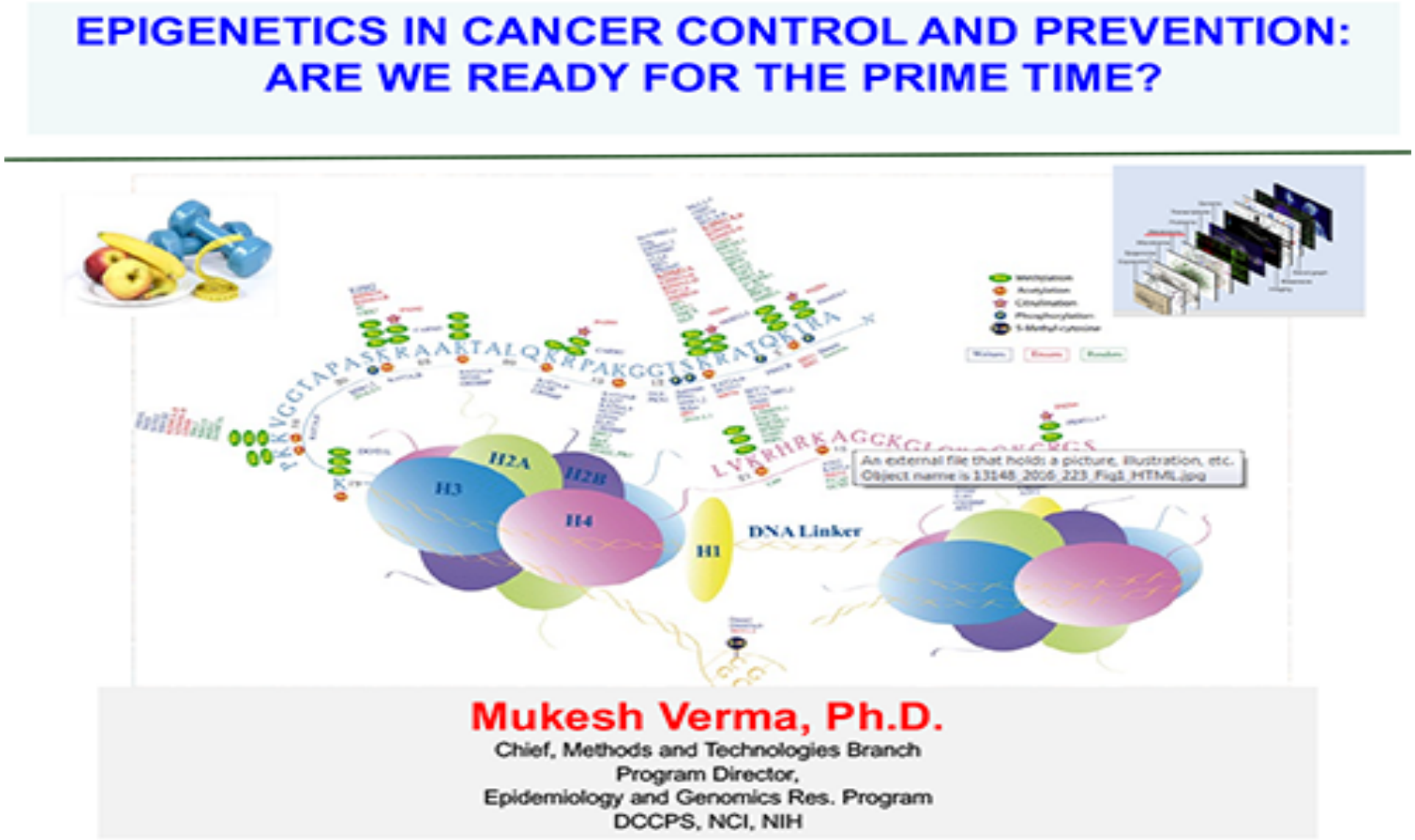
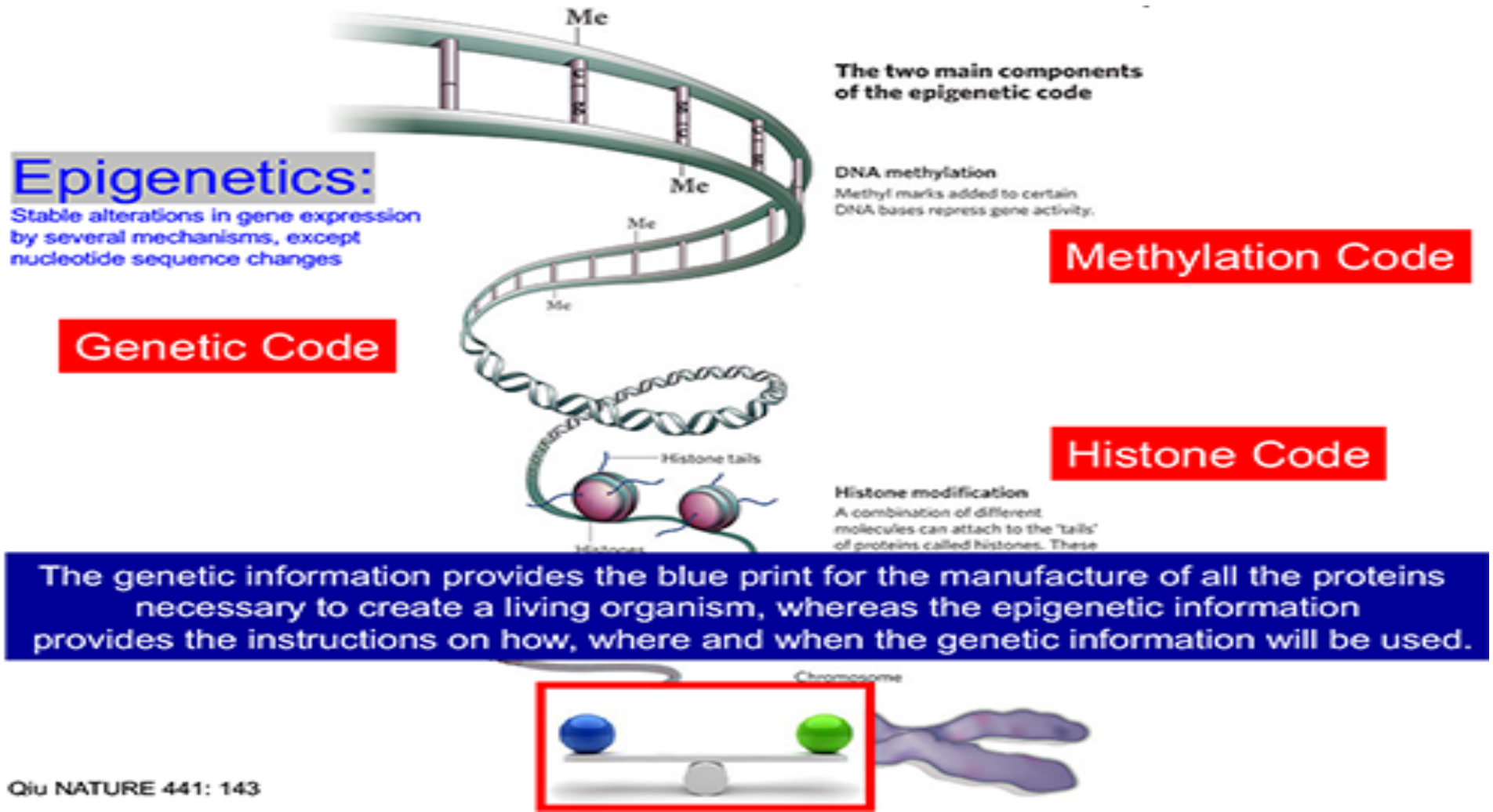
Epigenetics

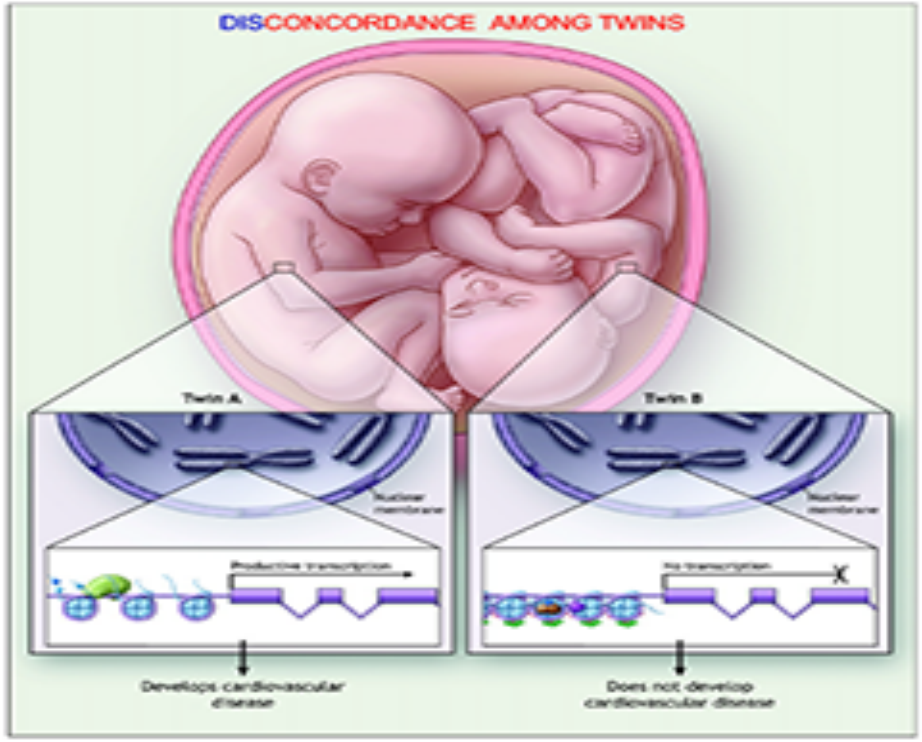


Epigenetics



DNA and destiny

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



Epigenetic predisposition to angiogenesneis? Individual? Populations?

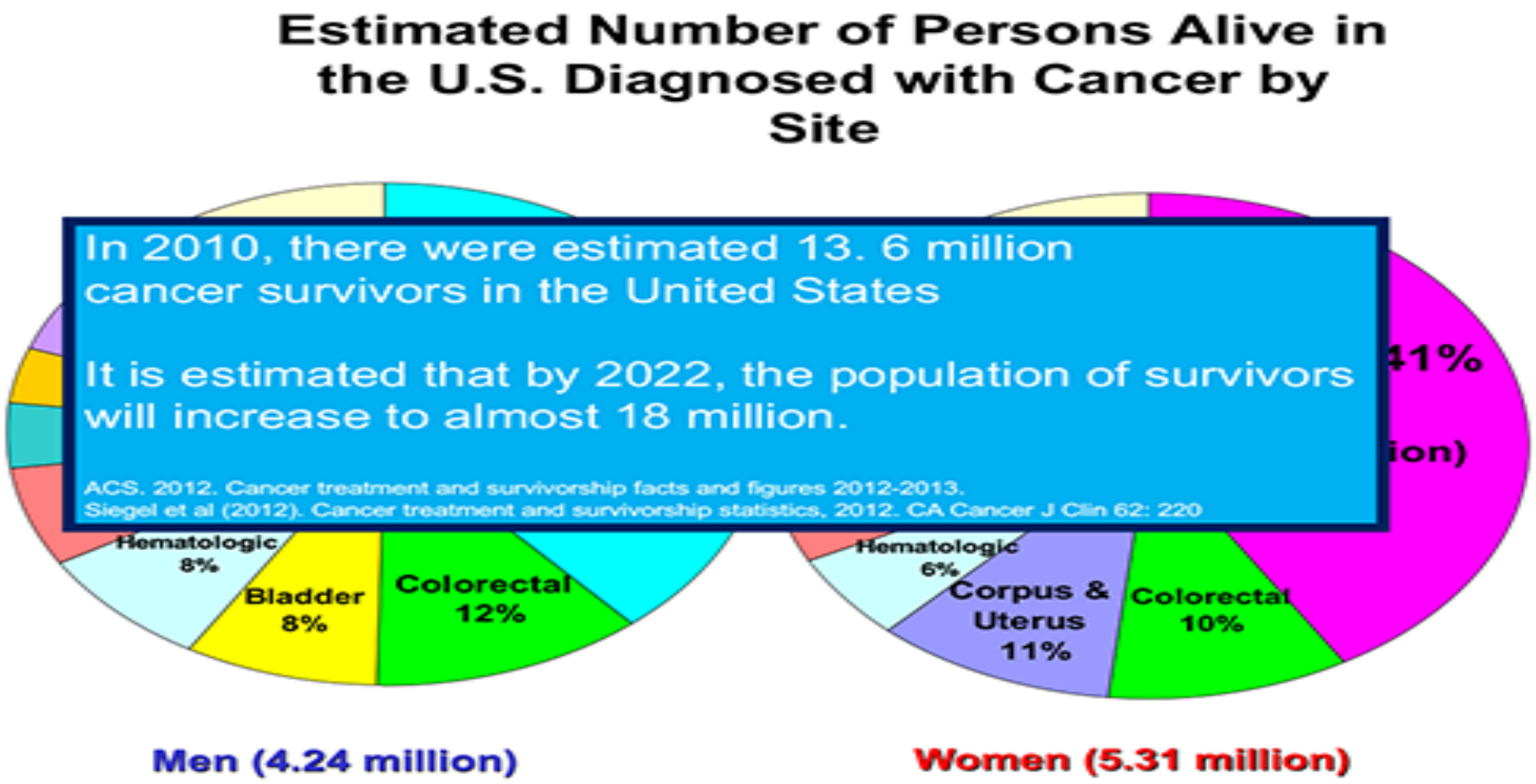
Pharmacogenomics and pharmacoepigenomics (personalized medicine)

Microenvironment, microbiome, and gene expression

GWAS and EWAS

Adapted from Matouk and Marsden Cir Res 102:873

Cancer Survivors



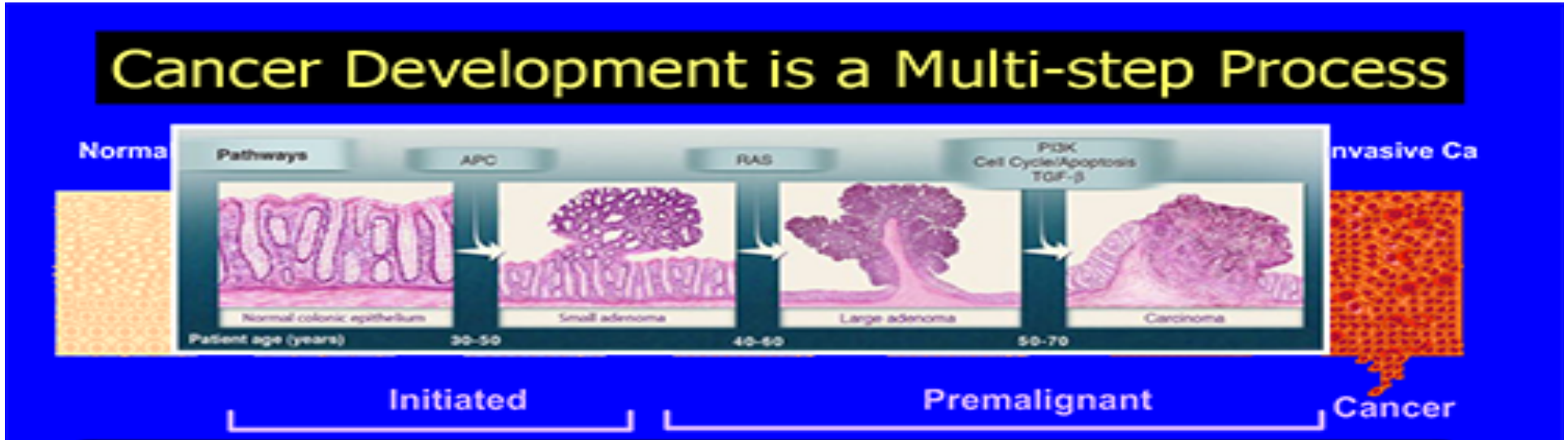
In 2010, there were estimated 13. 6 million cancer survivors in the United States

It is estimated that by 2022, the population of survivors will increase to almost 18 million.

ACS. 2012. Cancer treatment and survivorship facts and figures 2012-2013.
Siegel et al (2012). Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 62: 220

Cancer continuum

Cancer development



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.

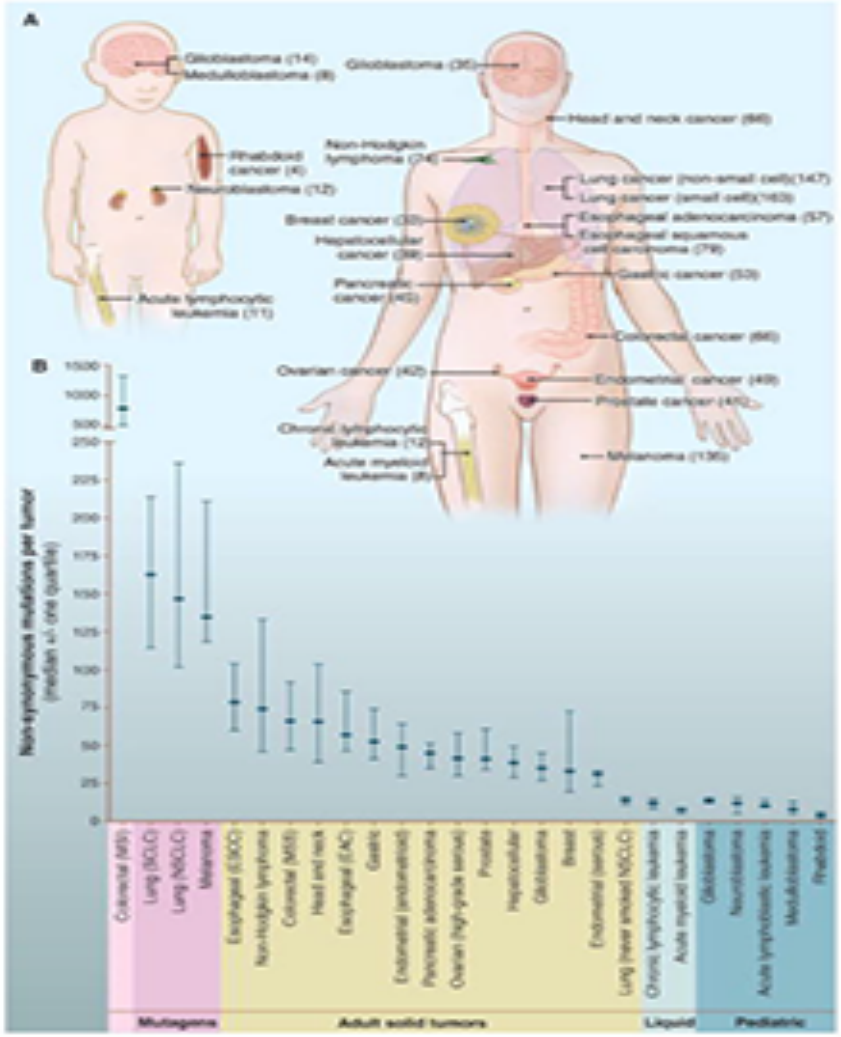
dapted from Vogelstein et al 2013

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

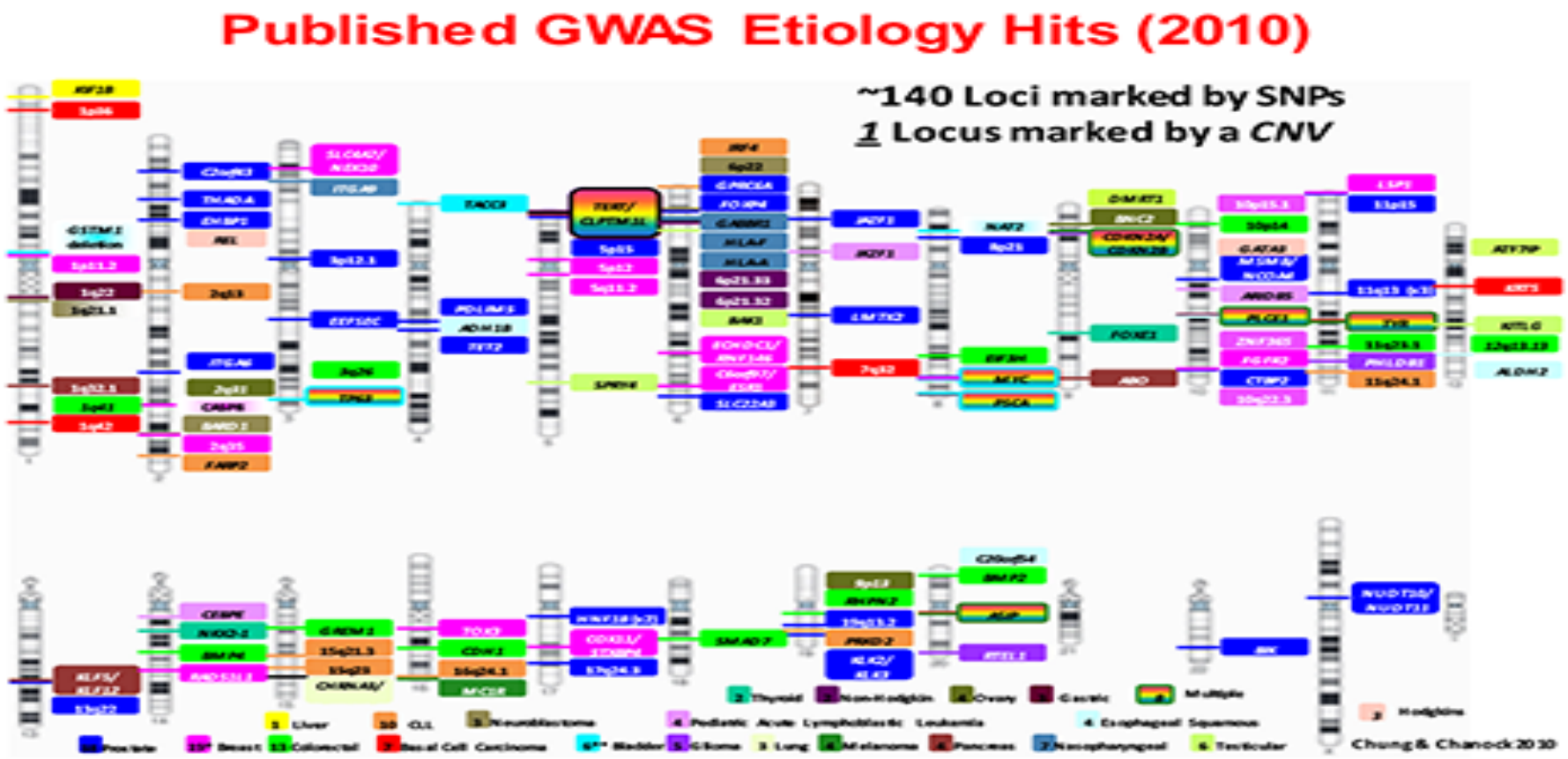


CANCER GENOME LANDSCAPE

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

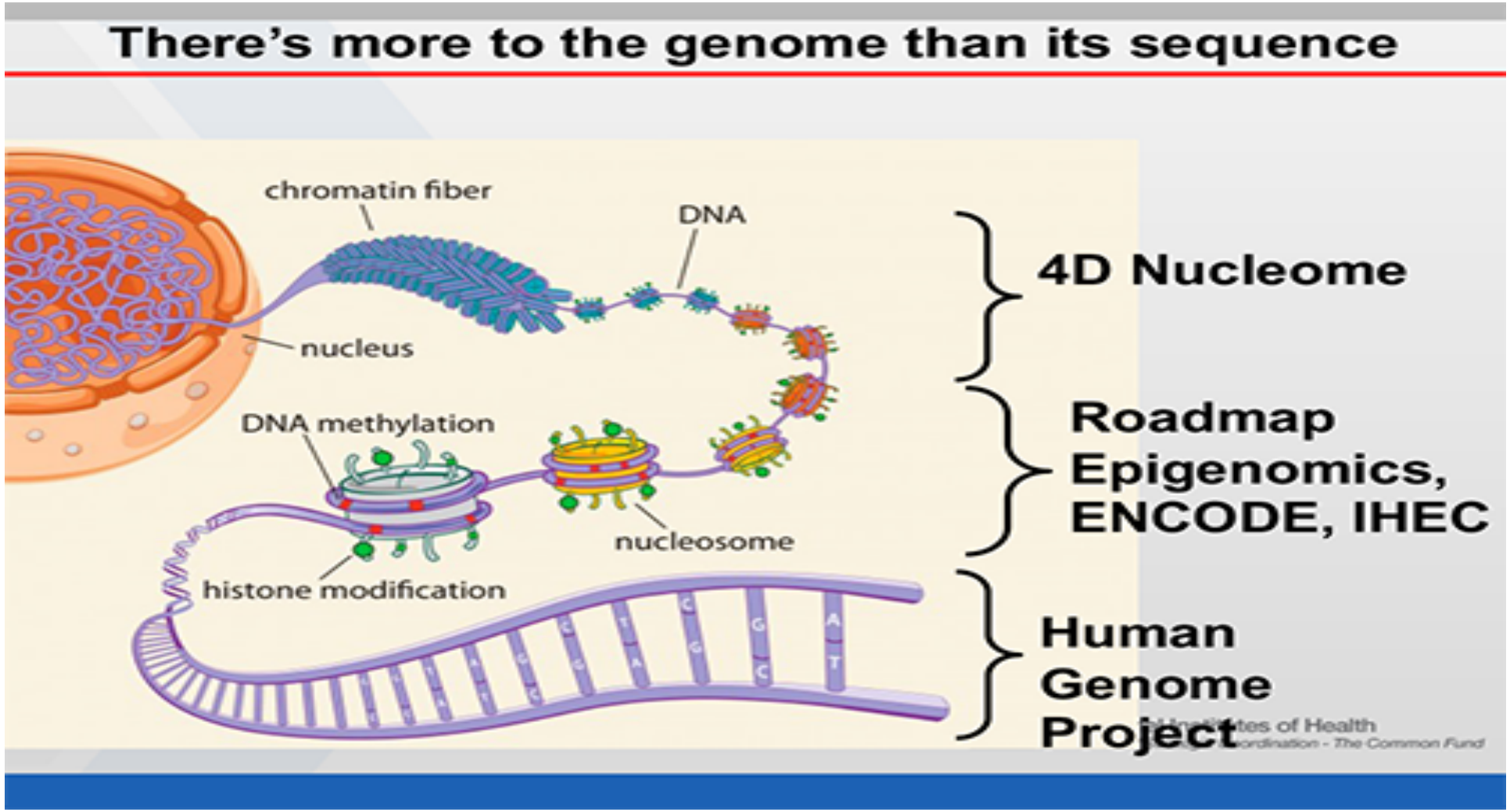
Adapted from Vogelstein and Kinzler (Science 2013)

GWAS hits


Published GWAS Etiology Hits (2010)
~140 Loci marked by SNPs
1 Locus marked by a CNV
Chung & Chanock 2010
Hindorff L, Gillanders EM, Manolio T.
Adapted from T. Manolio

Cancer genes

Genome sequence



Kornberg and nucleosome

Nucleosomes (Units of Chromatin)

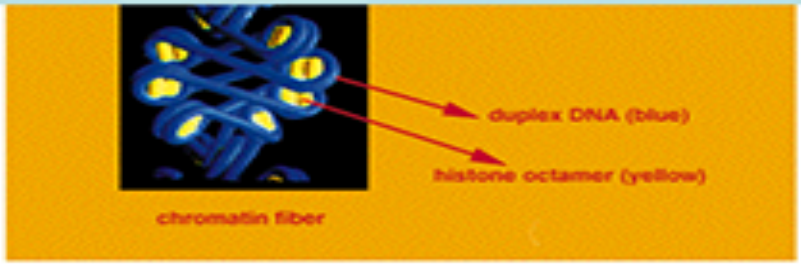
DNA
Histones H2a, H2b, H3, H4

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 octomeric complex of two of each of histone types

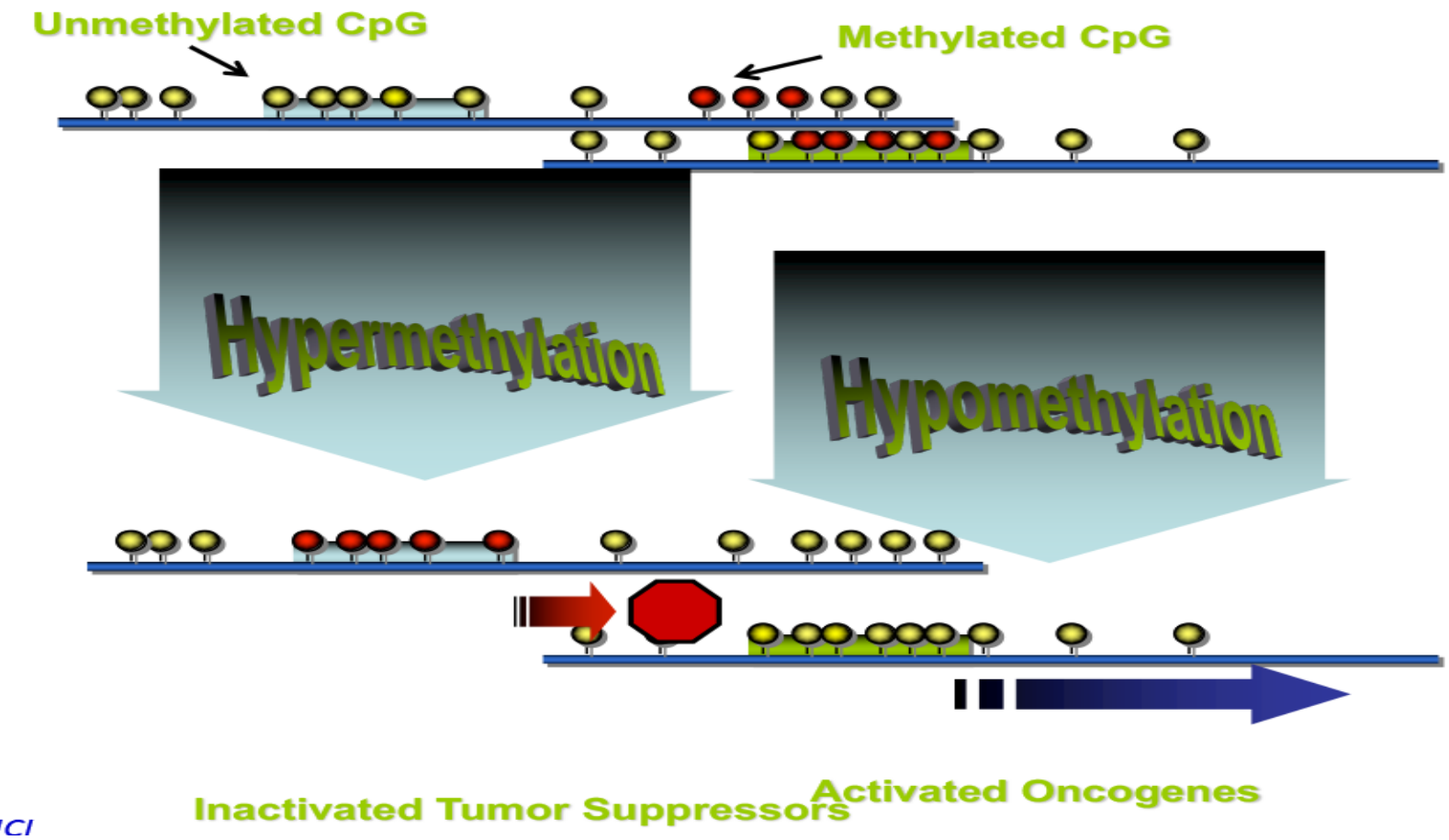
1974: Roger Kornberg discovers nucleosome who won Nobel Prize in 2006.



Nucleosome

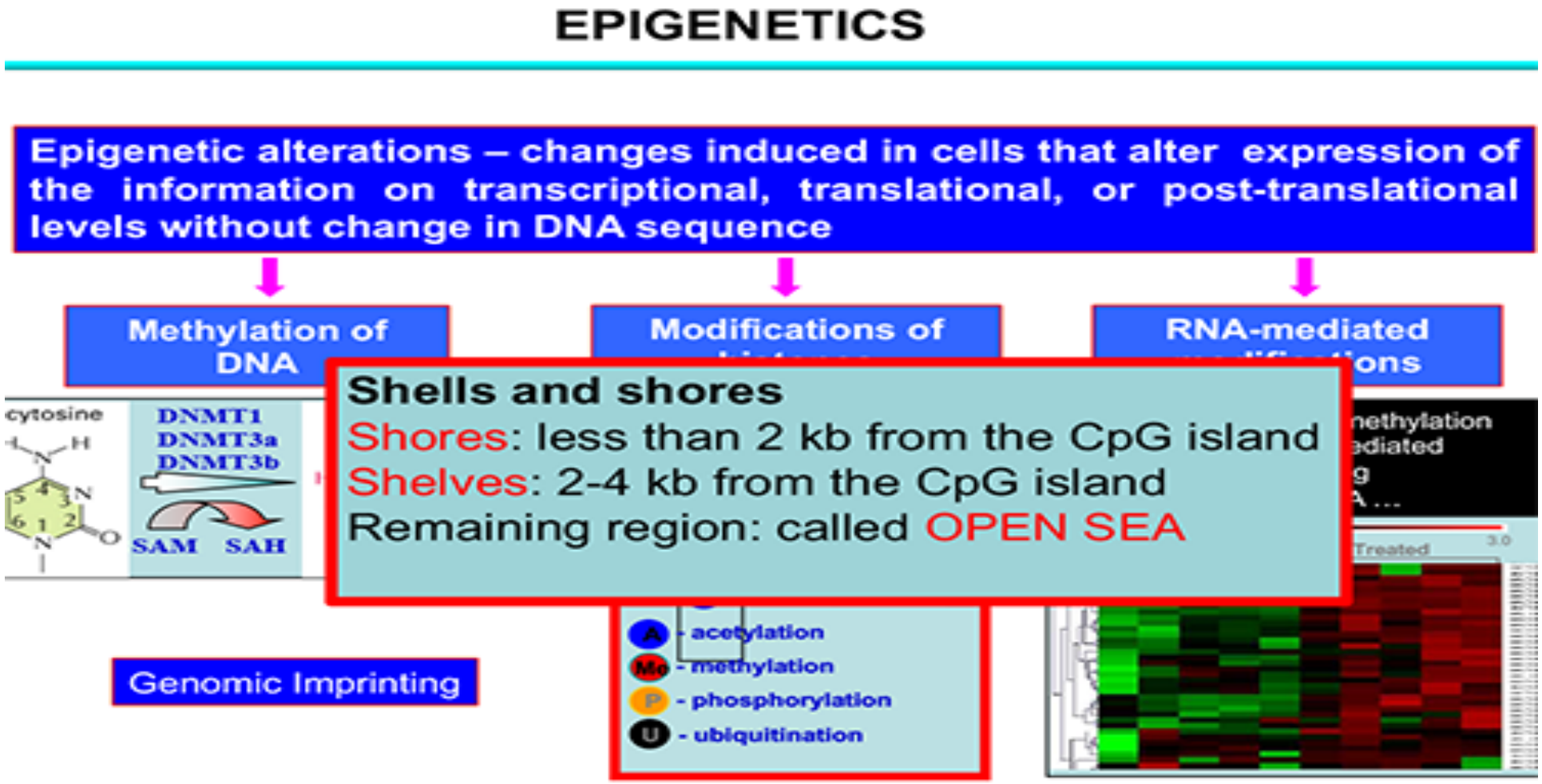
Shores are 0-2kb from islands
Shelves are 2-4 kb and enhancers are beyond shelves

DNA methylation

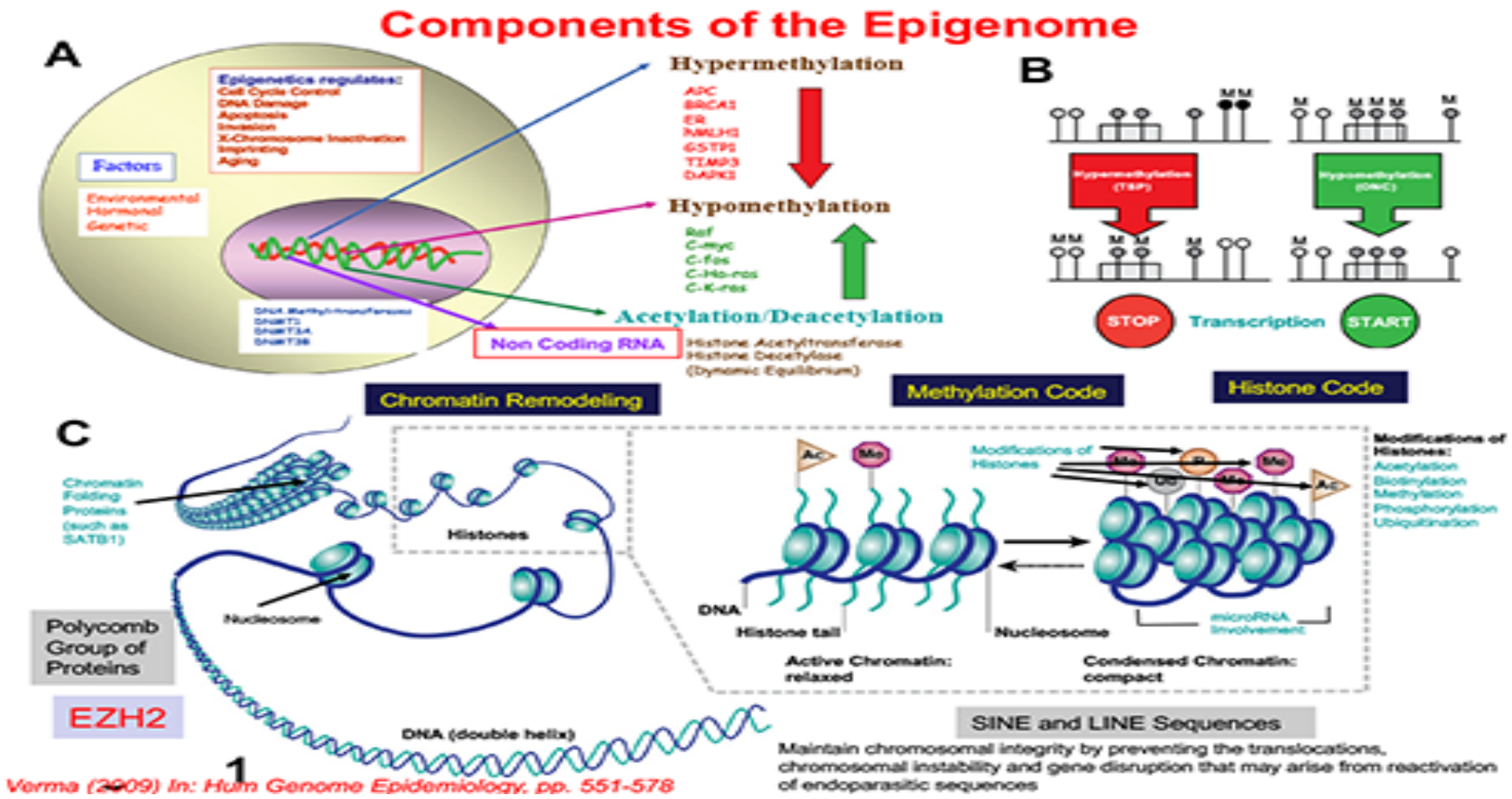


Asad Umar, NCI

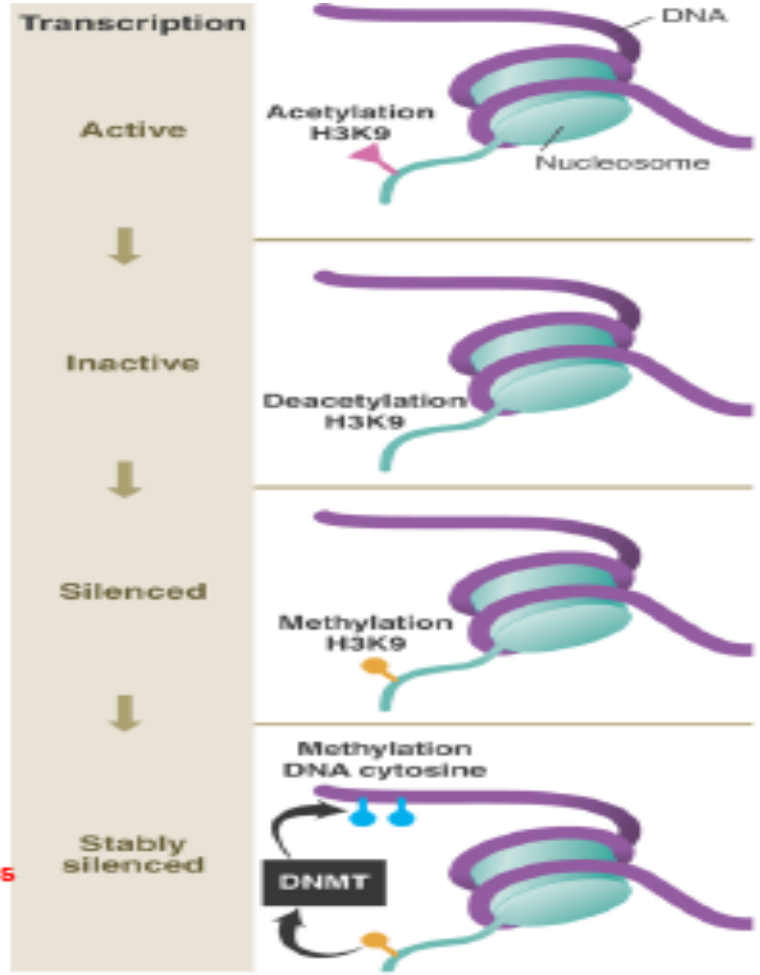
Epigenetics



Epigenome components

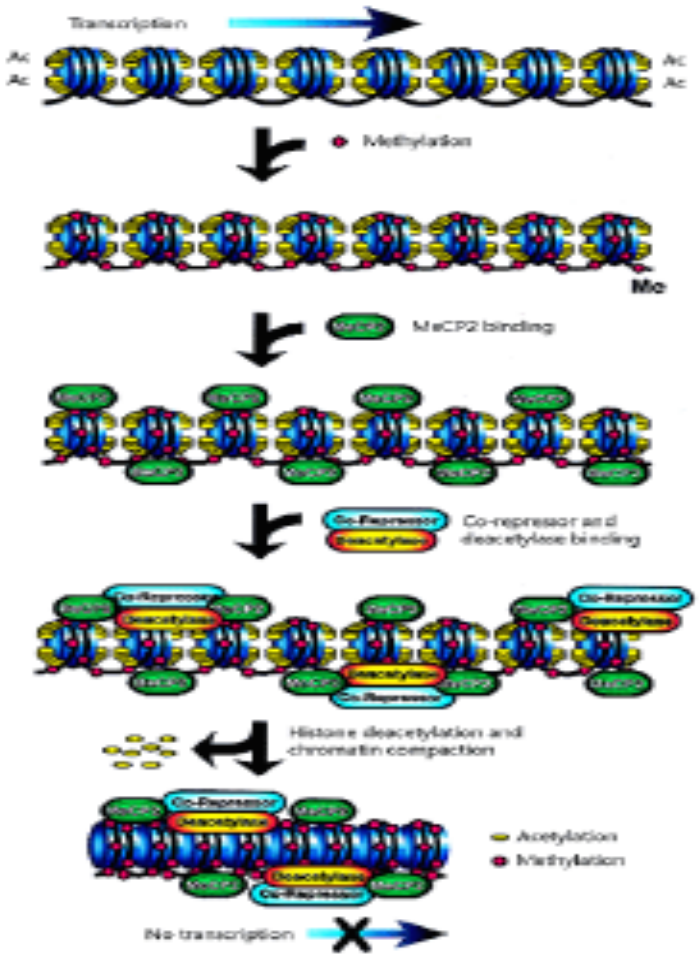


Methylation



Peter Jones

Andrew Feinberg

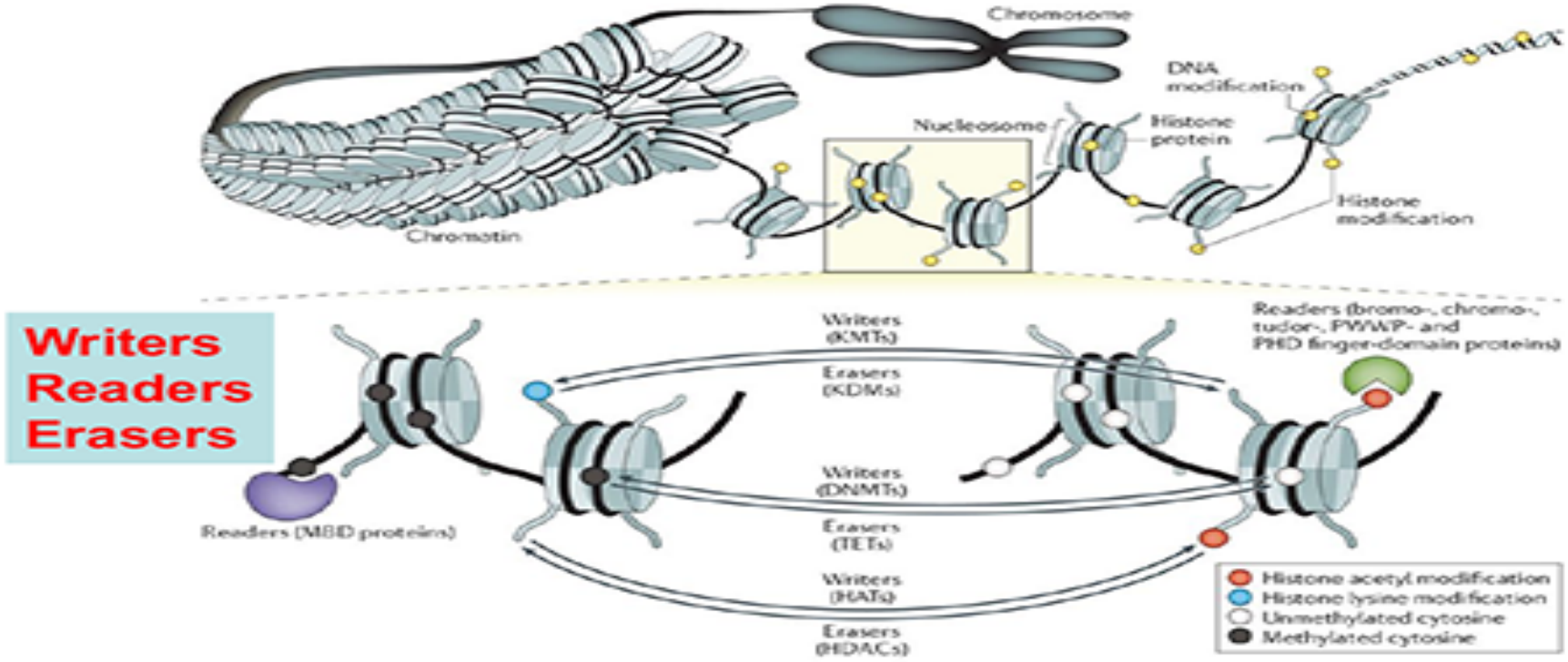


Jones and Laird. *Nat Rev Gen* 21: 163

Chromatin modifications

Figure 1 : Modulation of covalent modifications on chromatin.

From: Targeting the cancer epigenome for therapy

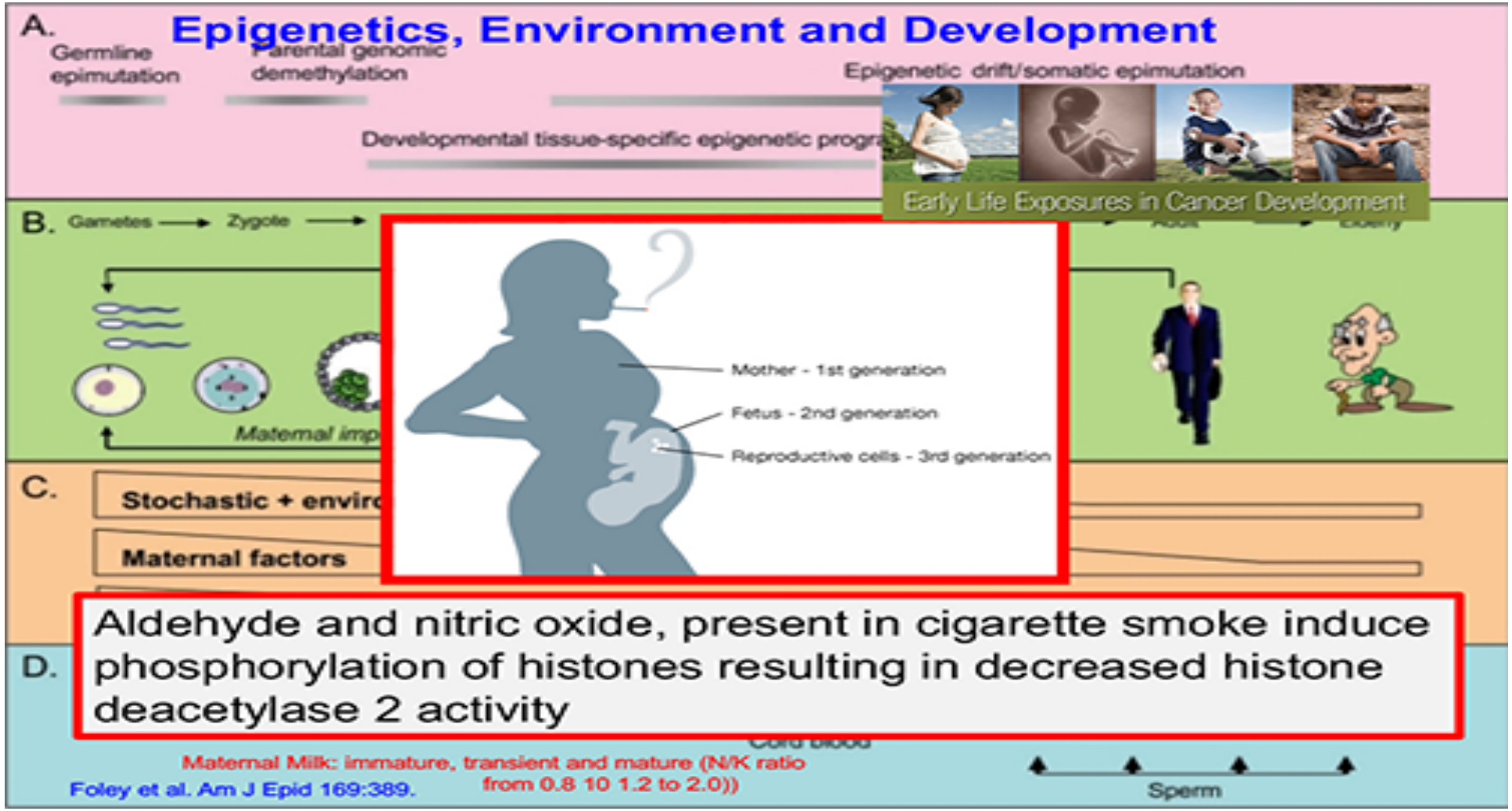


Ten-eleven translocation (TET) family of 5-methylcytosine oxidases.

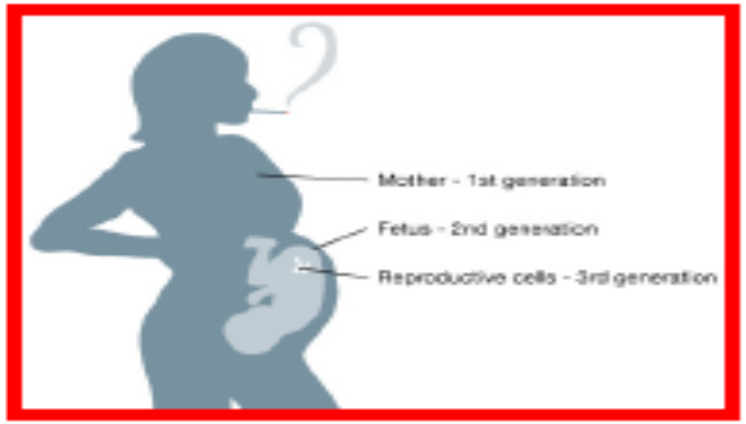
Exercise

Exercise is important for everyone
portant?
changes
• 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

Environment and development



Histone phosphorylation



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

Endogenous factors

PLoS One. 2016 May 12;11(5):e0155554. doi: 10.1371/journal.pone.0155554. eCollection 2016.

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

Rzehak P[1], Saffery R
Verduci E[8], Riva E[8].

Author inform

Abstract
Mounting evidence
profile in the blood
assesed 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[4,6], 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**

Cancer etiology

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

EGRP studies

29

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)

- 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.

Toxic substances and the epigenome

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

Environment and child health outcomes

ECHO

Scientific goal

ECHO Scientific Goal

Answer crucial questions about the effects of a **broad** range of **early environmental influences** on child health and development.

From
society
to
biology

Health outcomes throughout childhood and adolescence

https://www.nih.gov/echo/pediatric-cohorts

Developmental Life Stages

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

[RFP FY17]

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

ECHO advantages

Developmental Life Stages

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

Adolescence 12 years through 18 (or 21?) years

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

Epigenetics and behavior

Epigenetics and behavior (including emotions)

Transl Psychiatry. 2016 Mar 29;6:e765. doi: 10.1038/tp.2016.32.

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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

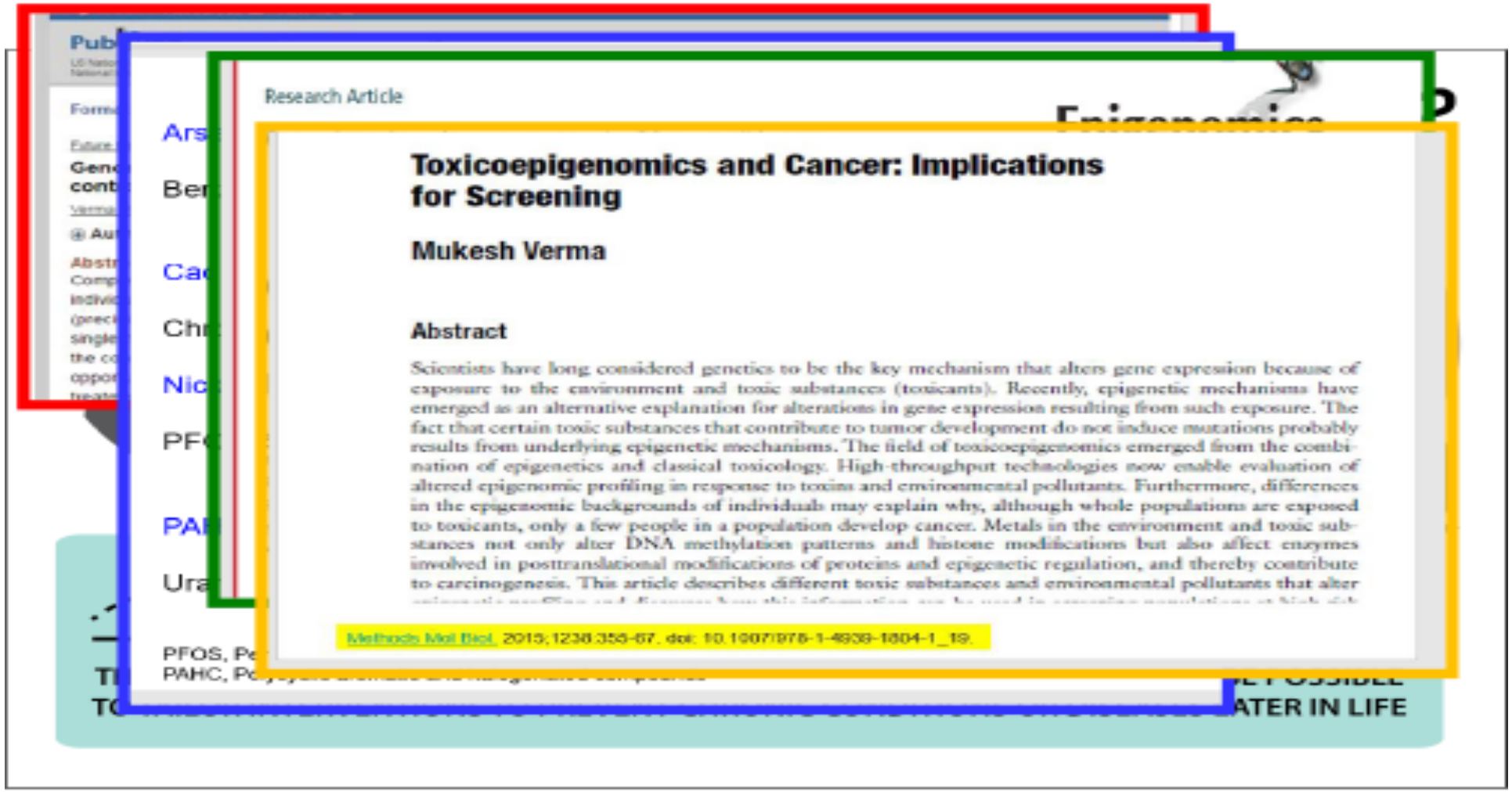
Open/close author information list

Abstract

Compelling evidence suggests that maternal mental health in pregnancy can influence fetal development. The imprinted genes, insulin-like growth factor 2 (IGF2) and H19, are involved in fetal growth and each is regulated by DNA methylation. This study aimed to determine the association between maternal mental well-being during pregnancy and differentially methylated regions (DMRs) of IGF2 (DMR0) and the IGF2/H19 imprinting control region (ICR) in newborn offspring. Maternal depression, anxiety and perceived stress were assessed at 28 weeks of pregnancy in the Barwon Infant Study (n=576). DNA methylation was measured in purified cord blood mononuclear cells using the Sequenom

within your DNA that can be controlled by you, by your emotions, beliefs and behavioral choices."

Toxico epigenomics



Research Article

Toxicoepigenomics and Cancer: Implications for Screening

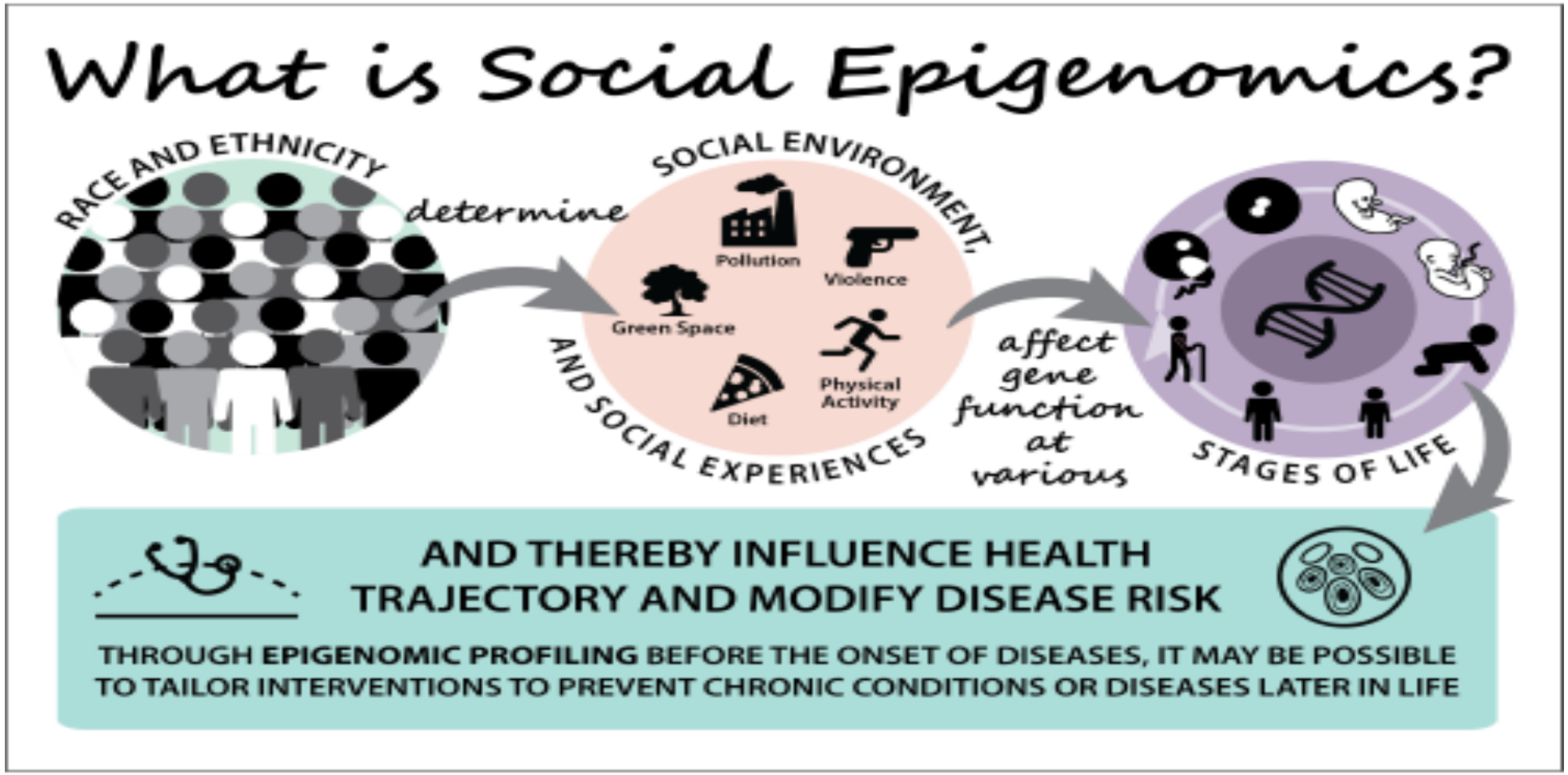
Mukesh Verma

Abstract

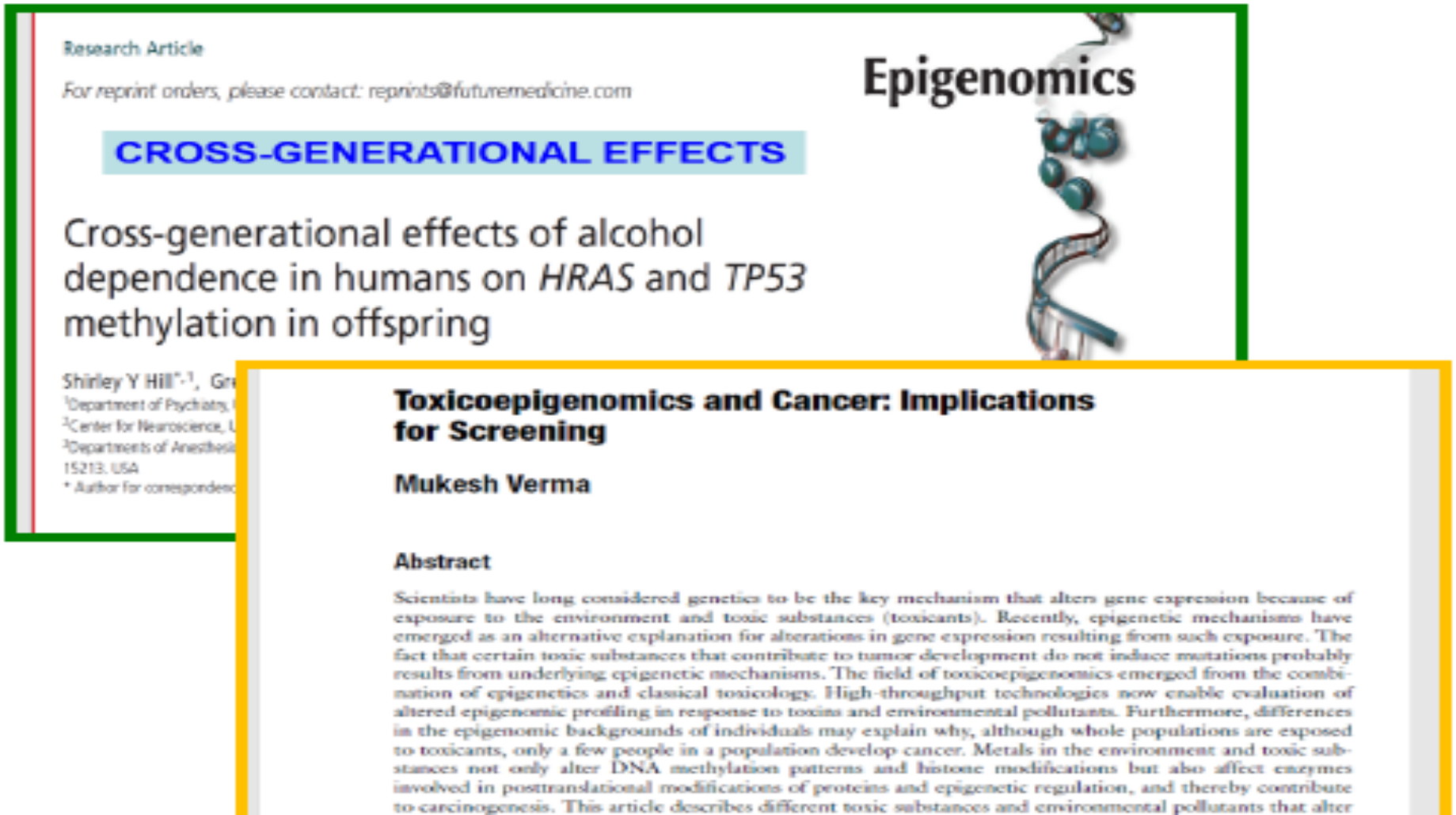
Scientists have long considered genetics to be the key mechanism that alters gene expression because of exposure to the environment and toxic substances (toxicants). Recently, epigenetic mechanisms have emerged as an alternative explanation for alterations in gene expression resulting from such exposure. 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

Methods Mol Biol. 2015;1238:355-67. doi: 10.1007/978-1-4939-1804-1_19.

Social epigenomics



Epigenomics



Research Article

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Epigenomics

CROSS-GENERATIONAL EFFECTS

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

Shirley Y Hill*,[1], Gr

[1]Department of Psychiatry,

[2]Center for Neuroscience, U

[3]Department of Anesthesi

15213, USA

* Author for correspondenc

Toxicoepigenomics and Cancer: Implications for Screening

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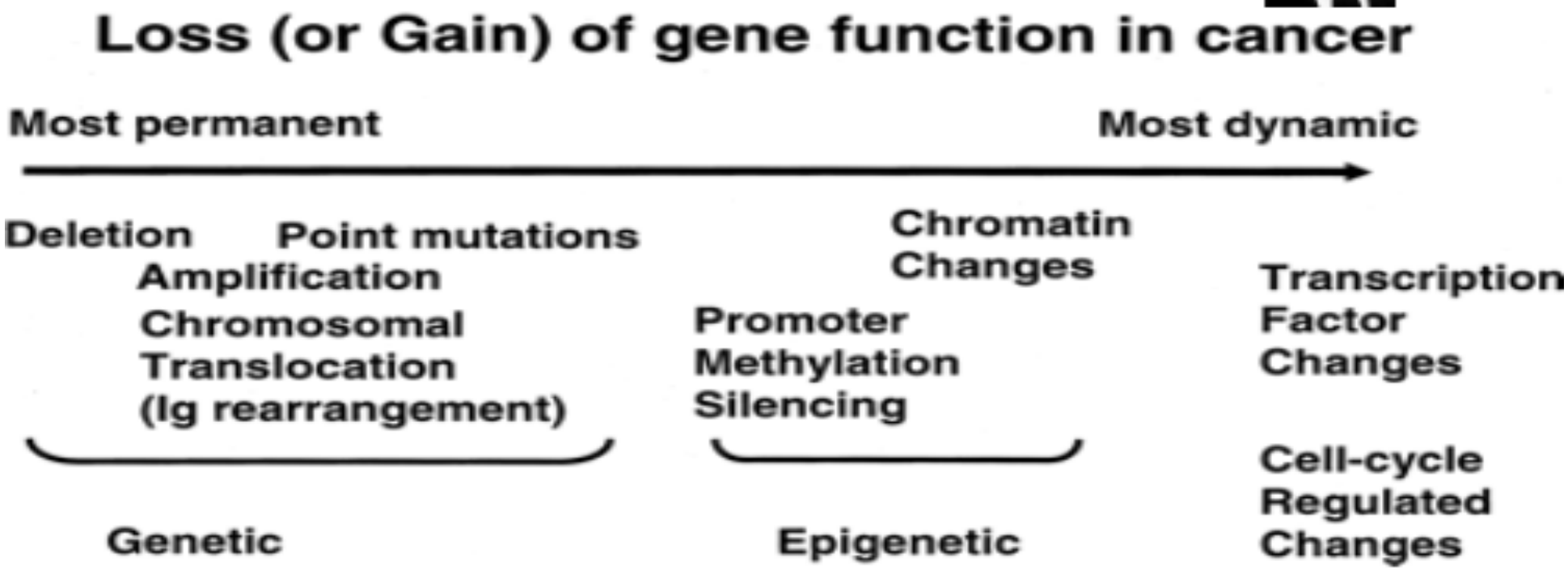
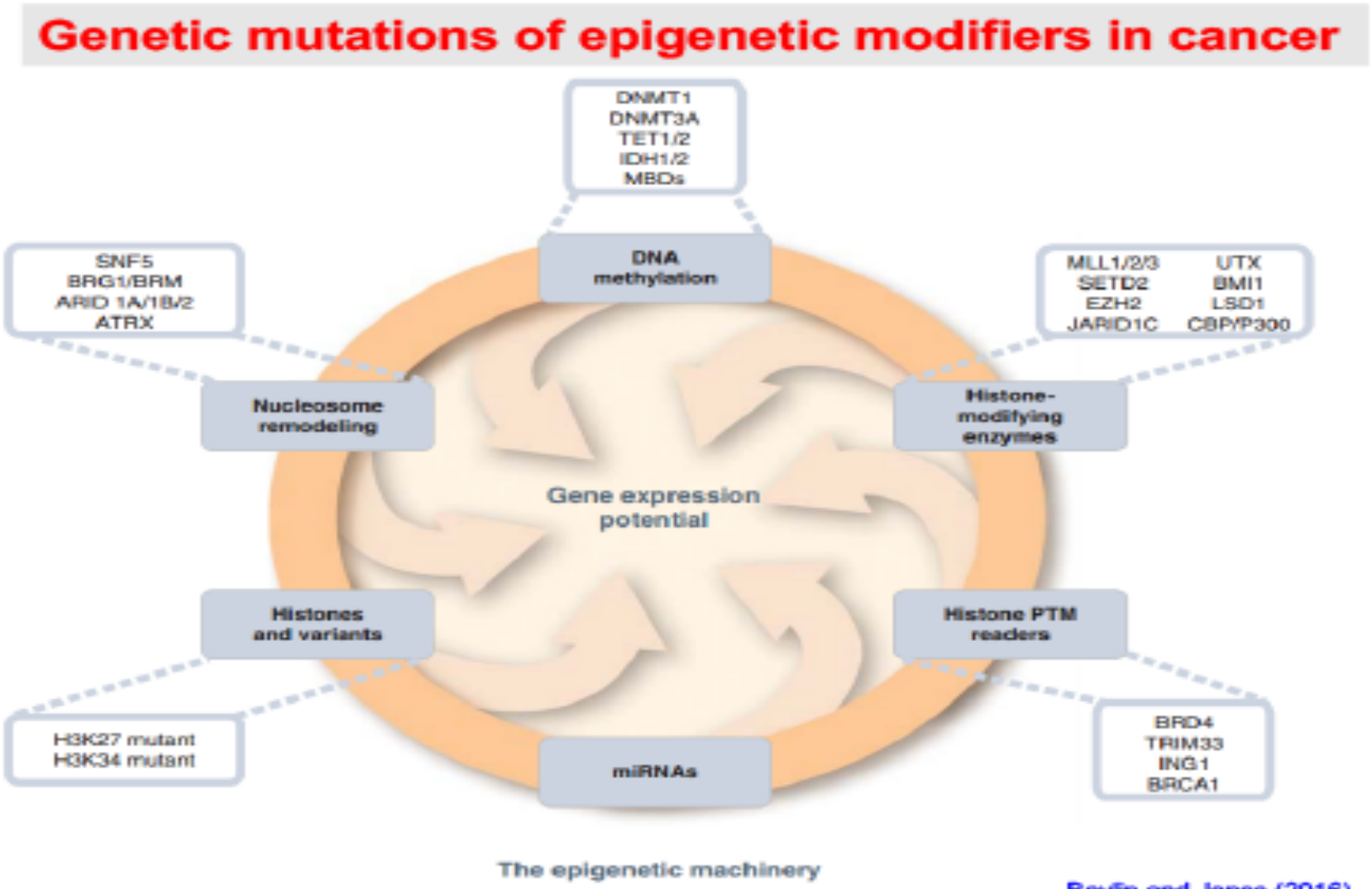
Loss (or gain) of gene function in cancer

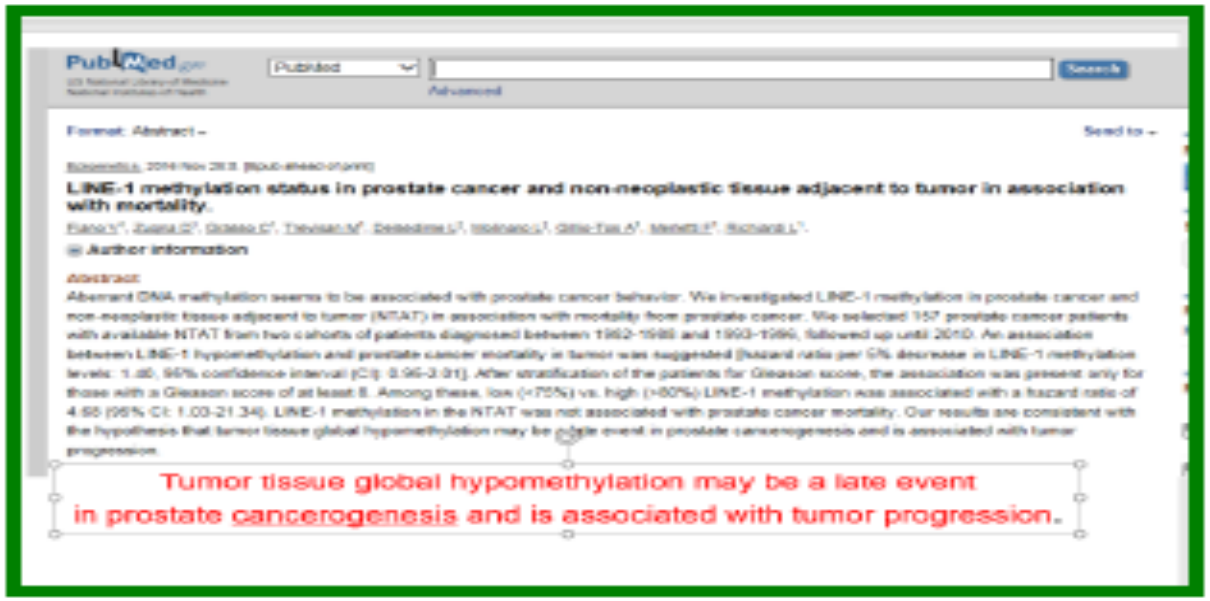


Genetic mutations



Baylin and Jones (2016)

Hypomethylation



PubMed.gov

Format: Abstract

Send to

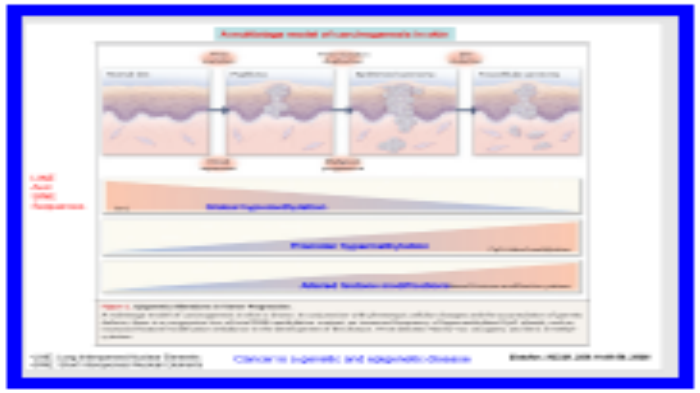
LINE-1 methylation status in prostate cancer and non-neoplastic tissue adjacent to tumor in association with mortality.

Author information

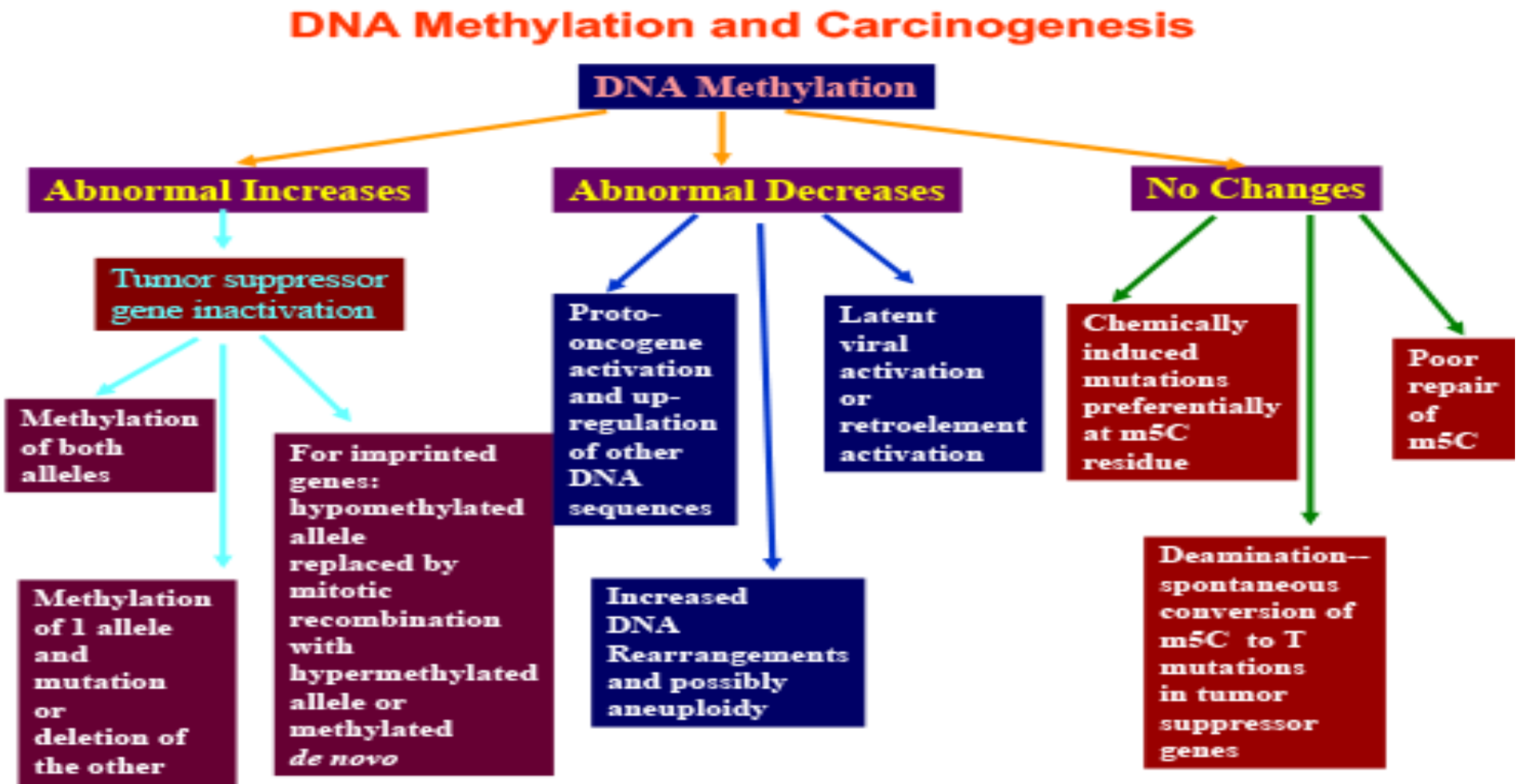
Abstract

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 1992-1998 and 1993-1999, 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: 1.46, 95% confidence interval (CI): 0.96-3.91). 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 4.65 (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.

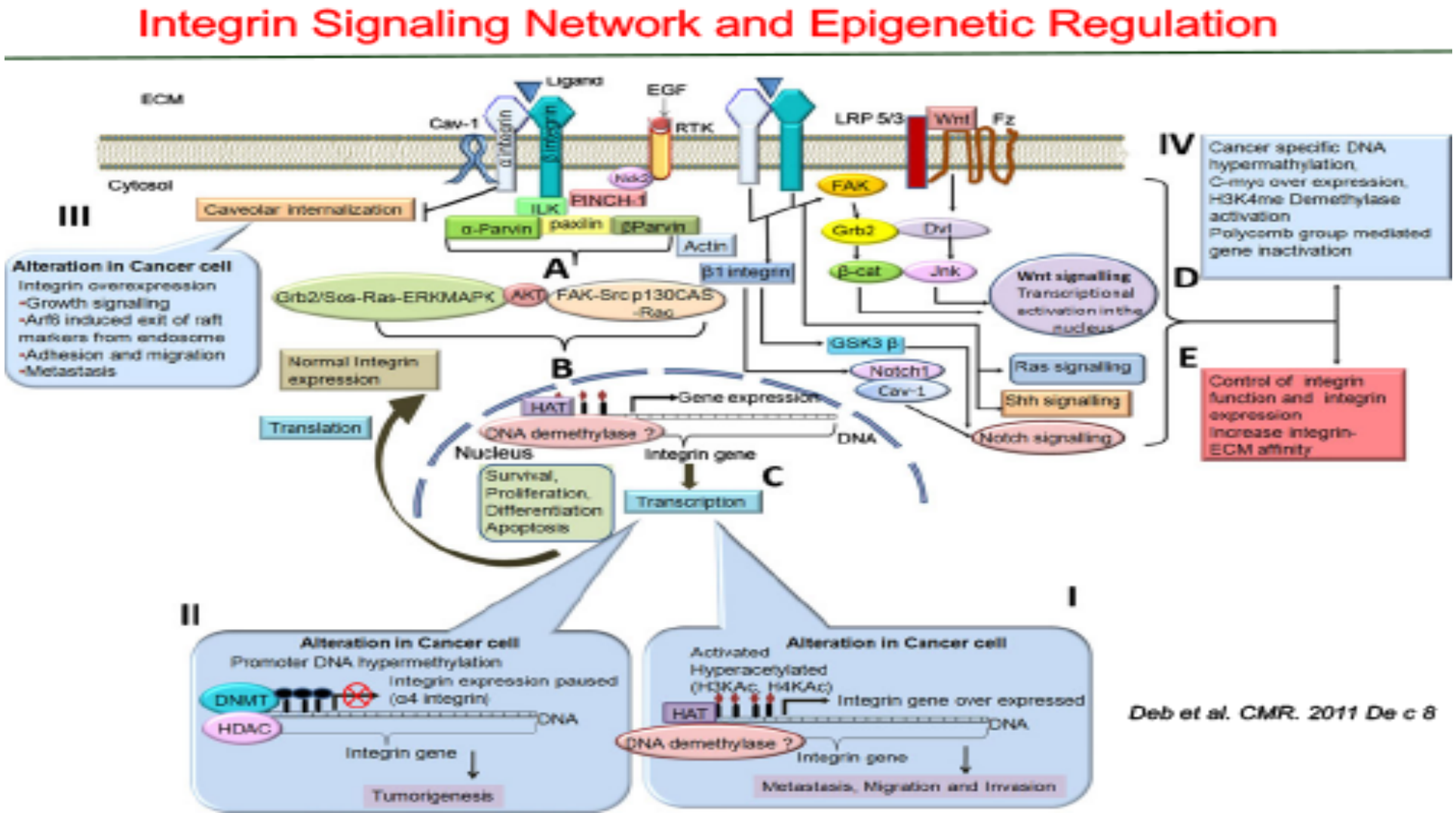
Tumor tissue global hypomethylation may be a late event in prostate cancerogenesis and is associated with tumor progression.



DNA methylation and carcinogenesis

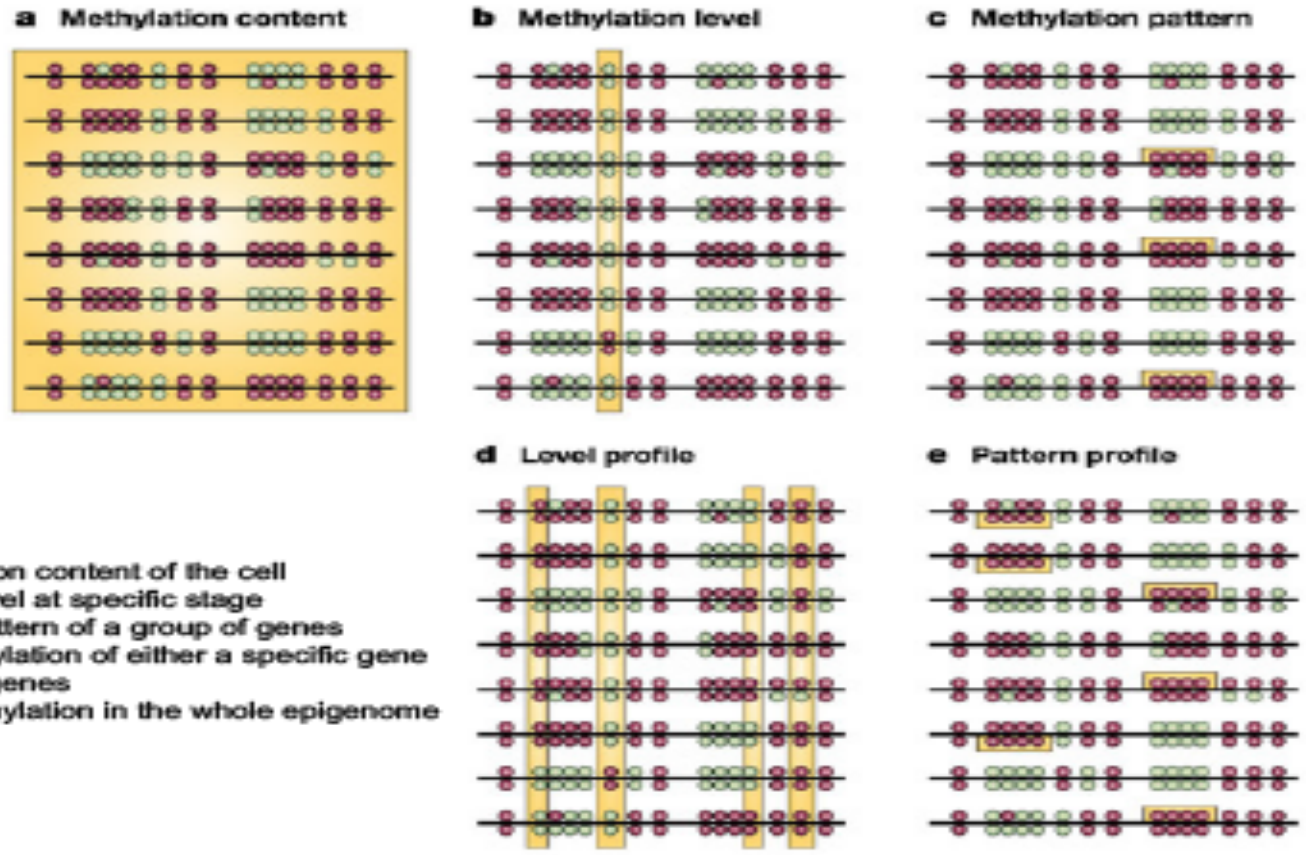


Integrin signaling



Deb et al. CMR. 2011 Dec 8

Methylation



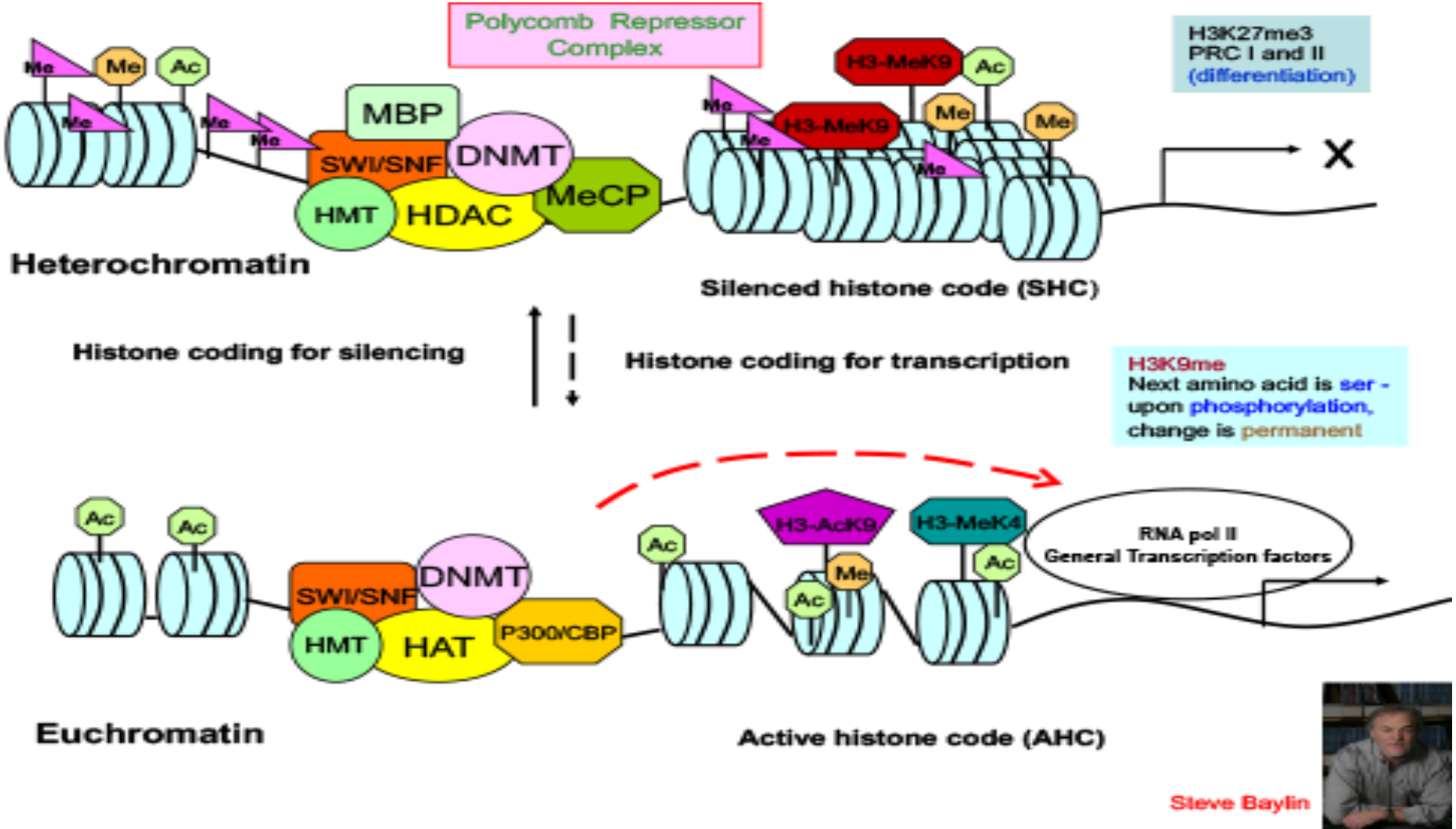
- Total methylation content of the cell
- methylation level at specific stage
- methylation pattern of a group of genes
- profile of methylation of either a specific gene or a number of genes
- pattern of methylation in the whole epigenome

To reduce
- false negative
- false positives

Laird *Nat Rev Cancer* 3:253

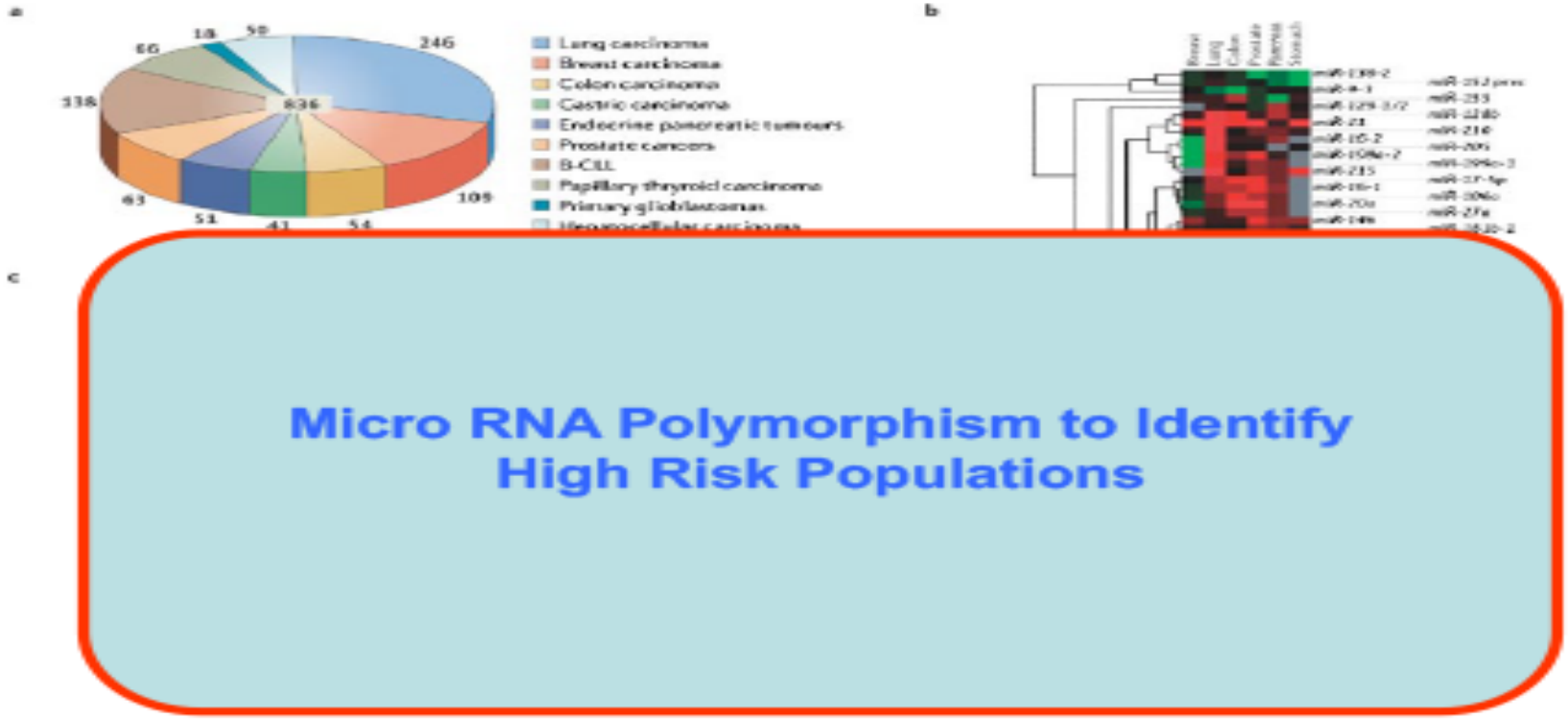
Nature Reviews | Cancer

Histone acetylation



Micro RNA signatures

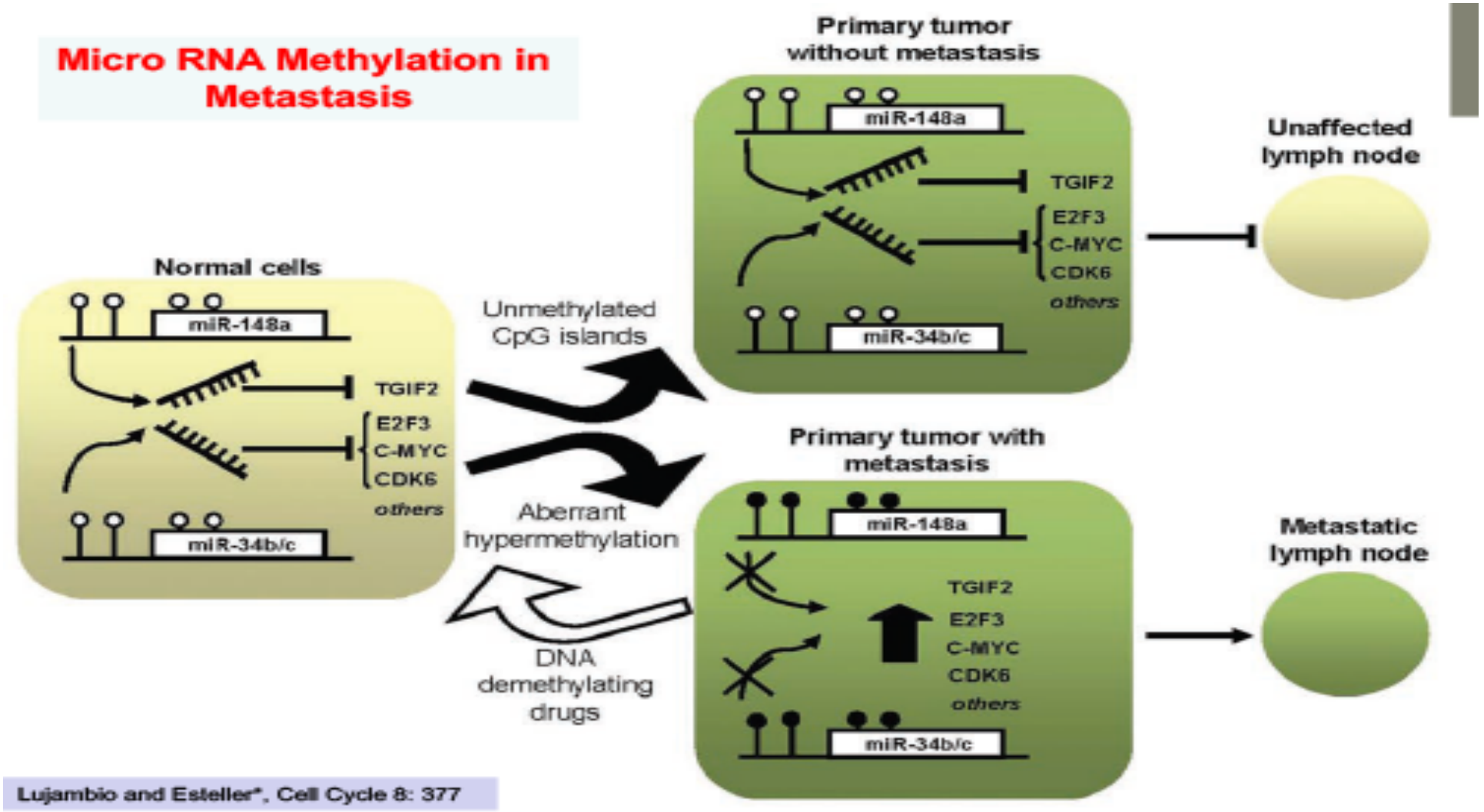
Mirco RNA Signatures in Human Cancers



Mir-31 inhibits metastasis in breast cancer

nature REVIEWS CANCER

Micro RNA methylation



Extracellular vesicles

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

BMC Clinical Pathology

REVIEW Open Access

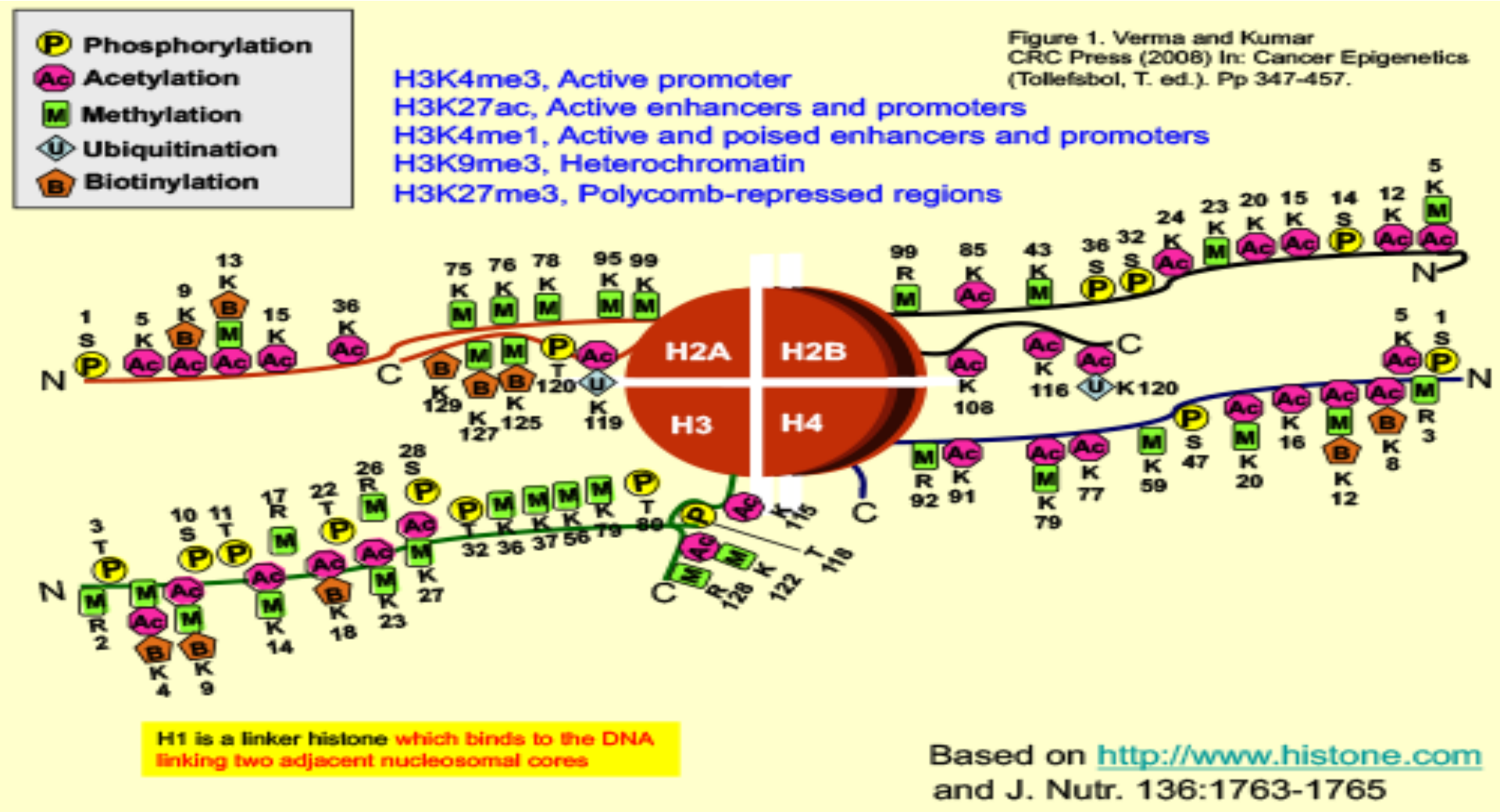
Extracellular vesicles: potential applications in cancer diagnosis, prognosis, and epidemiology

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,

Histone modifications



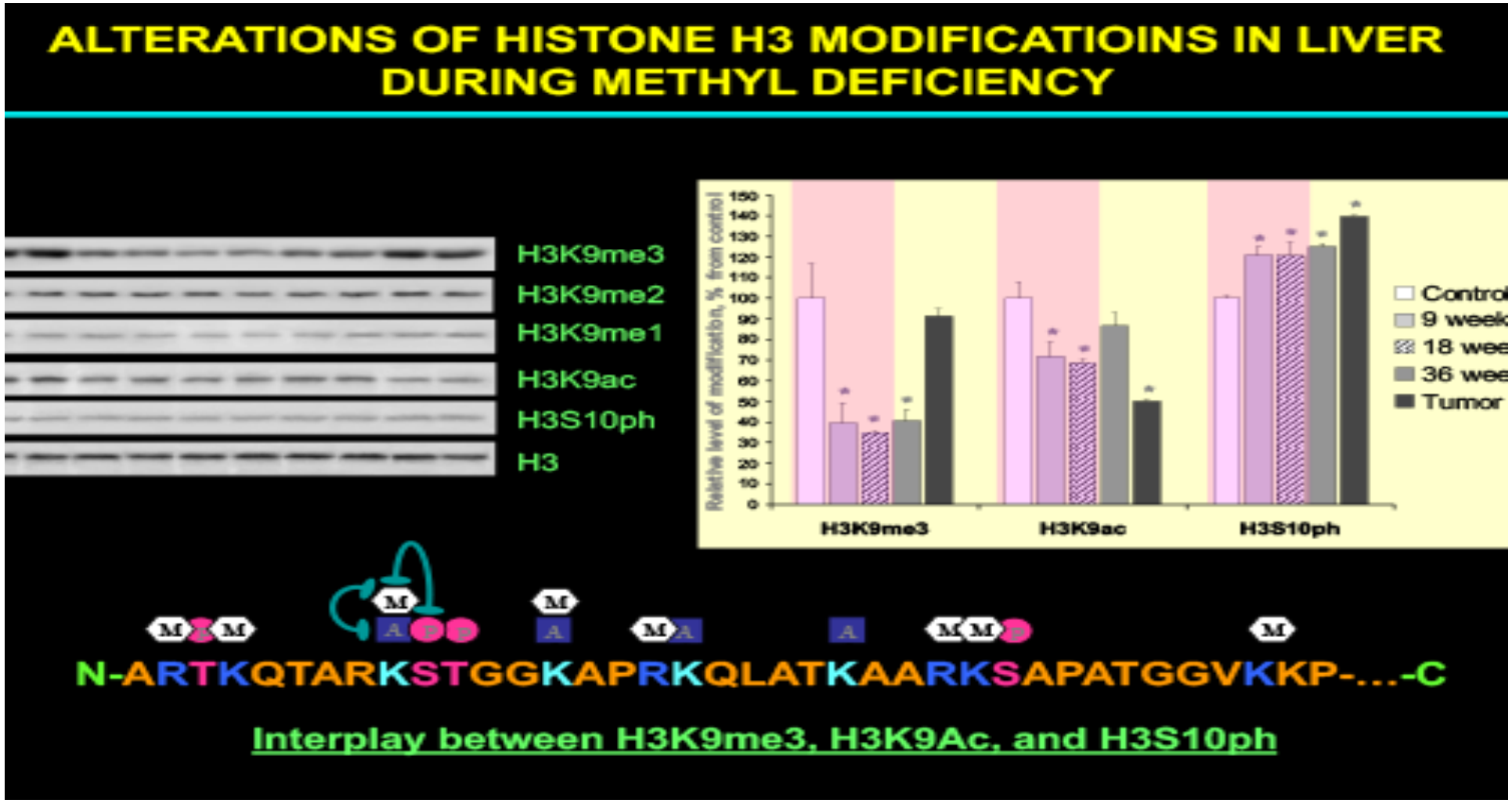
Histones

Clin Epigenetics. 2016; 8: 57

Activating: e.g. H3K4me3

Silencing: e.g. H3K9me3, H3K27me3

Histone H3 modifications

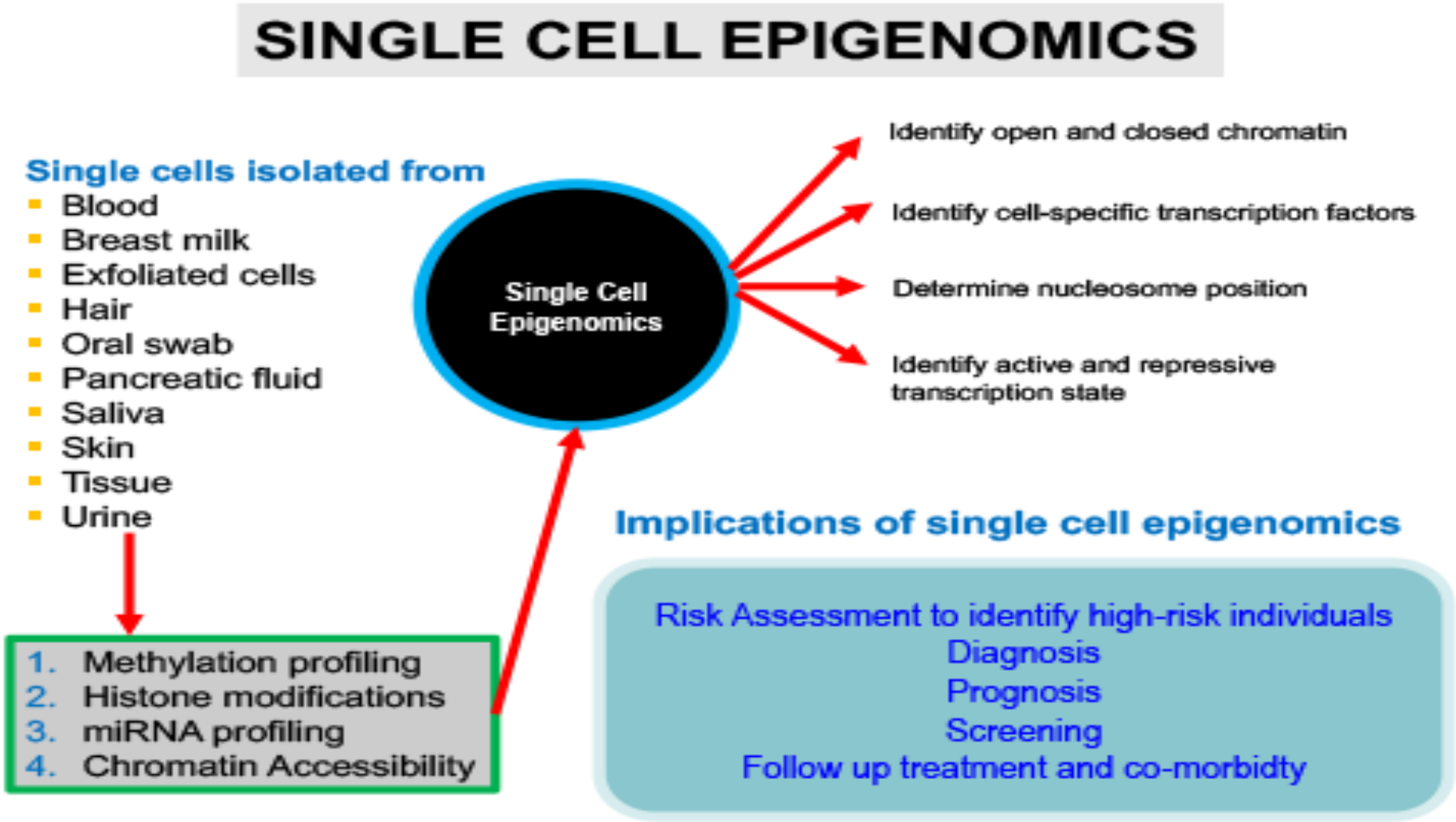


Epigenetic regulation

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

Single cell epigenomics



Histone modifications

20 Diagnosing Cancer Using Histone Modification Analysis

Mukesh Verma and Deepak Kumar

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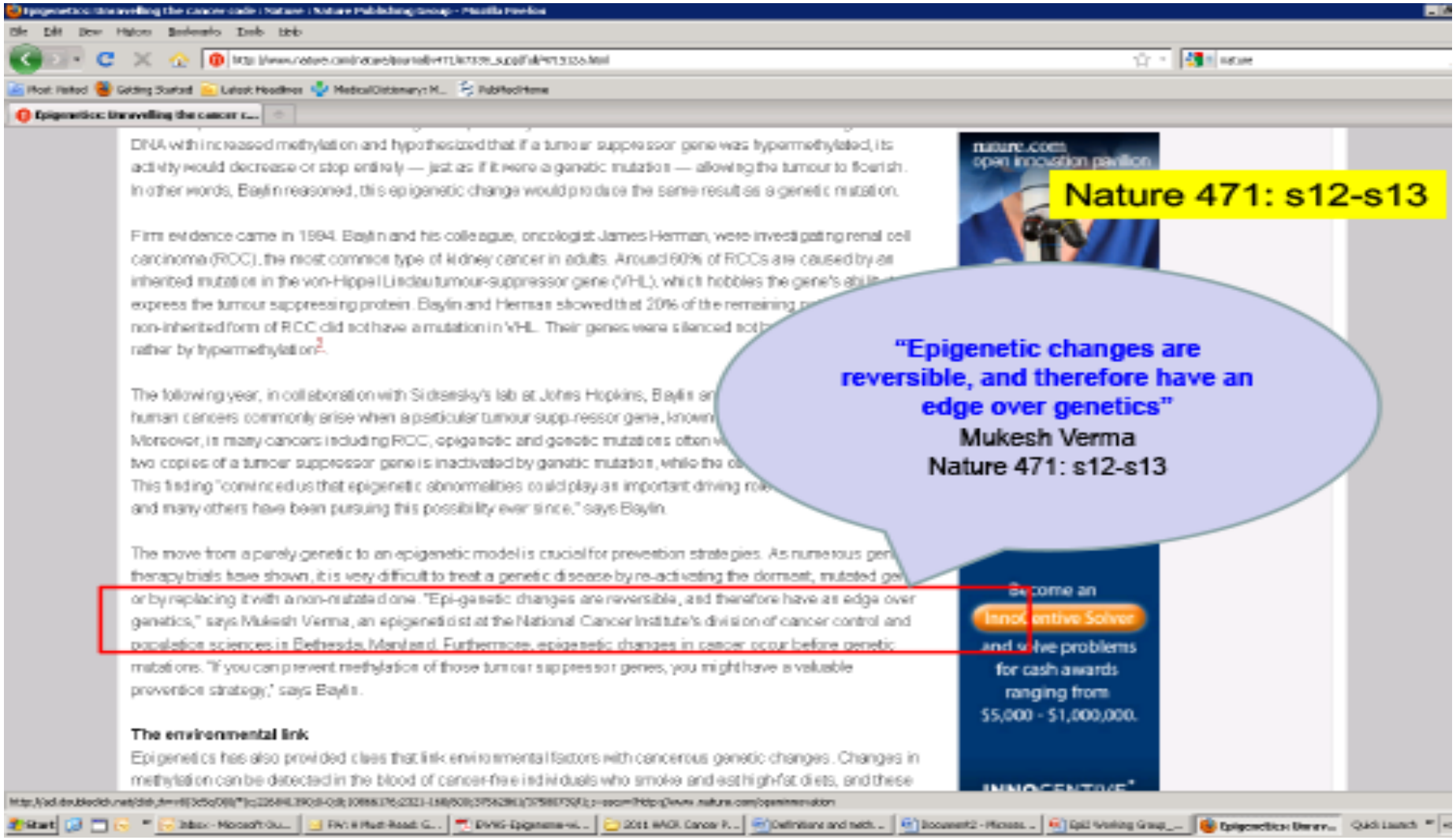
ISBN 9781420045796 - CAT# 45792

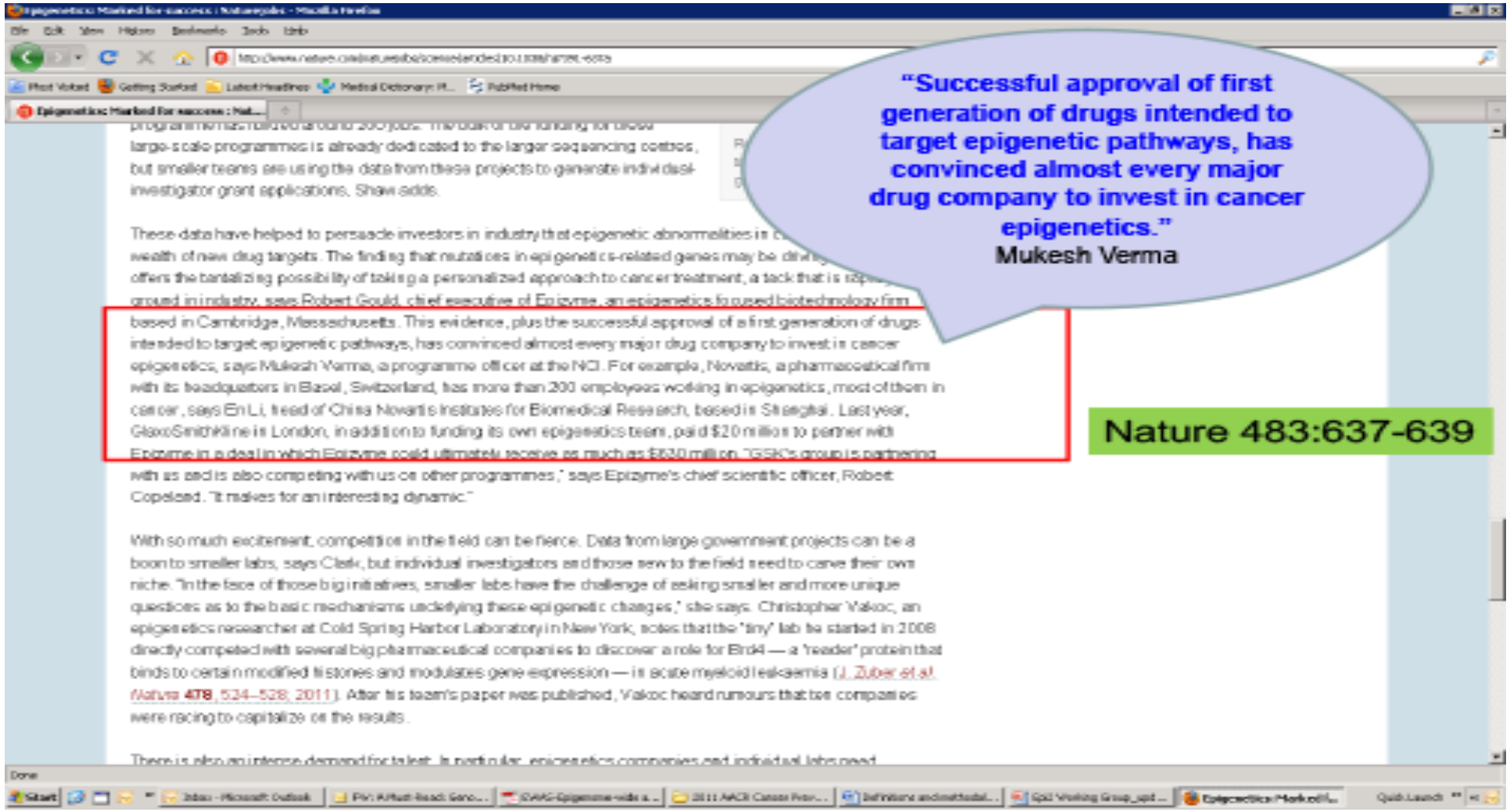
Books

Books edited by Mukesh Verma

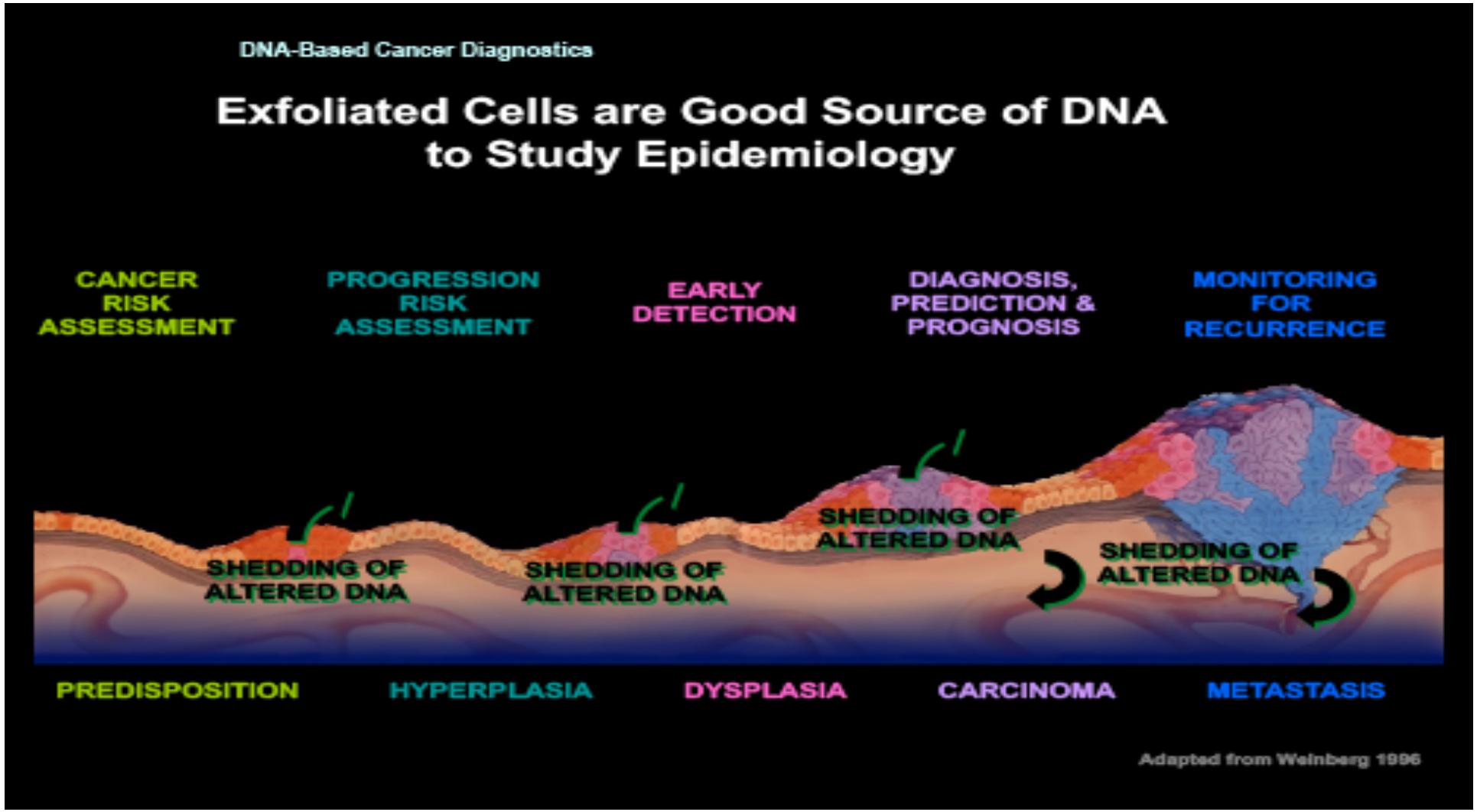
Epigenetic changes



Epigenetic drugs



Exfoliated cells



Tumors and epigenetics

Tumor Types and Genes Regulated by Epigenetic Mechanism

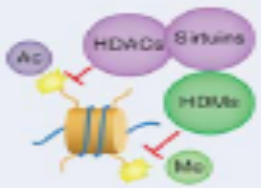
TUMOR LOCATION	GENE
Breast	p16, BRCA1, GSTP1, DAPK, CDH1, TIMP-3
Brain	p16, p14^{MRF}, MGMT, TIMP-3
Bladder	p16, DAPK, APC
Colon	p16, p14^{MRF}, CRBP1, MGMT, hMLH1, DAPK, TIMP-3, APC
Endometrium	hMLH1
Esophagus	p16, p14^{MRF}, GSTP1, CDH1APC
Head and Neck	p16, MGMT, DAPK
Kidney	p16, p14^{MRF}, MGMT,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

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) Cnu Rev Clin Sc 41: 585-607
Verma and Moore (2006) Cnu Rev Hemmat Oncol 60:9-18
Verma et al (2006) Mol Diag Therapy 10:1-15

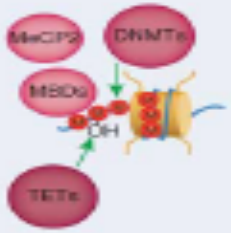
Histone enzymes

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

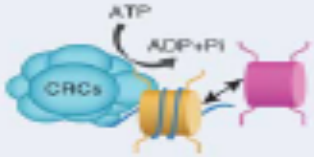


Category	Gene
HATs	CREBBP
	EP300
	MYST3
	MYST4
	KAT5
HMTs	MLL
	EZH2
	NSD1
	PRDM2
	SMYD3
	WHSC1

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



Category	Gene
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

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.

Epigenetic drugs

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

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

Epigenetical Nature 429 457

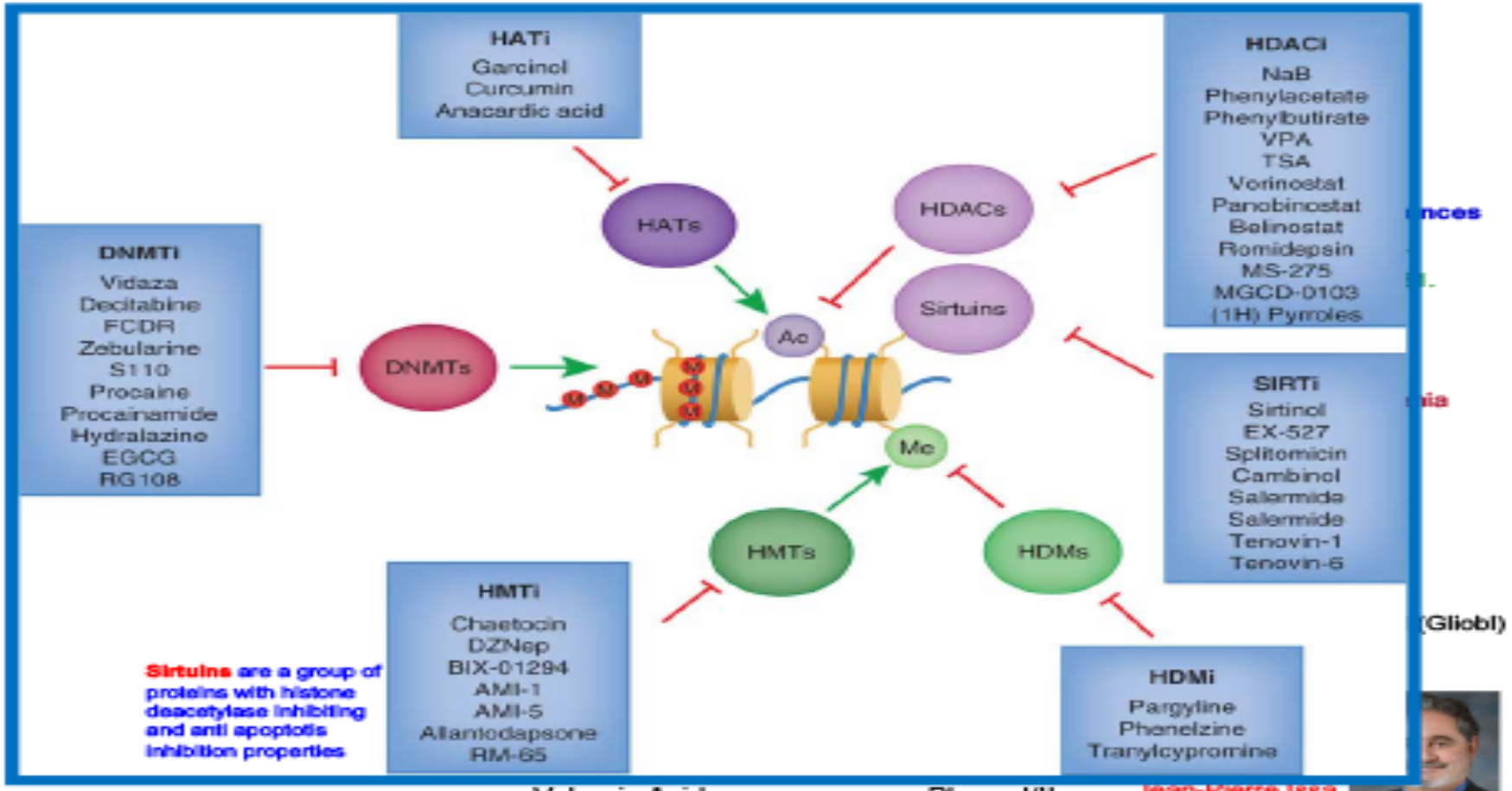
Adverse Experiences SAHA
Duvic et al. 2007.
Am J Hem 109:31.

- Dehydration
- Diarrhea
- Nausea
- Thrombocytopenia
- Vomiting

Vorinostat Phill (Glob)

Jean Pierre Issa
Temple Univ.

Methylation and acetylation enzymes



Eggr et al Nature. 429:457.

Valproic Acid

Phase I/II

Jean-Pierre Issa
Temple Univ.

HDAC inhibitors

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

  Thereby inducing

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

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

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

Phase I study

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.

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-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

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

Methylation Inhibitors in Clinical Trials (Clinicaltrials.gov)

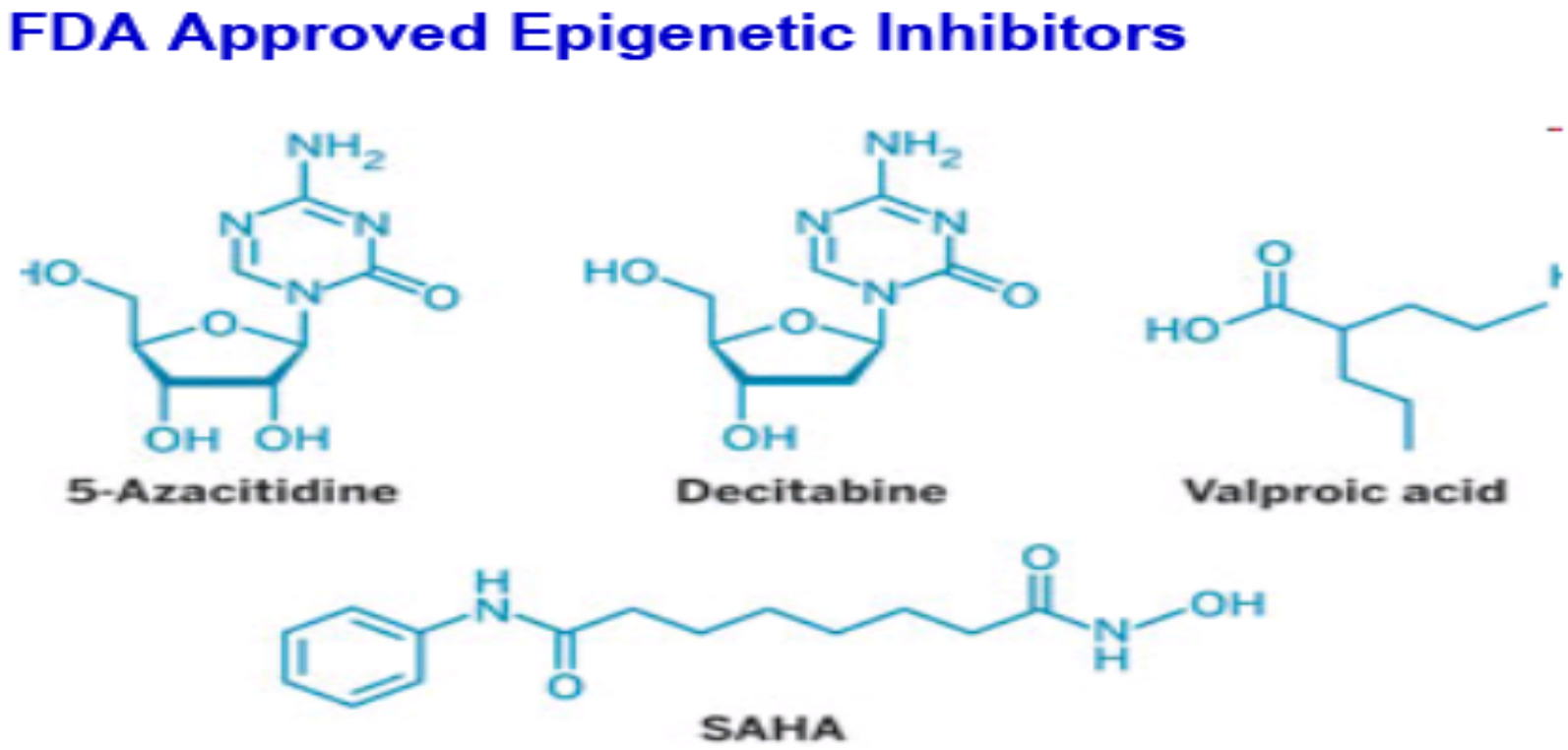
STATUS	STUDY
Completed	A Phase II Study of Epigenetic Therapy to Overcome Chemotherapy Resistance in Refractory Solid Tumors
Active Not Recruiting	Azacytidine and Valproic Acid in Patients With Advanced Cancers
Recruiting	Azacitidine With or Without MS-275 in Treating Patients With Myelodysplastic Syndromes, 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-2 b 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 Ado Met 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-2 b in Advanced Renal Cell Carcinoma

Total : 51 studies

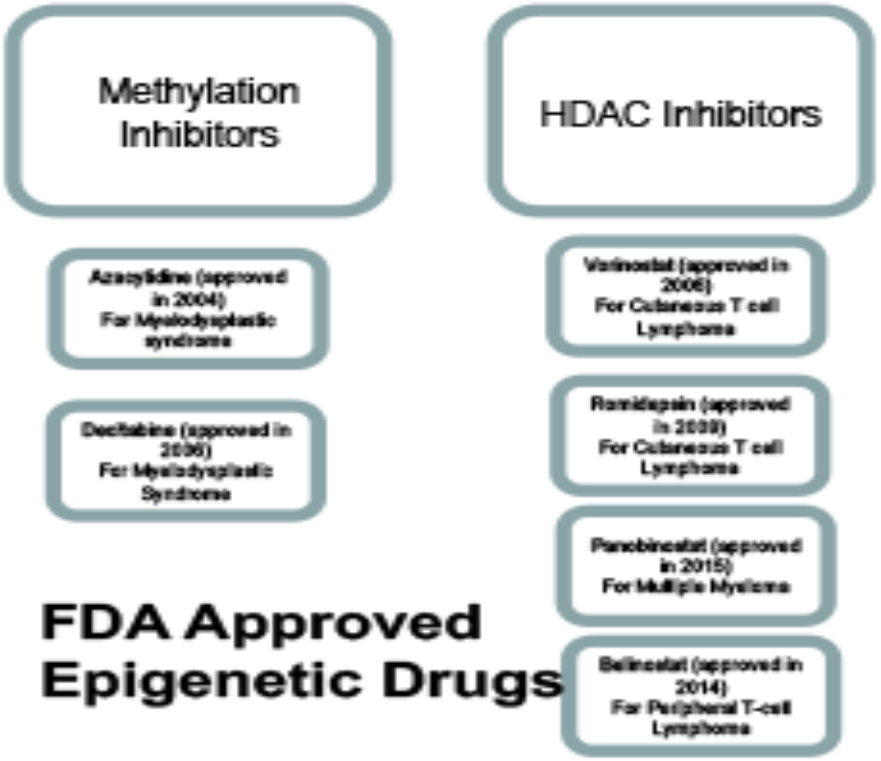
http://clinicaltrials.gov/ct2/results?term=methylation+inhibitors

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

Epigenetic inhibitors



Approved epigenetic drugs



Epigenetic drugs

Cancer type	Epigenetic therapy	Drug combination	Patient selection	Response	Pharmacodynamic target validation?*	Refs[1]
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
Epithelial ovarian cancer	Decitabine (demethylating agent)	Decitabine and carboplatin	Initial response by RECIST and/or CA125 criteria then progressing 6–12 months after previous platinum therapy	3 of 15 CA125 partial response; 1 of 15 RECIST partial response	Yes	78
Epithelial ovarian cancer	Decitabine (demethylating agent)	Decitabine and carboplatin	Progression or recurrence within 6 months of platinum-based compound	1 of 17 complete response; 5 of 17 partial response	Yes	77
Epithelial ovarian cancer	Azacytidine (demethylating agent)	Azacytidine and carboplatin	Progression or recurrence within 6 months of platinum-based compound	1 of 29 complete response; 3 of 29 partial response	Yes	90
Prostate cancer	Azacytidine (demethylating agent)	Azacytidine, LHRH analogue and anti-androgens	Progression on combined androgen blockade	19 of 34 PSADT >3 months; 11 of 34 PSADT >6 months; 9 of 34 PSADT >9 months	Yes	91
ER- and PR-positive breast cancer	Vorinostat (pan-deacetylase inhibitor)	Vorinostat and tamoxifen	Progression or recurrence on any aromatase inhibitors or completed tamoxifen for 1 year	8 of 34 partial response	Yes	92
Epithelial ovarian cancer	Belinostat (pan-deacetylase inhibitor)	Belinostat and carboplatin	Recurrence at ≤6 months of last platinum and taxol treatment	2 of 27 objective response	No	93
Epithelial ovarian cancer	Belinostat (pan-deacetylase inhibitor)	Belinostat, carboplatin and paclitaxel	Platinum-refractory or -resistant disease	15 of 35 objective response	No	94

EGFR, epidermal growth factor receptor; ER, oestrogen receptor; LHRH, luteinizing-hormone-releasing hormone; PR, progesterone receptor; PSADT, prostate specific antigen doubling time; RECIST, response evaluation criteria in solid tumours. *Pharmacodynamic validation refers to whether there was evidence of epigenetic responses in surrogate or tumour tissue from patients. [1]Publications were identified using Pubmed Search terms: HDAC inhibitor, decitabine or 5-azadeoxycytidine or azacytidine or 5-azacytidine or demethylating agent and cancer. Only clinical trials of solid tumours that used a chemotherapy agent that patients are already known to be resistant to are included.

Combination therapy

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

Low doses of DNA-demethylating agents

Cell PRESS

Cancer Cell
Article

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

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

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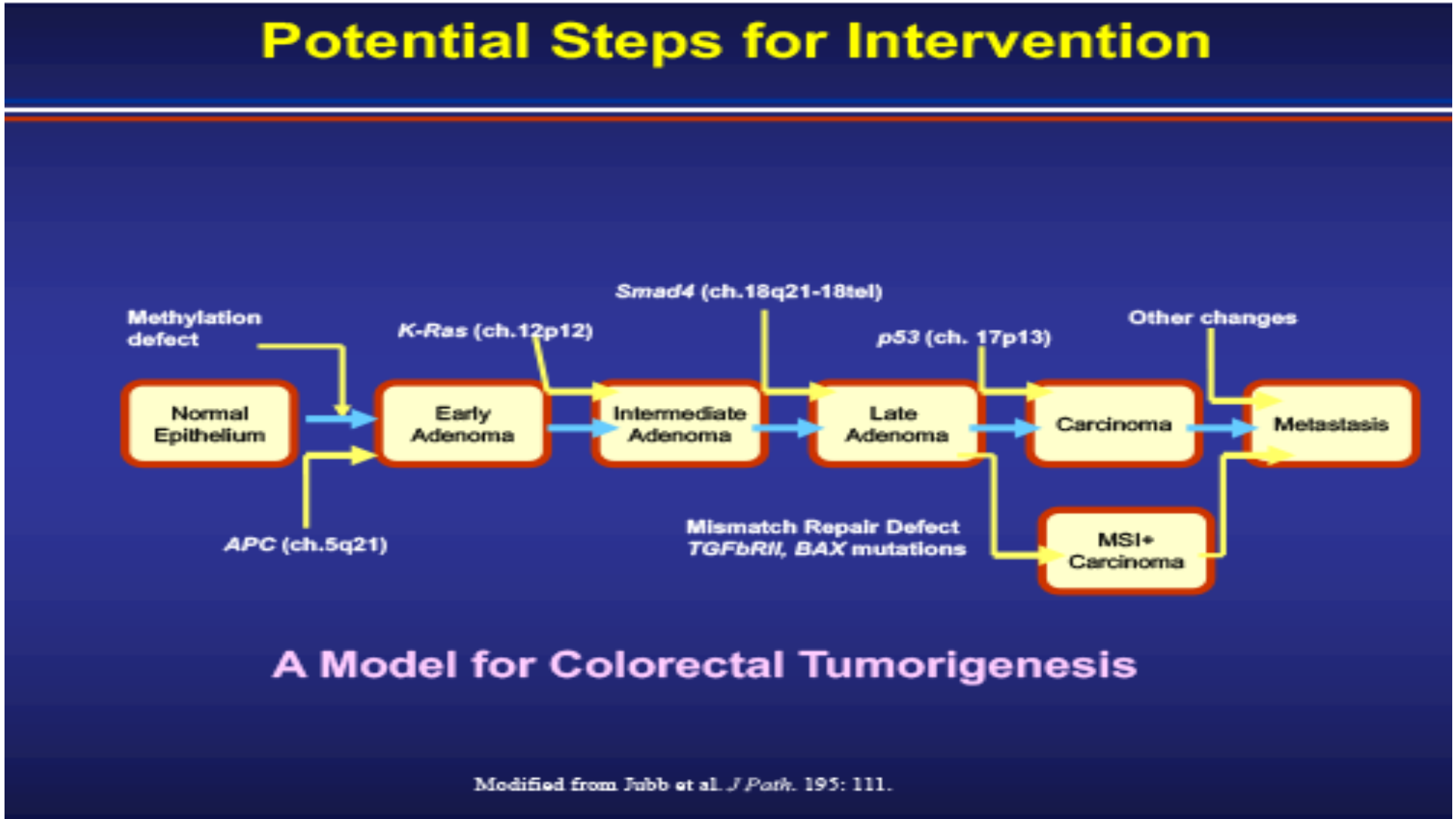
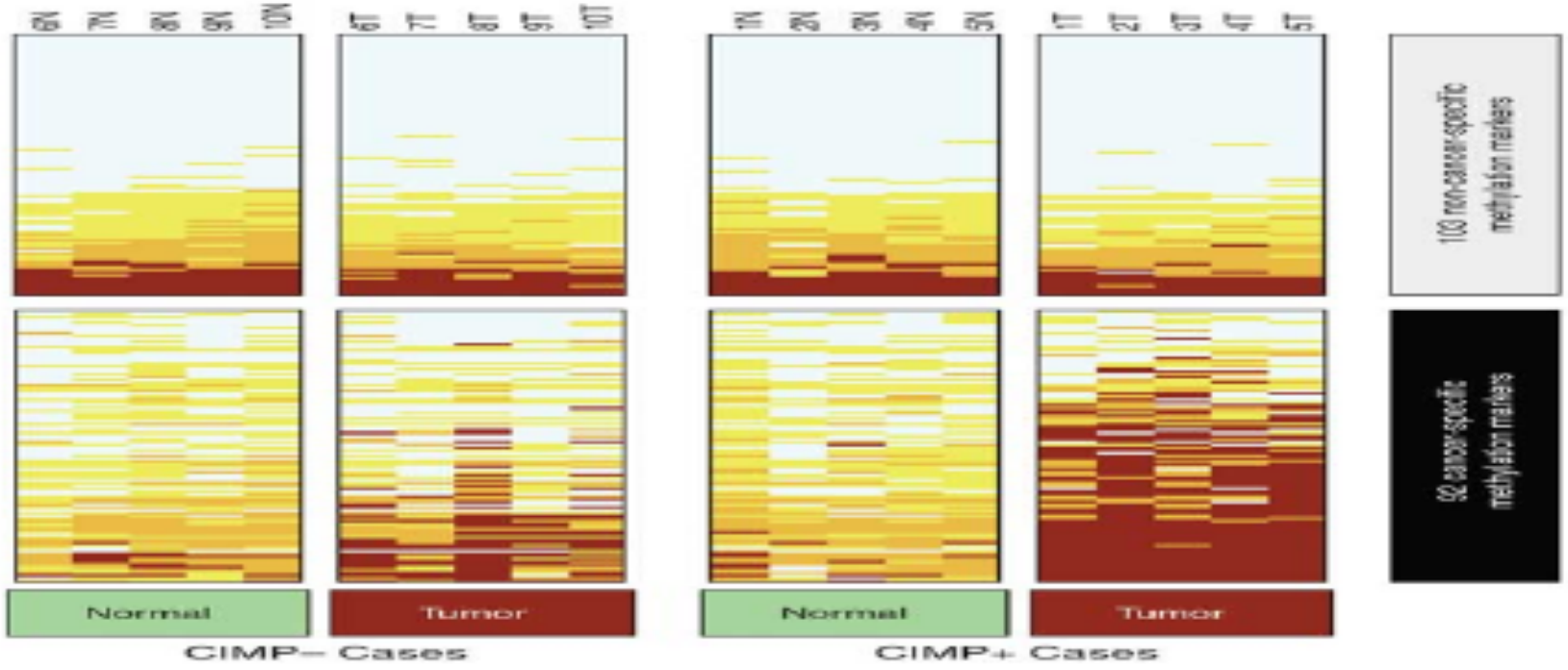
DOI 10.1016/j.ccr.2011.12.029

Intervention



Microsatellite instability

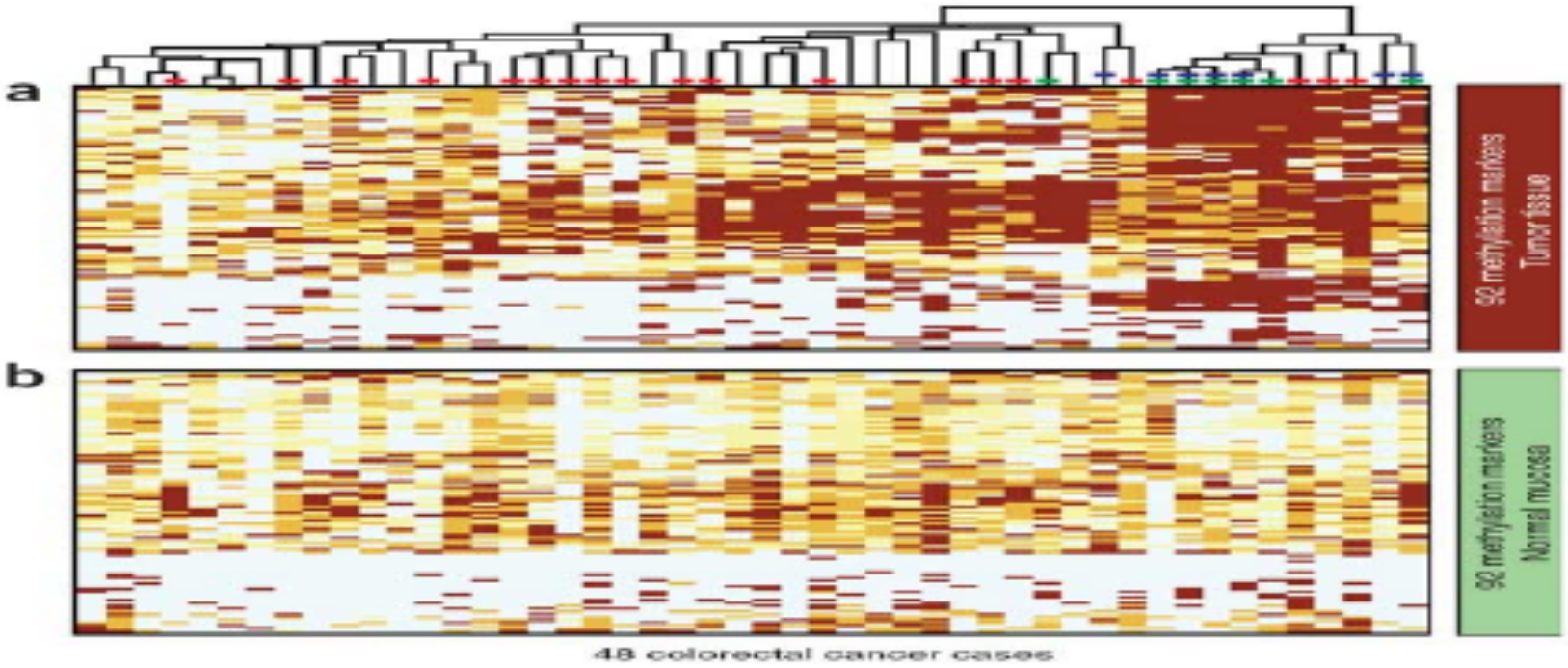
CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer



Nature Genetics **38**, 787 - 793

Tumor clusters

Identification of tumor clusters.

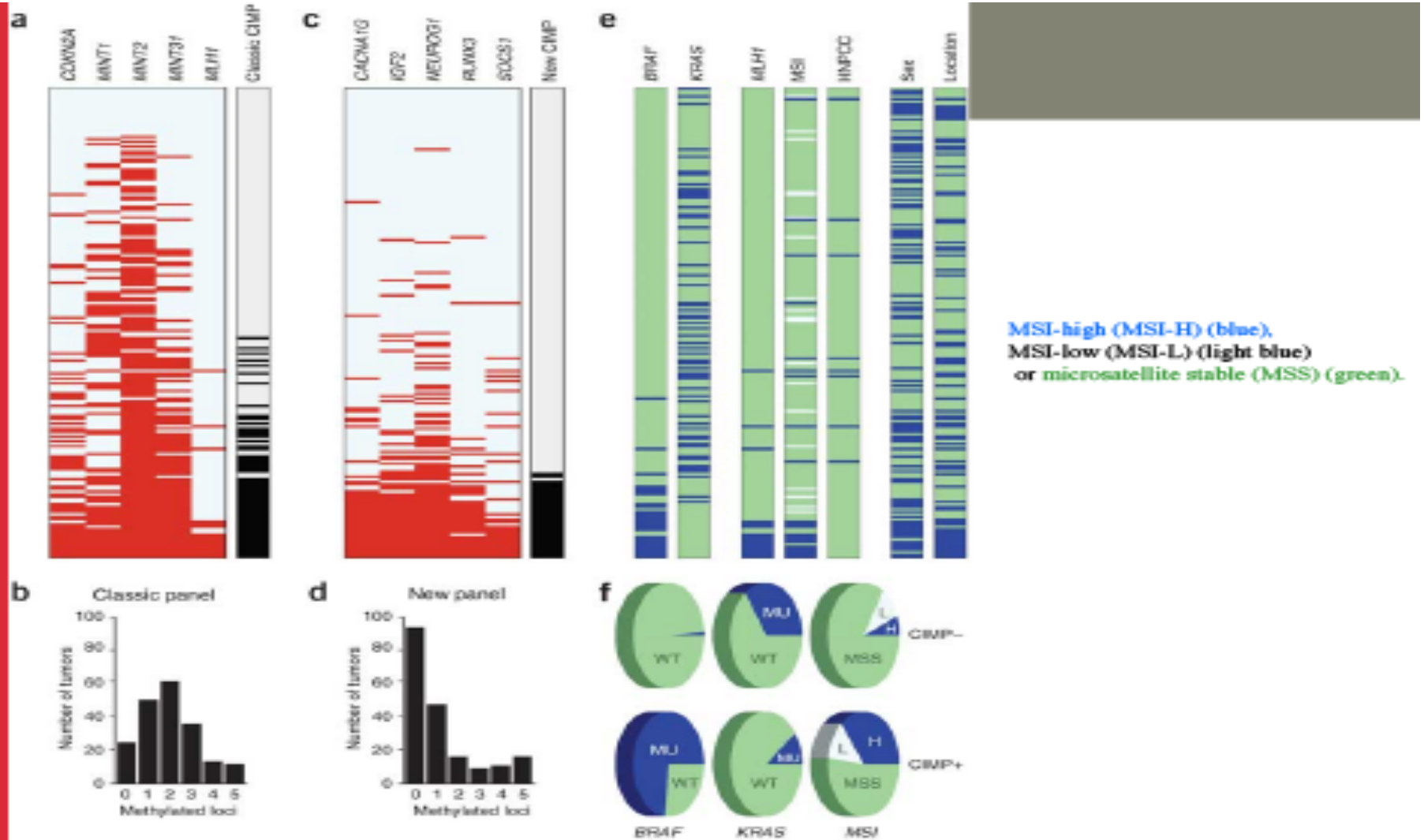


KRAS mutation indicated by a red rectangle overlaying the branch,
BRAF mutations indicated by a green rectangle
MSI-H cases designated with a blue rectangle.

48 Colorectal tumors

Nature Genetics **38**, 787 - 793

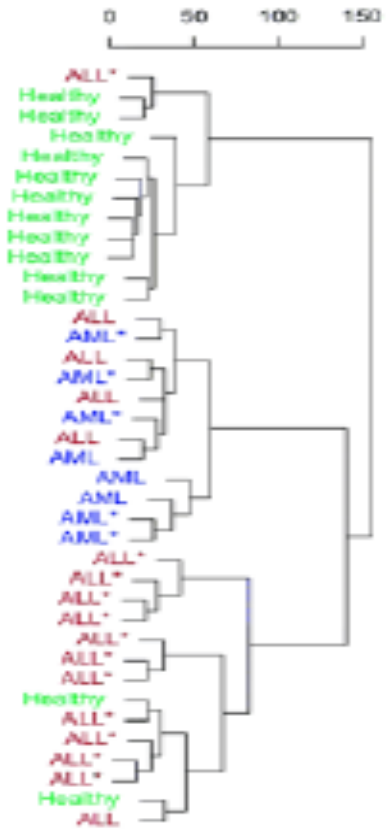
Genetic analysis



Methylation analysis

National Cancer Institute

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

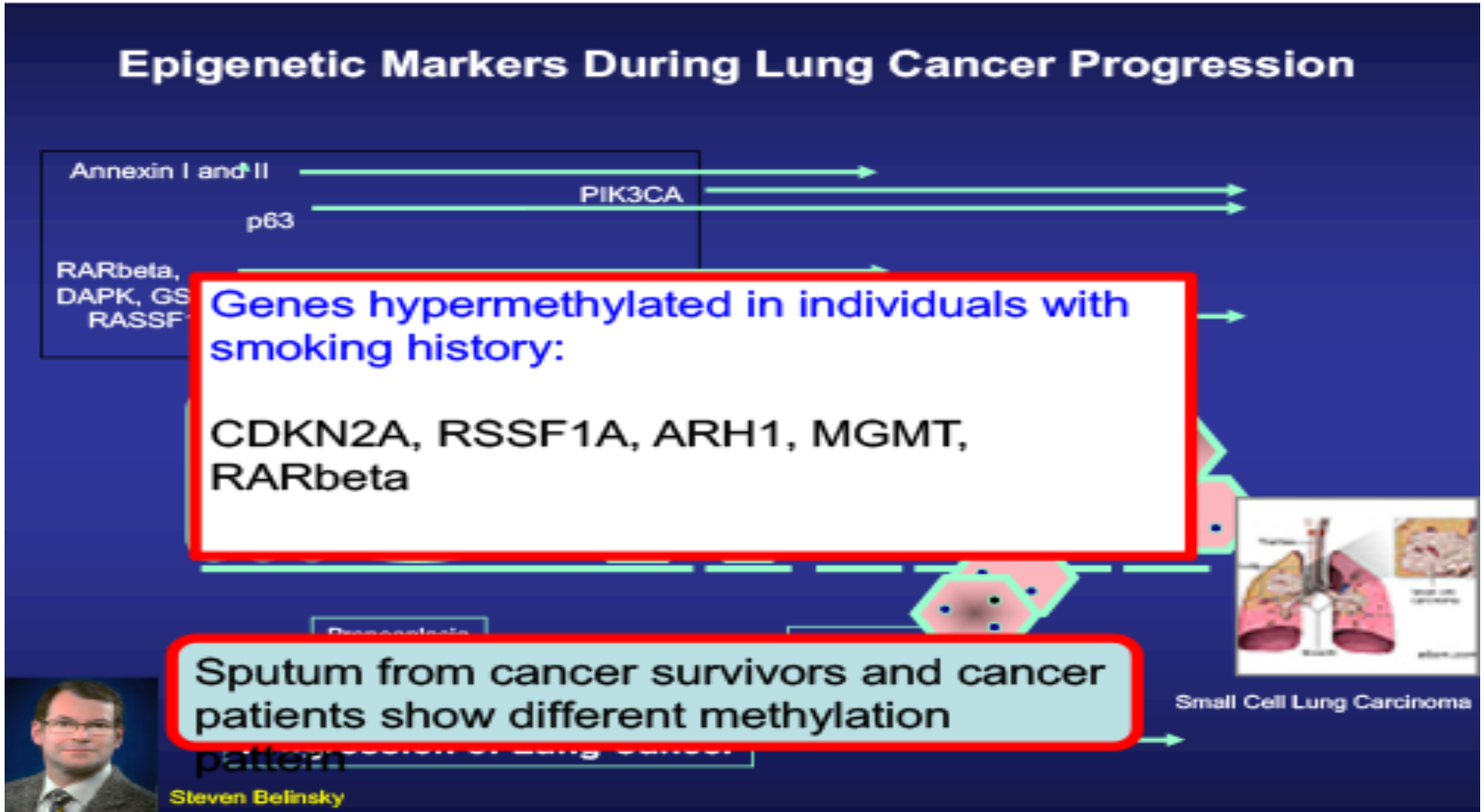


AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia

Lymphoma

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

Epigenetic markers



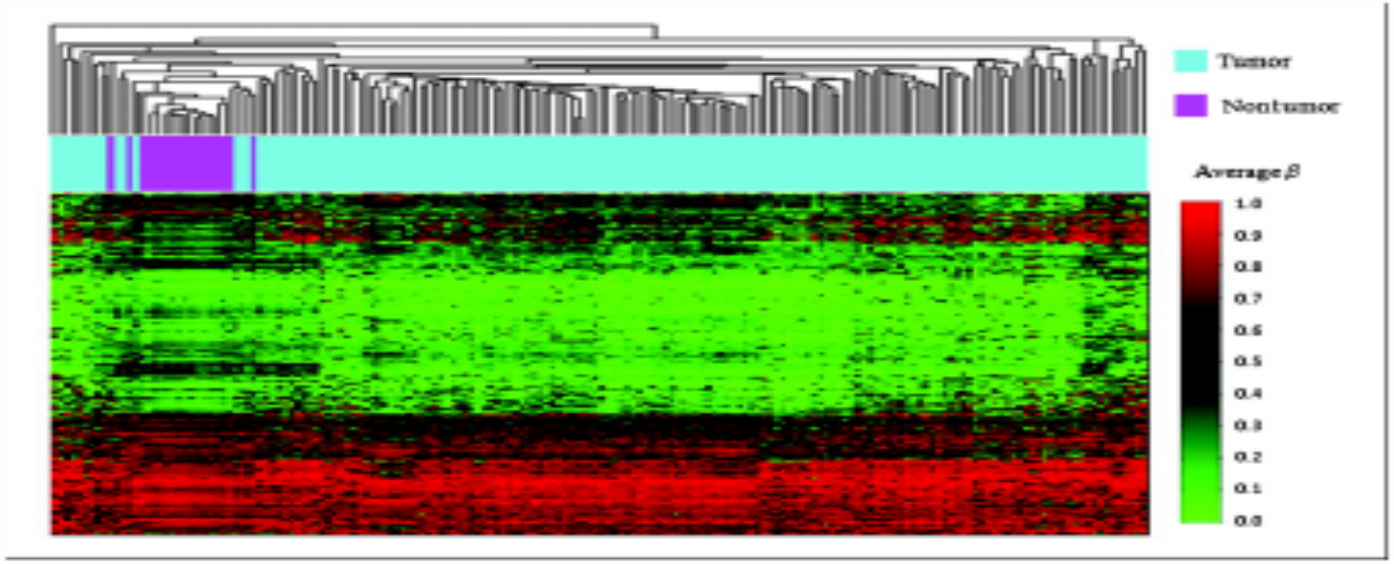
Mesothelioma

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

ASBESTOS

MESOTHELIOMA

Non-Mutagenic carcinogen



803 cancer related genes

158 pleural mesothelioma with minimum mutation

18 normal pleura

Prediction of survival

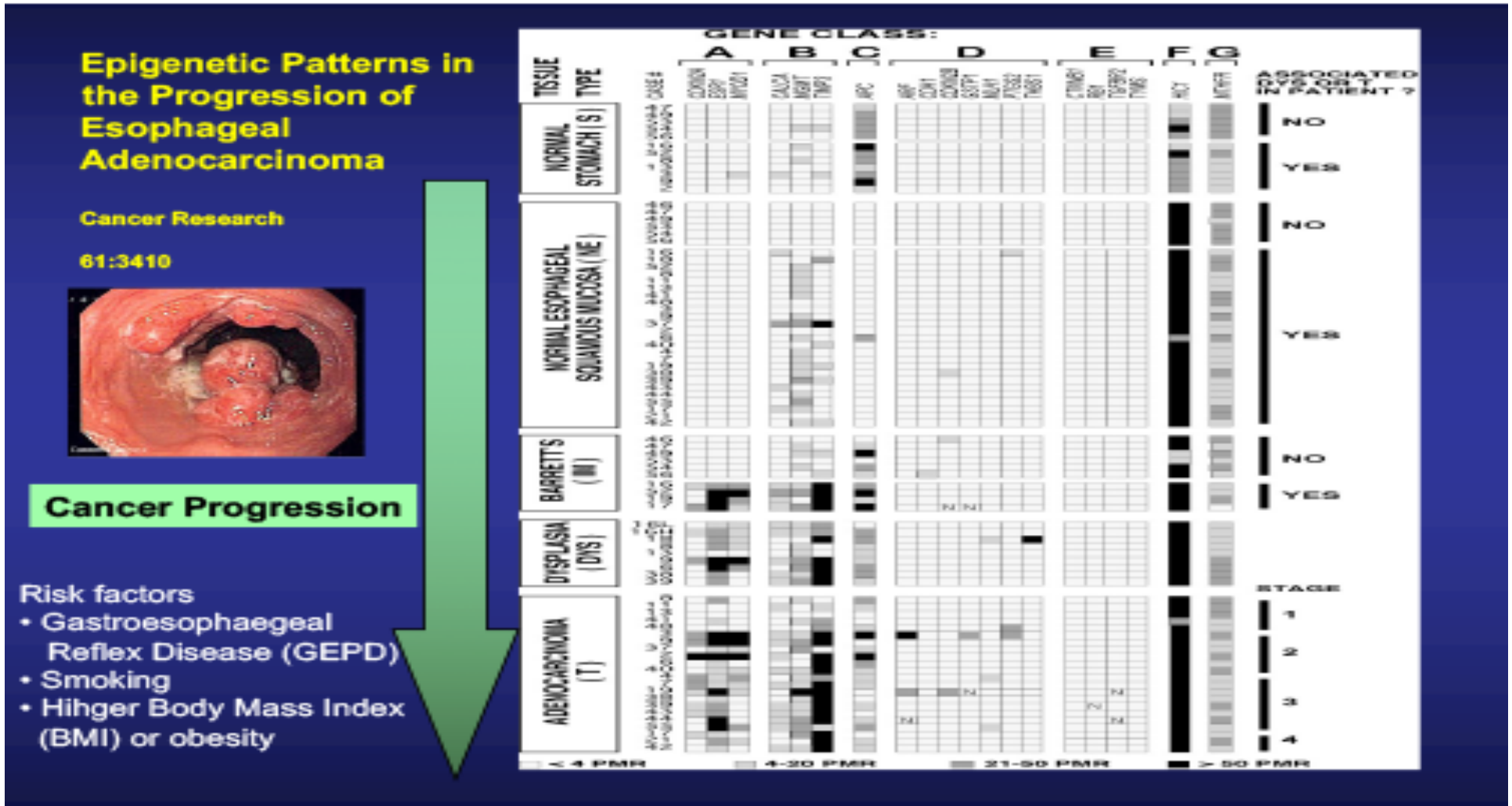
Christensen, B. C. et al. Cancer Res 2009;69:227-234

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

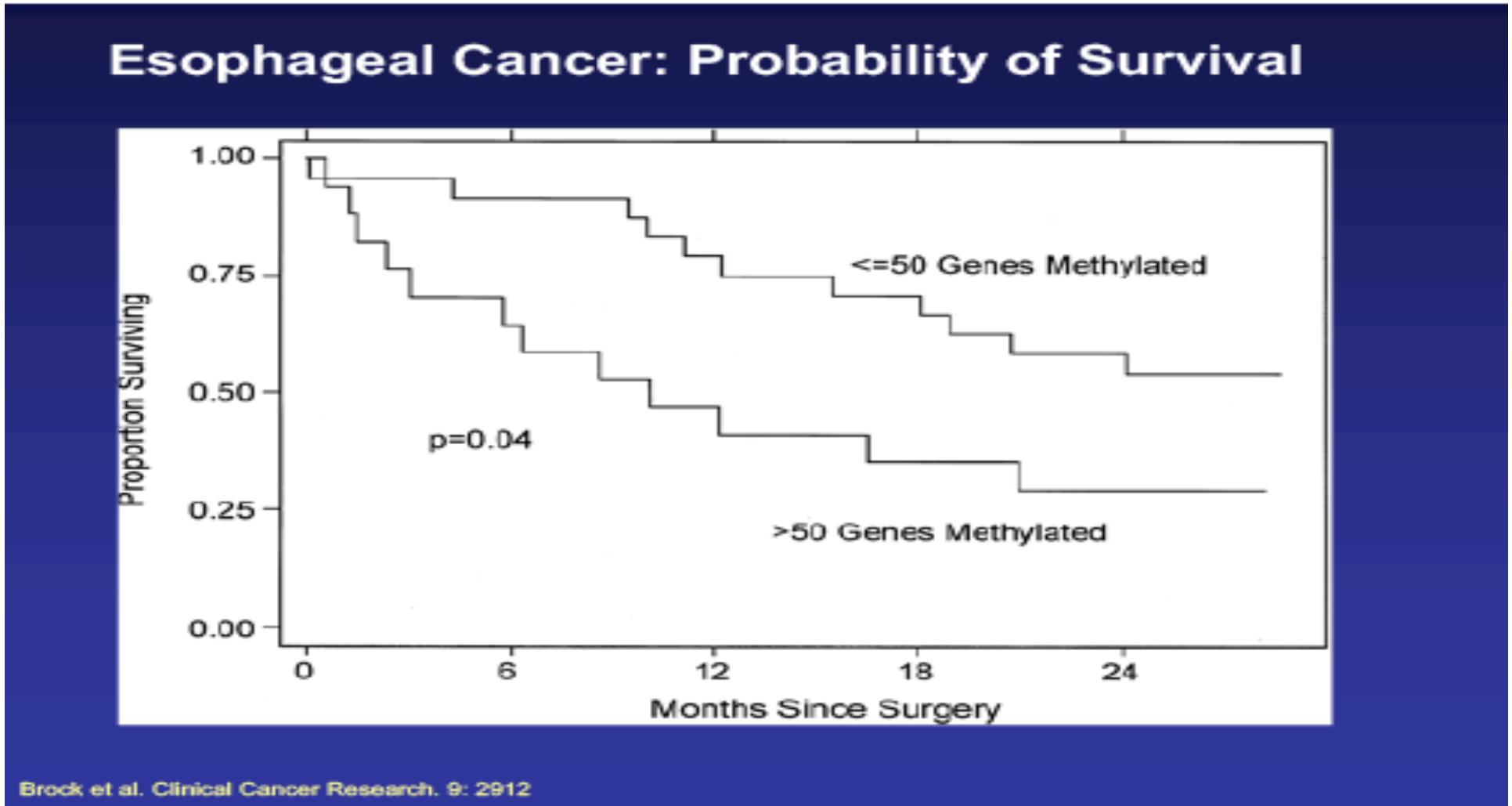
Cancer Research

Copyright ©2009 Amer

Epigenetic pattern



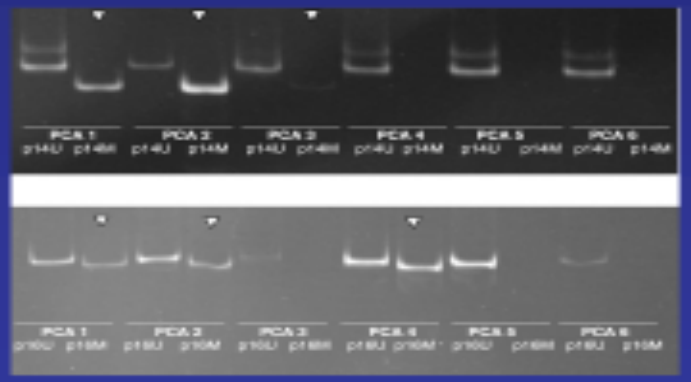
Esophageal cancer

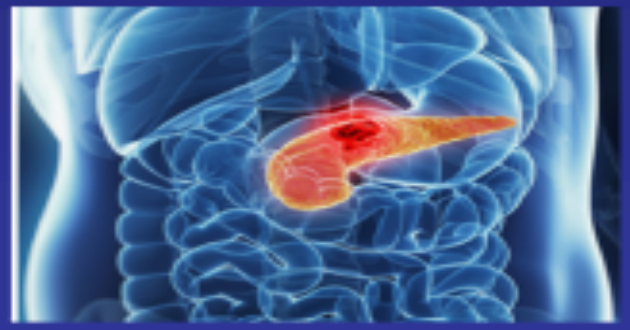


Pancreatic cancer

Pancreatic Cancer: Methylation of p14ARF and p16INK4a

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



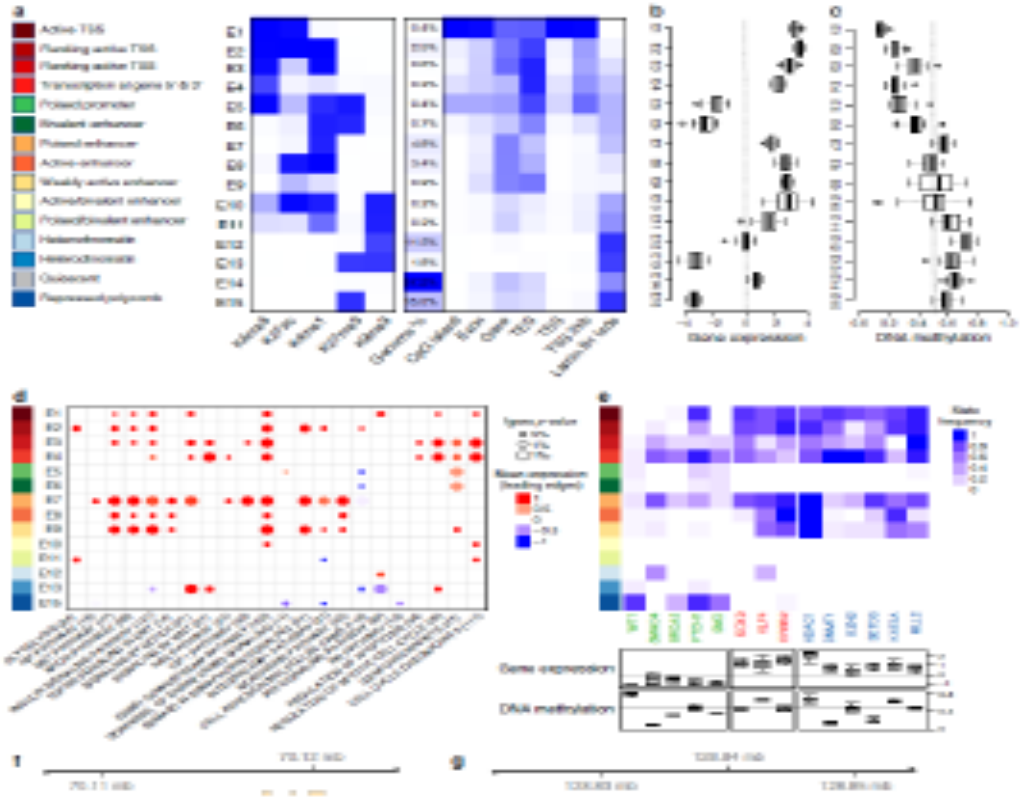


Sample: Pancreatic Fluid

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

Chromatin states

Distinct chromatin states of human PDAC



NATURE COMMUNICATIONS | (2018) 9:1978

Breast cancer

Breast Cancer Response to Tamoxifen Treatment by ESR1 Methylation

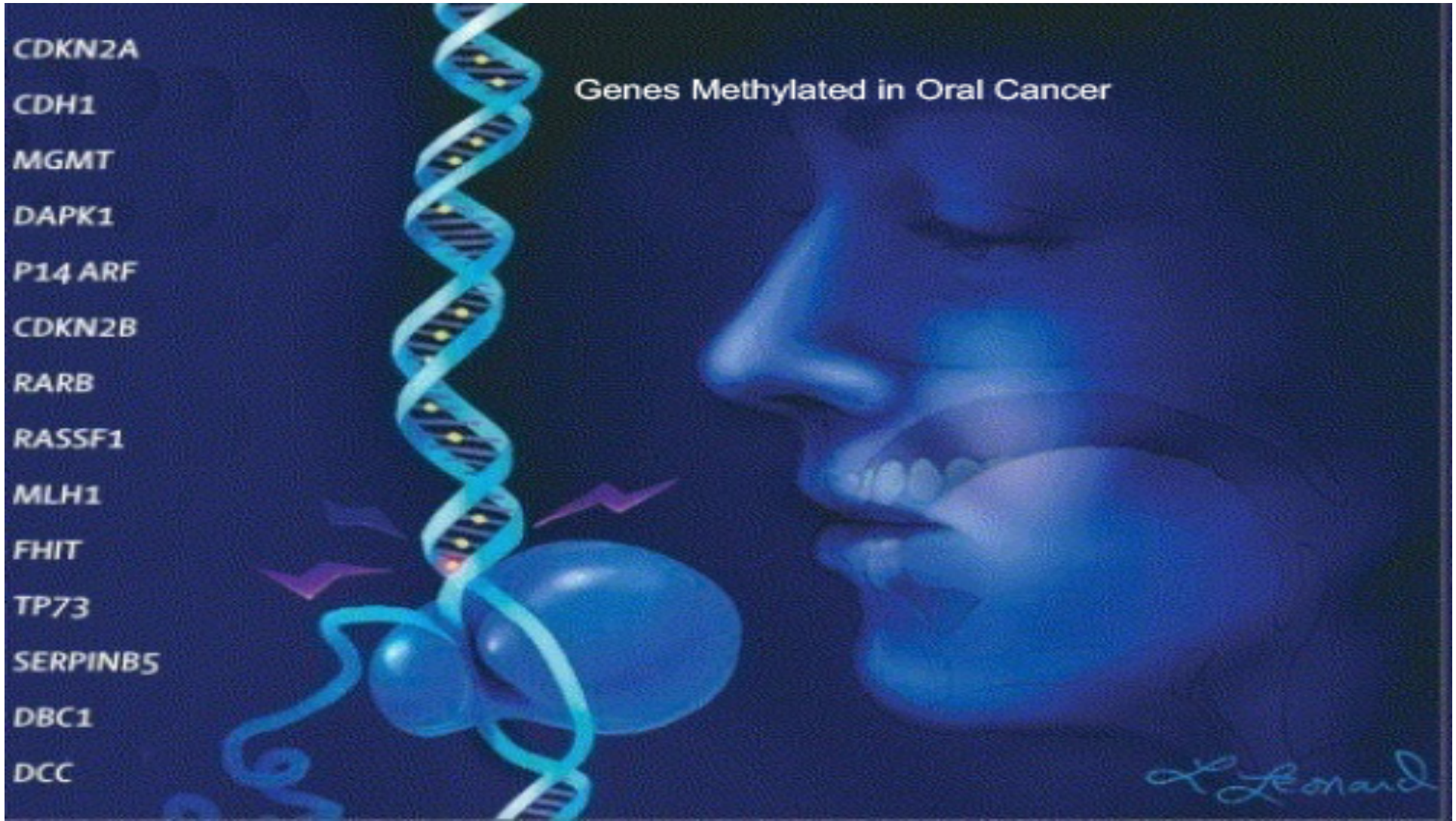
Preinvasive lesions, often designated as "in situ" or "intraepithelial neoplasia" falls in the domain of prevention.

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

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

Widschwendter et al. *Cancer Research* 4, 3807

Methylated genes



Immune system and epigenetics

Immune System and Epigenetics

Shin HJ et al.
Links STAT4 expression in human T cells is regulated by DNA methylation but not by promoter polymorphism.
J Immunol.175(11):7143-50.

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

Gasche JA, Hoffmann J, Boland CR, Goel A.
Interleukin-6 promotes tumorigenesis by altering DNA **methylation** in oral **cancer** cells.
Int J **Cancer**. 2011 Sep 1;129(5):1053-63.

Fujisawa T, Joshi BH, Puri RK.
Histone modification enhances the effectiveness of IL-13 receptor targeted immunotoxin in murine models of human pancreatic **cancer**.
J Transl Med. 2011 Apr 8;9:37.

Tahara T et al.
Association between IL-17A, -17F and MIF polymorphisms predispose to CpG island hyper-**methylation** in gastric **cancer**.
Int J Mol Med. 2010 Mar;25(3):471-7.

Biomarkers

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

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

ion in Prostate Cancer

rug detoxification enzyme which

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

G (Frankfurt, Prime Standard: ECX),

agnostics company, today announced

a non-exclusive licensing agreement

arker

Quest Diagnostics Incorpora

leading provider of diagnost

services.

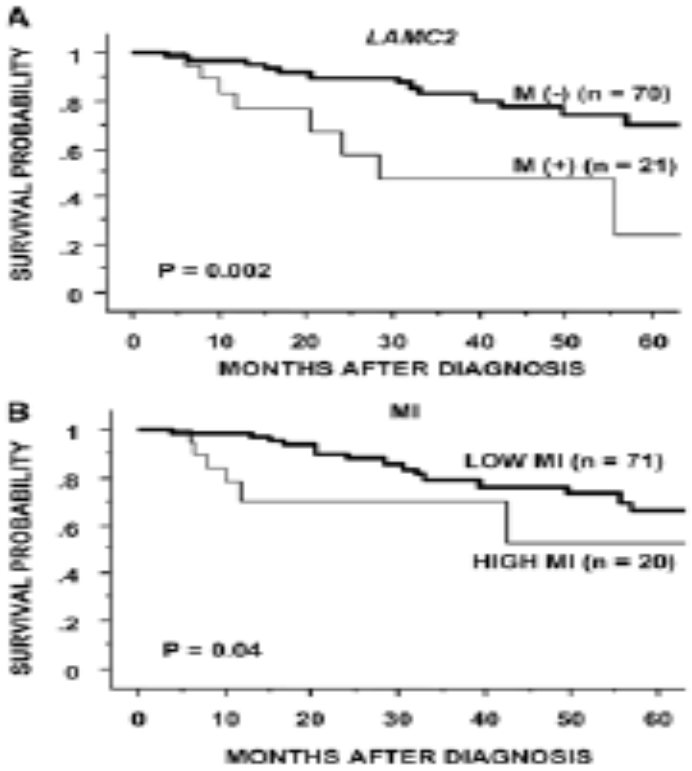
Methyl-Profiler™ DNA Methylation PCR ARRAYS

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

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

Bladder cancer methylation

Bladder Cancer
Methylation of LAMC2 in Exfoliated Cells Isolated from Urine

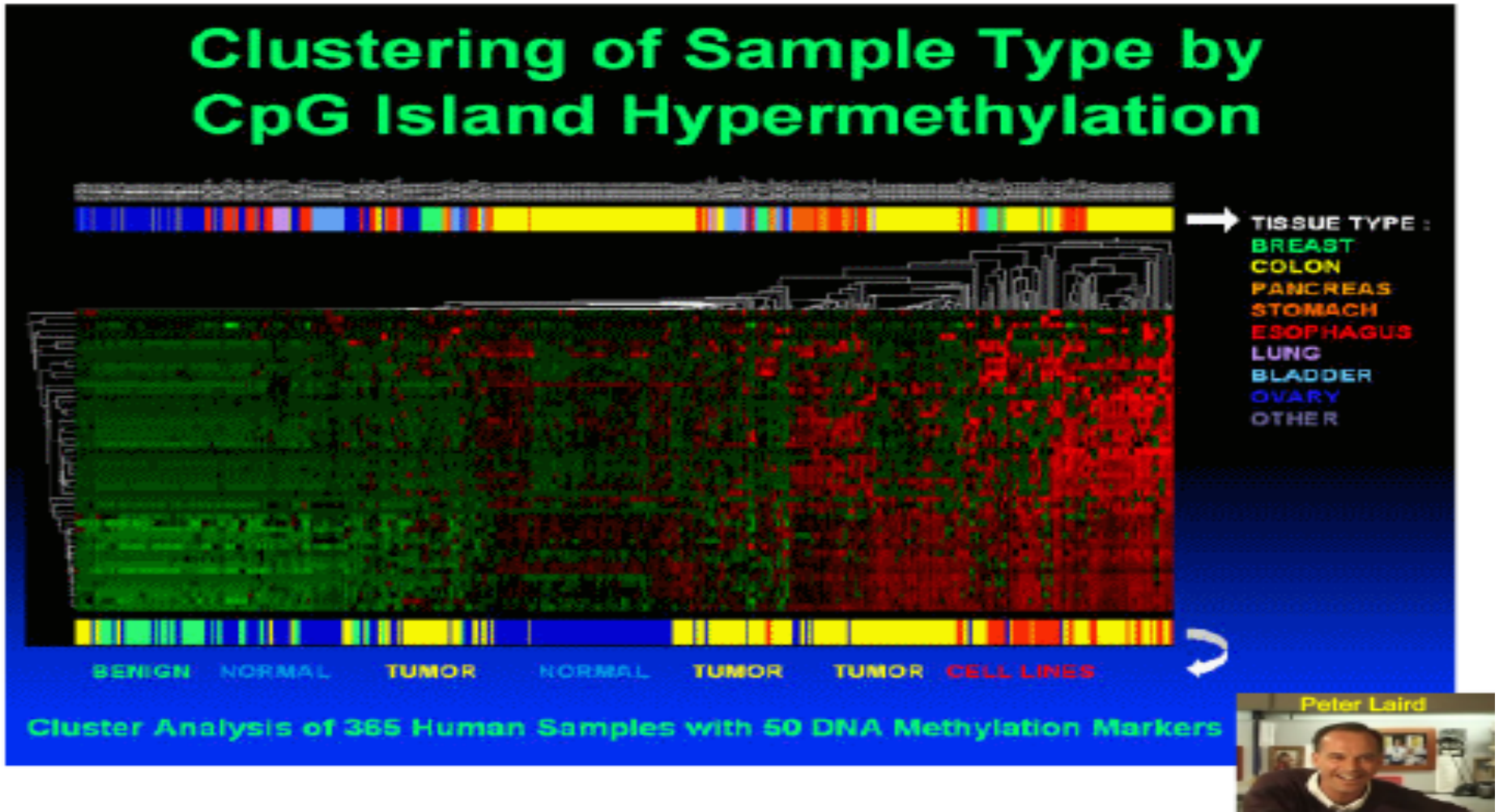


Another Study:
Schistosomes and Bladder Cancer

MI, Methylation Index

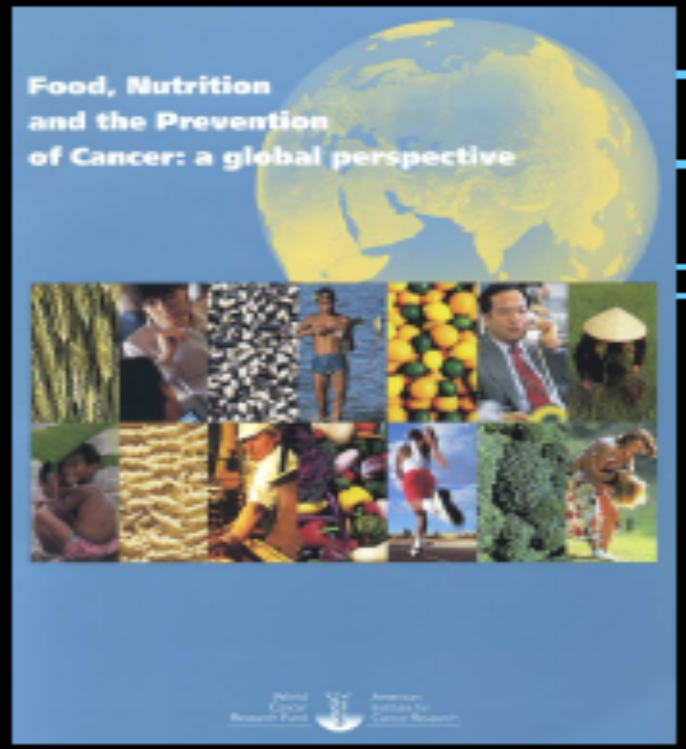
(Sathyanarayana et al. *Can Res* 64: 1425)

CpG island hypermethylation



Diet and cancer

DIET AND CANCER: FOCUS ON PREVENTION



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.

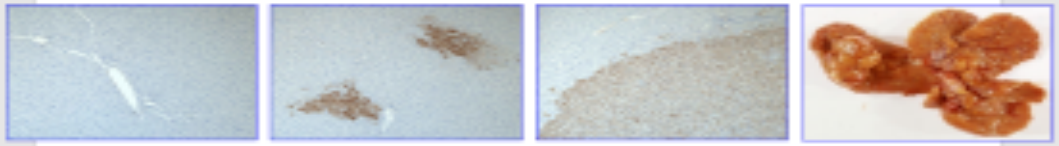
- Understanding the determinants of the earliest detectable phenotypes in initiated cells
- Uncovering the molecular mechanisms of action of dietary nutrients leading to cancer formation and prevention
- Defining effects of dietary compounds not only on cancer cells but on normal and precancerous cells
- 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 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)

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

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.

Come see the new improvements to the interface!

Format: Abstract

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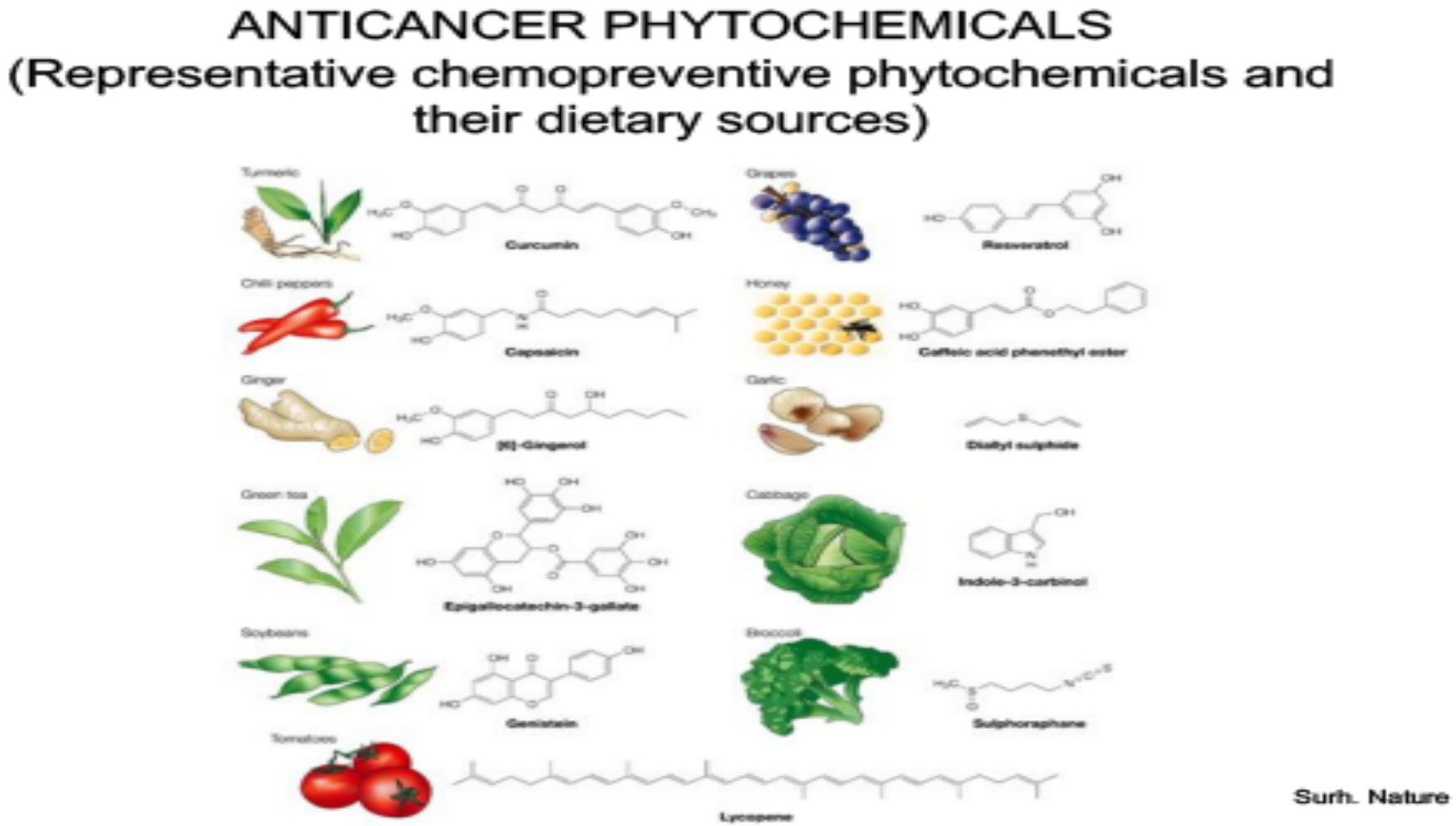
Association of TNFRSF12A Methylation With Prognosis in Hepatocellular Carcinoma With History of Alcohol Consumption.

Author information

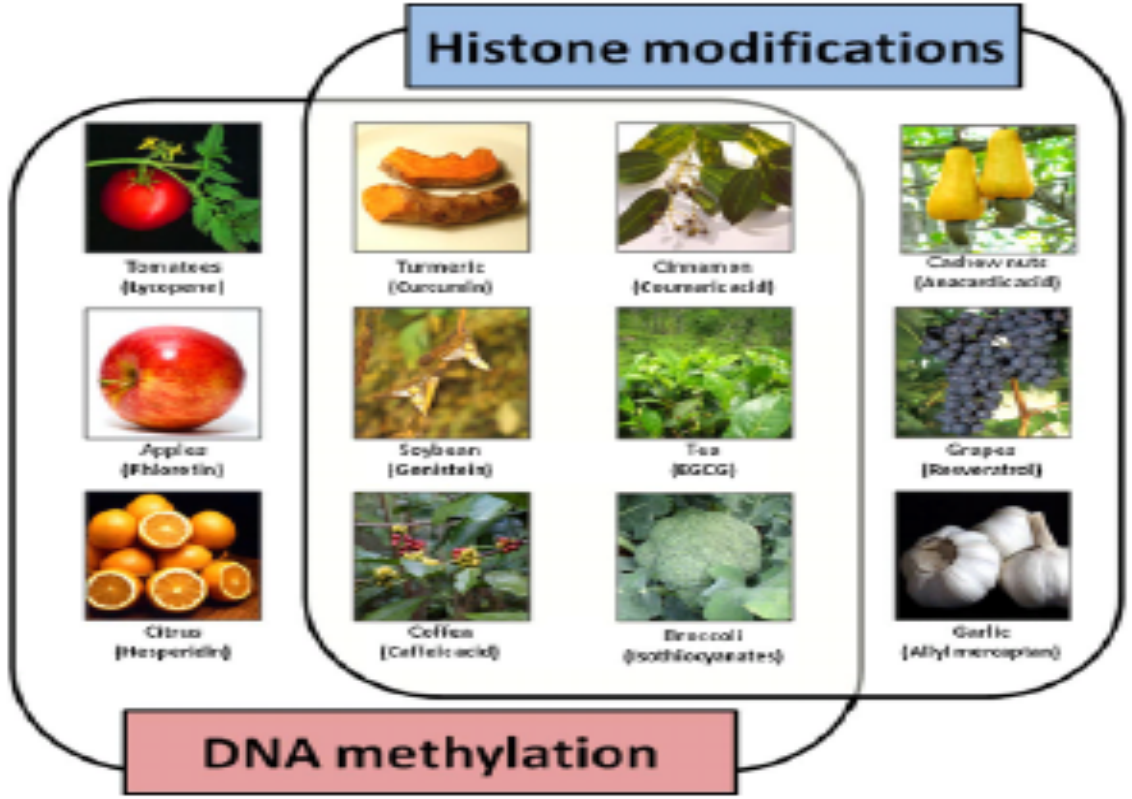
Abstract

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-cg05519447 and cg06806280-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 cg05519447 and cg06806280 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 cg06806280 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



Epigenetic foods



Research opportunities

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?

Christopher Plass (Heidelberg)

Nancy Kiviat (Seattle)

Christine Ambrosone (Roswell Park, Buffalow)

Research challenges

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?

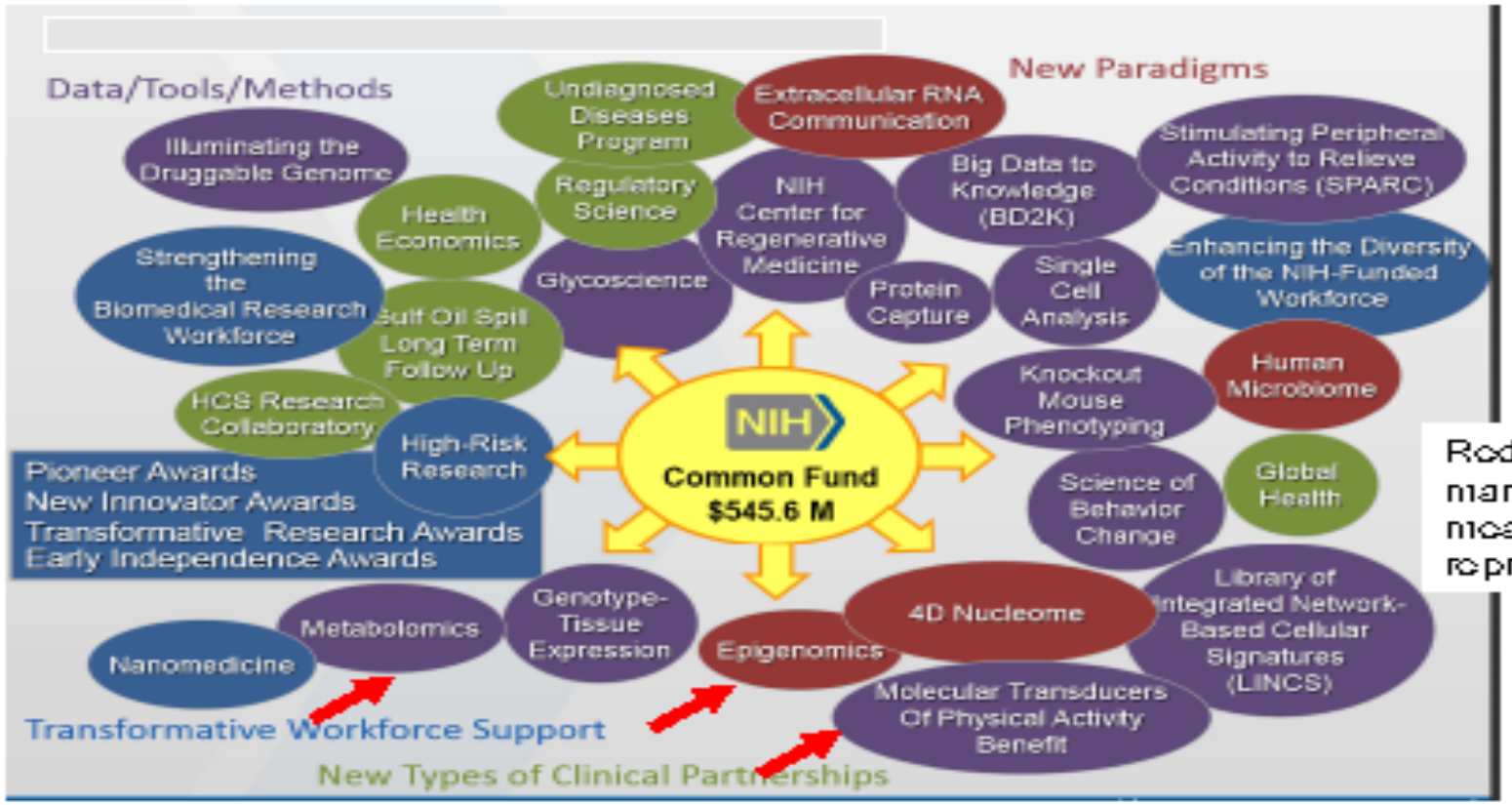
How much microbiome-specific metabolites can affect epigenetic regulation? How effective are probiotics in cancer prevention?

How to address challenges

National Cancer Institute

How are we addressing these challenges?

NIH common fund



Red arrow marks means that I represent NCI

Personalized medicine

Mini review

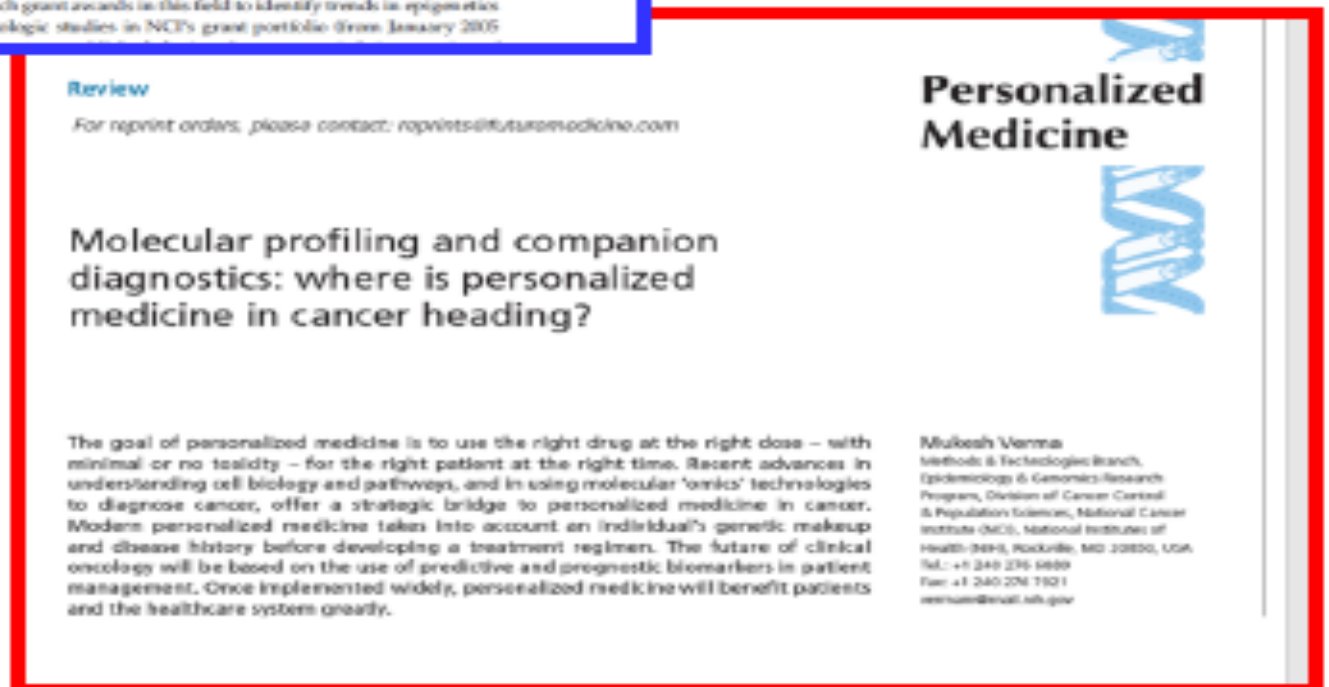
Cancer Epidemiology, Biomarkers & Prevention

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 Pitch[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

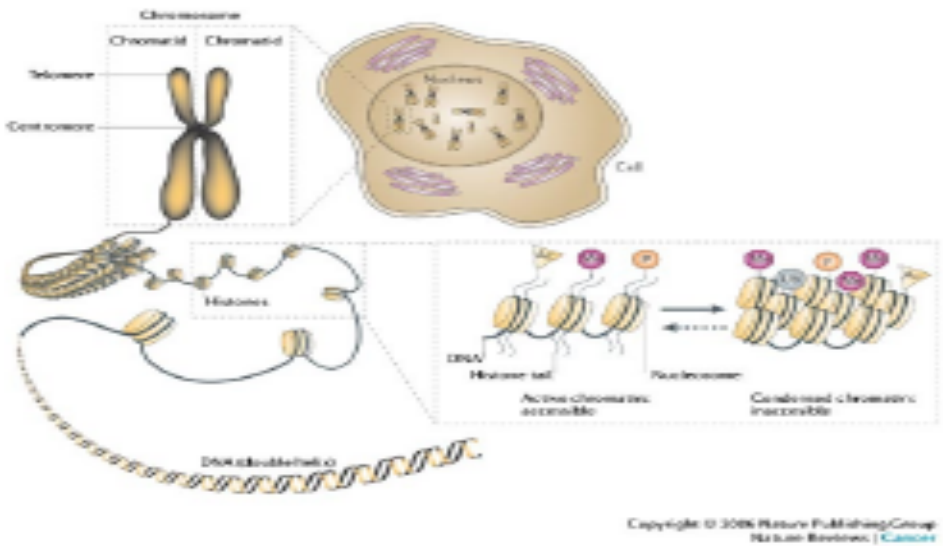
Molecular profiling and companion diagnostics: where is personalized medicine in cancer heading?

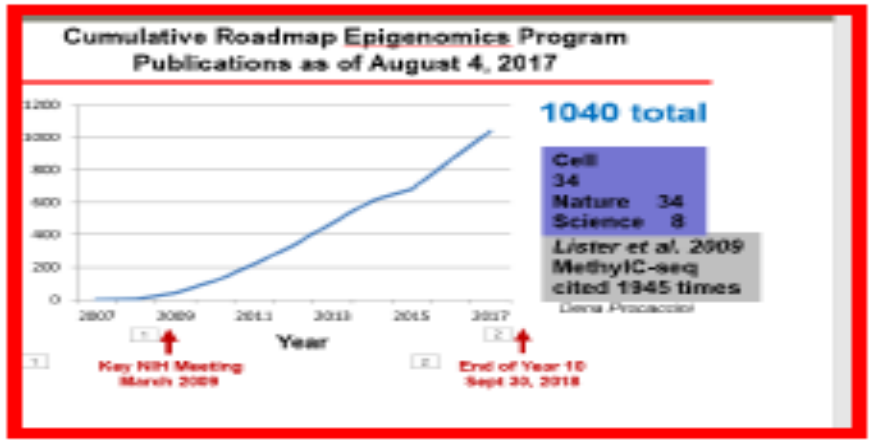
The goal of personalized medicine is to use the right drug at the right dose – with minimal or no toxicity – for the right patient at the right time. Recent advances in understanding cell biology and pathways, and in using molecular 'omics' technologies to diagnose cancer, offer a strategic bridge to 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 6609
Fax: +1 240 276 7021

Epigenetics roadmap

Epigenetics Roadmap



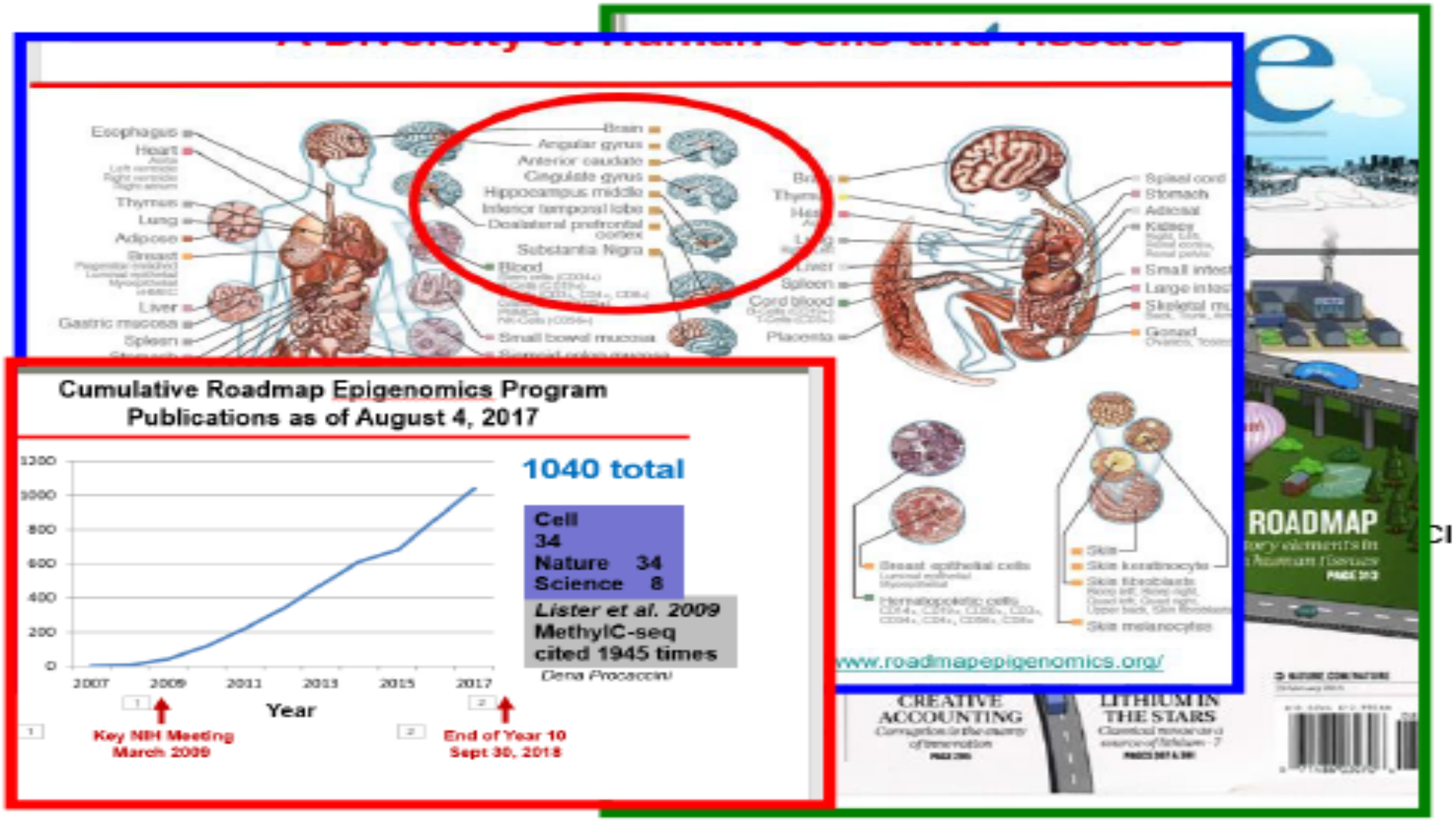


Epigenetically Regulated Diseases: Several cancers, autoimmune disorders, reproductive disorders, and neurobehavioral and cognitive dysfunctions

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/

Roadmap


Cumulative Roadmap Epigenomics Program
Publications as of August 4, 2017
1040 total
Cell 34
Nature 34
Science 8
Lister et al. 2009
MethylC-seq
cited 1945 times
Dena Procaccini
Year
Key NIH Meeting
March 2009
End of Year 10
Sept 30, 2018
www.roadmapepigenomics.org/
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The International Human Epigenome Consortium (IHEC) unites scientists from all over the world working together to achieve one common goal:
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Research

Molecular profiling

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Published OnlineFirst December 10, 2013; DOI: 10.1158/1055-9965.EPI-13-0573

Cancer Epidemiology, Biomarkers & Prevention

Review

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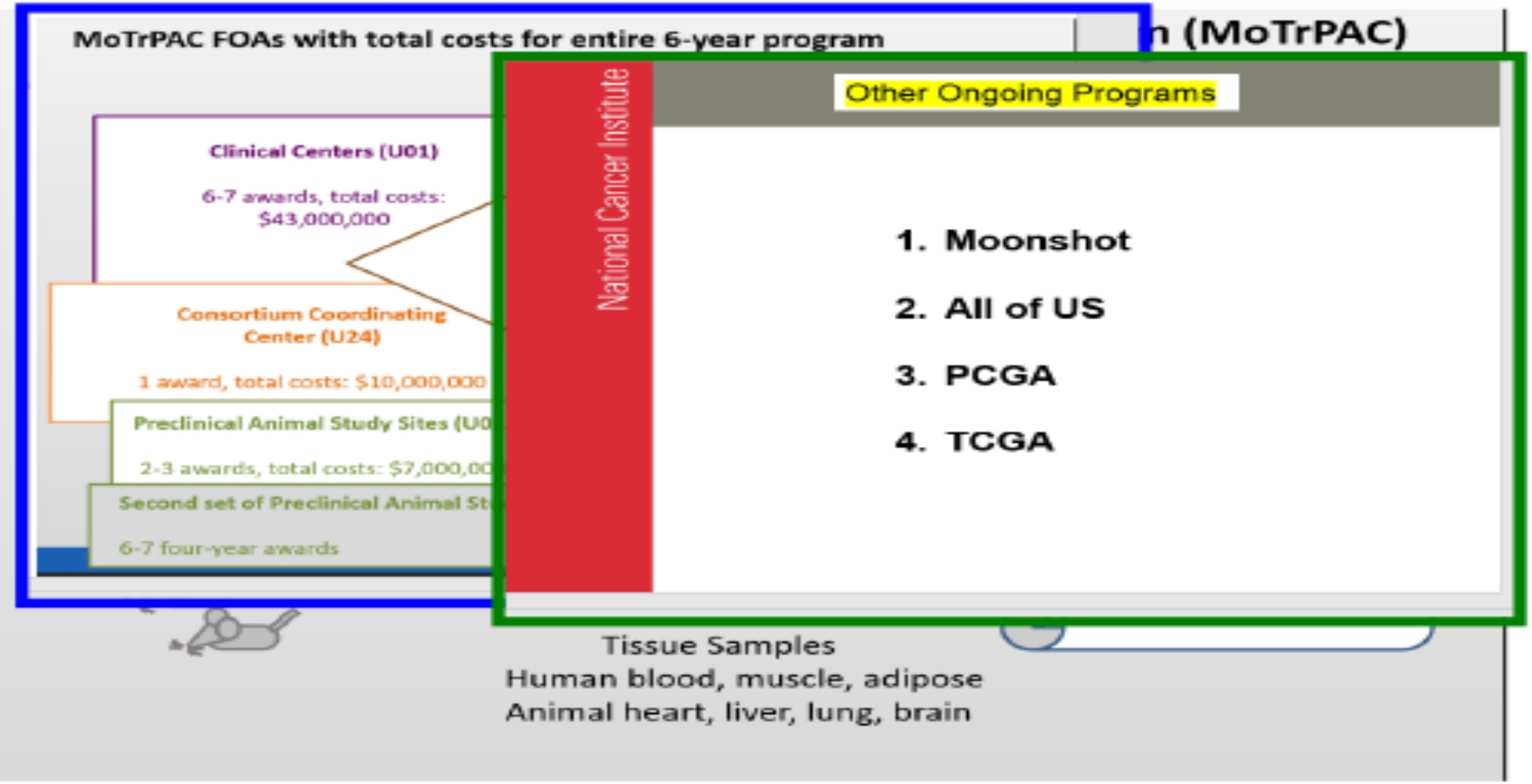
Mukesh Verma

vide insights into
gly, investigators
current progress
literature and the
ds in epigenetics

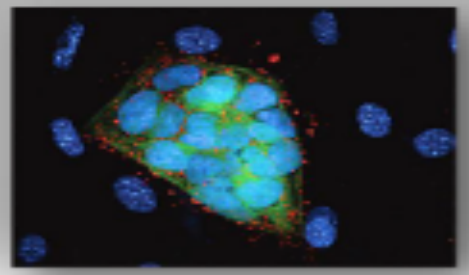
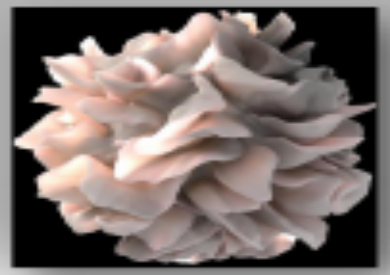
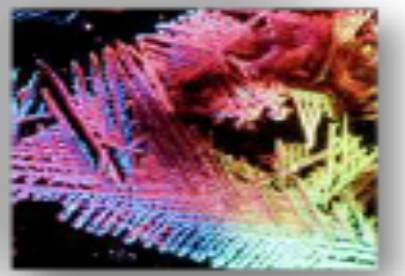
research. We present a summary of the epidemiologic studies in NCI's grant portfolio from January 2005 through December 2012 and in the scientific literature published during the same period, irrespective of support from the NCI. Blood cells and tumor tissue were the most commonly used biospecimens in these studies, although buccal cells, cervical cells, sputum, and stool samples were also used. DNA methylation profiling was the focus of the majority of studies, but several studies also measured microRNA profiles. We

Ongoing programs



NIH

NIH...

Mukesh Verma, PhD
vermam@mail.nih.gov

Turning Discovery Into Health