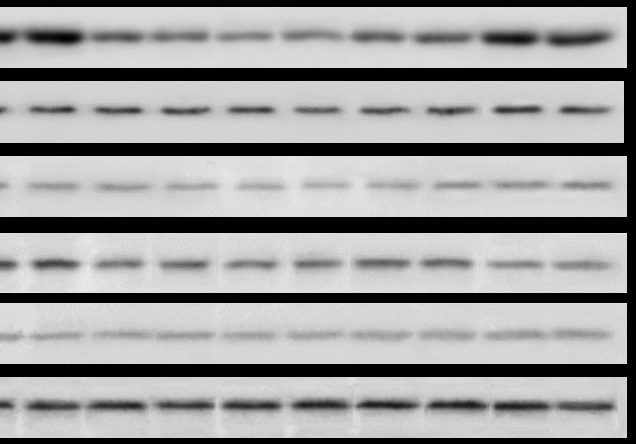
Histone mapping

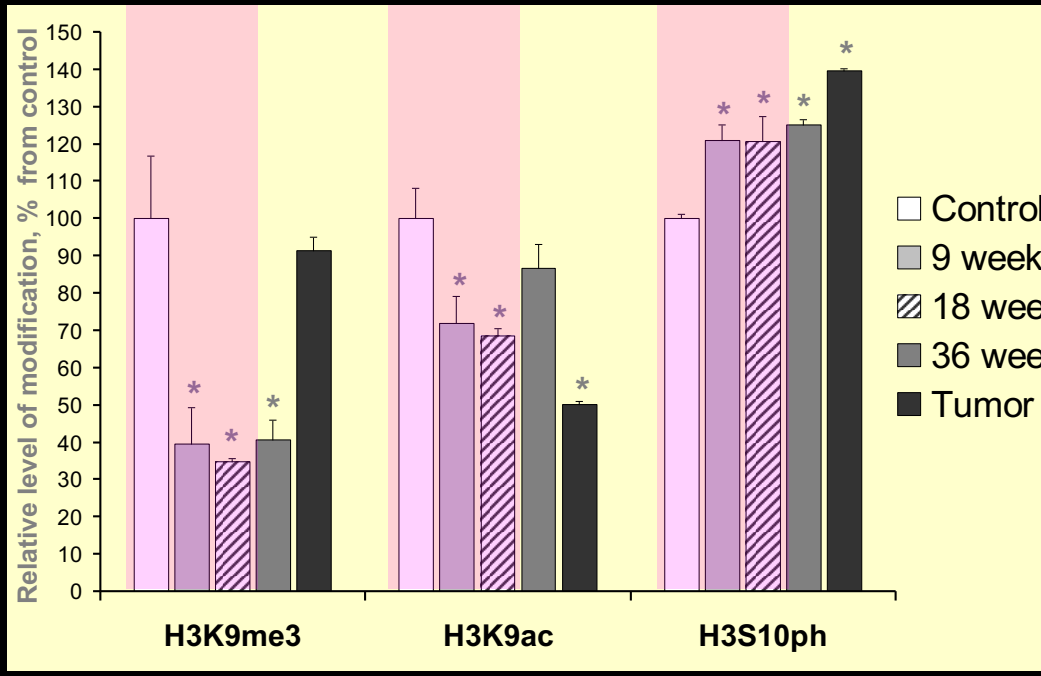


Weikun Xia et al. Science 2019;365:353-360

Science AAAS

ALTERATIONS OF HISTONE H3 MODIFICATIOINS IN LIVER DURING METHYL DEFICIENCY





N-ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKP-…-C

Interplay between H3K9me3, H3K9Ac, and H3S10ph

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

20 Diagnosing Cancer Using Histone Modification Analysis

Mukesh Verma and Deepak Kumar

CONTENTS

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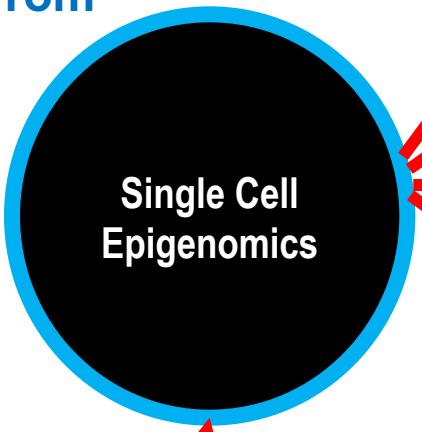
ISBN 9781420045796 - CAT# 45792

SINGLE CELL EPIGENOMICS

Single cells isolated from

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



Identify open and closed chromatin

Identify cell-specific transcription factors

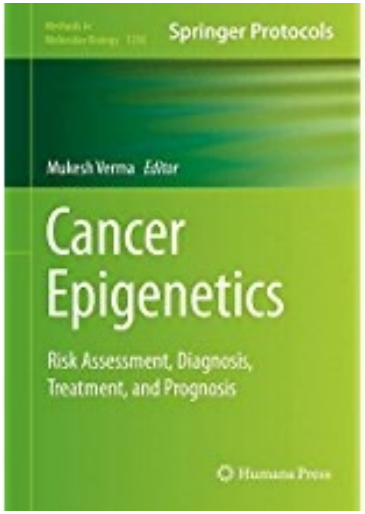
Determine nucleosome position

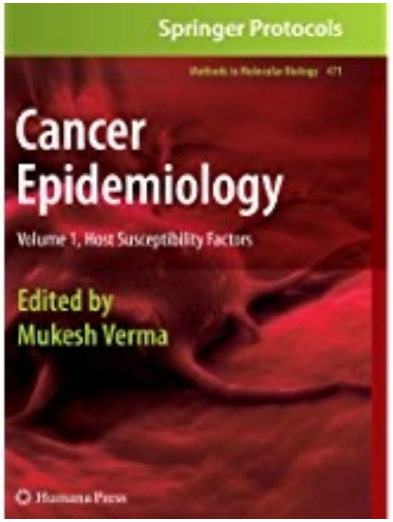
Identify active and repressive transcription state

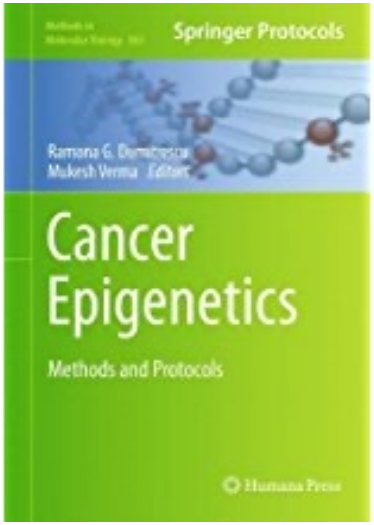
1. Methylation profiling
2. Histone modifications
3. miRNA profiling
4. Chromatin Accessibility

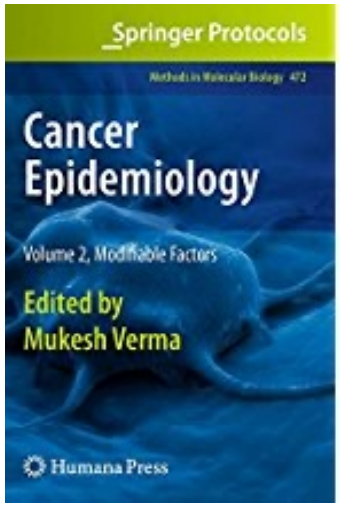
Implications of single cell epigenomics

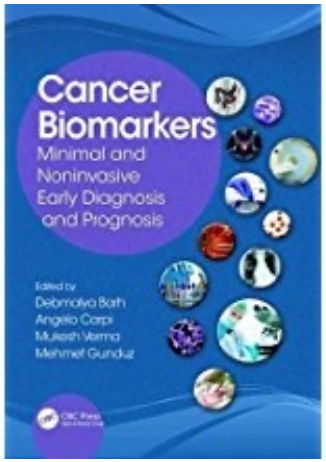
Risk Assessment to identify high-risk individuals
Diagnosis
Prognosis
Screening
Follow up treatment and co-morbidity

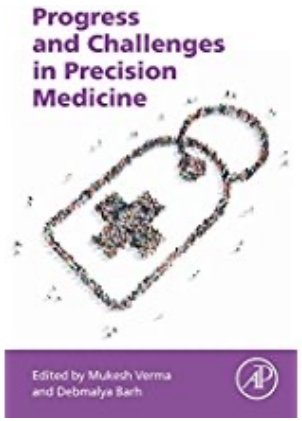


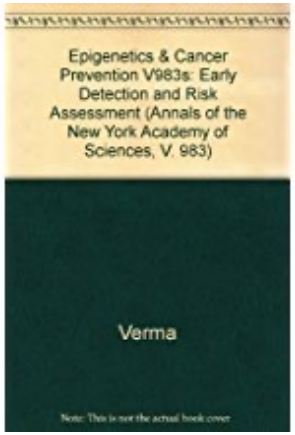












Books edited by Mukesh Verma

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, Baylin reasoned, this epigenetic change would produce the same result as a genetic mutation.

Firm evidence came in 1994. Baylin 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 abilit express the tumour suppressing protein. Baylin and Herman showed that 20% of the remaining p non-inherited form of RCC did not have a mutation in VHL. Their genes were silenced not b rather by hypermethylation[2].

The following year, in collaboration with Sidransky's lab at Johns Hopkins, Baylin an human cancers commonly arise when a particular tumour supp-ressor gene, known Moreover, in many cancers including RCC, epigenetic and genetic mutations often w two copies of a tumour suppressor gene is inactivated by genetic mutation, while the ot This finding "convinced us that epigenetic abnormalities could play an important driving role and many others have been pursuing this possibility ever since," says Baylin.

The move from a purely genetic to an epigenetic model is crucial for prevention strategies. As numerous gen therapy trials have shown, it is very difficult to treat a genetic disease by re-activating the dormant, mutated ge or by replacing it with a non-mutated one. "Epi-genetic 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 Baylin.

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

Nature 471: s12-s13

"Epigenetic changes are reversible, and therefore have an edge over genetics"
Mukesh Verma
Nature 471: s12-s13

programme has funded around 200 jobs. The bulk of the funding for these large-scale programmes is already dedicated to the larger sequencing centres, but smaller teams are using the data from these projects to generate individual-investigator grant applications, Shaw adds.

These data have helped to persuade investors in industry that epigenetic abnormalities in c... wealth of new drug targets. The finding that mutations in epigenetics-related genes may be drivin... offers the tantalizing possibility of taking a personalized approach to cancer treatment, a tack that is rapi... ground 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 En Li, head of China Novartis Institutes for Biomedical Research, based in Shanghai. 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. "GSK'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."

"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

Nature 483:637-639

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 Vakoc, 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 Brd4 — 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, Vakoc heard rumours that ten companies were racing to capitalize on the results.

There is also an intense demand for talent. In particular, epigenetics companies and individual labs need

Tumor Types and Genes Regulated by Epigenetic Mechanism

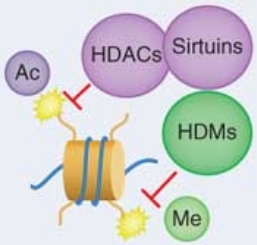
TUMOR LOCATION	GENE
Breast	p16, BRCA1, GSTP1, DAPK, CDH1, TIMP-3
Brain	p16, $p14^{ARF}$, MGMT, TIMP-3
Bladder	p16, DAPK, APC
Colon	p16, $p14^{ARF}$, CRBP1, MGMT, hMLH1, DAPK, TIMP-3, APC
Endometrium	hMLH1
Esophagus	p16, $p14^{ARF}$, GSTP1, CDH1APC
Head and Neck	p16, MGMT, DAPK
Kidney	p16, $p14^{ARF}$,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

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

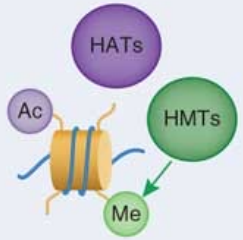
Verma and Srivastava (2002). Lancet Oncol. 3: 755-363;
Verma et al (2004) Crit. Rev. Clin. Sc. 41: 585-607;
Verma and Manne (2006). Crit. Rev. Hematol. Oncol. 60: 9-18;
Verma et al (2006). Mol. Diag. Therapy. 10: 1-15.

Examples of epigenetic modifications and affected genes

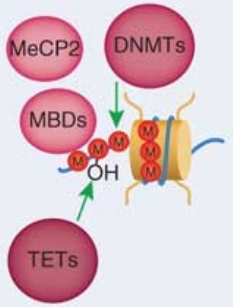
Category	Gene
HDACs	*HDAC1*
	HDAC2
	HDAC6
Sirtuins	*SIRT1*
	SIRT2
	SIRT3
	SIRT7
HDMs	*KDM1A*
	KDM2B
	KDM4C
	KDM5A
	KDM5B
	KDM5C
	KDM6A
	KDM6B



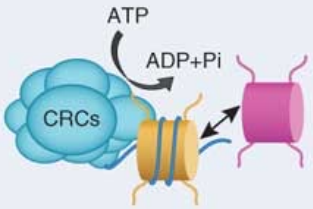
Category	Gene
HATs	*CREBBP*
	EP300
	MYST3
	MYST4
	KAT5
HMTs	*MLL*
	EZH2
	NSD1
	PRDM2
	SMYD3
	WHSC1



Category	Gene
DNMTs	*DNMT1*
	DNMT3A
	DNMT3B
(2OG)-Fe(II)–dependent oxygenases	*TET1*
	TET2
Methyl-CpG binding proteins	*MBD1*
	MBD2
	MBD3
	MBD4
	MECP2



Histone variants	*H2AFZ*
Chromatin remodeling factors	*ARID1A*
	CHD5
	CHD7
	MTA1
	MTA2
	MTA3
	SMARCA2
	SMARCA4
	SNF5



3CACC6

Category	Gene
Histone modification readers	*ING1*
	ING2
	ING3
	ING4
	ING5

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

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

- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting

Vorinostat Ph II (Gliobl)

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

Eggr et al Nature. 429:457.

Jean-Pierre Issa
Temple Univ.

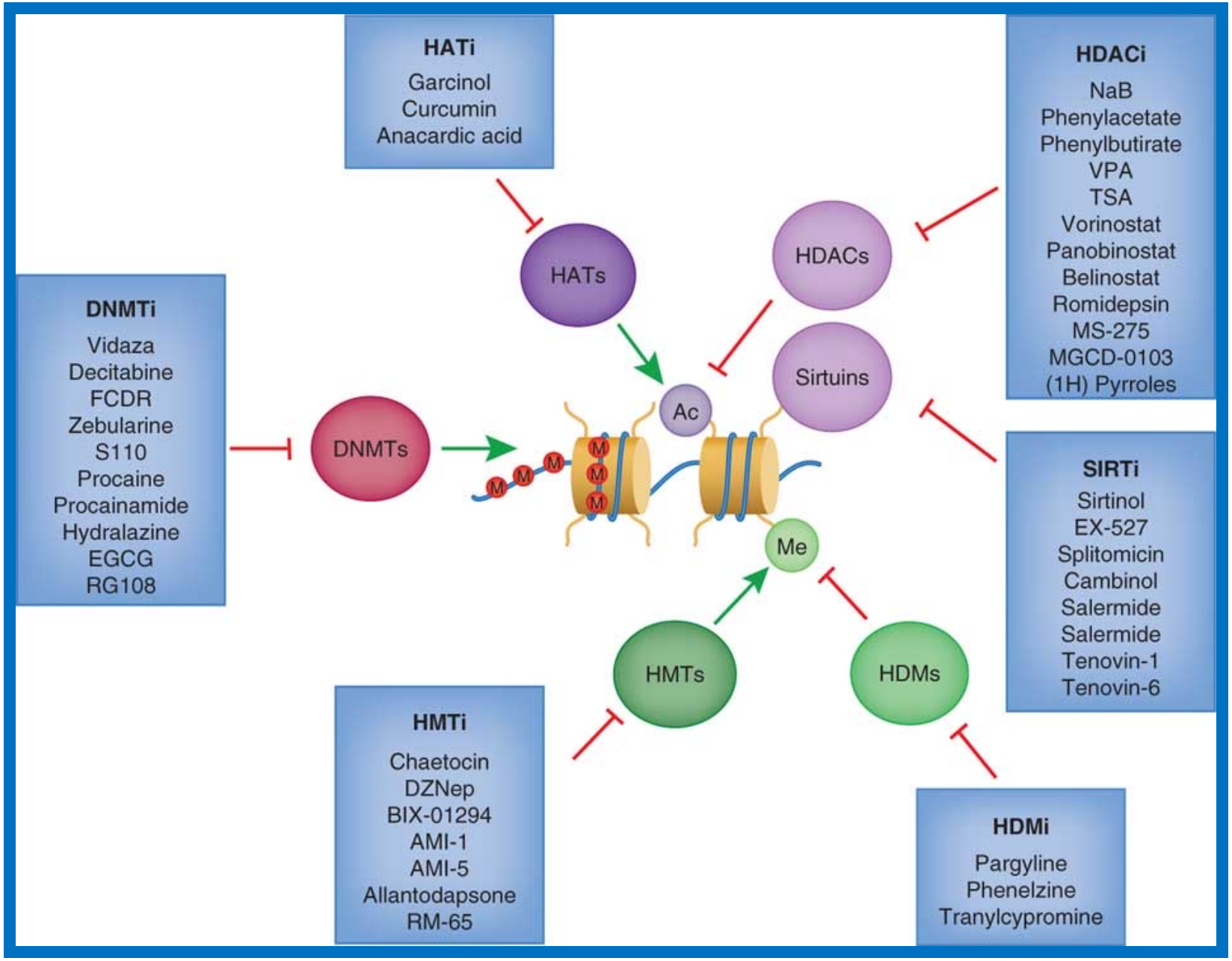

HATi
Garcinol
Curcumin
Anacardic acid
HDACi
NaB
Phenylacetate
Phenylbutirate
VPA
TSA
Vorinostat
Panobinostat
Belinostat
Romidepsin
MS-275
MGCD-0103
(1H) Pyrroles
HATs
HDACs
DNMTi
Vidaza
Decitabine
FCDR
Zebularine
S110
Procaine
Procainamide
Hydralazine
EGCG
RG108
Sirtuins
DNMTs
Ac
M
Me
SIRTi
Sirtinol
EX-527
Splitomicin
Cambinol
Salermide
Salermide
Tenovin-1
Tenovin-6
HMTs
HDMs
HMTi
Chaetocin
DZNep
BIX-01294
AMI-1
AMI-5
Allantodapsone
RM-65
HDMi
Pargyline
Phenelzine
Tranylcypromine

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

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

5 Azacytidine
S.C. daily for 10 days

+

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

28 Days Cycle

55 people with Advanced cancer Median age 60

Peripheral blood
- Pyrosequencing
- Chip

Analysis
Day 1, 10 and 28

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

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

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

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

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

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

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

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

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

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

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

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

Histone Inhibitors in Clinical Trials (Clinicaltrials.gov)

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

Total : 84 studies

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

Methylation Inhibitors in Clinical Trials (Clinicaltrials.gov)

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

Total : 51 studies

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

Schering-Plough (Decitabine {5-aza-Deoxycytadine} Trial for melanoma) (8 hrs to inactivate DNMT1)
Bristol-Myers Squibb (other compounds)

FDA Approved Epigenetic Drugs

Methylation Inhibitors

- **Azacytidine** (approved in 2004) For Myelodysplastic syndrome
- **Decitabine** (approved in 2006) For Myelodysplastic Syndrome

HDAC Inhibitors

- **Vorinostat** (approved in 2006) For Cutaneous T cell Lymphoma
- **Romidepsin** (approved in 2009) For Cutaneous T cell Lymphoma
- **Panobinostat** (approved in 2015) For Multiple Myeloma
- **Belinostat** (approved in 2014) For Peripheral T-cell Lymphoma

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‡
Gastrointestinal stromal tumours	Panobinostat (pan-deacetylase inhibitor)	Panobinostat 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	3 of 15 CA125		

AML subtypes and combination therapy

Pharmaceutical Participation

AML Subtype	Drug	Company
Tet2/WTI	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

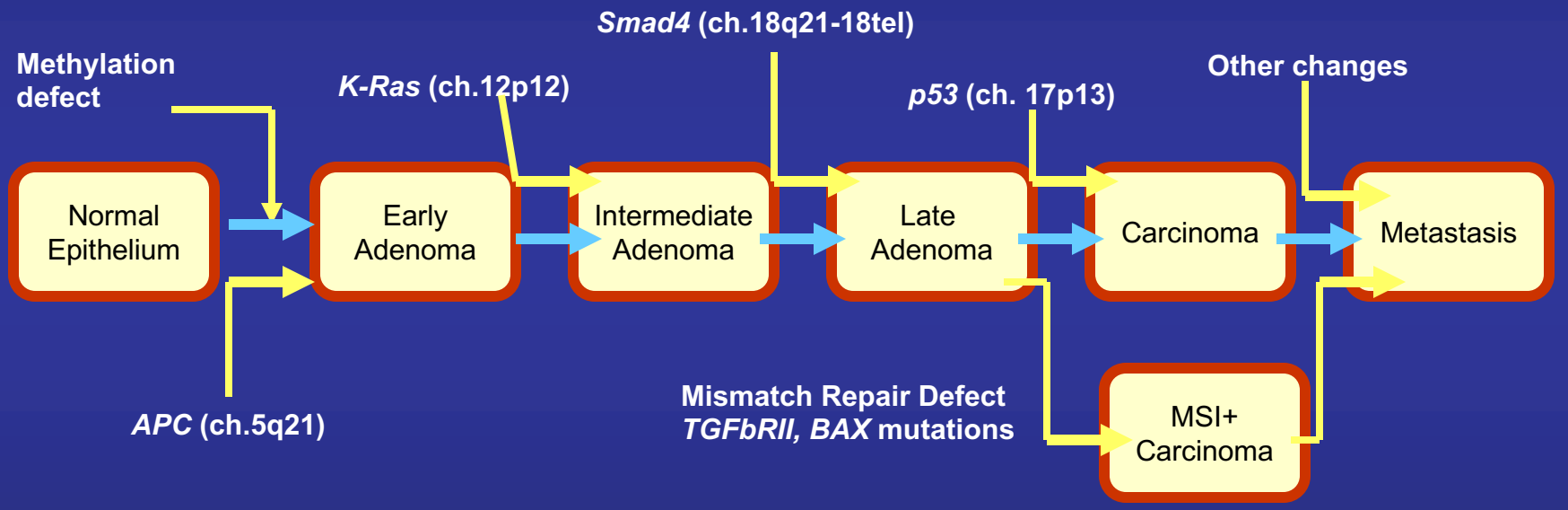
Epigenetic Therapy for Colorectal Cancer

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

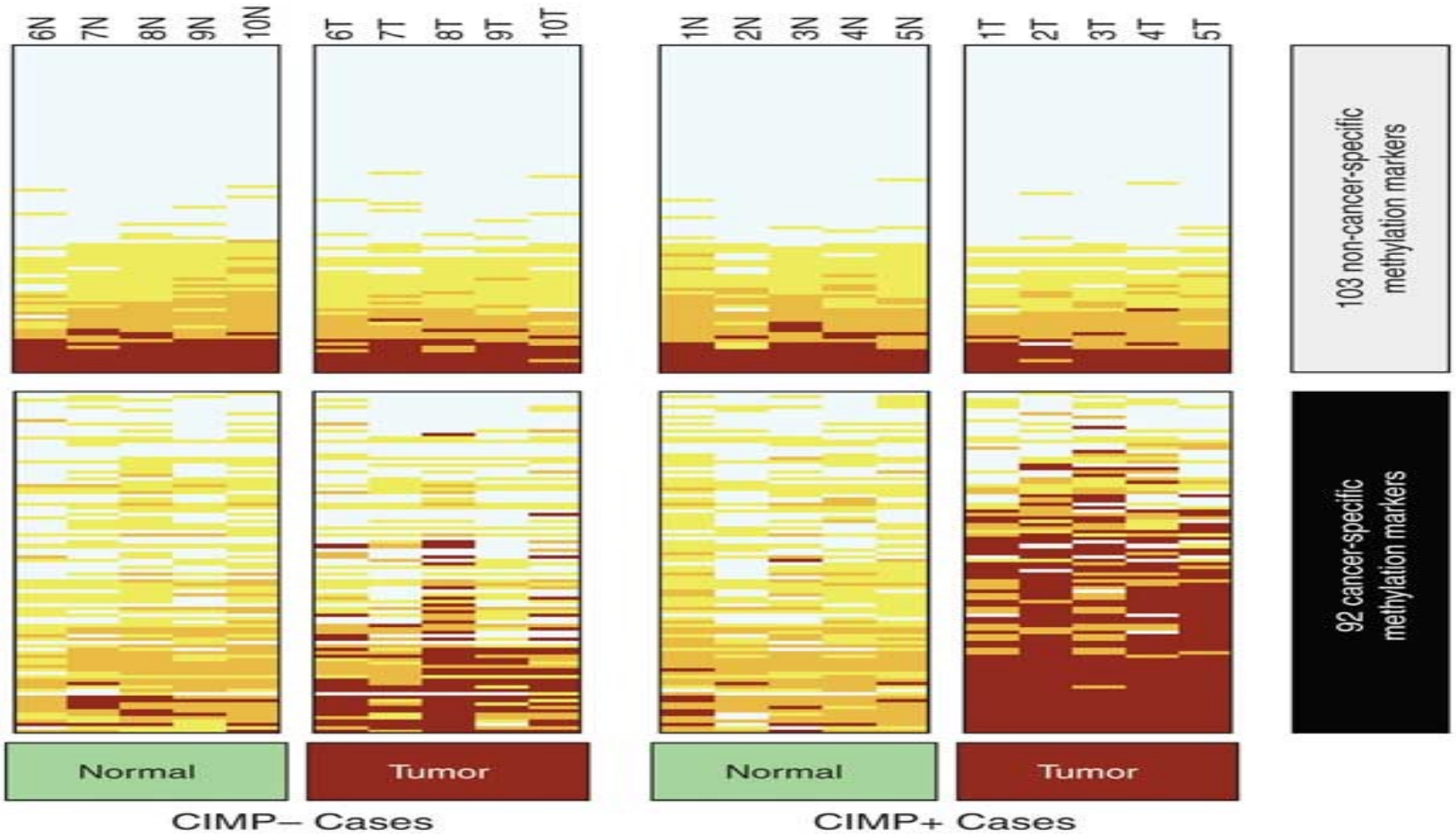
Potential Steps for Intervention



A Model for Colorectal Tumorigenesis

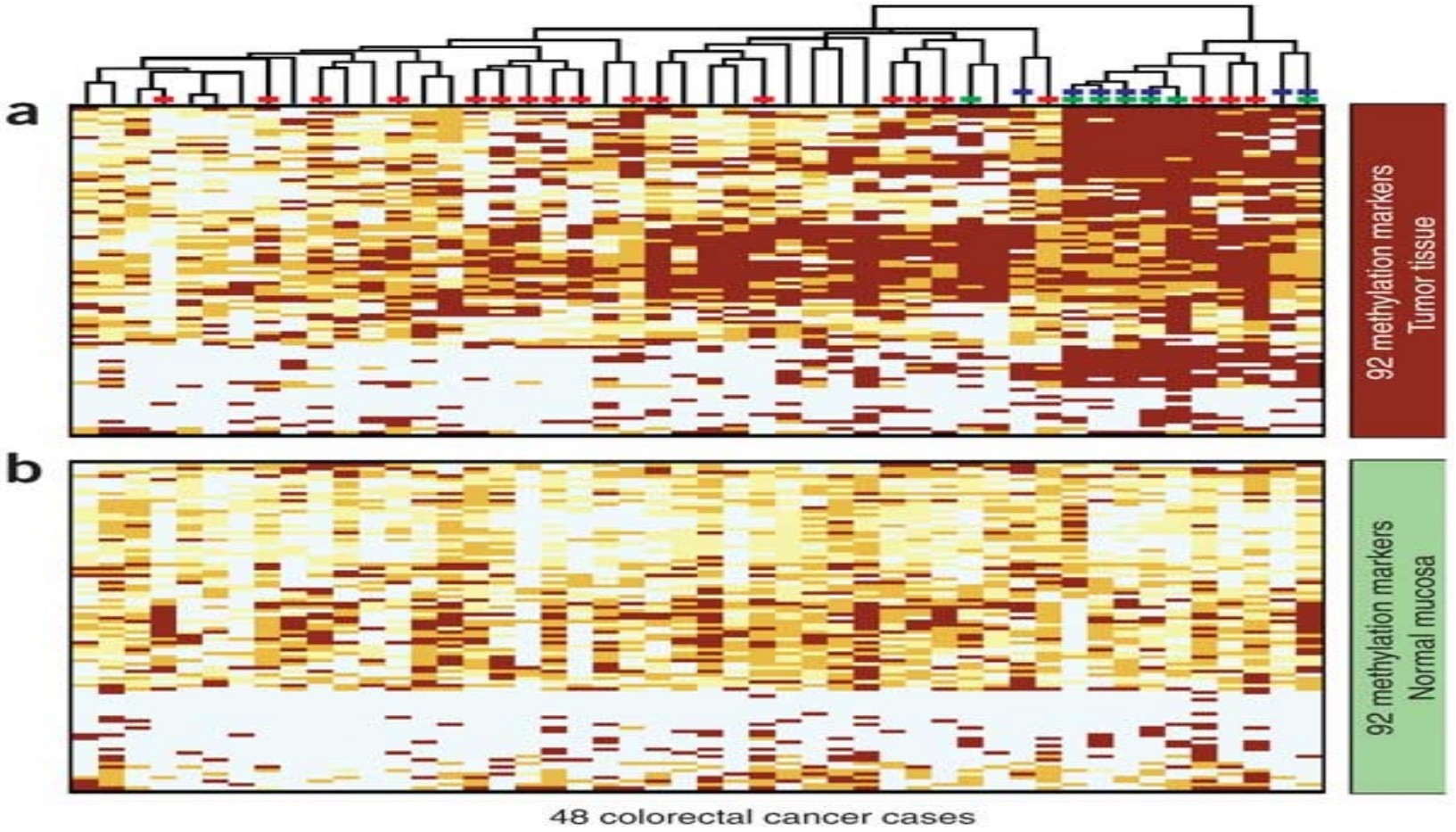
Modified from Jubb et al. *J Path*. 195: 111.

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



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

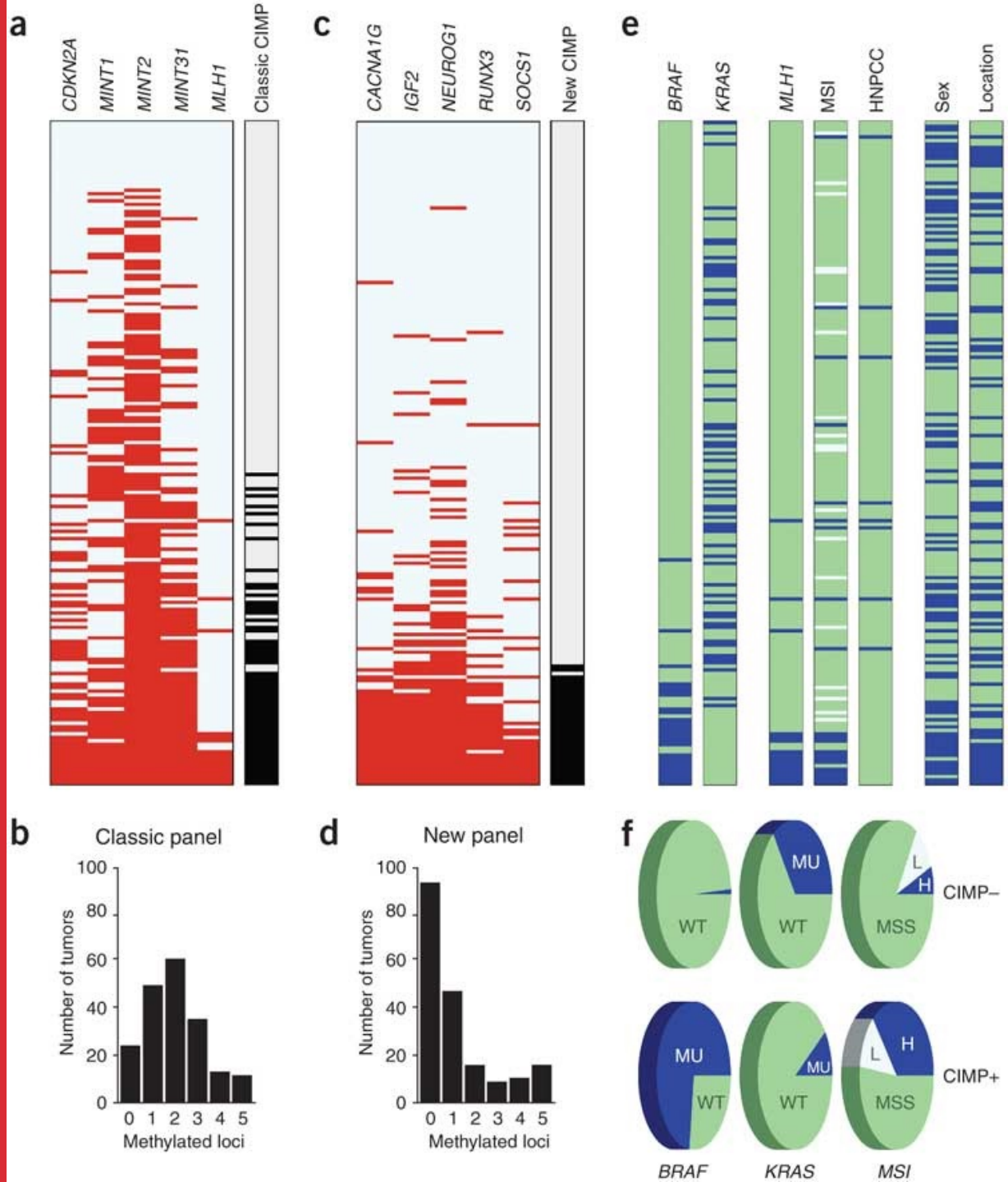
Identification of tumor clusters.



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

48 Colorectal tumors

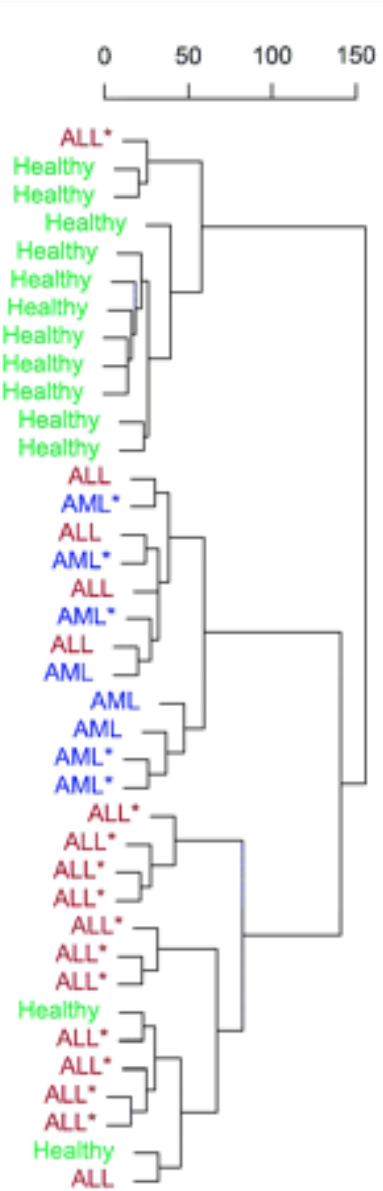
Nature Genetics ***38****, 787 - 793*



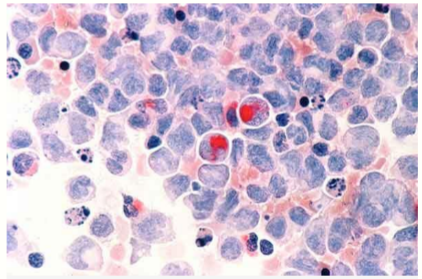
MSI-high (MSI-H) (blue),
MSI-low (MSI-L) (light blue)
or microsatellite stable (MSS) (green).

National Cancer Institute

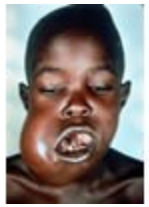
Prediction of Tumor Class based on Methylation Analysis (AML and ALL)



AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia



Human cells with acute myelocytic leukemia.
Credit: National Cancer Institute



Lymphoma

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

Epigenetic Markers During Lung Cancer Progression

Annexin I and II

PIK3CA

p63

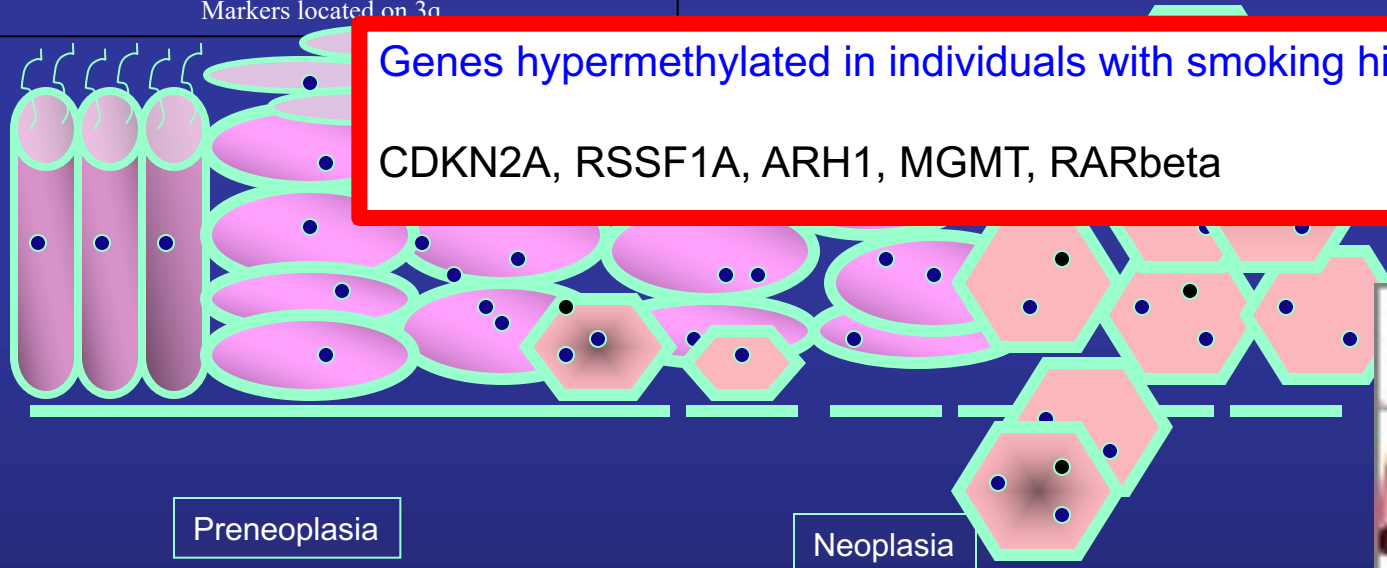
RARbeta,
DAPK, GSTP1, FHIT
RASSF1, MGMT

Markers located on 3q

Sputum from cancer survivors and cancer patients show different methylation pattern

Genes hypermethylated in individuals with smoking history:

CDKN2A, RSSF1A, ARH1, MGMT, RARbeta



Preneoplasia

Neoplasia

Small Cell Lung Carcinoma

Progression of Lung Cancer

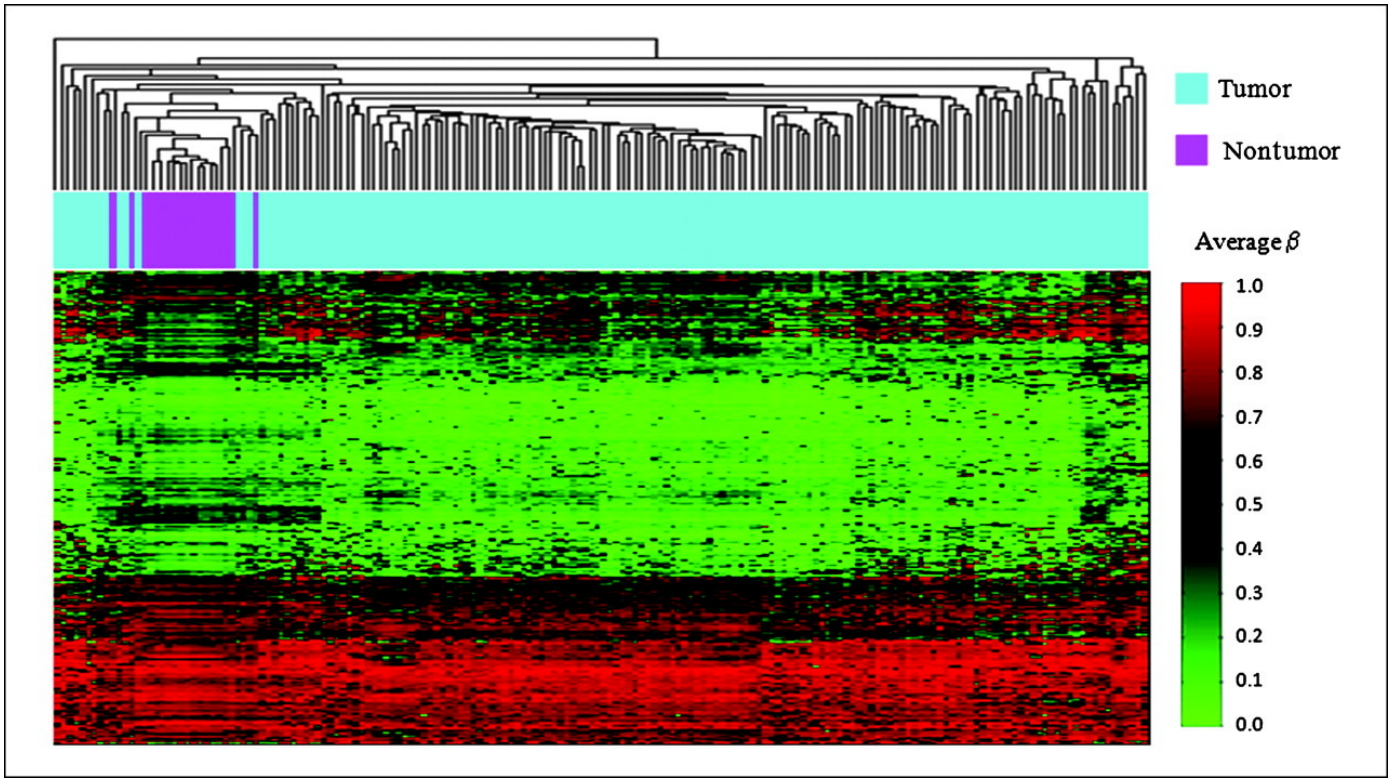
Steven Belinsky

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

ASBESTOS

MESOTHELIOMA

Non-Mutagenic carcinogen



803 cancer related genes

158 pleural mesothelioma with minimum mutation

18 normal pleura

Prediction of survival

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

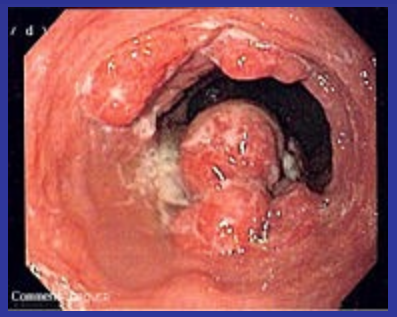
Epigenetic Profiles Distinguish Pleural Mesothelioma from Normal Pleura and Predict Lung Asbestos Burden and Clinical Outcome

Cancer Research

Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

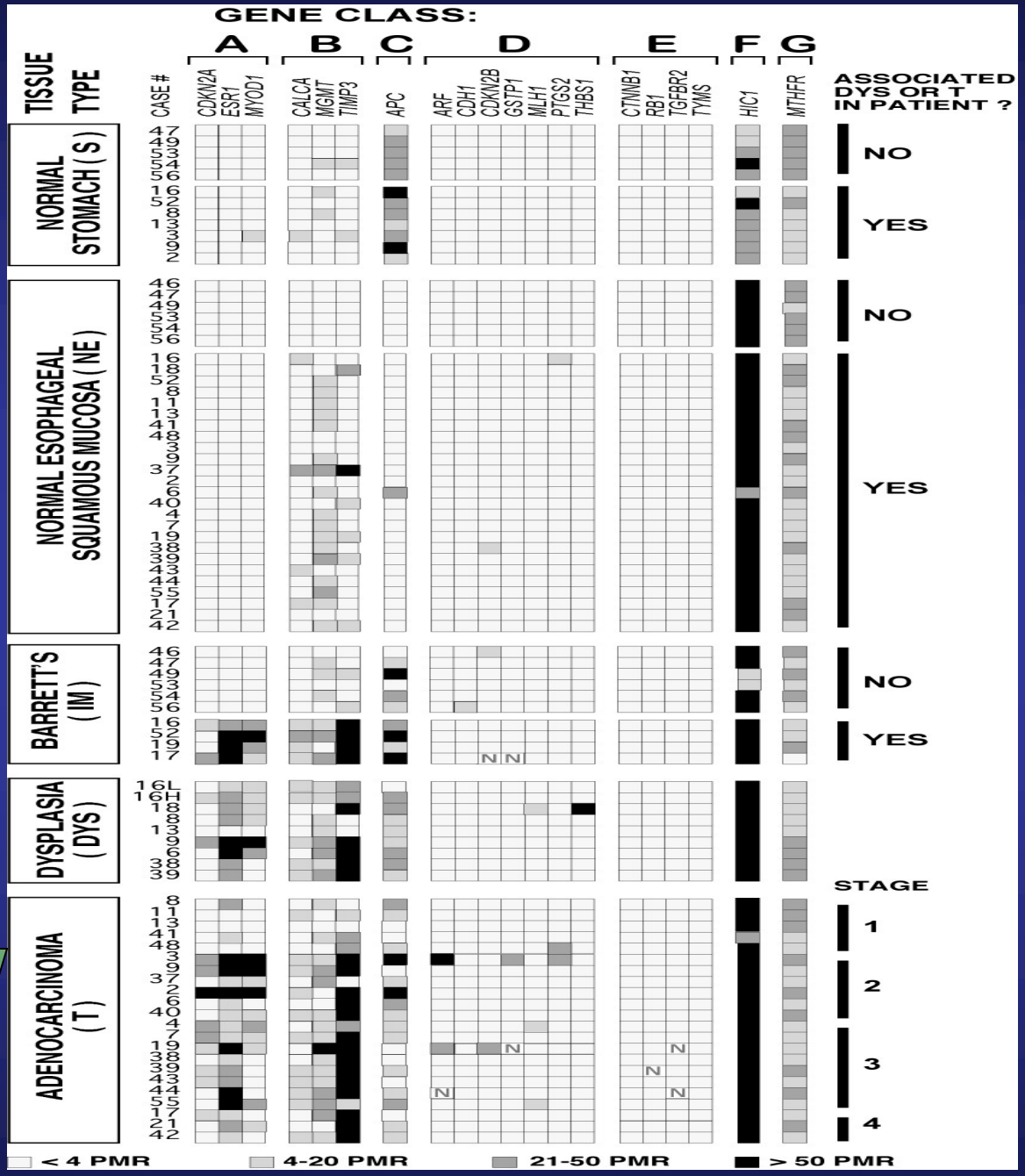
Cancer Research

61:3410

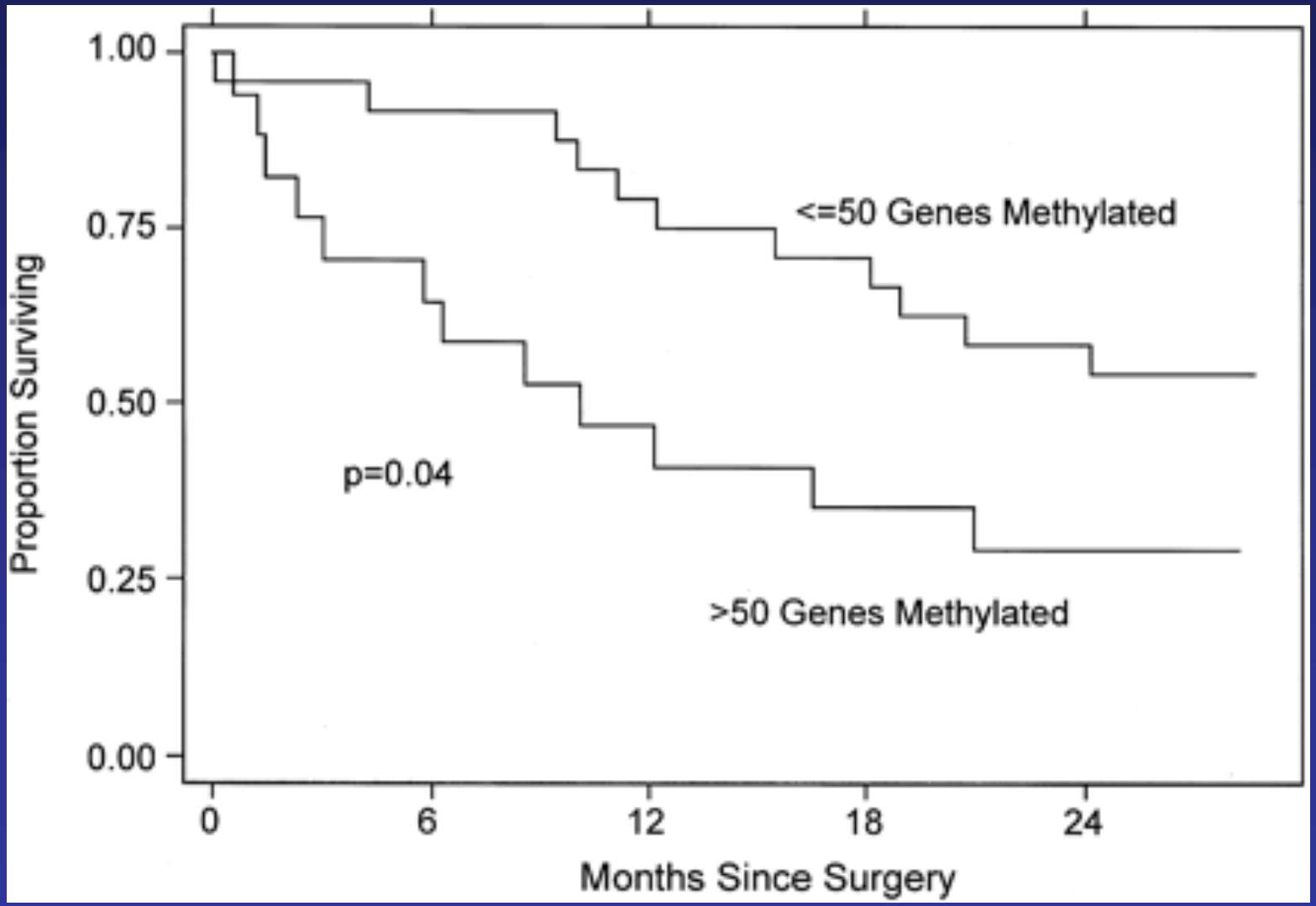


Cancer Progression

Risk factors
- Gastroesophaegeal Reflex Disease (GEPD)
- Smoking
- Hihger Body Mass Index (BMI) or obesity



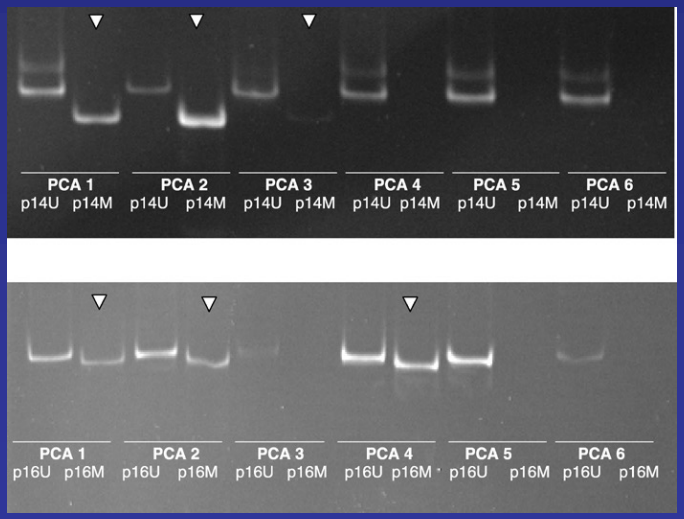
Esophageal Cancer: Probability of Survival

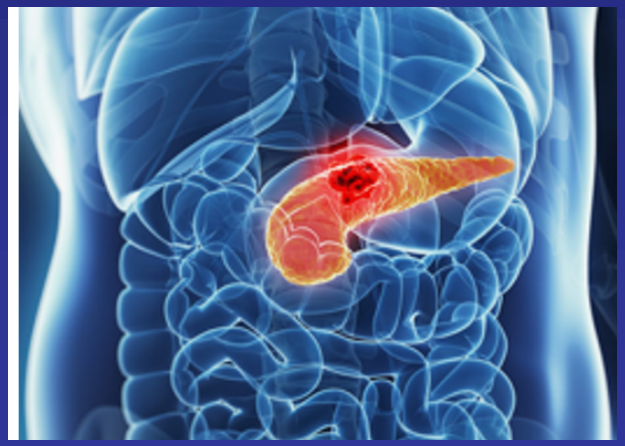


Brock et al. Clinical Cancer Research. 9: 2912

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

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

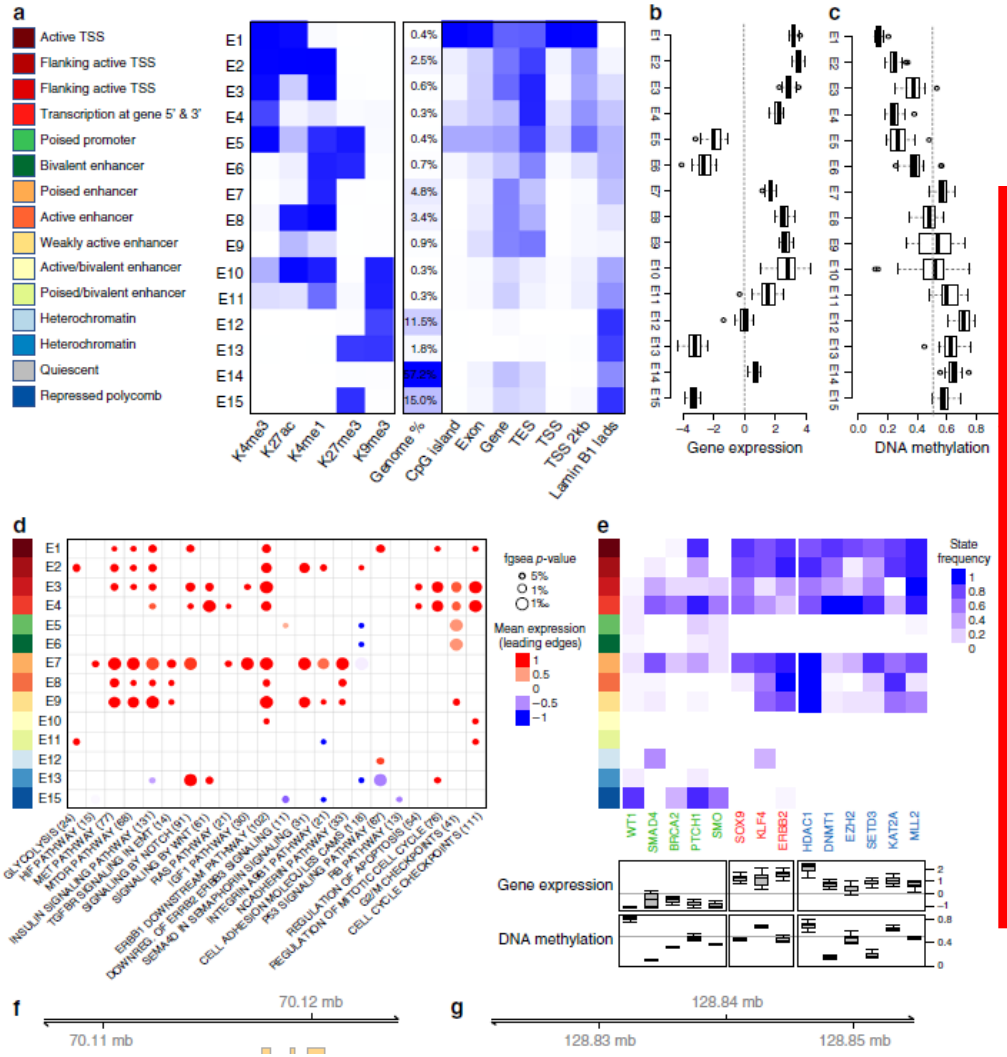




Sample: Pancreatic Fluid

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

Distinct chromatin states of human PDAC

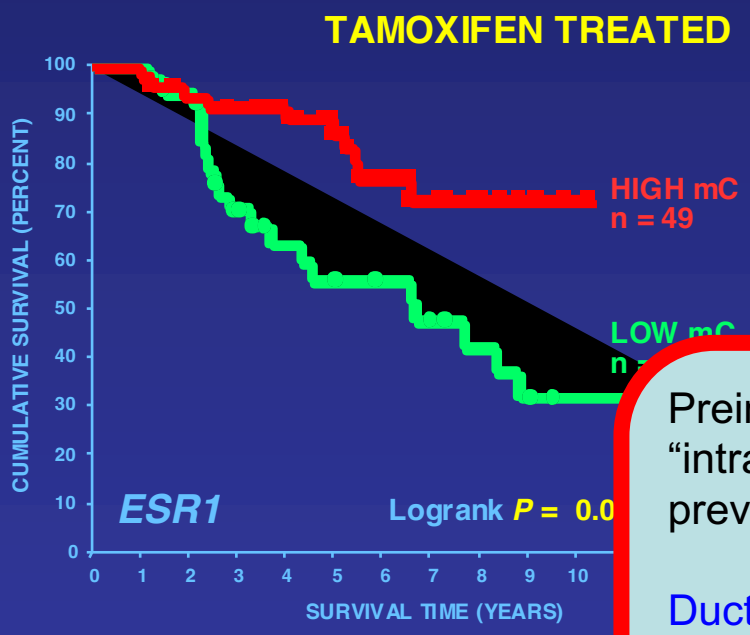


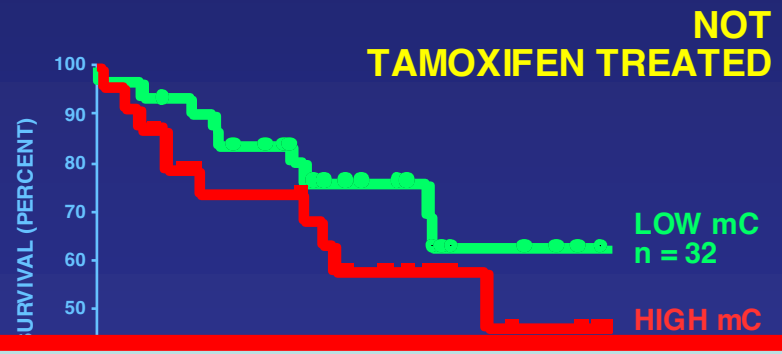
Chromatin modifications to identify active and poised enhancers and active transcription start sites by chromatin immunoprecipitation (TSS)

Methylation profiling by methylation -seq, and active transcription sites by RNAseq

Open chromatin sites corresponded to lower methylation levels and higher gene expression.

Breast Cancer Response to Tamoxifen Treatment by ESR1 Methylation



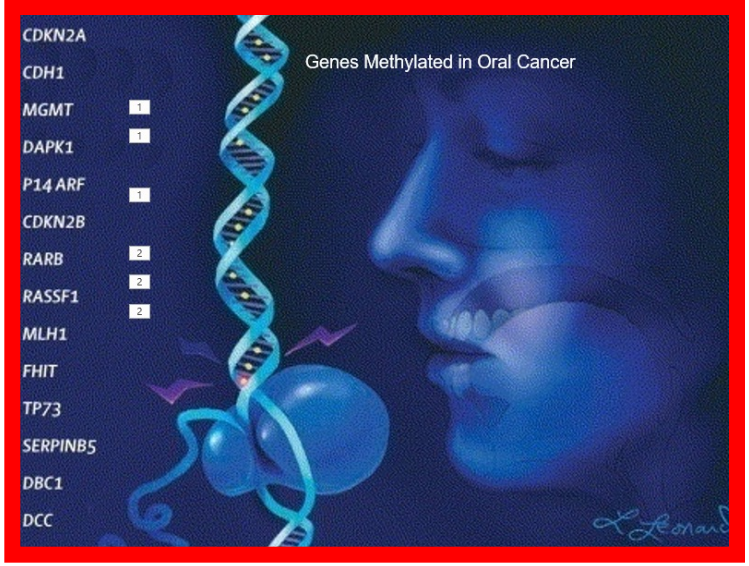


Preinvasive lesions, often designated as “in situ” or “intraepithelial neoplasia” falls in the domain of prevention.

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.

Widschwendter et al. *Cancer Research* 4, 380



Chapter 21

Epigenetic Regulation of HIV, AIDS, and AIDS-Related Malignancies

Mukesh Verma

Abstract

Although epigenetics is not a new field, it
research have not been explored fully. To d
immunodeficiency virus (HIV) and AID
between the virus and the host, involvemer

Methods Mol Biol. 2015;1238:381-403. doi: 10.1007/978-1-

Epigenetic Biomarkers in Liver Cancer

Krishna K. Banaudha and Mukesh Verma

Abstract

Liver cancer (hepatocellular carcinoma or HCC) is a major cancer worldwide. Research in this field is needed to identify biomarkers that can be used for early detection of the disease as well as new approaches to its treatment. Epigenetic biomarkers provide an opportunity to understand liver cancer etiology and evaluate novel epigenetic inhibitors for treatment. Traditionally, liver cirrhosis, proteomic biomarkers, and the presence of hepatitis viruses have been used for the detection and diagnosis of liver cancer. Promising results from microRNA (miRNA) profiling and hypermethylation of selected genes have raised hopes of identifying new biomarkers. Some of these epigenetic biomarkers may be useful in risk assessment and for screening populations to identify who is likely to develop cancer. Challenges and opportunities in the field are discussed in this chapter.

Key words Biogenesis, Biomarkers, Epigenetics, Epidemiology, Hepatitis, Liver cancer, miRNA, Treatment

Methods Mol Biol. 2015;1238:65-76. doi: 10.1007/978-1-4939-1804-1_4.

Immune System and Epigenetics

T cell methyltransferase in Lupus

- The levels of DNA methyltransferase go down in T cells in lupus patients compared to age and sex-matched controls
- Hypomethylation of re

MicroRNA-29b contributes to
indirectly targeting DNA met
Qin H et al. J Dermatol Sci. 2

Ethnicity-specific epigenetic
Coit P et al. Epigenetics Chr

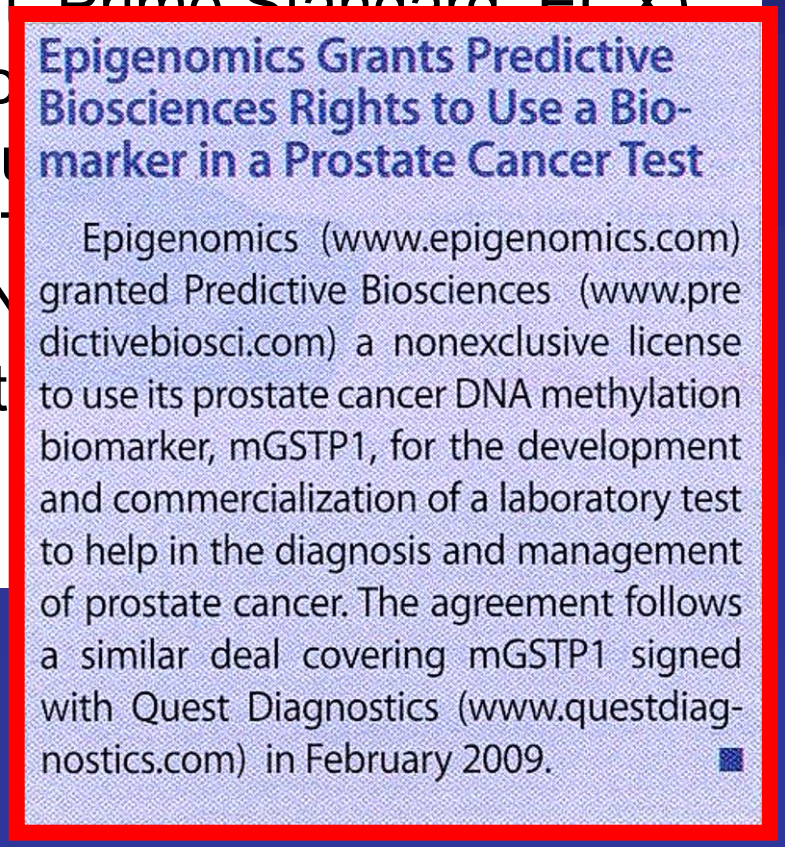
Lupus: The Immunological
J Investig Dermatol Symp Pr

Microbiome in human gastrointestinal tract [Marcin Klapczynski/Getty Images]

GSTP1 Methylation in Prostate Cancer

GSTP1 codes for the drug detoxification enzyme which

Berlin, Germany and Seattle, WA, U.S.A., February 25, 2009 - Epigenomics AG (Frankfurt, Prime Standard: ECX) a cancer molecular diagnostics co that it has entered into a non-exclu for its proprietary biomarker mGST Quest Diagnostics Incorporated (N leading provider of diagnostic test services.

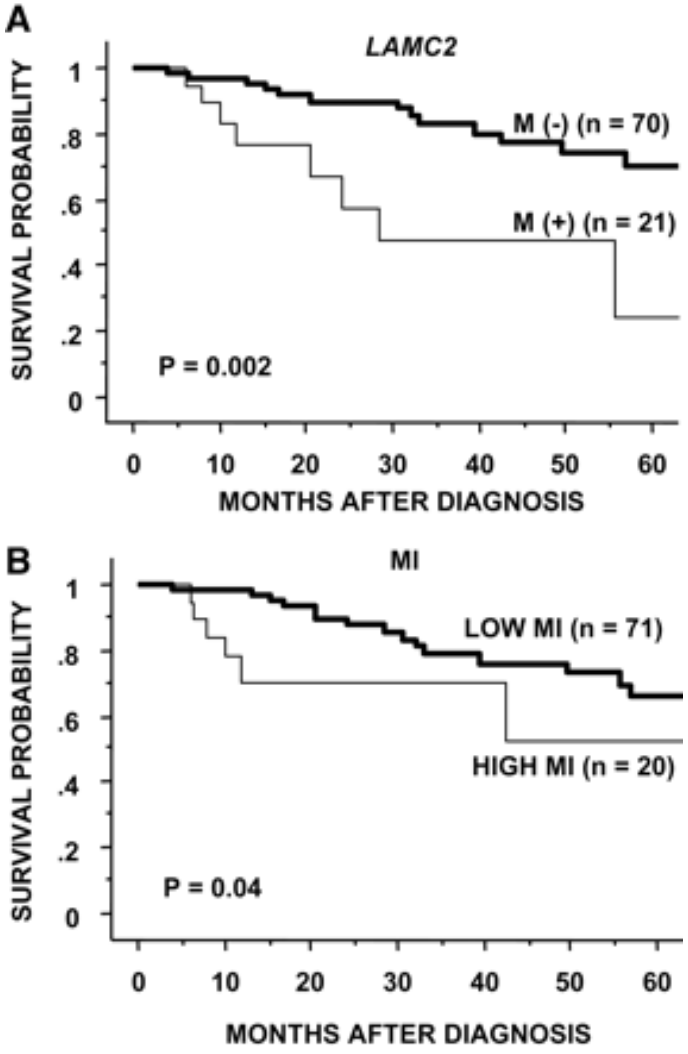


Epigenomics Grants Predictive Biosciences Rights to Use a Biomarker in a Prostate Cancer Test

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.

Bladder Cancer

Methylation of LAMC2 in Exfoliated Cells Isolated from Urine

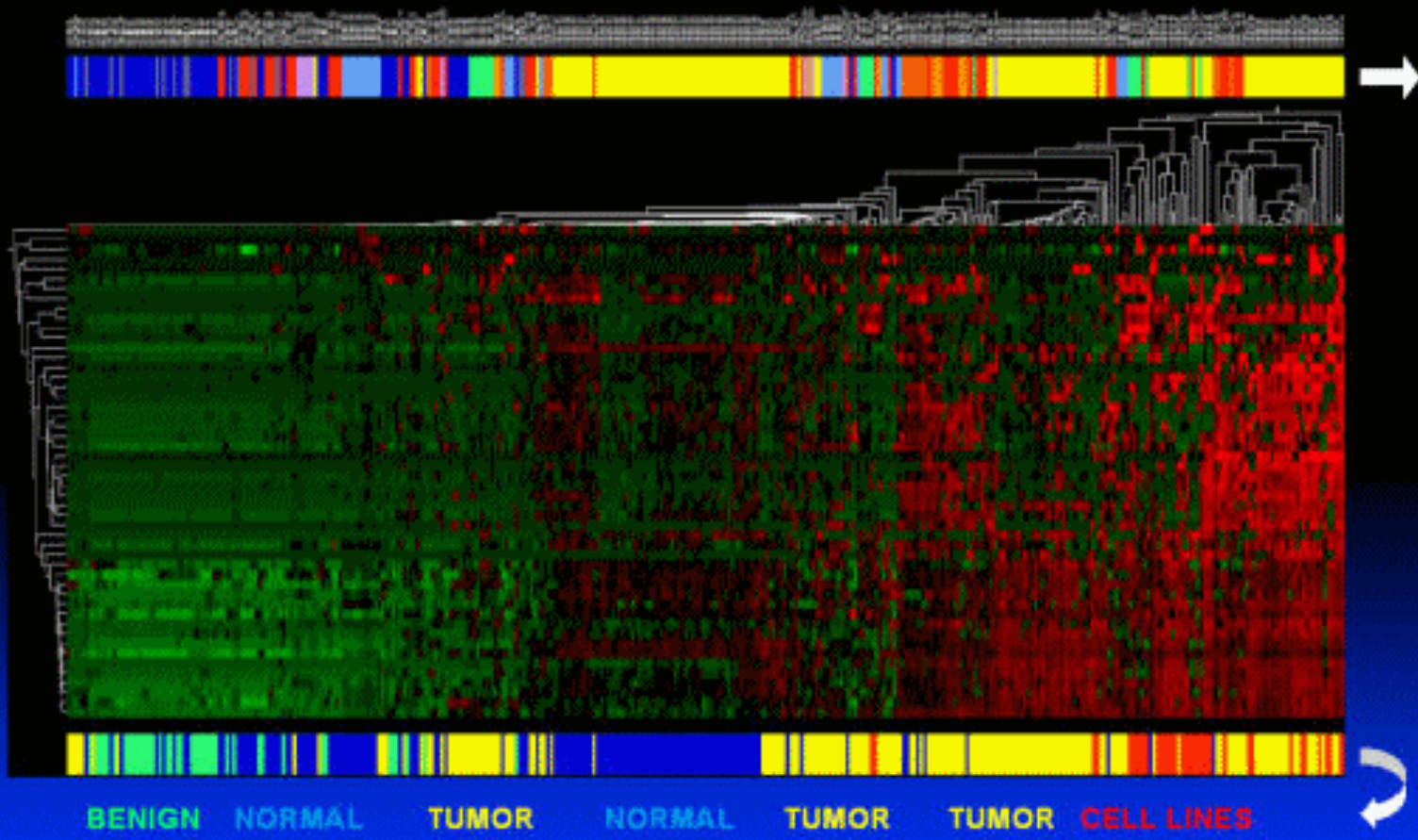


Another Study:
Schistosomes and Bladder Cancer

MI, Methylation Index

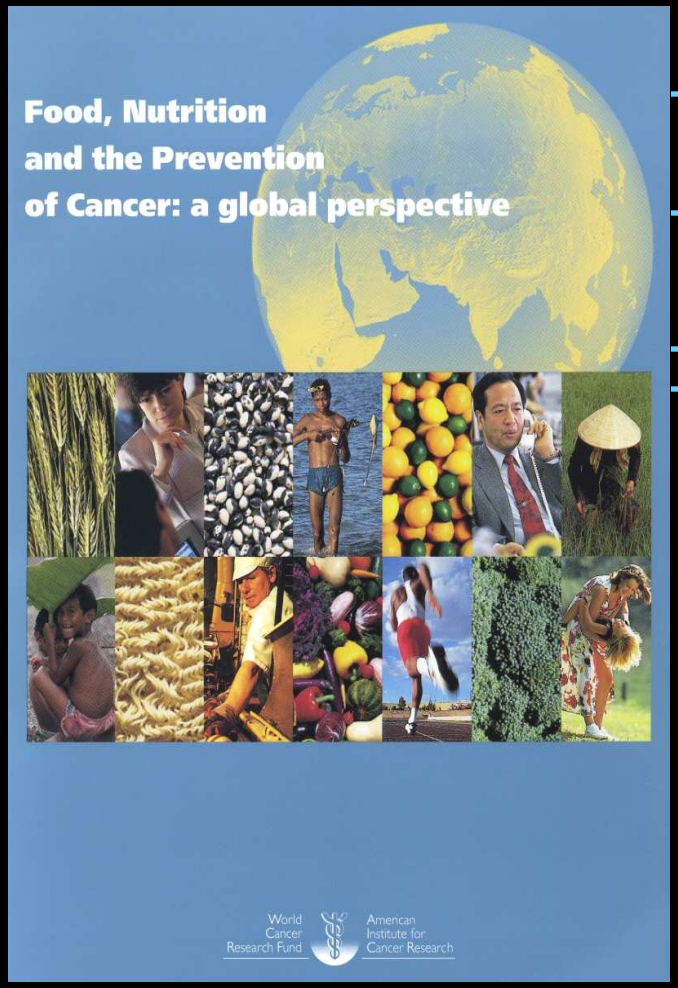
(Sathyanarayana et al. *Can Res* 64: 1425)

Clustering of Sample Type by CpG Island Hypermethylation



Cluster Analysis of 365 Human Samples with 50 DNA Methylation Markers

DIET AND CANCER: FOCUS ON PREVENTION



Igor Pogribny

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.

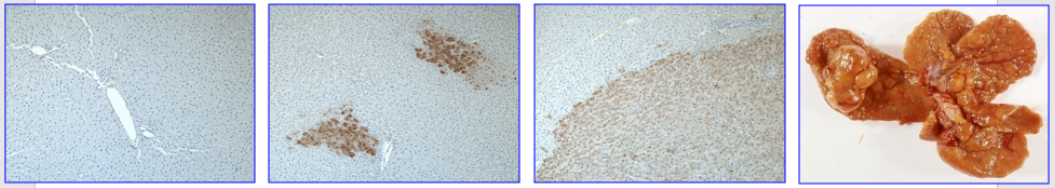
Between 30% and 40% of cancer cases throughout the world are preventable by feasible dietary means.

- 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-DEFICIENT MODEL OF ENDOGENOUS HEPATOCARCINOGENESIS

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

Normal tissue | 36 weeks, GSTπ-foci | >54 weeks, GSTπ-tumor | Liver tumor



Igor et al. 2007 (personal communication)

Nutr Cancer. 2016 Jul;68(5):719-33. doi: 10.1080/01635581.2016.1180410. Epub 2016 Jun 8.

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

Moreno FS[1], Heidor R[1], Pogribny IP[2].

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 its successful treatment. This indicates that the prevention of HCC may be the most promising strategy in reducing its incidence and mortality. Emerging evidence indicates that numerous nutrients and nonnutrient dietary bioactive components can reduce the occurrence and/or delay the development of HCC through modifications of deregulated epigenetic mechanisms. This review examines the existing knowledge on the epigenetic mechanism-based studies in in vitro and in vivo models of HCC on the chemopreventive potential of epigenetic food components, including dietary methyl-group donors, epigallocatechin-3-gallate, 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.

ssociation of TNFRSF12A ...

Come see the new improvements to the interface!

Format: Abstract Send to

Front Genet. 2020 Jan 9;10:1299. doi: 10.3389/fgene.2019.01299. eCollection 2019.

Association of TNFRSF12A Methylation With Prognosis in Hepatocellular Carcinoma With History of Alcohol Consumption.

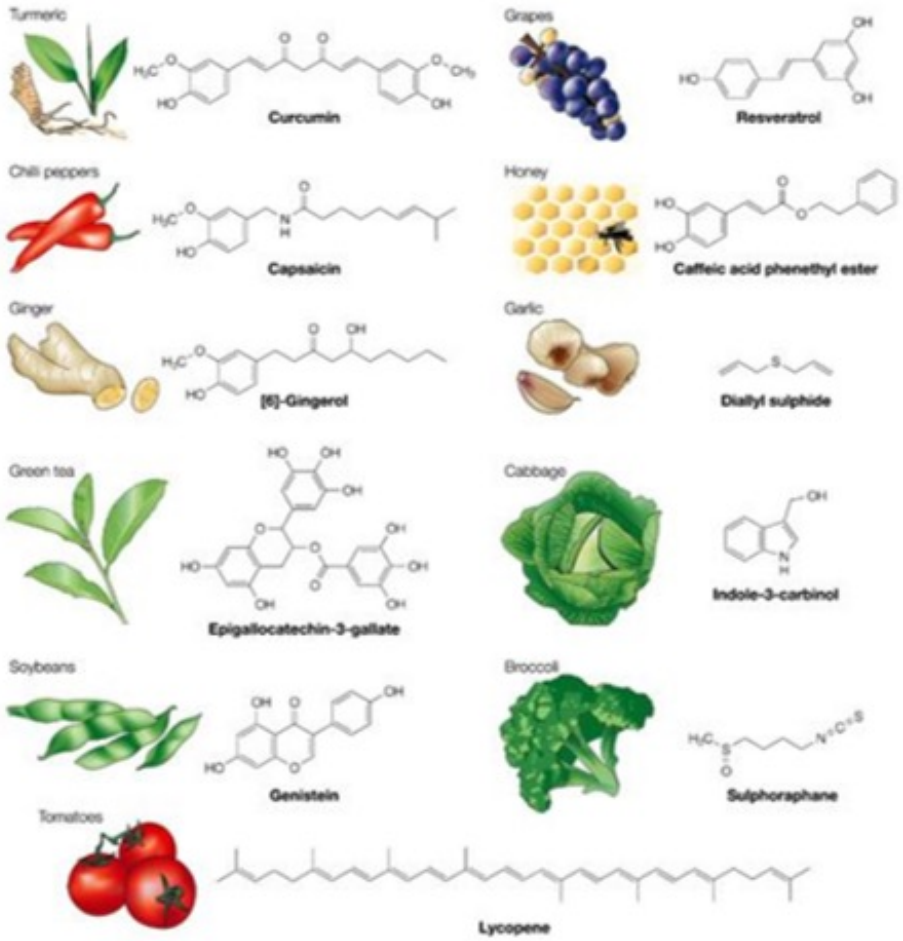
Wang Y[1,2], Zhang S[3], Xie X[4], Chen Z[5], Wu L[4], Yu Z[4], Guo X[1,3], Chen G[4].

Author information

Abstract

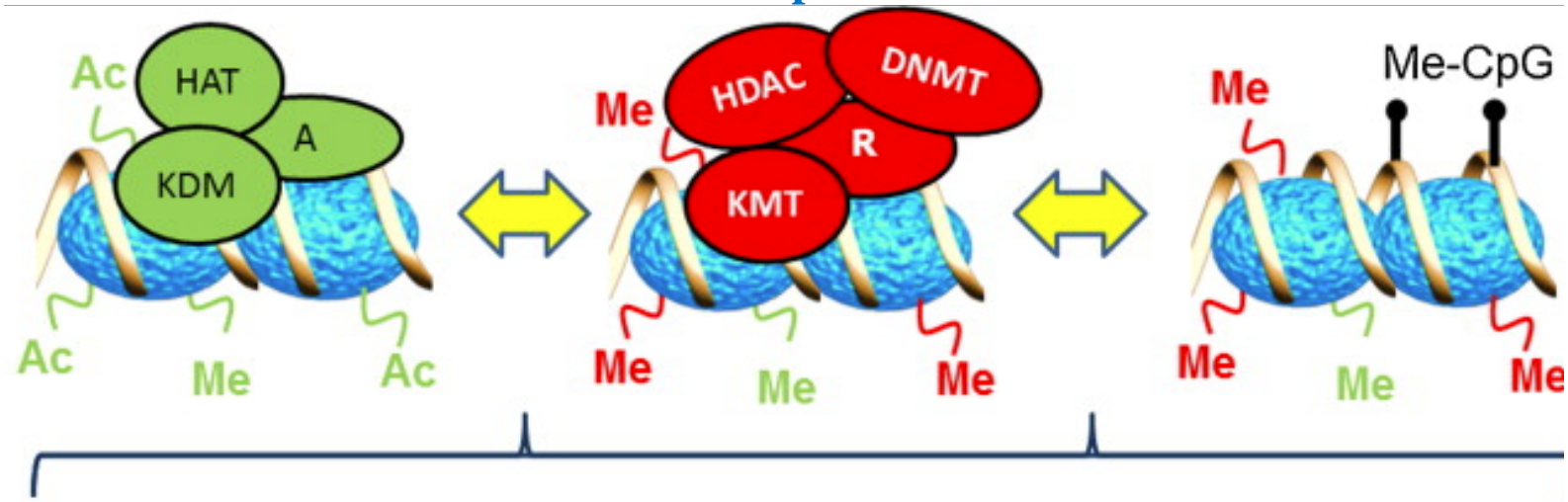
Hepatocellular carcinoma (HCC) is the third leading cause of cancer related death worldwide with a poor prognosis. Alcoholic liver disease accounts for approximately one-third of all HCC cases. Current evidence proved that aberrant over-expression of TNFRSF12A correlates with the severity of disease, making it a likely indicator of disease a more aggressive 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 TNFRSF12A affect the occurrence, development and prognosis of HCC were under warranted. Thus, in this study we mined two publicly available datasets to detect the association between DNA methylation level of CpG sites in gene TNFRSF12A and the development of HCC in those with alcohol abuse history. Finally, we discovered that the hypomethylation of two methylation sites-cg00510447 and cg26808293-could identify HCC from other non-HCC liver diseases. Also, hypomethylation of these two sites could identify alcoholic cirrhosis from other non-hepatocellular carcinoma liver diseases. Most important, the prognostic analysis revealed that the hypomethylation of cg00510447 and cg26808293 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, hypomethylation of cg26808293 signified poor prognosis. The further mechanism analysis revealed that the DNA methyltransferases DNMT3L might regulate TNFRSF12A 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 alcoholic liver disease into HCC.

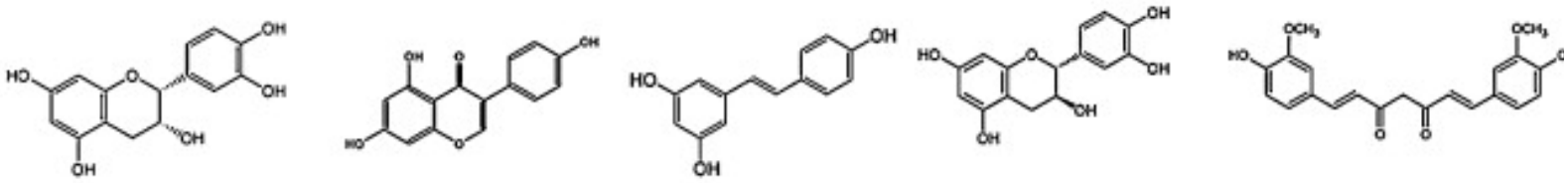
ANTICANCER PHYTOCHEMICALS
(Representative chemopreventive phytochemicals and their dietary sources)



Surh. Nature

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

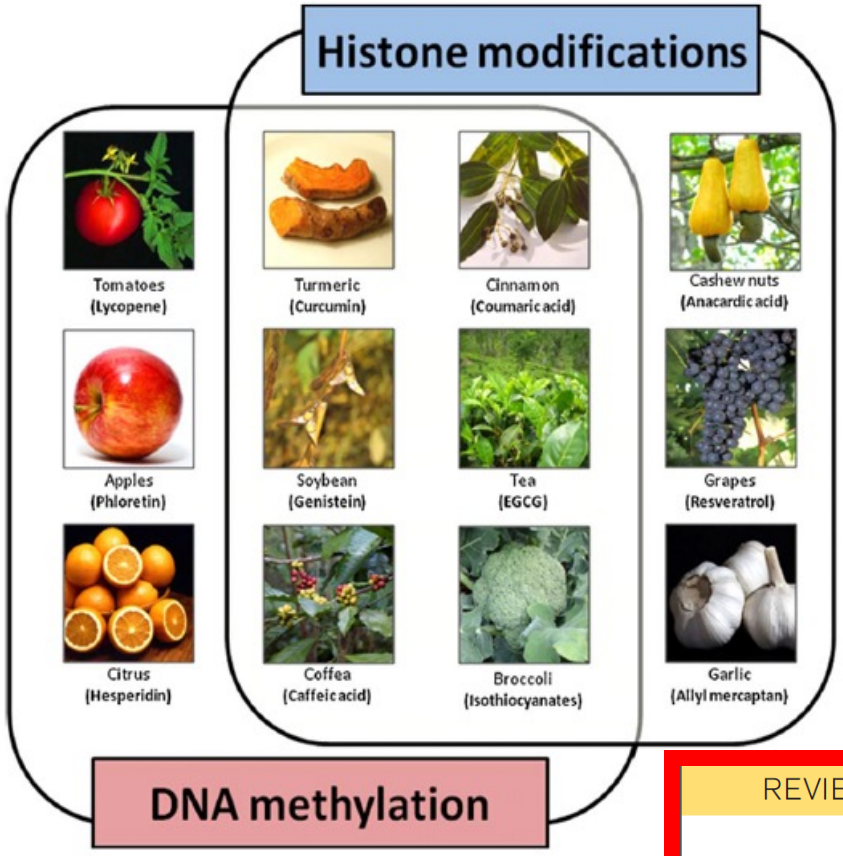




Epicatechin Genistein Resveratrol Catechin Curcumin

Green tea Soy Grape Cocoa Curcuma

Pharm Res 65: 565-576.



2. Illustration depicting major plants with evidence for epigenetic modifications. The figure illustrates photographs from n

REVIEW

CURRENT OPINION

Cancer control and prevention: nutrition and epigenetics

Mukesh Verma

Purpose of review
To evaluate recent developments in nutritional epigenomics and related challenges, opportunities, and implications for cancer control and prevention.

Recent findings
Cancer is one of the leading causes of death worldwide, and understanding the factors that contribute to cancer development may facilitate the development of strategies for cancer prevention and control. Cancer development involves genetic and epigenetic alterations. Genetic marks are permanent, whereas epigenetic marks are dynamic, change with age, and are influenced by the external environment. Thus, epigenetics provides a link between the environment, diet, and cancer development. Proper food selection is imperative for better health and to avoid cancer and other diseases. Nutrients either contribute directly to cancer prevention or support the repair of genomic and epigenomic damage caused by exposure to cancer-causing agents such as toxins, free radicals, radiation, and infectious agents. Nutritional epigenomics provides an opportunity for cancer prevention because selected nutrients have the potential to reverse cancer-associated epigenetic marks in different tumor types. A number of natural foods and their

Myplate.gov

Explore the MyPlate Food Groups

Fruits
Focus on whole fruits
Learn more

Vegetables
Vary your veggies
Learn more

Grains
Make half your grains whole grains
Learn more

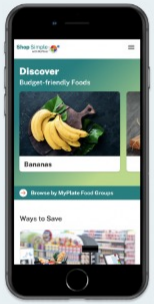
Protein Foods
Vary your protein routine
Learn more

Dairy
Move to low-fat or fat-free dairy milk or yogurt (or lactose-free dairy or fortified soy versions)
Learn more

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



Shop Simple with MyPlate

Find savings in your area and discover new ways to prepare budget-friendly foods.

Learn more

MyPlate on Alexa

Get MyPlate nutrition tips on Amazon Alexa devices or the free Alexa app.

Learn more

Start Simple with MyPlate App

Build healthy eating habits one goal at a time! Download the *Start Simple with MyPlate* app today.

Learn more

https://www.myplate.gov/

Mobile food record

Ahmad et al doi: 10.1145/2986035.2986038; (Zhu et al 2022)

National Cancer Institute

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?

How to use epigentic age information in cancer control?

National Cancer Institute

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?

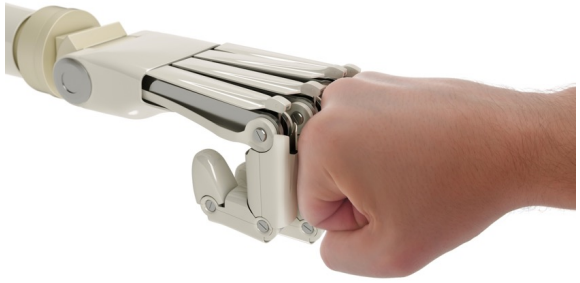
How to avoid activity of DNMT and HDAC inhibitors on normal cell functions?

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

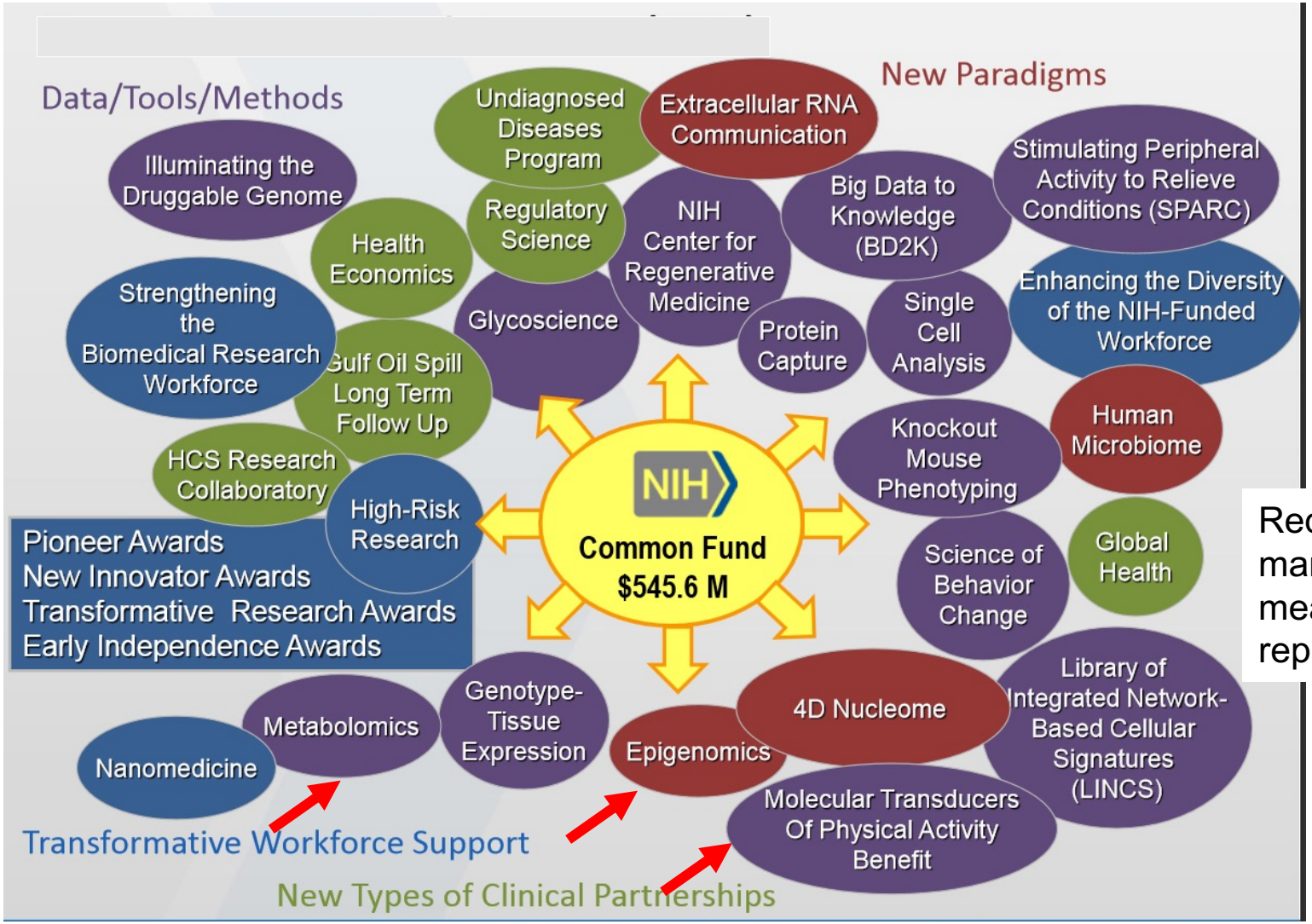
How to target cancer stem cells using epigenetic approaches?

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

National Cancer Institute

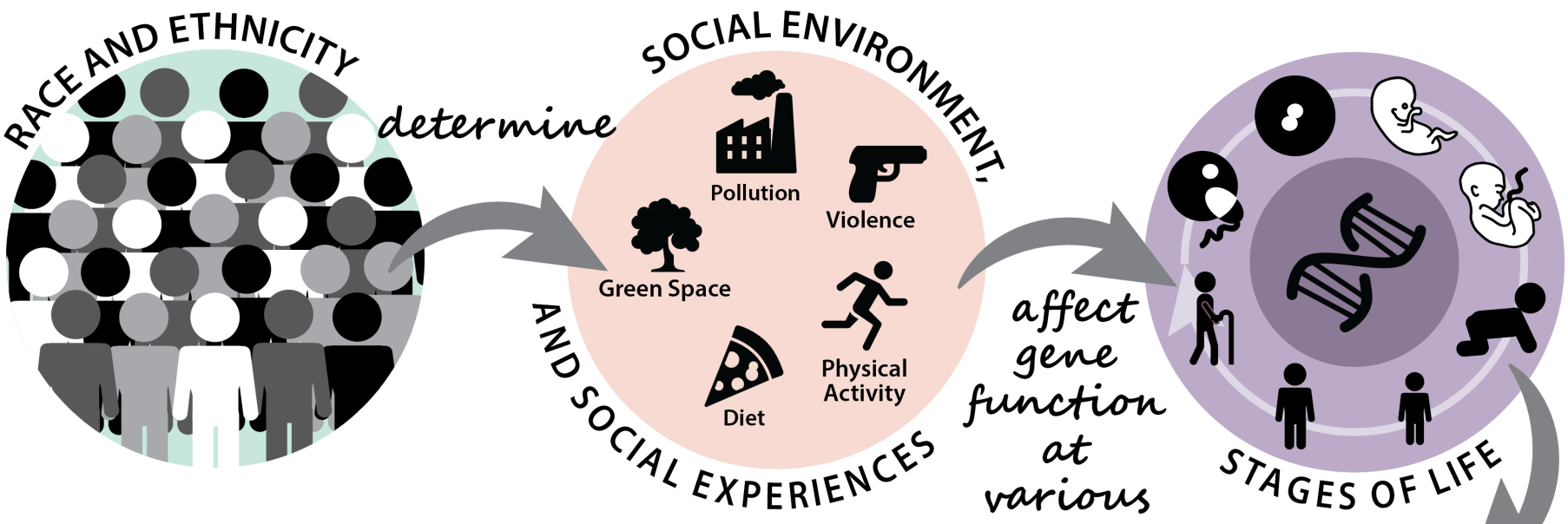


How are we addressing these challenges?



Red arrow marks means that I represent NCI

What is Social Epigenomics?



AND THEREBY INFLUENCE HEALTH TRAJECTORY AND MODIFY DISEASE RISK

THROUGH **EPIGENOMIC PROFILING** BEFORE THE ONSET OF DISEASES, IT MAY BE POSSIBLE TO TAILOR INTERVENTIONS TO PREVENT CHRONIC CONDITIONS OR DISEASES LATER IN LIFE

Minireview

Cancer Epidemiology, Biomarkers & Prevention

Epigenetic Research in Cancer Epidemiology: Trends, Opportunities, and Challenges

Mukesh Verma[1], Scott Rogers[1], Rao L. Divi[1], Sheri D. Schully[1], Stefanie Nelson[1], L. Joseph Su[1], Sharon A. Ross[2], Susan Pilch[3], Deborah M. Winn[1], and Muin J. Khoury[1,4]

Abstract

Epigenetics is emerging as an important field in cancer epidemiology that promises to provide insights into gene regulation and facilitate cancer control throughout the cancer care continuum. Increasingly, investigators are incorporating epigenetic analysis into the studies of etiology and outcomes. To understand current progress and trends in the inclusion of epigenetics in cancer epidemiology, we evaluated the published literature and the National Cancer Institute (NCI)–supported research grant awards in this field to identify trends in epigenetics research. We present a summary of the epidemiologic studies in NCI's grant portfolio (from January 2005

Review

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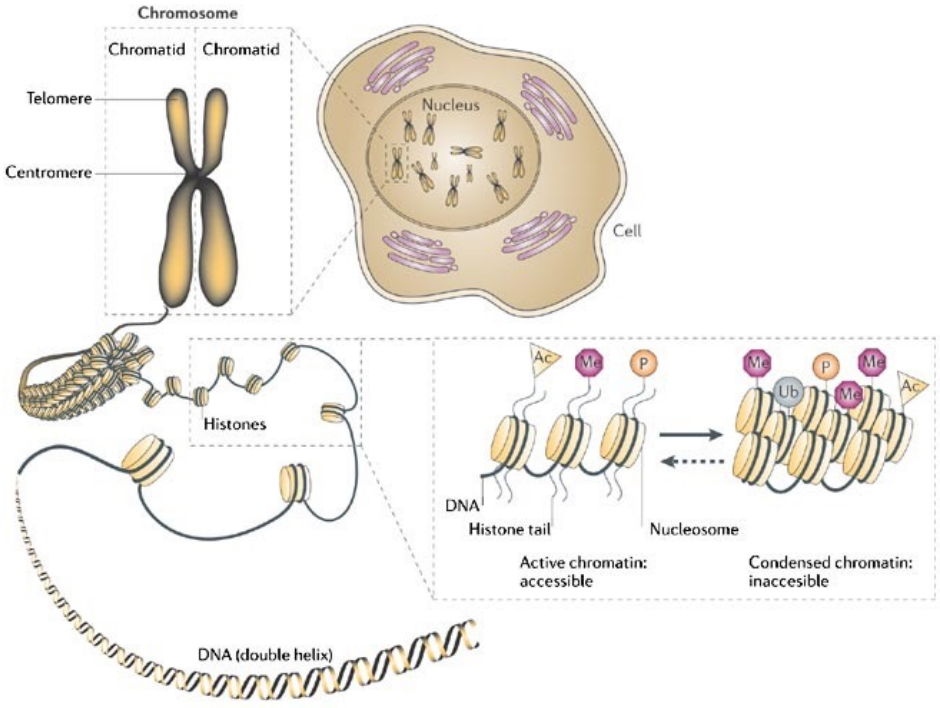
Personalized Medicine

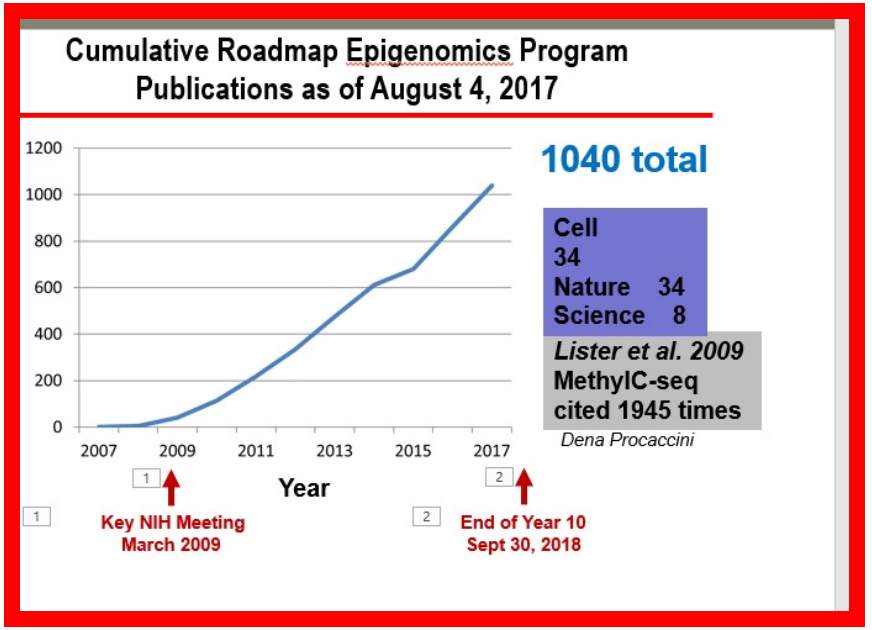
Molecular profiling and companion diagnostics: where is personalized medicine in cancer heading?

The goal of personalized medicine is to use the right drug at the right dose – with minimal or no toxicity – for the right patient at the right time. Recent advances in understanding cell biology and pathways, and in using molecular 'omics' technologies to diagnose cancer, offer a strategic bridge to personalized medicine in cancer. Modern personalized medicine takes into account an individual's genetic makeup and disease history before developing a treatment regimen. The future of clinical oncology will be based on the use of predictive and prognostic biomarkers in patient management. Once implemented widely, personalized medicine will benefit patients and the healthcare system greatly.

Mukesh Verma
Methods & Technologies Branch, Epidemiology & Genomics Research Program, Division of Cancer Control & Population Sciences, National Cancer Institute (NCI), National Institutes of Health (NIH), Rockville, MD 20850, USA
Tel.: +1 240 276 6889
Fax: +1 240 276 7921
vermam@mail.nih.gov

Epigenetics Roadmap

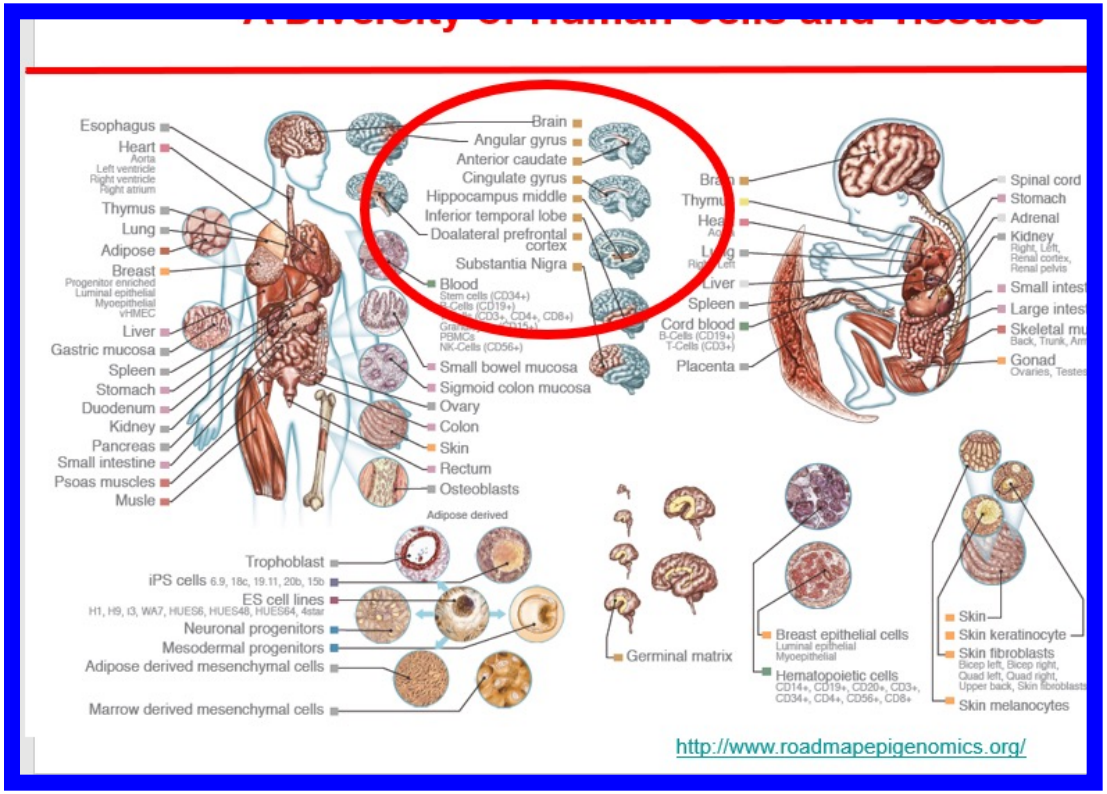




Epigenetically Regulated Diseases:
Several cancers, autoimmune disorders, reproductive disorders, and neurobehavioral and cognitive dysfunctions

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/



http://www.roadmapepigenomics.org/

Epigenomics Program Budget

(RM 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

= RFA released

8-Year Total $219 M
(plus $3M JUMPSTART)

INTERNATIONAL HUMAN EPIGENOME CONSORTIUM

1000 Epigenomes
DNA Methylation, Histone Marks,
Histone Variants, RNA,
Chromatin Associated Proteins
1000 epigenomes @ $100,000 each
$100,000,000

Reference	Stem Cells	Environment	Disease	Aging
Normal Human Cell Types, Model Organisms	Human Embryonic Stem Cells, iPS Cells	Infections, Toxins, Nutrition, Stress	Cancer, Obesity, Atherosclerosis, Autoimmune, Autism, Psychiatric, Asthma, Addiction	

Data Coordination, Access, and Archiving
Quality Control
$30,000,000

Translating Discoveries into Improved Human Health

Mechanism/Function | Prevention | Diagnosis/Biomarkers | Therapies

http://ihec-epigenomes.org/

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

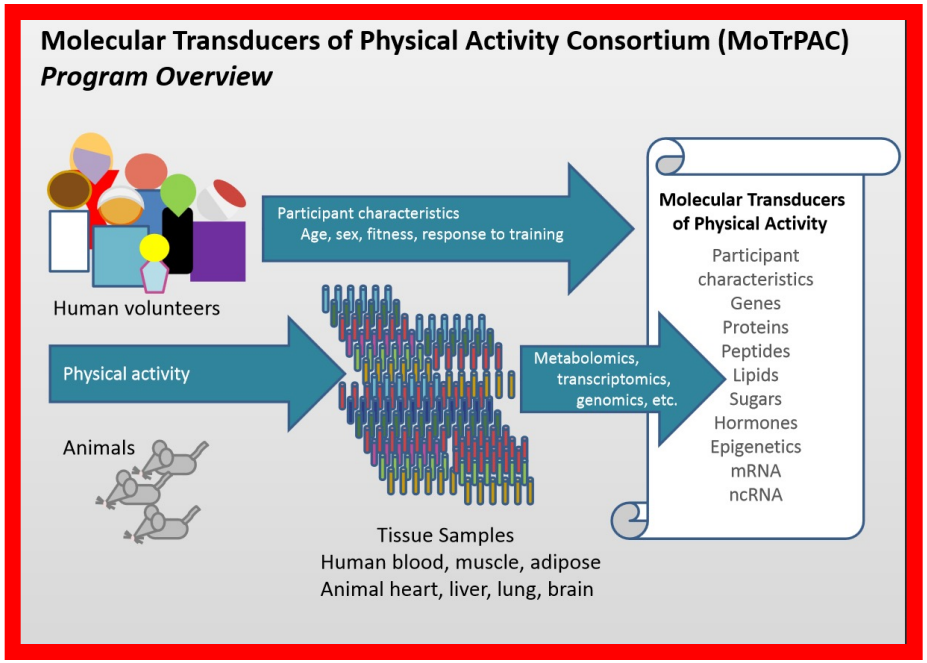
Australia
Austria
Canada
China
Euro. Comm. (EU)
France
Germany
Israel
Italy
Japan
Korea
Netherlands
Norway
Poland
Singapore
Spain
Sweden
Switzerland
UK
USA

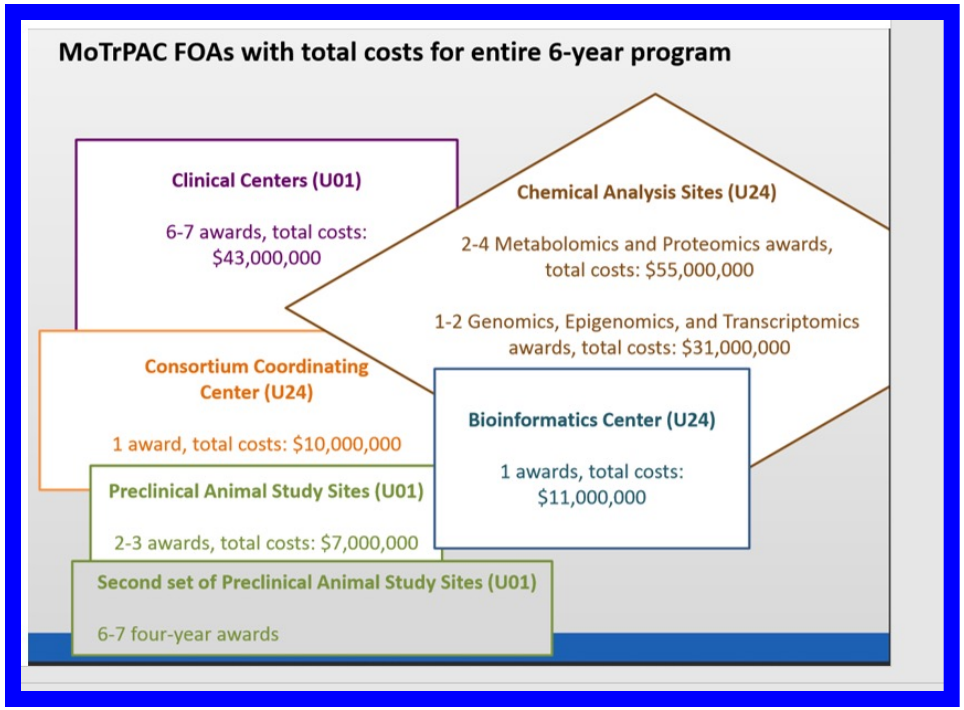
Funding Agencies
Consiglio Nazionale delle Ricerche, Italy
European Science Foundation
Genome British Columbia, Canada
German Research Foundation, Germany
National Natural Science Foundation, China
Netherlands Genomic Initiative, Netherlands
NIH, USA
Wellcome Trust, UK

Industrial Participants
Affymetrix
Genoscope
Novartis

Other
AACR

Publishers
Nature
Science


Molecular Transducers of Physical Activity Consortium (MoTrPAC)
Program Overview
Human volunteers
Participant characteristics
Age, sex, fitness, response to training
Physical activity
Animals
Tissue Samples
Human blood, muscle, adipose
Animal heart, liver, lung, brain
Metabolomics, transcriptomics, genomics, etc.
Molecular Transducers of Physical Activity
Participant characteristics
Genes
Proteins
Peptides
Lipids
Sugars
Hormones
Epigenetics
mRNA
ncRNA


MoTrPAC FOAs with total costs for entire 6-year program
Clinical Centers (U01)
6-7 awards, total costs: $43,000,000
Chemical Analysis Sites (U24)
2-4 Metabolomics and Proteomics awards, total costs: $55,000,000
1-2 Genomics, Epigenomics, and Transcriptomics awards, total costs: $31,000,000
Consortium Coordinating Center (U24)
1 award, total costs: $10,000,000
Bioinformatics Center (U24)
1 awards, total costs: $11,000,000
Preclinical Animal Study Sites (U01)
2-3 awards, total costs: $7,000,000
Second set of Preclinical Animal Study Sites (U01)
6-7 four-year awards

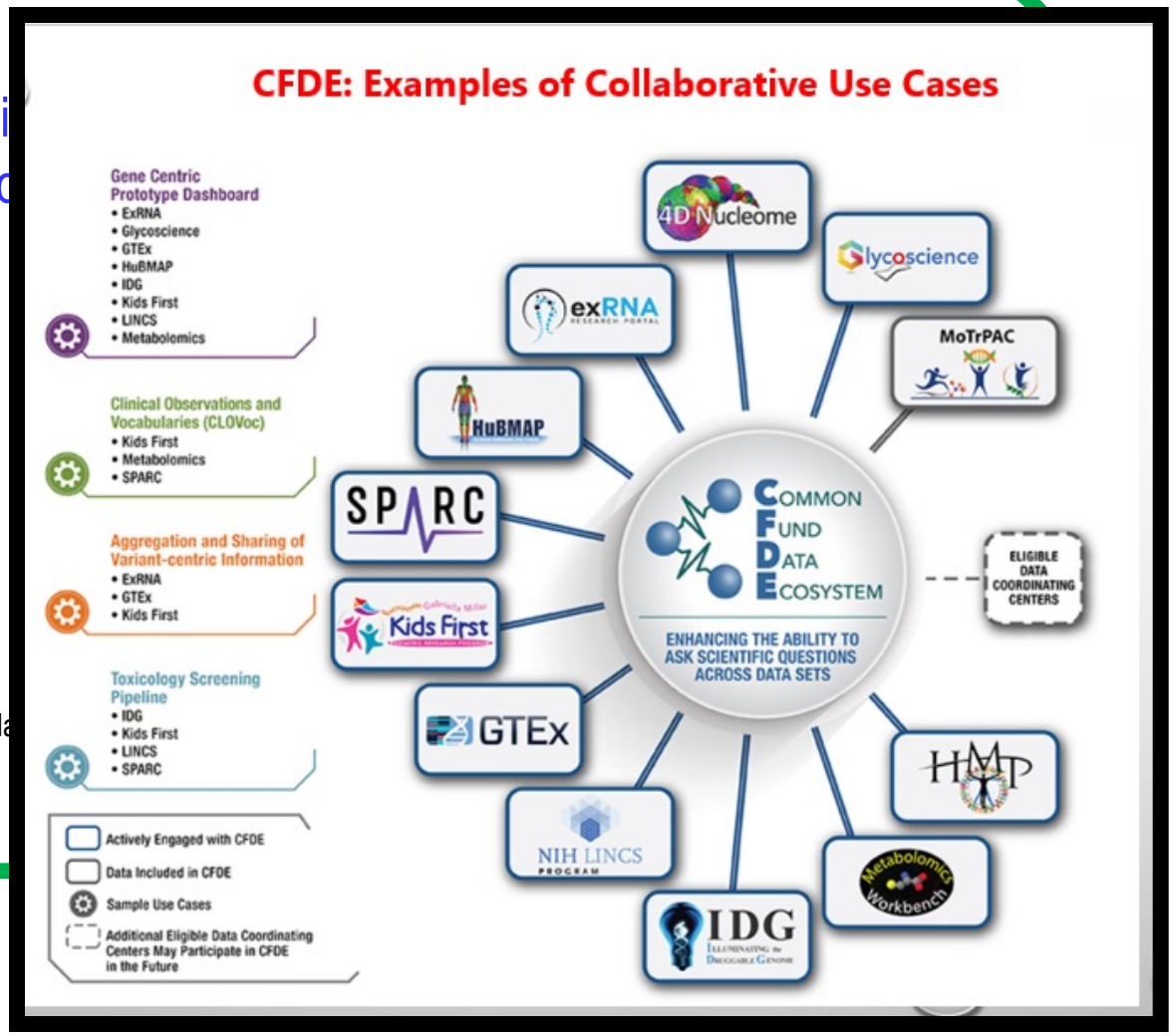

National Cancer Institute
Other Ongoing Programs
1. Moonshot
2. All of US
3. PCGA
4. TCGA

Common fund data ecosystem

An online portal that wi
multiple Common Fund
environment.

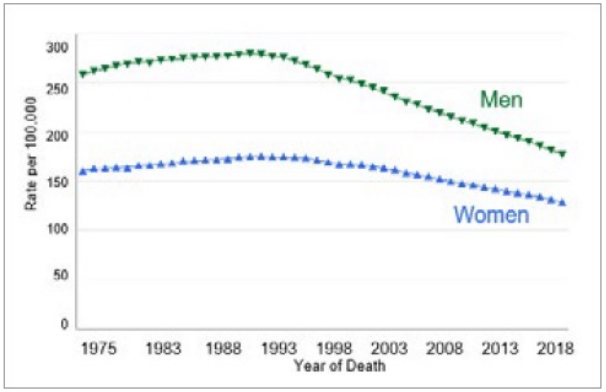
Follow FAIR principles
F = Findable
A = Accessible
I = Interoperable
R = Reusable

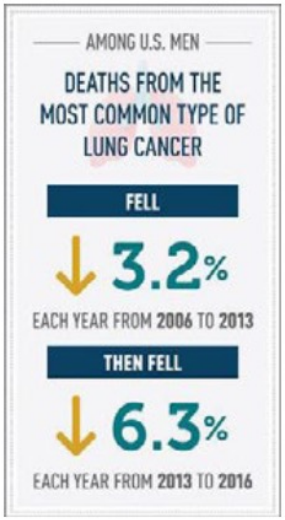
commonfund.nih.gov/da

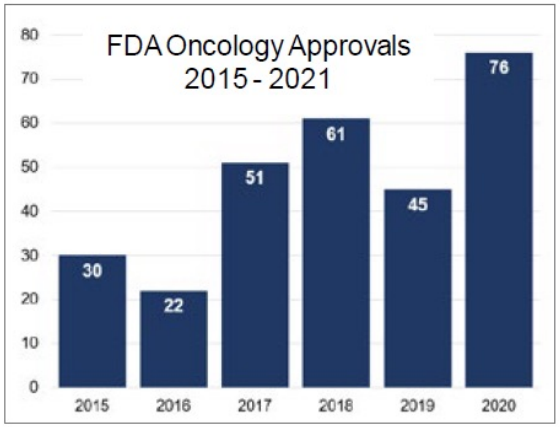


Remarkable progress in cancer research

Declining cancer mortality,
even in lung cancer and melanoma

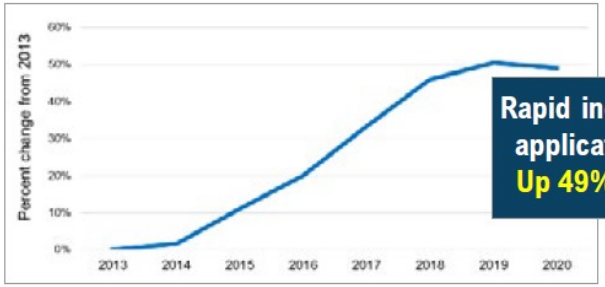






Average number of yearly FDA approvals 2006-2010: **13**

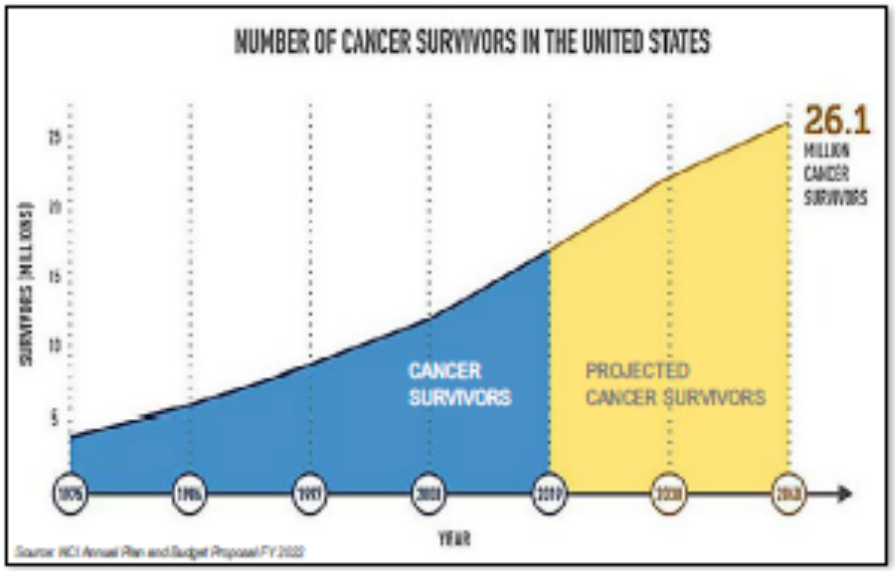
7 approvals ***in lung cancer alone***, in one month in 2020



Rapid increase in R01 applications to NCI:
Up 49% since 2013

Cancer Letters 5 November 2021

Number of Cancer Survivors in U.S. Grows to 18M



- **18.1 million cancer survivors** in the U.S. (as of January 2022)
- **26 million by 2040** (projected)
- More than **two-thirds are 65+**

For more: Miller KD, et al. Cancer treatment and survivorship statistics, 2022. *CA: A Cancer Journal for Clinicians*. June 23, 2022.

Cancer Letters September 9, 2022

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.**