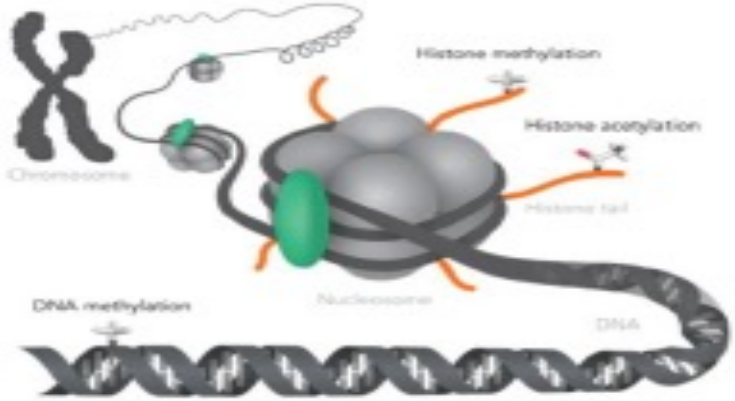
Epigenetics and cancer

National Cancer Institute

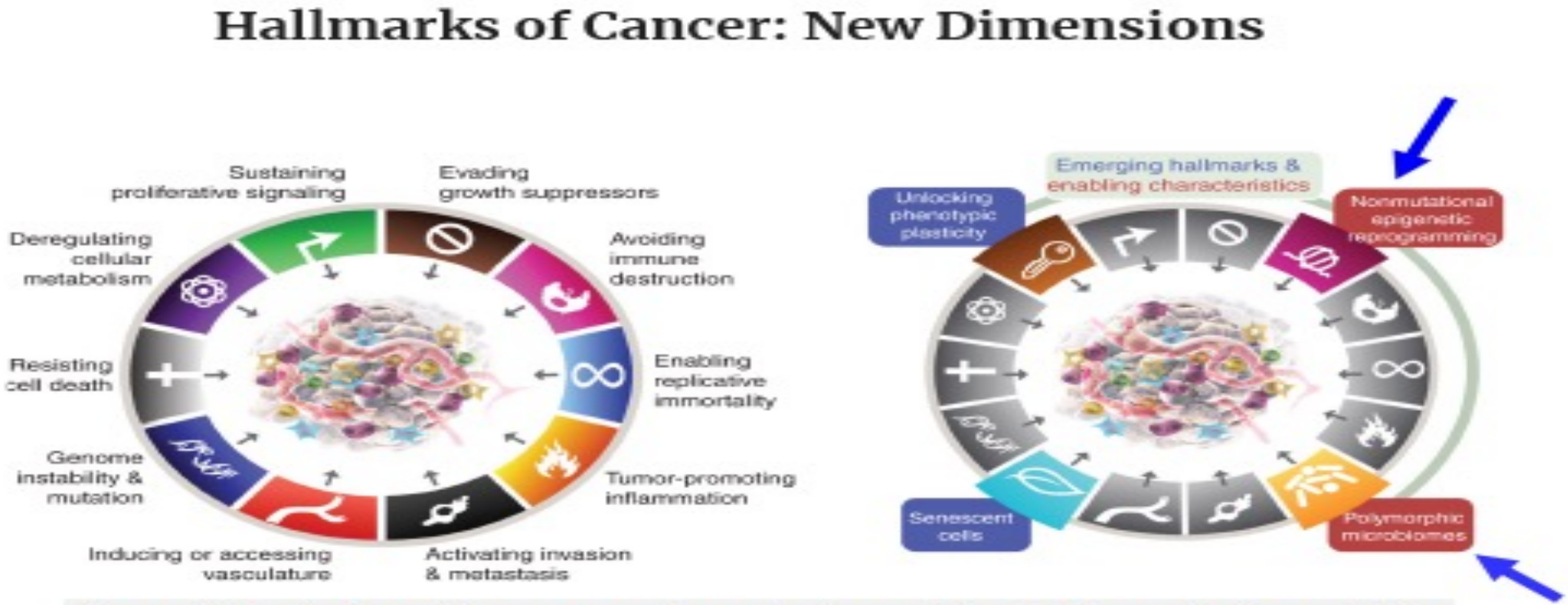
Epigenetics and Cancer



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

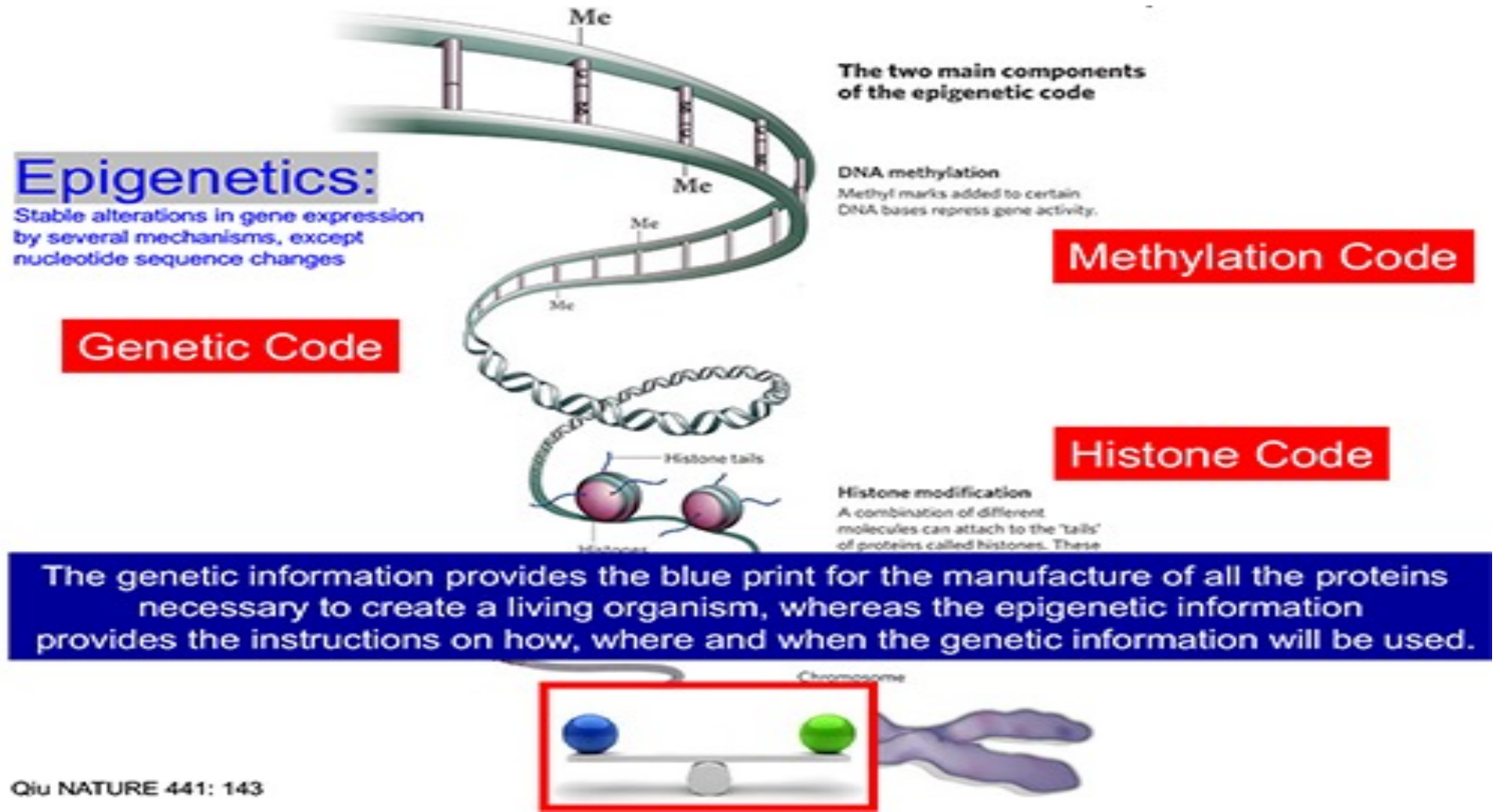
Fig. Credit: https://horizondiscovery.com/en/applications/cell-line/epigenetics

Hallmarks of cancer



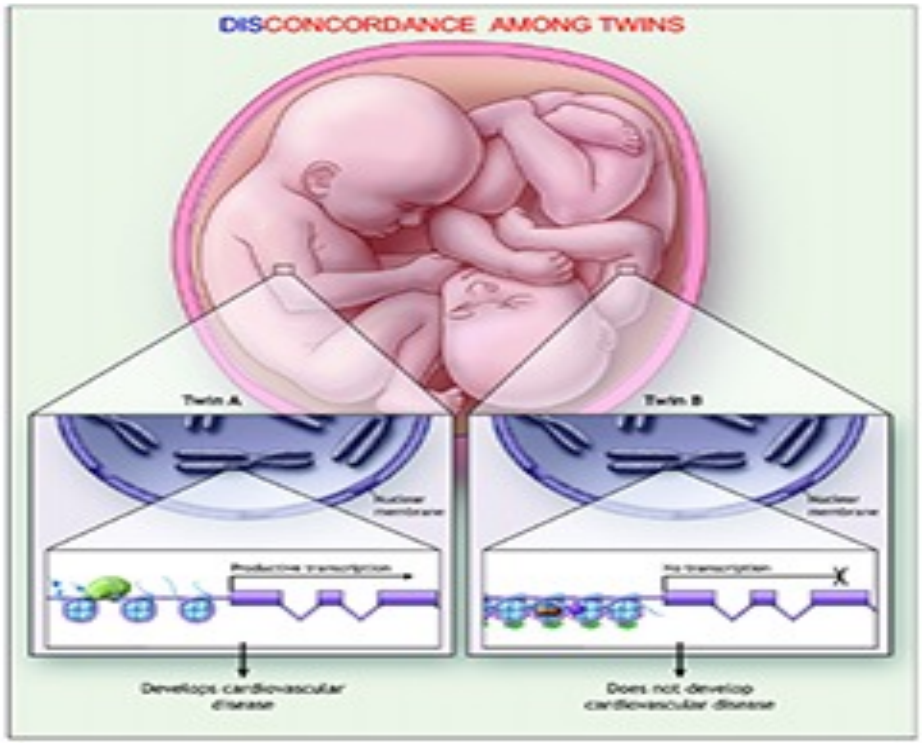
Epigenetics

Epigenetics



DNA and destiny

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



Epigenetic predisposition to angiogenesneis? Individual? Populations?

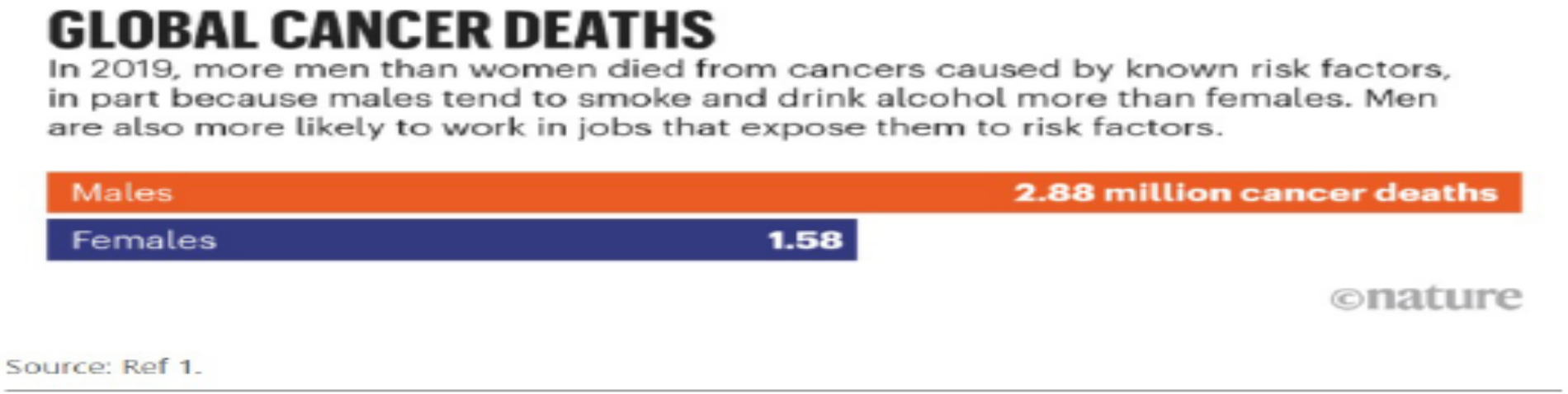
Pharmacogenomics and pharmacoepigenomics (personalized medicine)

Microenvironment, microbiome, and gene expression

GWAS and EWAS

Adapted from Matouk and Marsden Cir Res 102:873

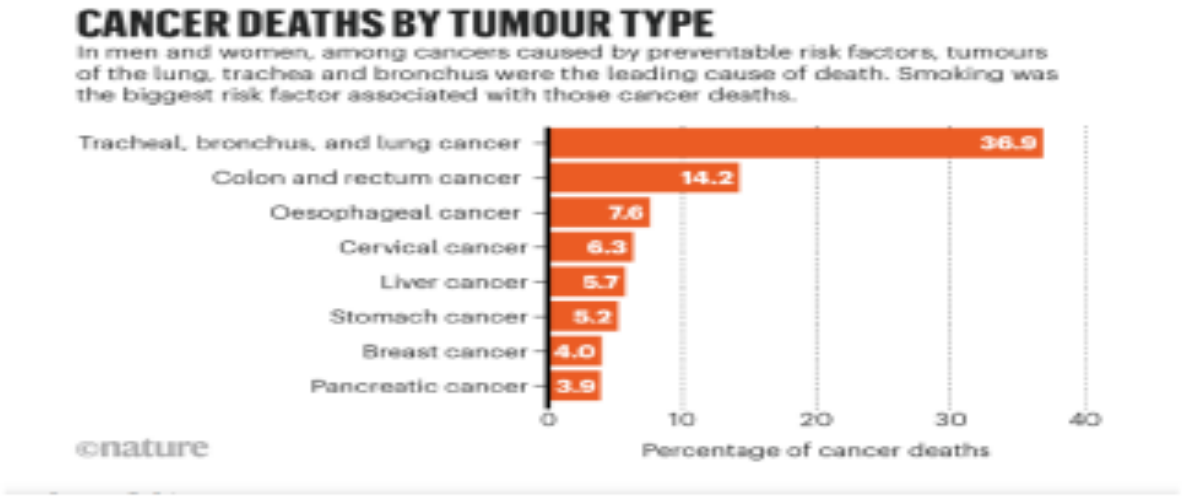
Global cancer deaths



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

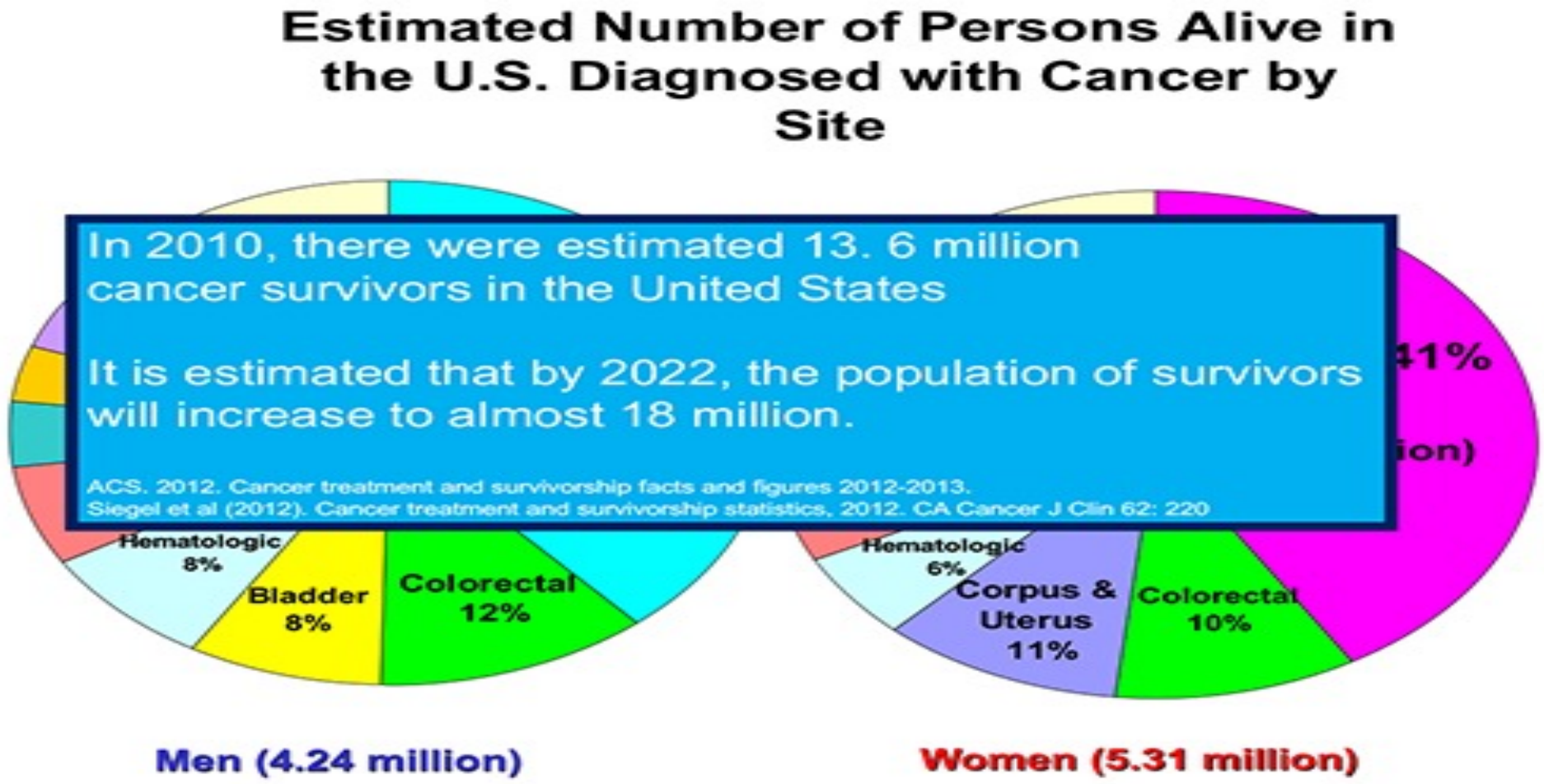
GBD 2019 Cancer Risk Factors Collaborators *Lancet* **400**, 563–591 (2022).

Cancer tumor deaths



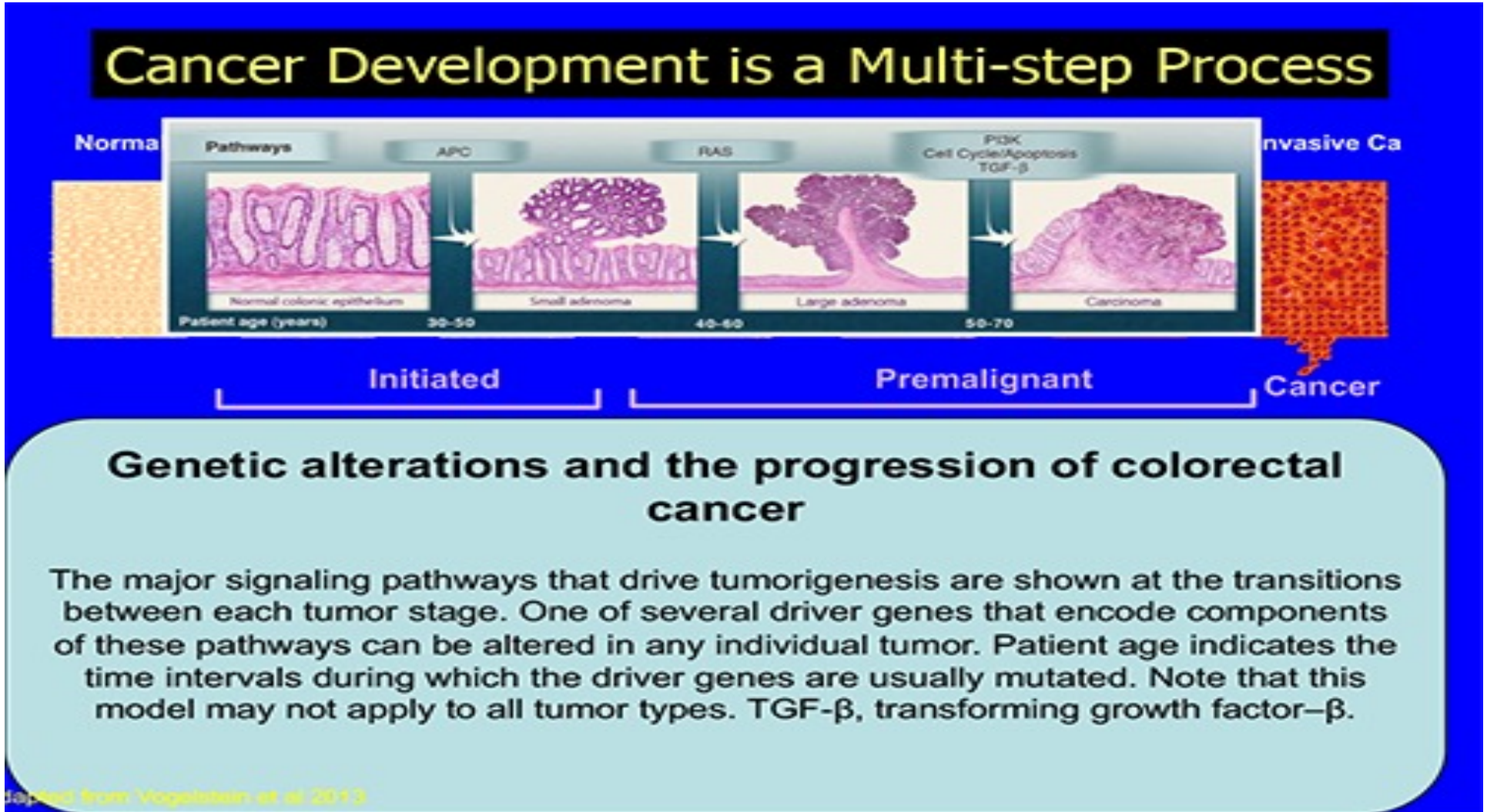
doi: https://doi.org/10.1038/d41586-022-02355-

Cancer Survivors

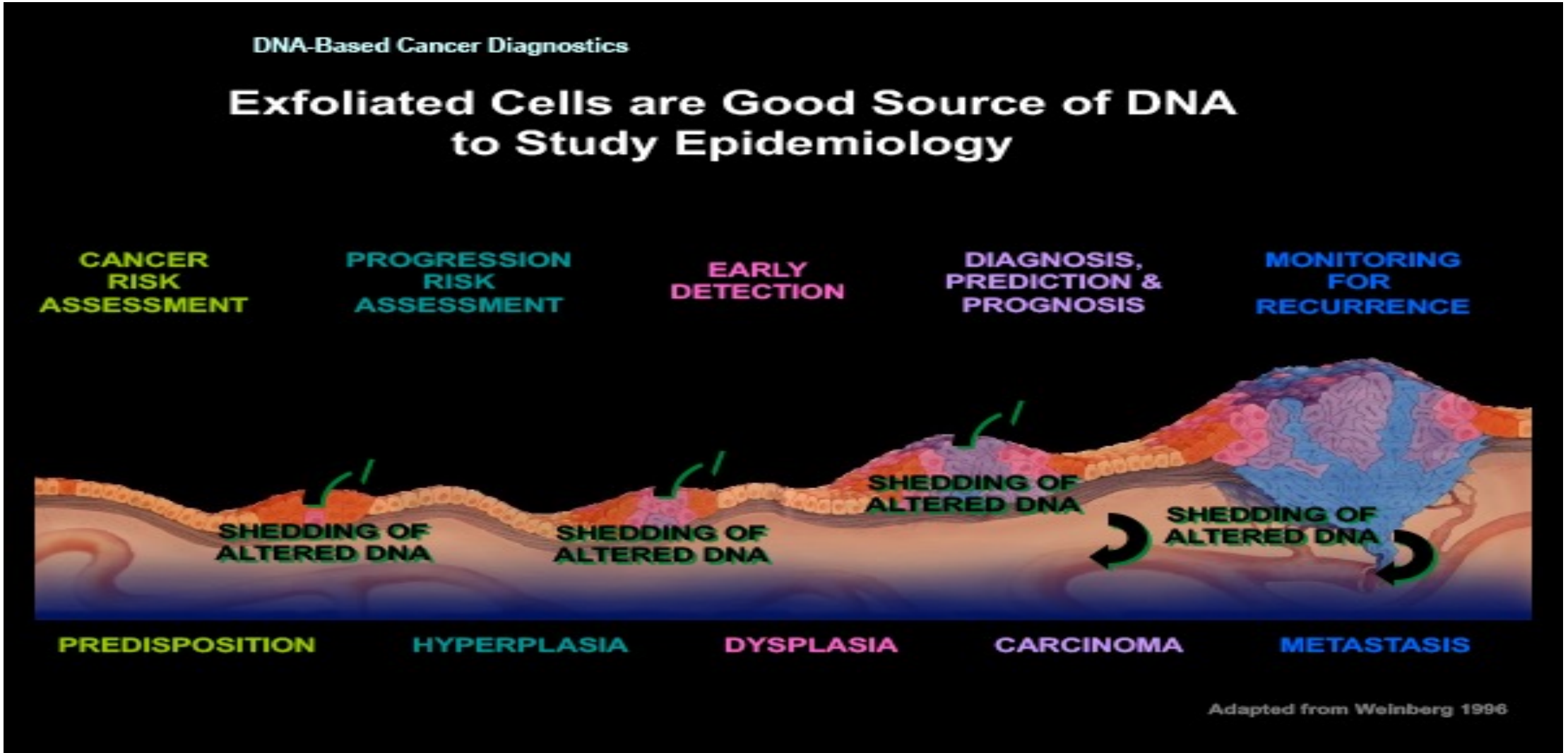


Cancer continuum

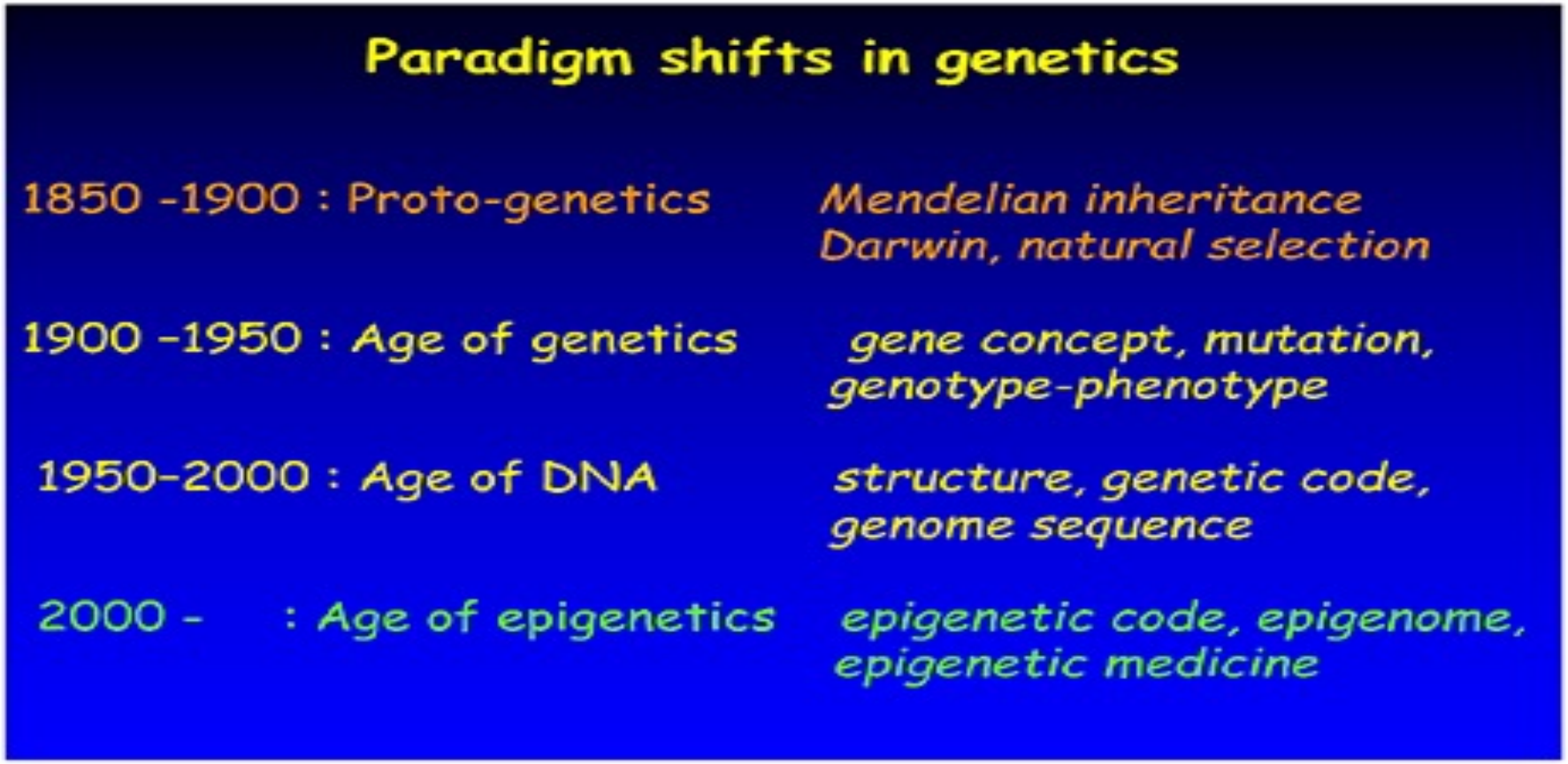
Cancer development



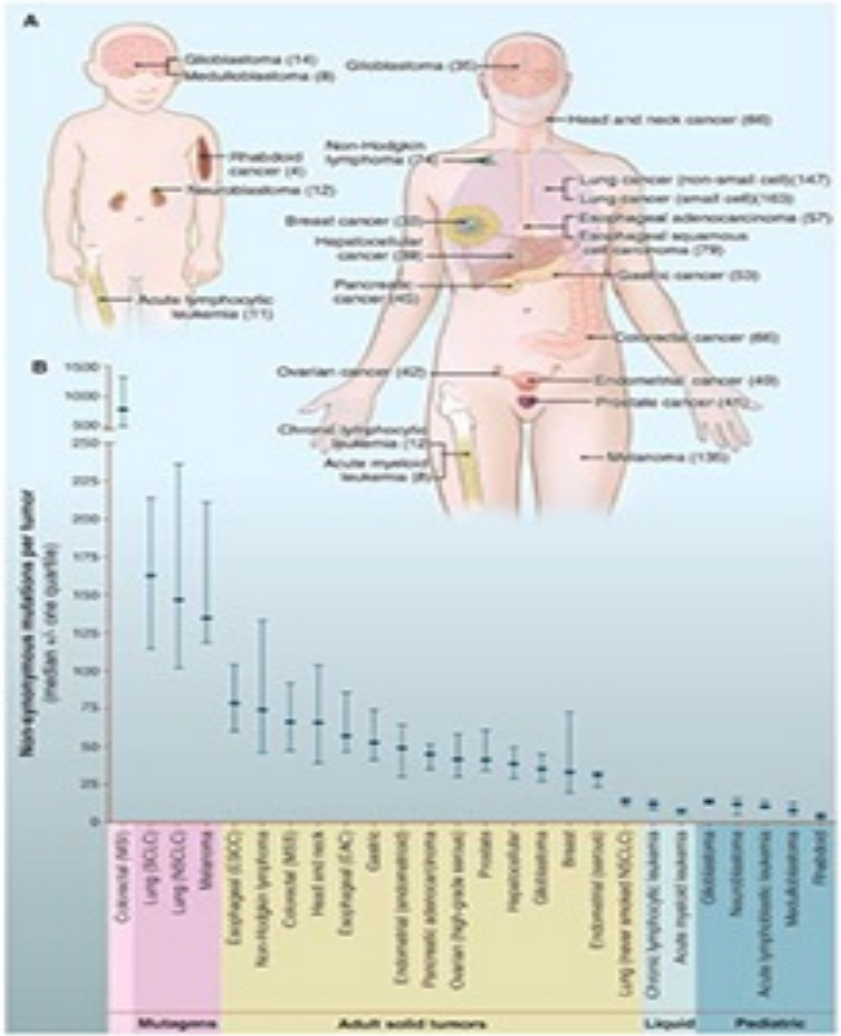
DNA sources


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
SHEDDING OF ALTERED DNA
SHEDDING OF ALTERED DNA
SHEDDING OF ALTERED DNA
SHEDDING OF ALTERED DNA
PREDISPOSITION
HYPERPLASIA
DYSPLASIA
CARCINOMA
METASTASIS
Adapted from Weinberg 1996

Paradigm shift



Genome landscape

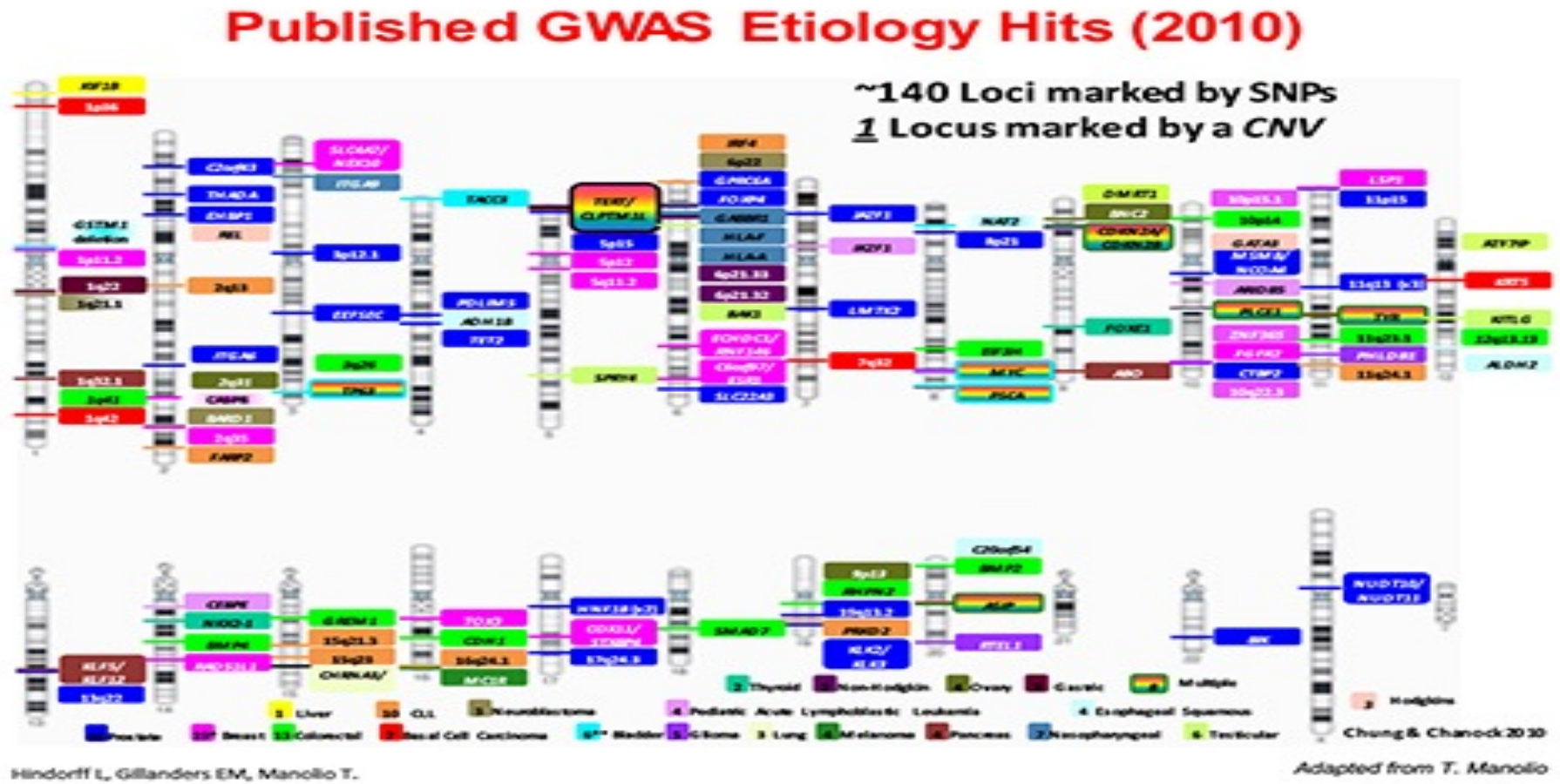


CANCER GENOME LANDSCAPE

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

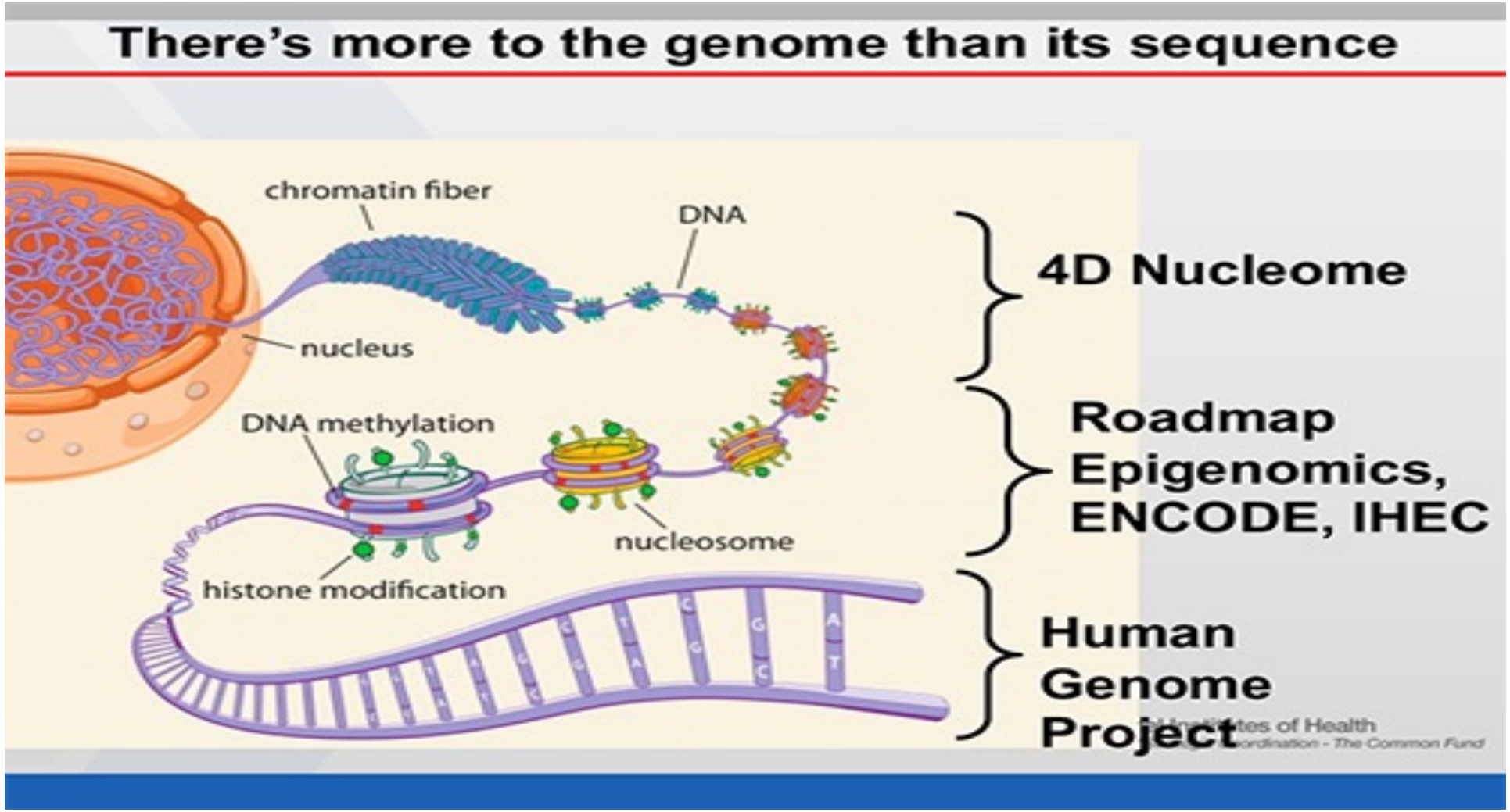
Adapted from Vogelstein and Kinzler (Science 2013)

GWAS hits

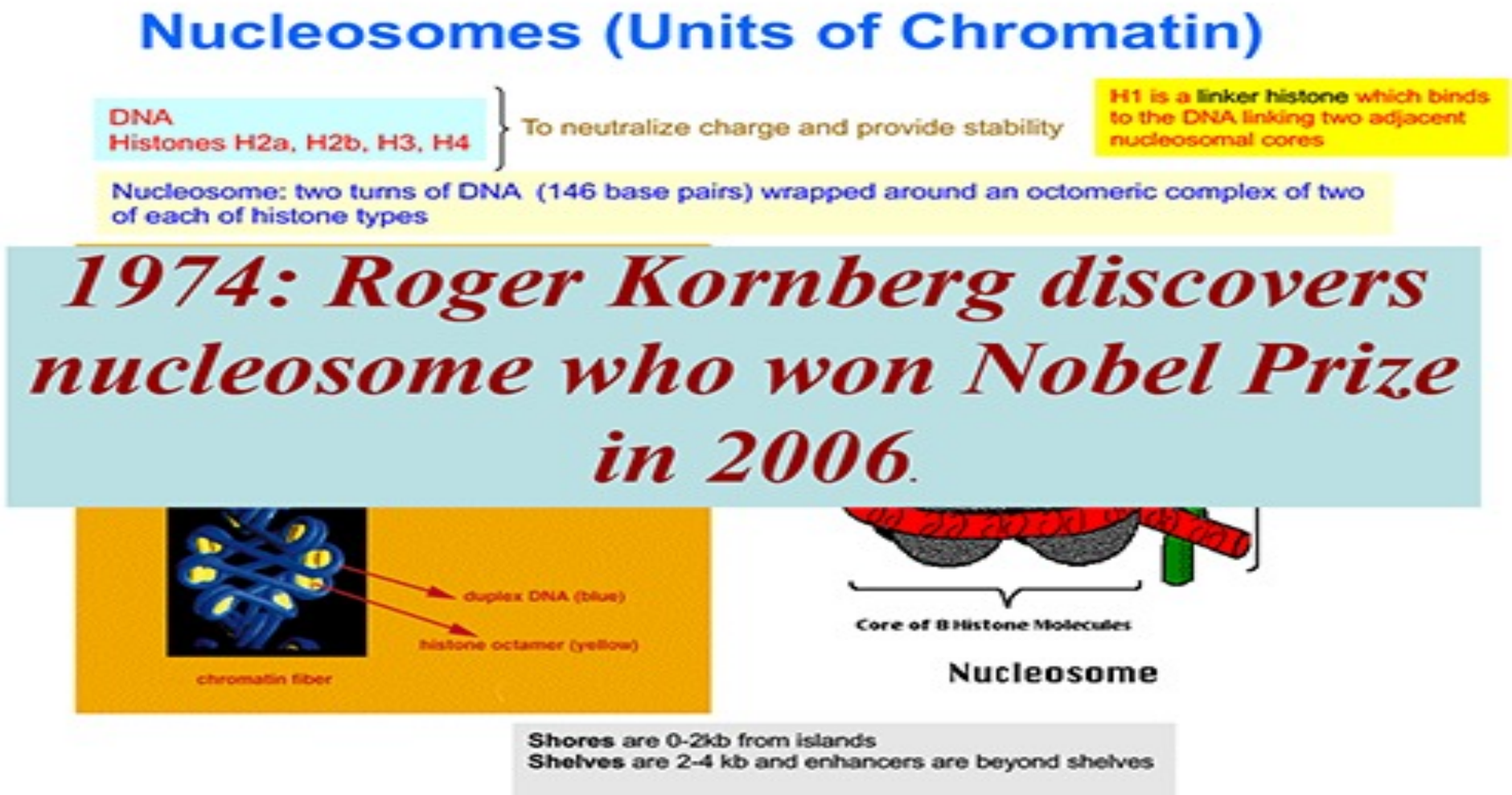

Published GWAS Etiology Hits (2010)
~140 Loci marked by SNPs
1 Locus marked by a CNV
Chung & Chanock 2010
Hindorff L, Gillanders EM, Manolio T.
Adapted from T. Manolio

Genome associations

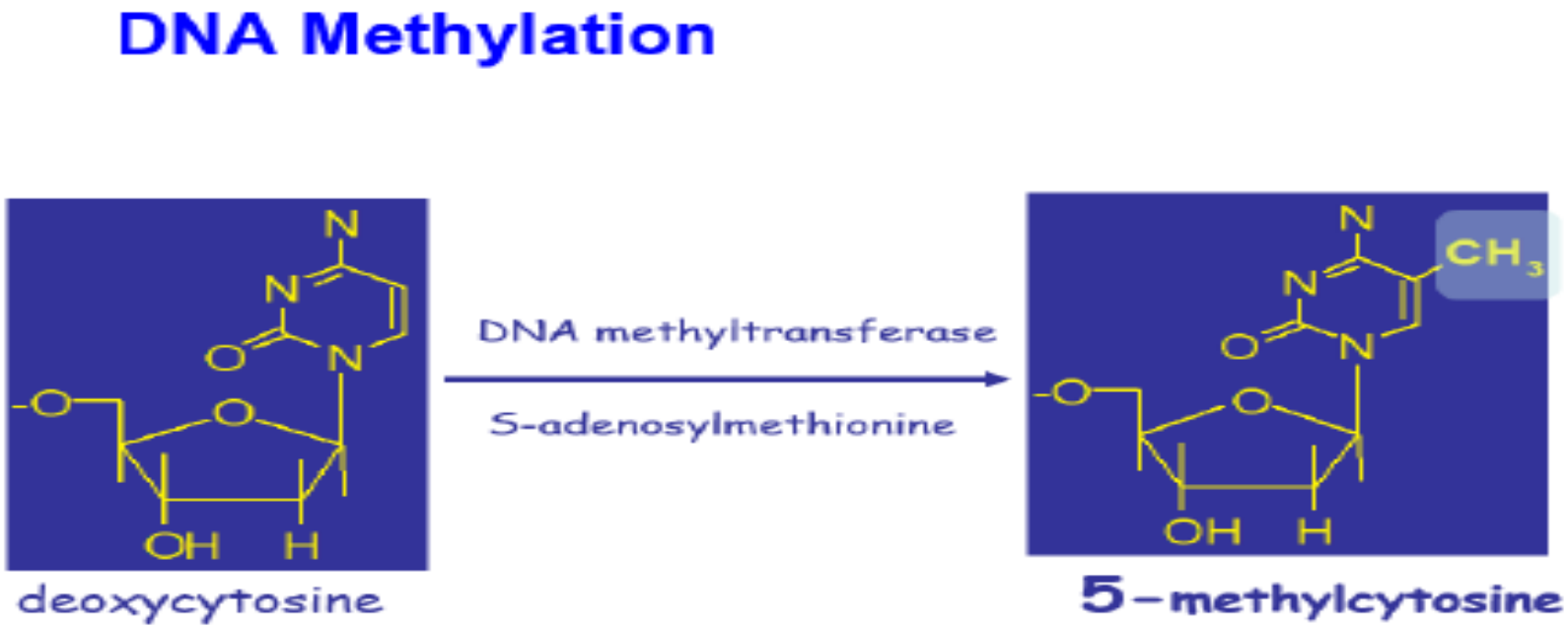
Genome sequence



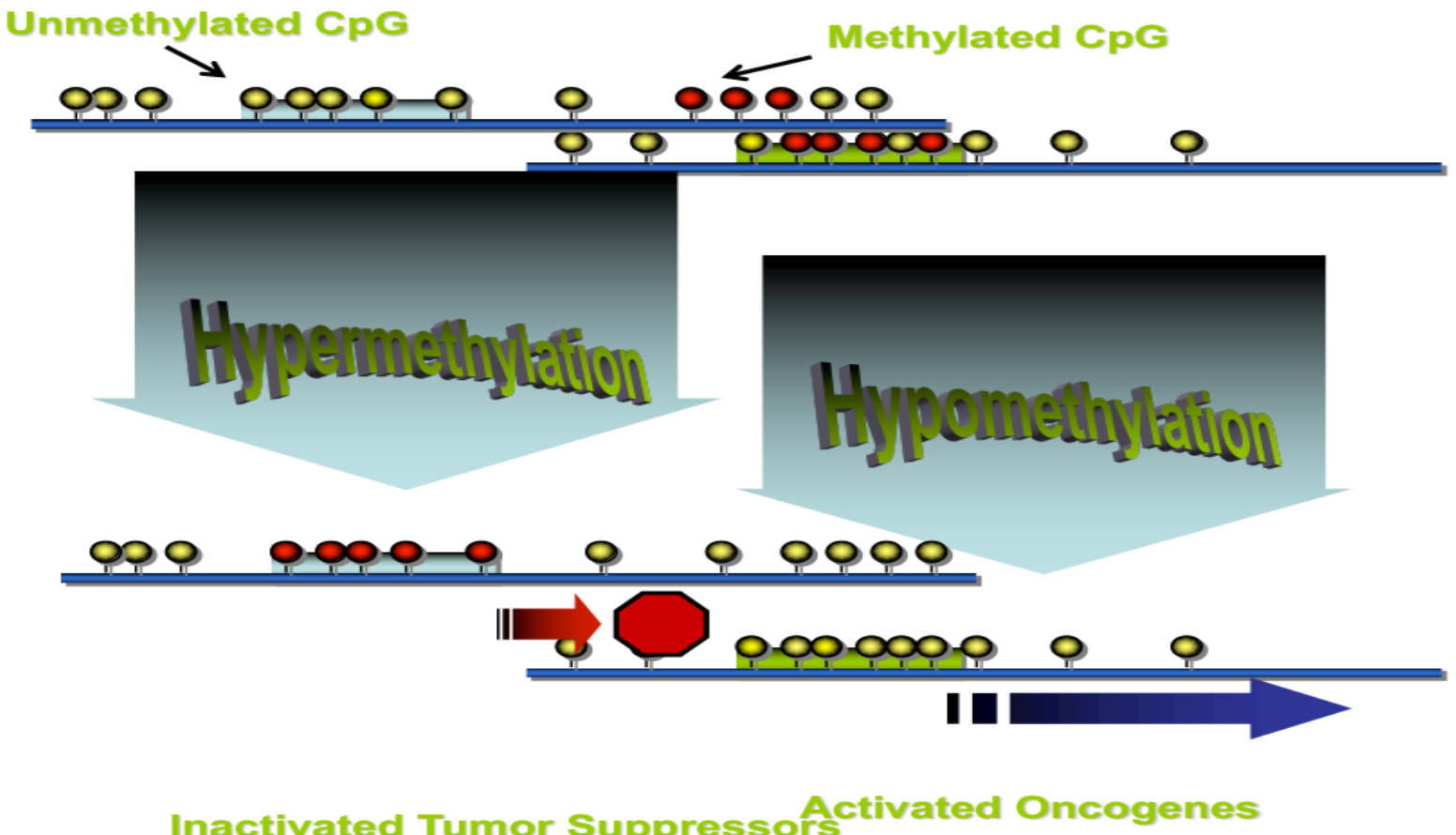
Kornberg and nucleosome



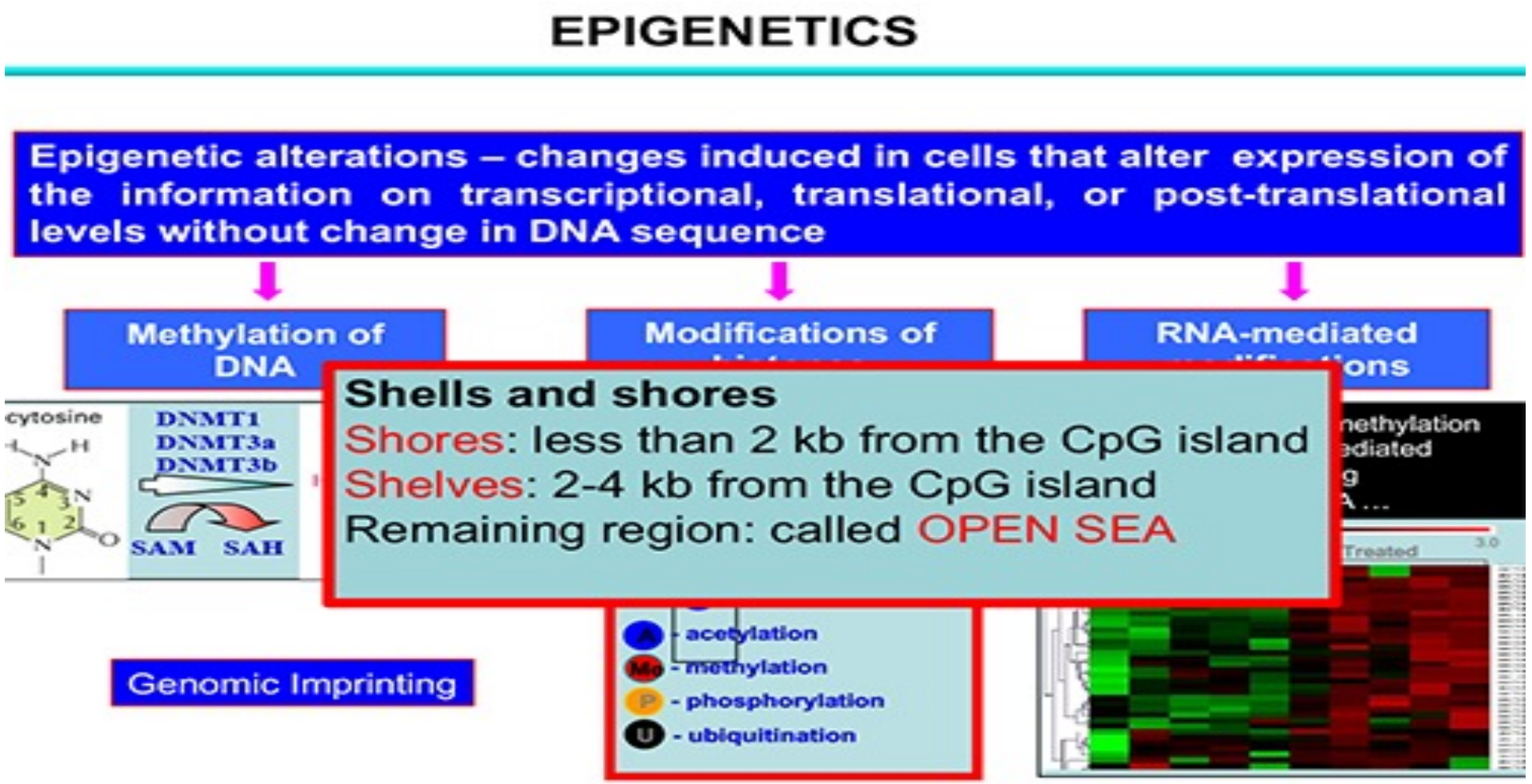
DNA methylation



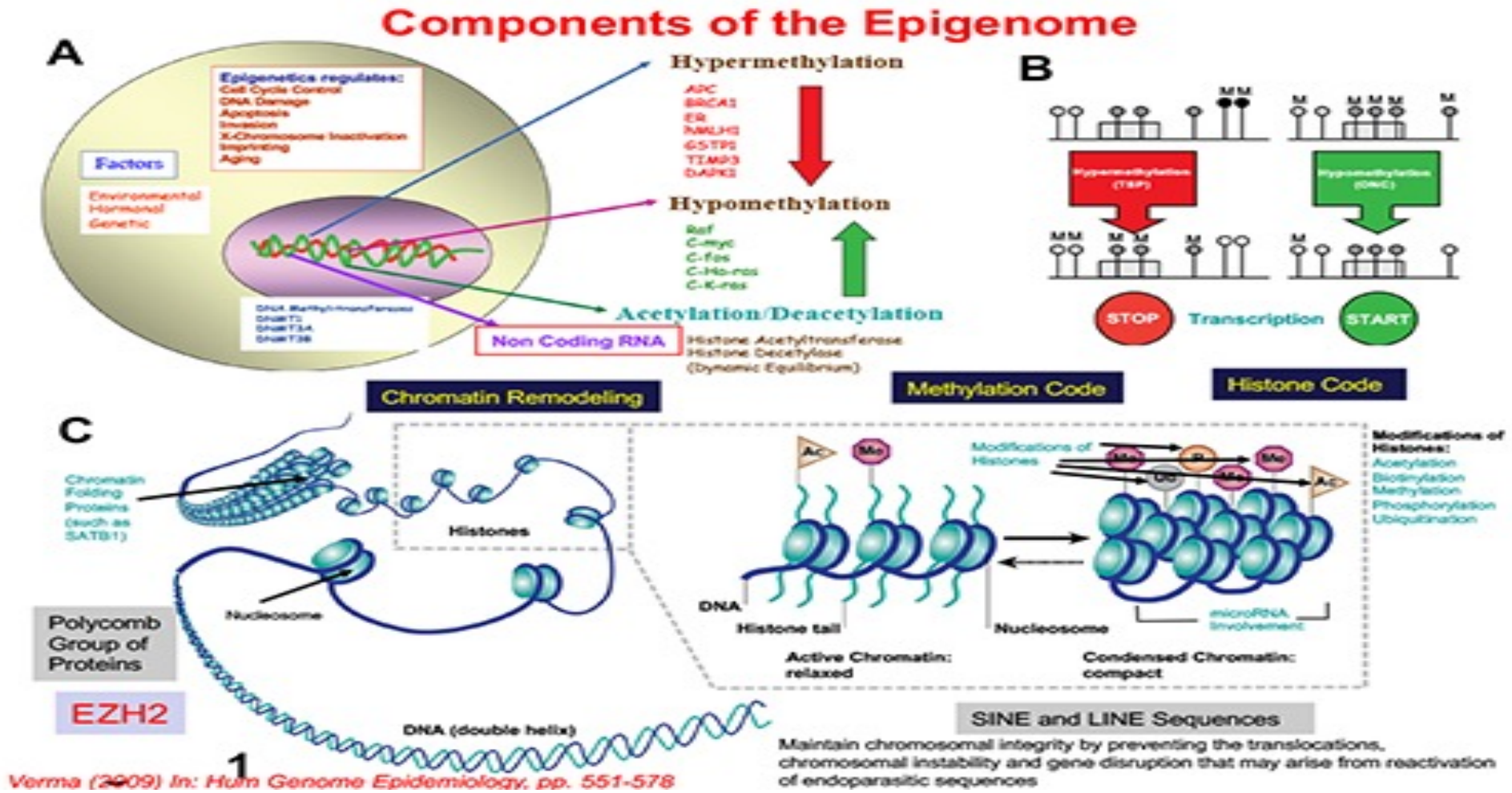
DNA methylation



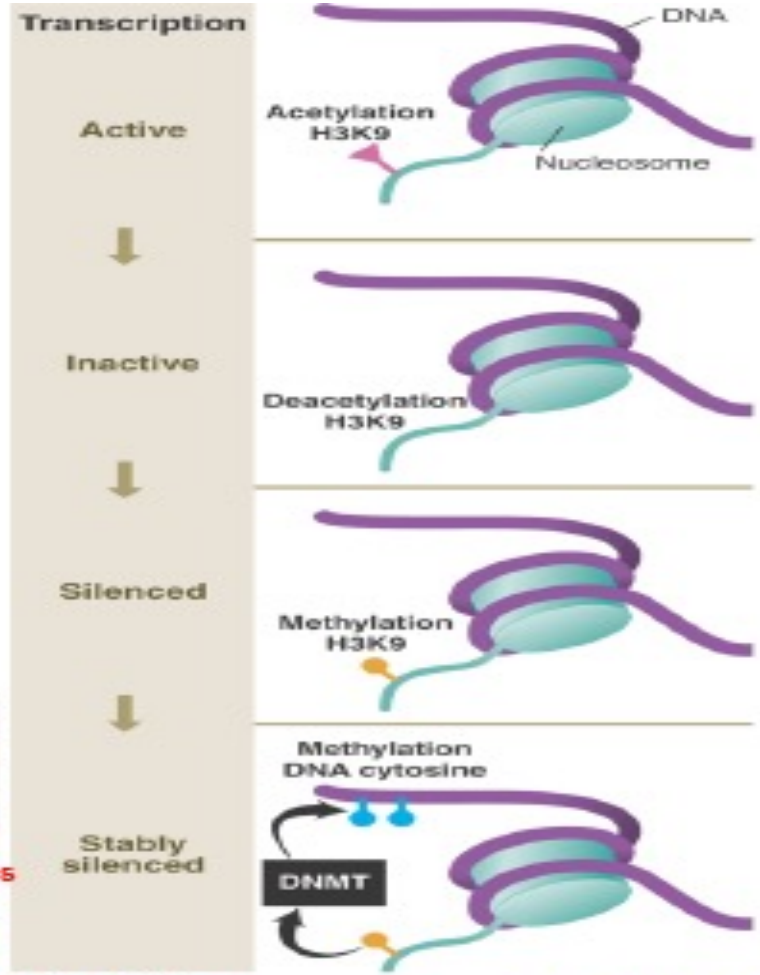
Epigenetics



Epigenome components

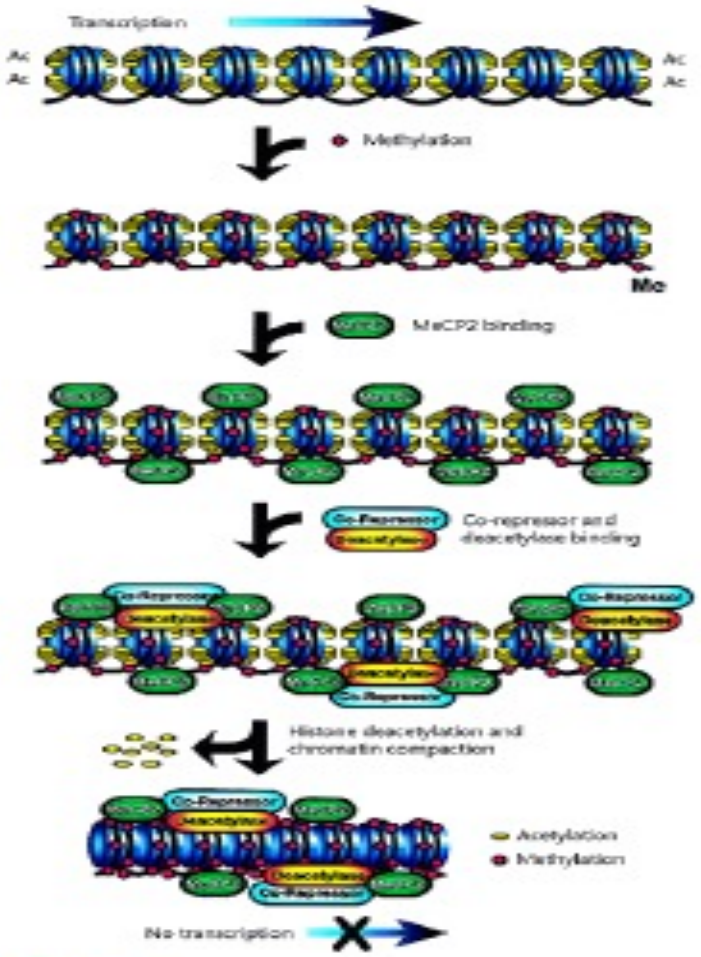


Methylation




Peter Jones


Andrew Feinberg

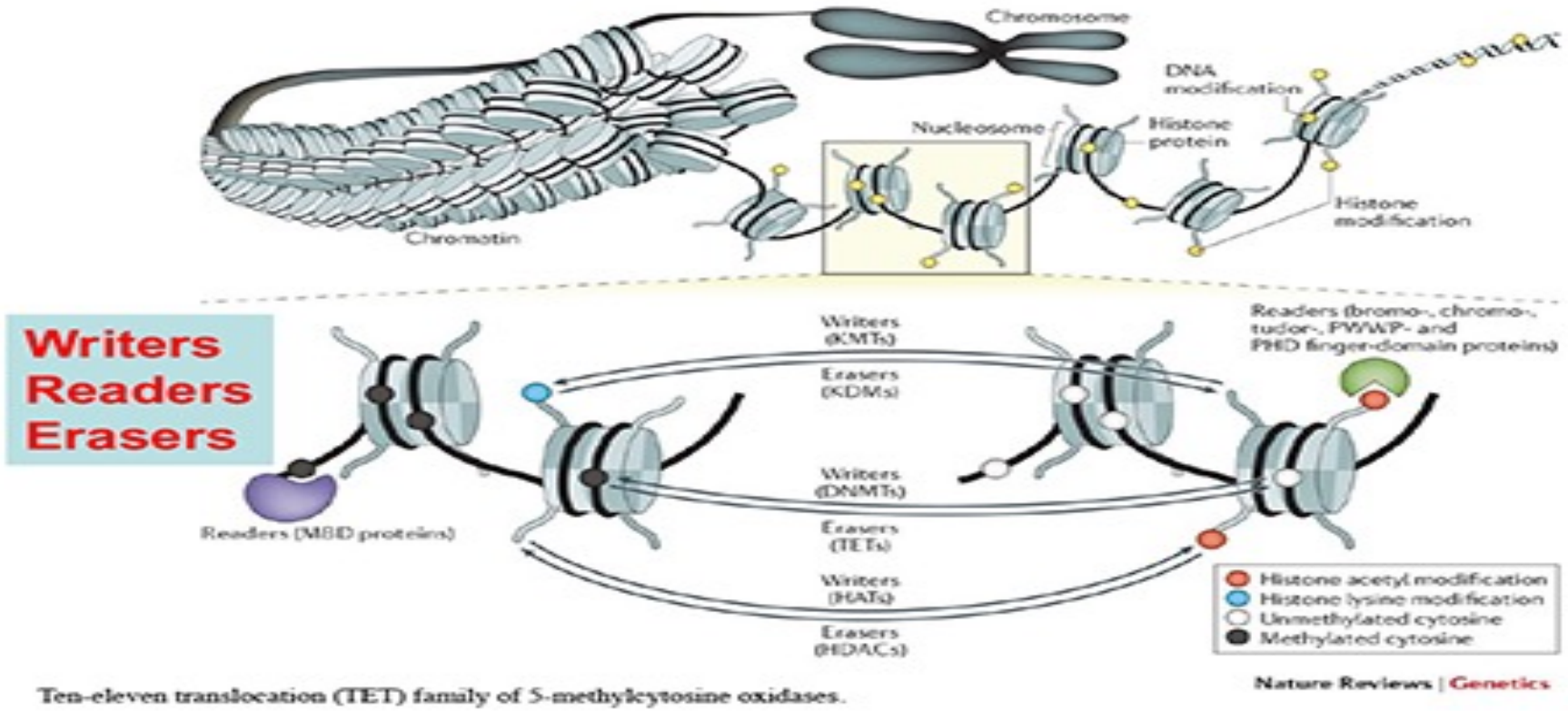


Jones and Laird. *Nat Rev Gen* 21: 163

Chromatin modifications

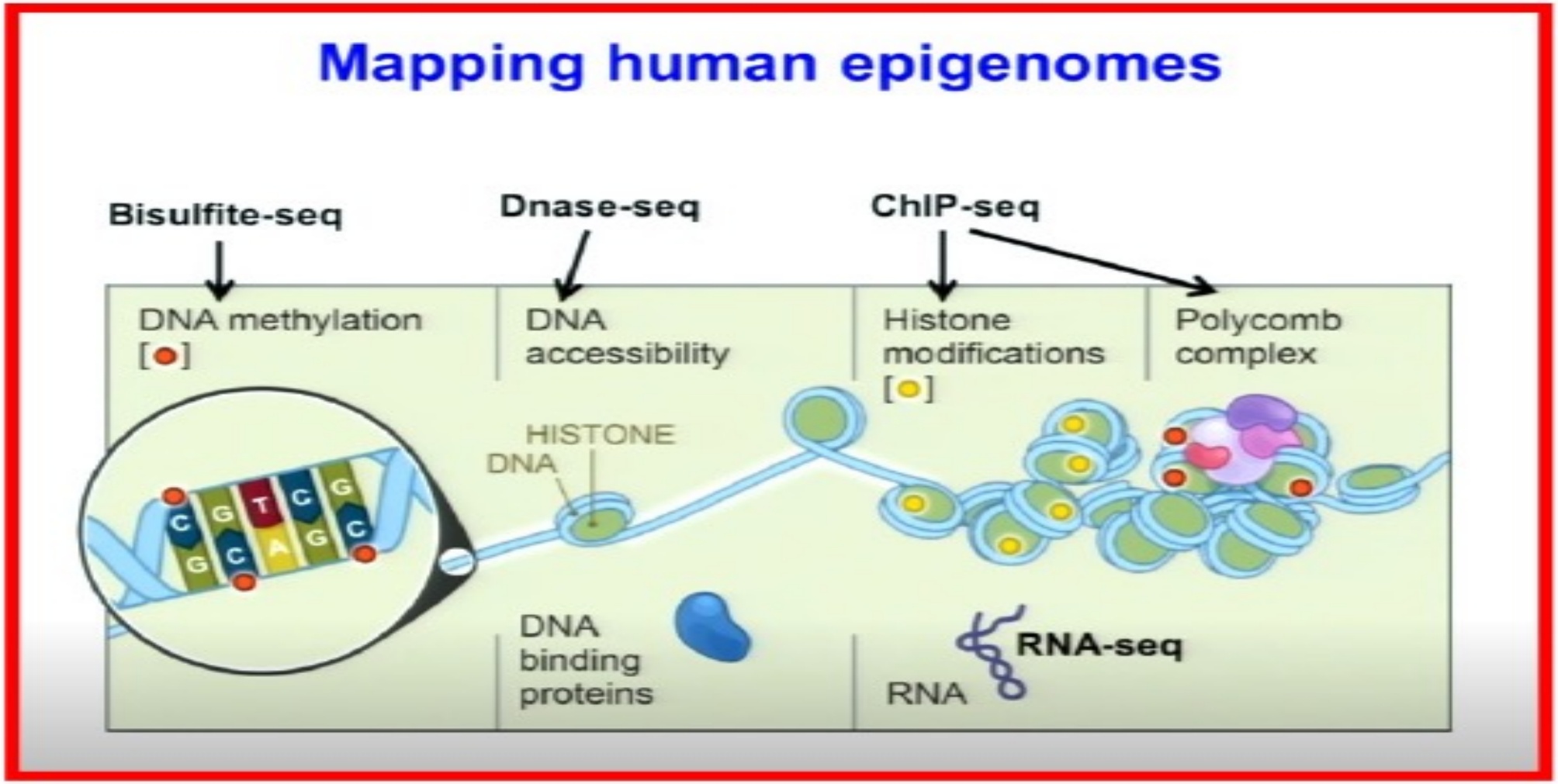
Figure 1 : Modulation of covalent modifications on chromatin.

From: Targeting the cancer epigenome for therapy



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

Epigenomes


Mapping human epigenomes
Bisulfite-seq
Dnase-seq
ChIP-seq
DNA methylation
[●]
DNA
accessibility
Histone
modifications
[●]
Polycomb
complex
HISTONE
DNA
C G T C G
G C A G C
DNA
binding
proteins
RNA-seq
RNA

Genome versus epigenome

Genome vs. epigenome – why is it important?

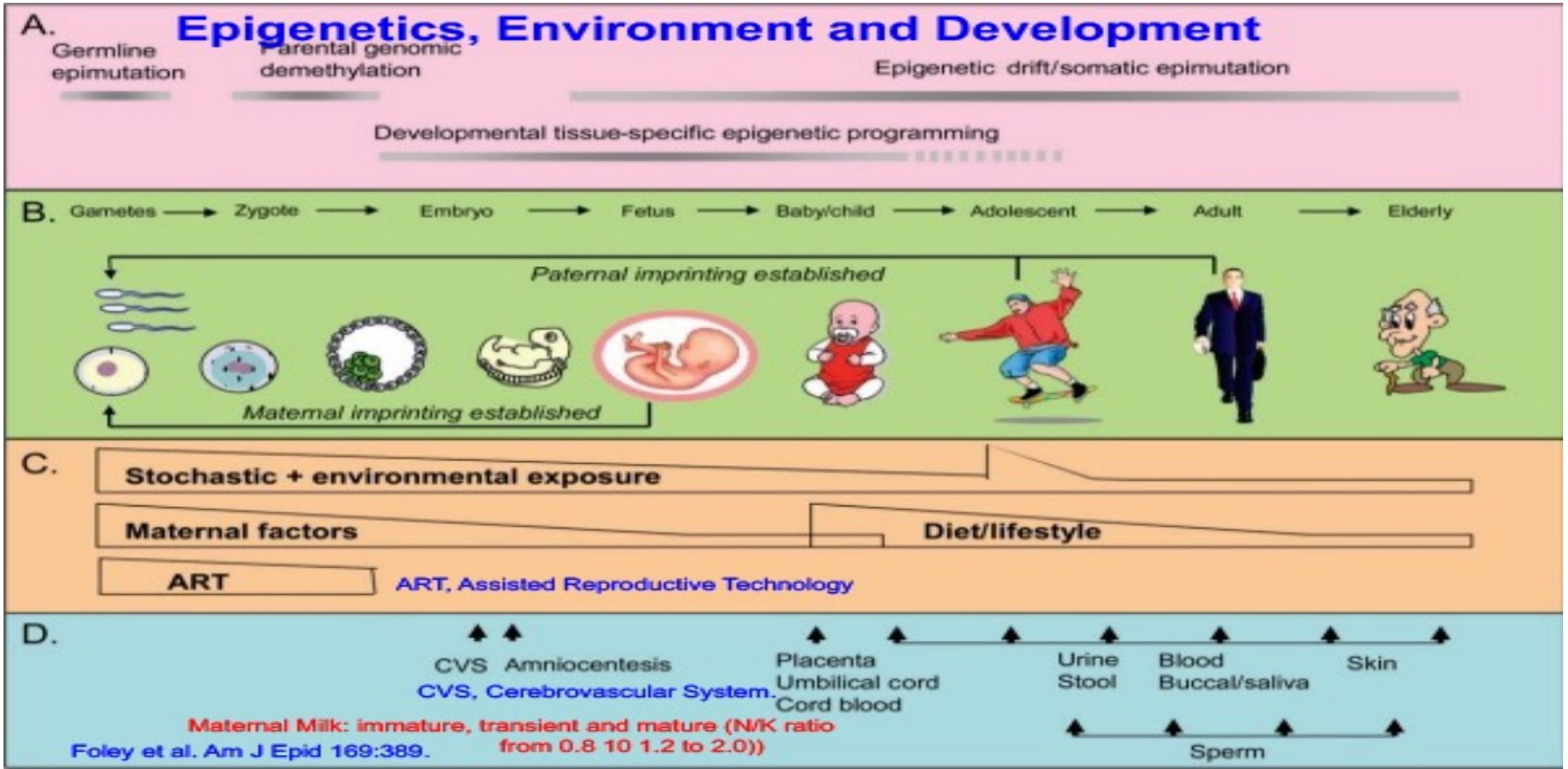
- **Genome is generally constant; epigenome changes**
  - Age
  - Diet
  - Disease
  - Lifestyle
  - Environment

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

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

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

Epigenetics, environment and development



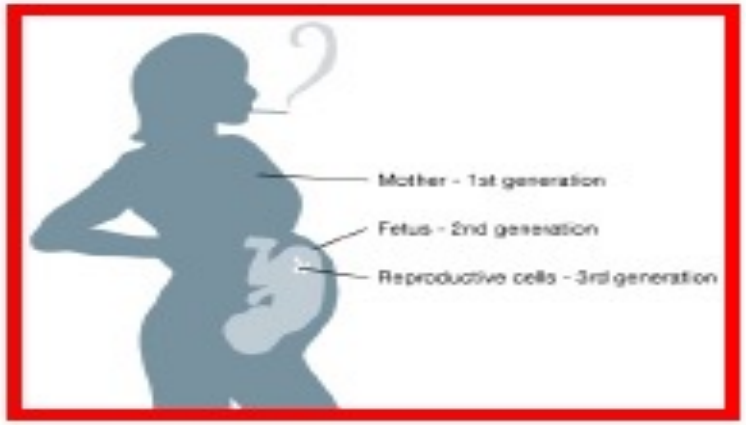
Toxic substances

Key toxic substances affecting the epigenome

Arsenic	Induces 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

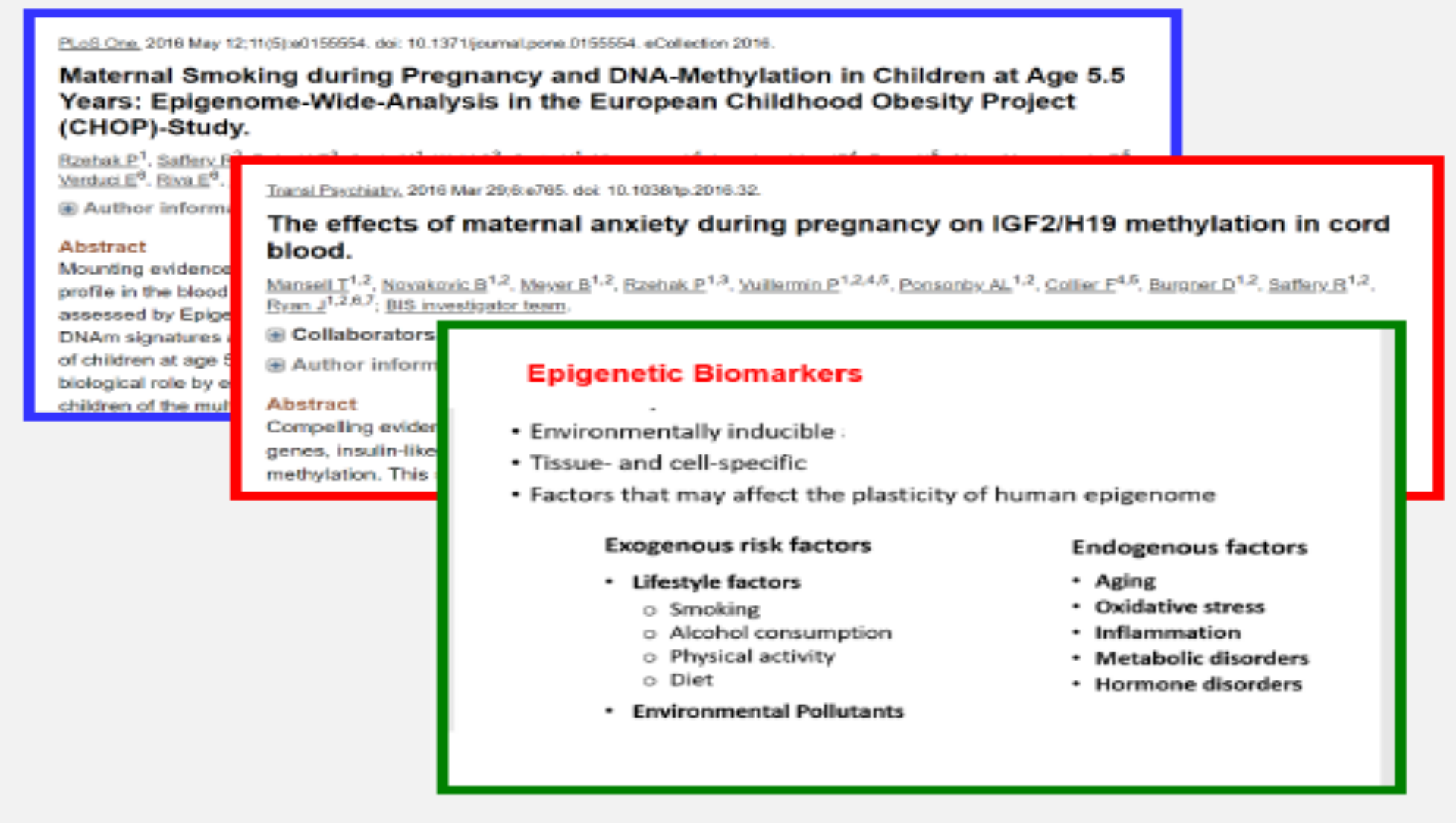
PFOS, Perfluorooctane sulfonate
PAHC, Polycyclic aromatic and halogenated compounds

Histone phosphorylation



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

Endogenous factors



Behavior

Epigenetics and behavior (including emotions)

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

Epigenetics and behavior

Epigenome-Wide Association Study of Aggressive Behavior

Translational 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.

Collaborators (11)

Author information

Abstract

Chapter 29

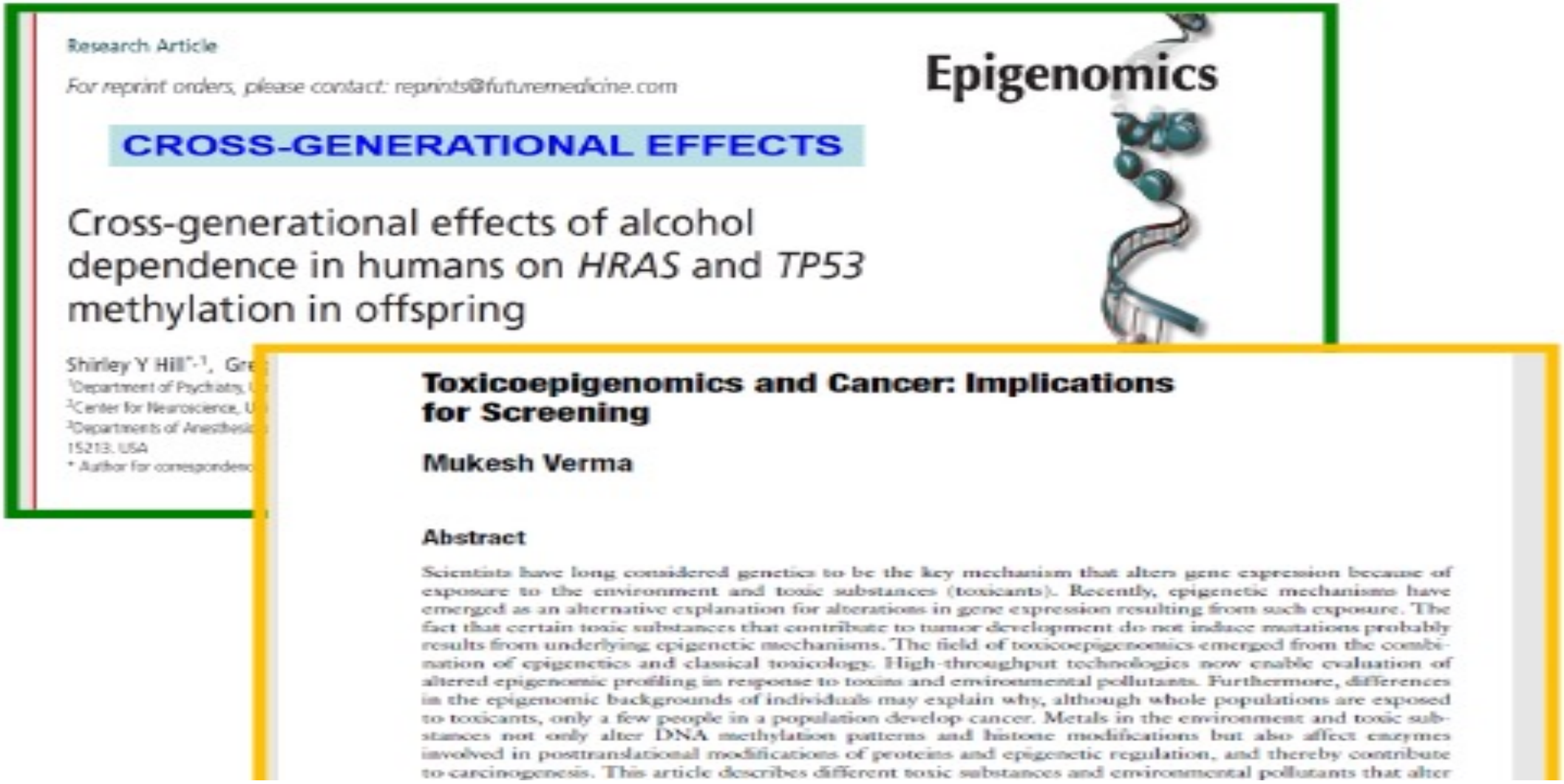
Epigenetic Regulation in Biopsychosocial Pathways

Kristin Litzelman and Mukesh Verma

Cancer Prevention Fellow

Abstract

Cross-generational effects



Research Article

For reprint orders, please contact: reprints@futuremedicine.com

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]Department of Anesthesi

15213, USA

* Author for correspondenc

Epigenomics

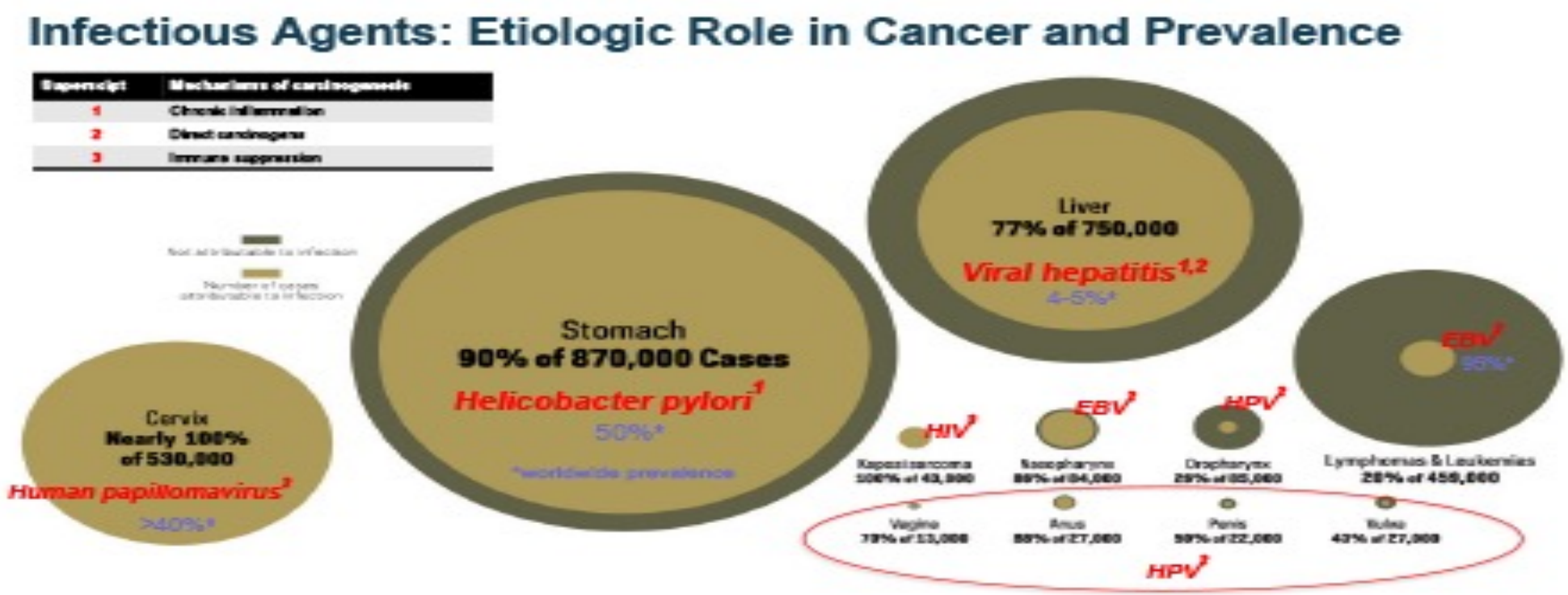
Toxicoepigenomics and Cancer: Implications for Screening

Mukesh Verma

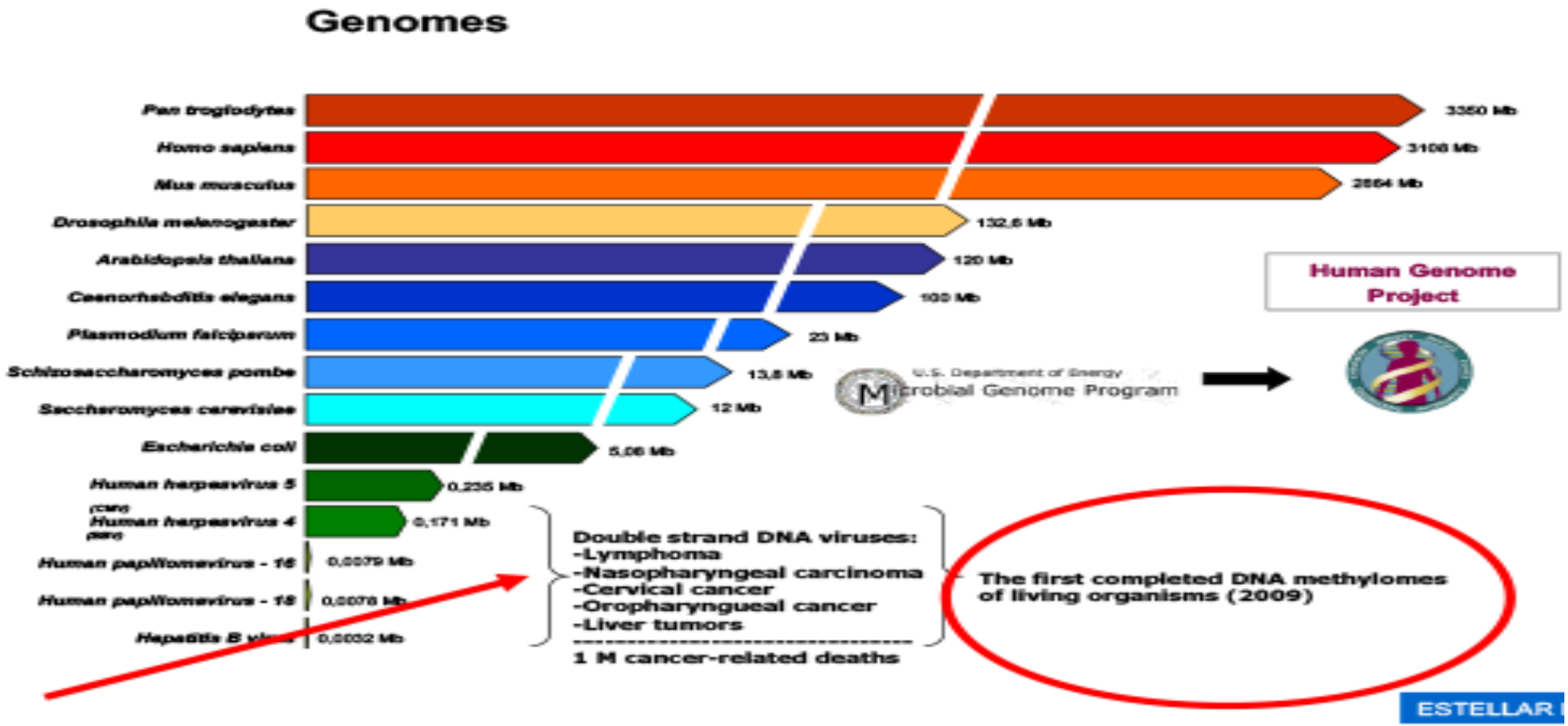
Abstract

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

Infectious agents



Genomes


Genomes
Pan troglodytes
Homo sapiens
Mus musculus
Drosophila melanogaster
Arabidopsis thaliana
Caenorhabditis elegans
Plasmodium falciparum
Schizosaccharomyces pombe
Saccharomyces cerevisiae
Escherichia coli
Human herpesvirus 5
Human herpesvirus 4
Human papillomavirus - 16
Human papillomavirus - 18
Hepatitis B virus
3350 Mb
3100 Mb
2664 Mb
132,6 Mb
120 Mb
100 Mb
23 Mb
13,8 Mb
12 Mb
5,06 Mb
0,235 Mb
0,171 Mb
0,0079 Mb
0,0078 Mb
0,0032 Mb
Human Genome Project
U.S. Department of Energy
Microbial Genome Program
Double strand DNA viruses:
-Lymphoma
-Nasopharyngeal carcinoma
-Cervical cancer
-Oropharyngueal cancer
-Liver tumors
1 M cancer-related deaths
The first completed DNA methylomes of living organisms (2009)
ESTELLAR

Oncogenic viruses and bacteria

Oncogenic viruses, bacteria and epigenetics

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

Int. J. Cancer: **113**, 440–445 (2005)

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

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

Ming-Fang Wu[1,2], Ya-Wen Cheng[2,3], Ji-Ching Lai[4], Min-Chih Hsu[4], Jung-Ta Chen[5], Wen-Shan Liu[6], Ming-Chih Chiou[2,3], Chih-Yi Chen[7] and Huei Lee[3,4]*

[1]*Dep... Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan*

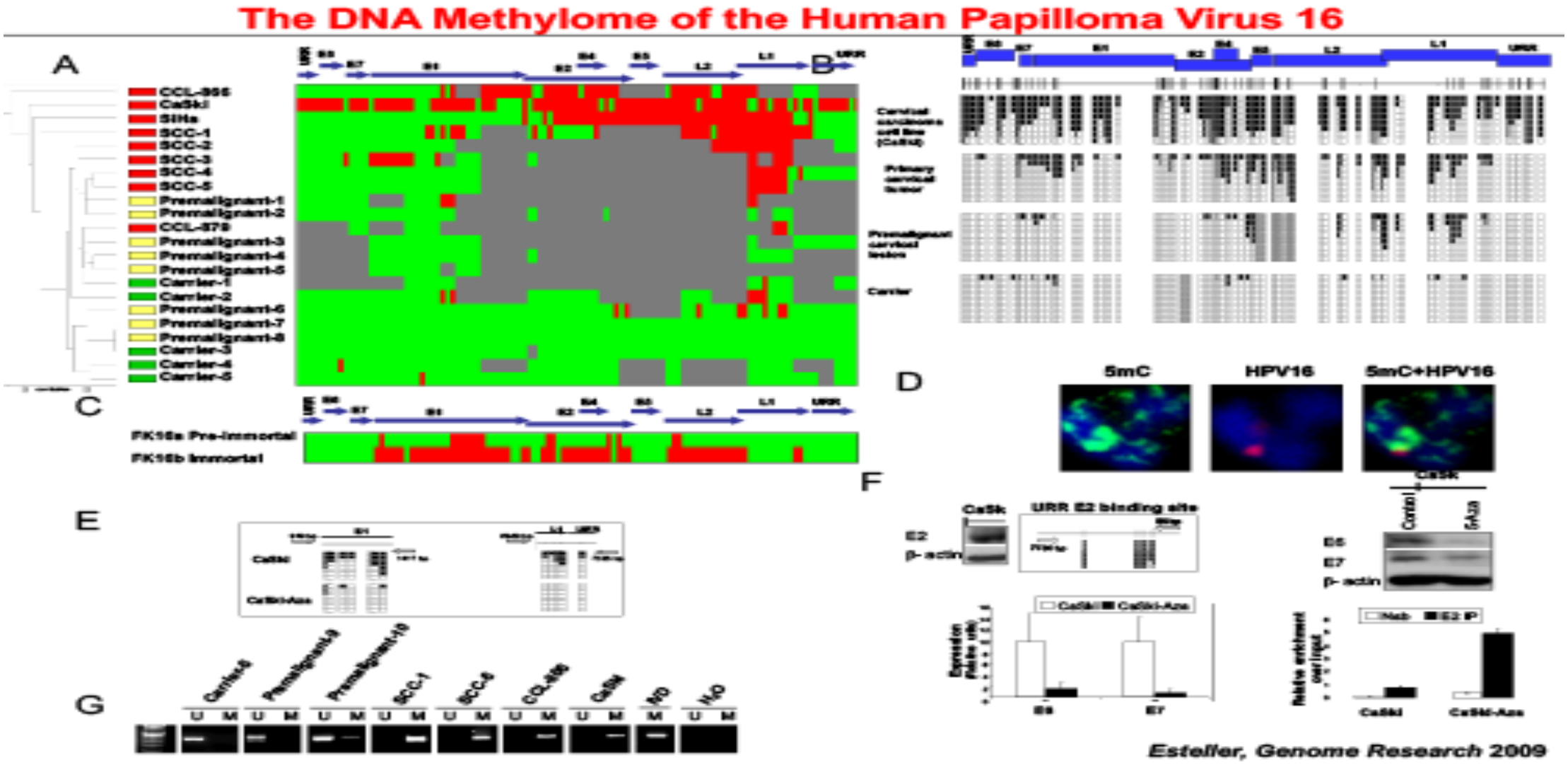
LANA

Complete methylome of HPV, EBV, and HBV.

Esteller M. Genome Research. 2009. 19: 438

EBNA

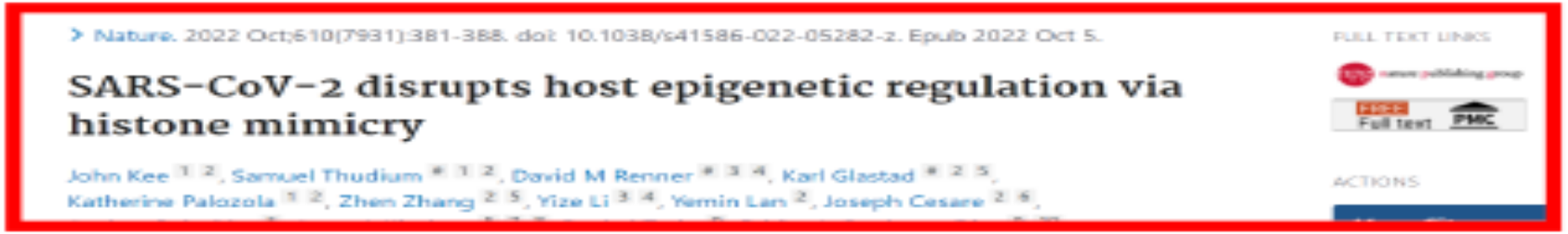
DNA methylome



Infection and cancer

Infection and Cancer: New and Emerging Associations

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



Risk Assessment

Understanding Cancer Etiology and Risk Assessment

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

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

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

Need follow up (for longitudinal studies) for several years

Challenge: Expensive, data sharing

Advantage: Essential to identify risk factors for cancer

Special populations

39

Special Populations in EGRP

African-American men & women

South American women

Asian-American & Asian men & women

Latin-American/Hispanics

African men & women

Alaskan & Hawaiian Natives

Middle-Eastern populations

American-Indian, incl. Navajo

Rural South

Chinese

EGRP Studies Are Everywhere

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

2.3 Million Subjects
Cohorts, CGN and Family Registries

Cohort consortium

- 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.

Environment and child health outcomes

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]

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

ECHO research

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

Loss (or gain) of gene function in cancer

Loss (or Gain) of gene function in cancer

Most permanent → Most dynamic

Genetic

- Deletion
- Point mutations
- Amplification
- Chromosomal Translocation (Ig rearrangement)

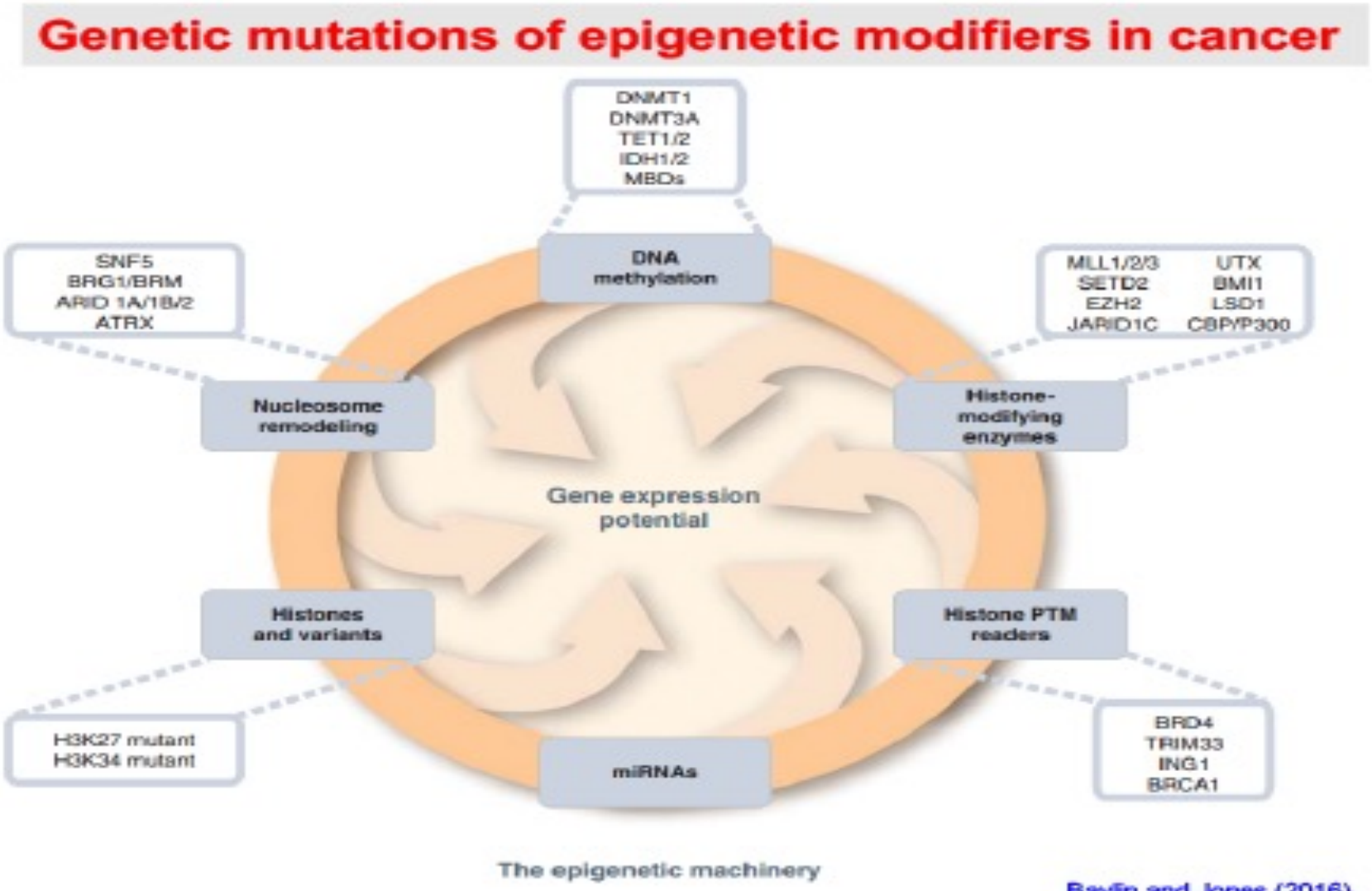
Epigenetic

- Chromatin Changes
- Promoter Methylation Silencing

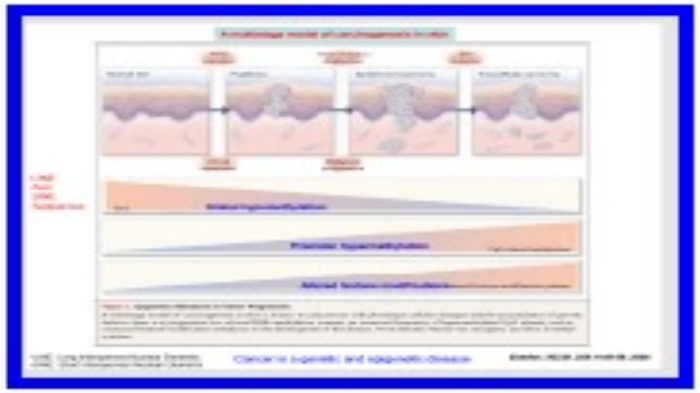
Transcription Factor Changes

Cell-cycle Regulated Changes

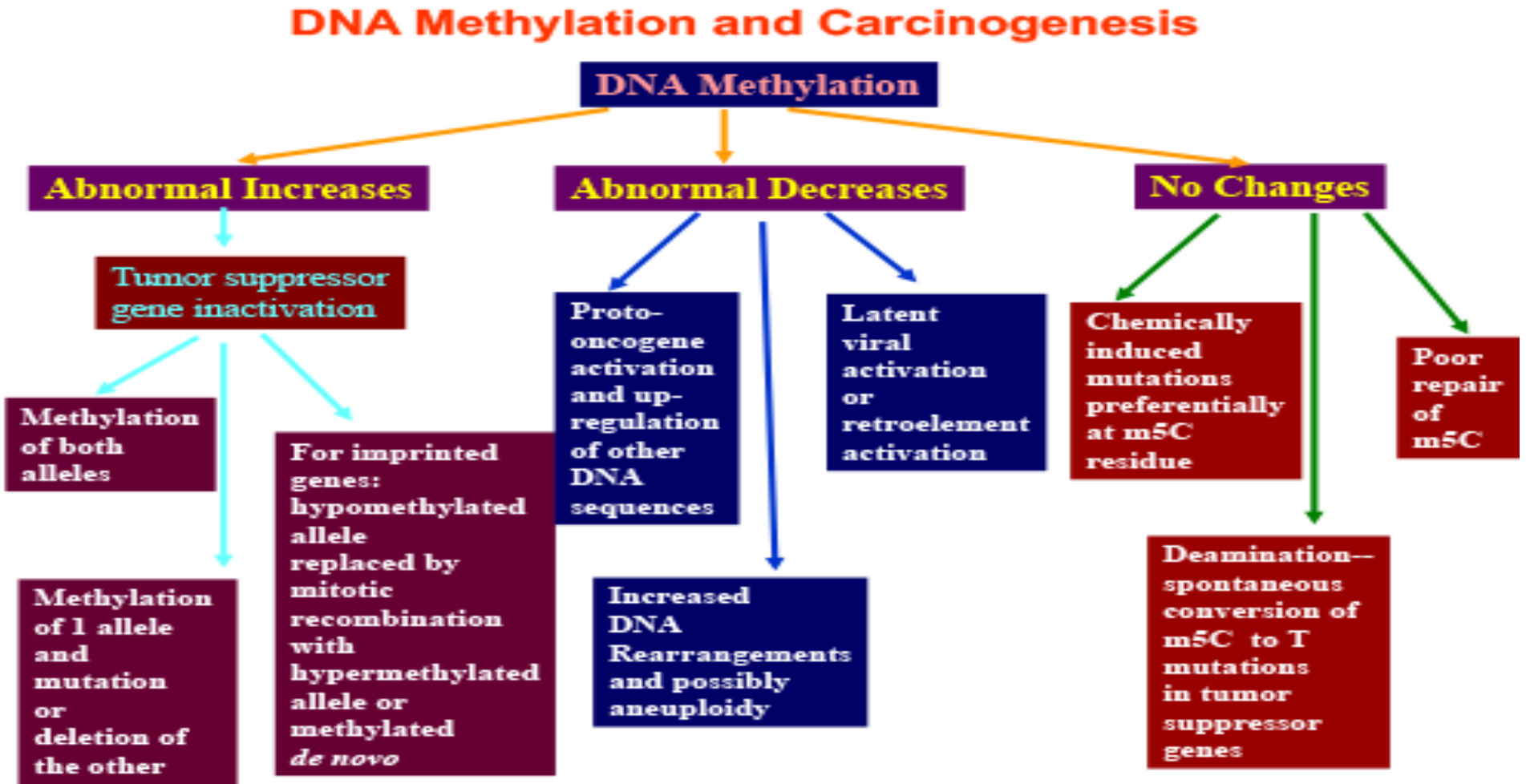
Genetic mutations



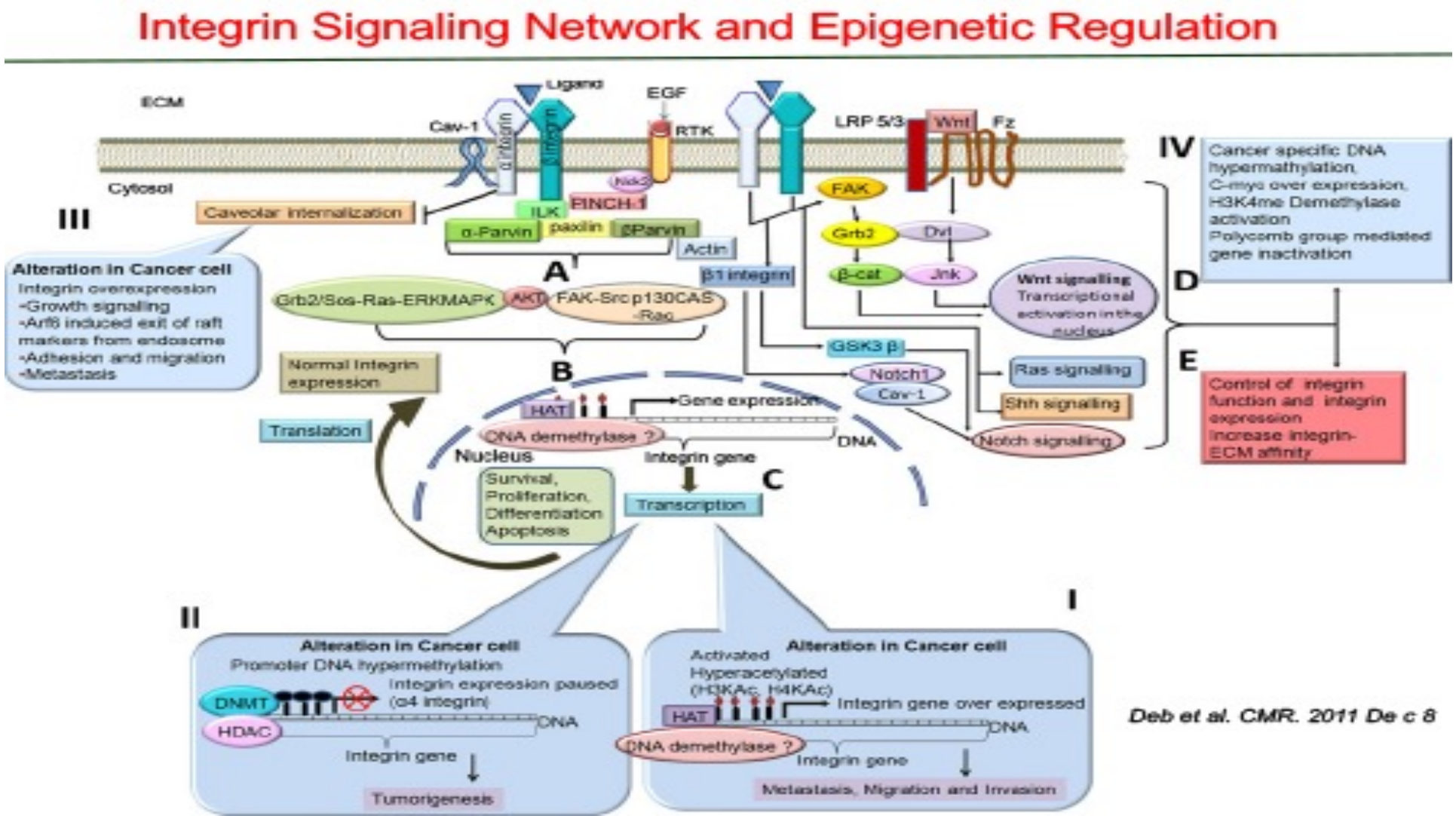
Hypomethylation



DNA methylation and carcinogenesis

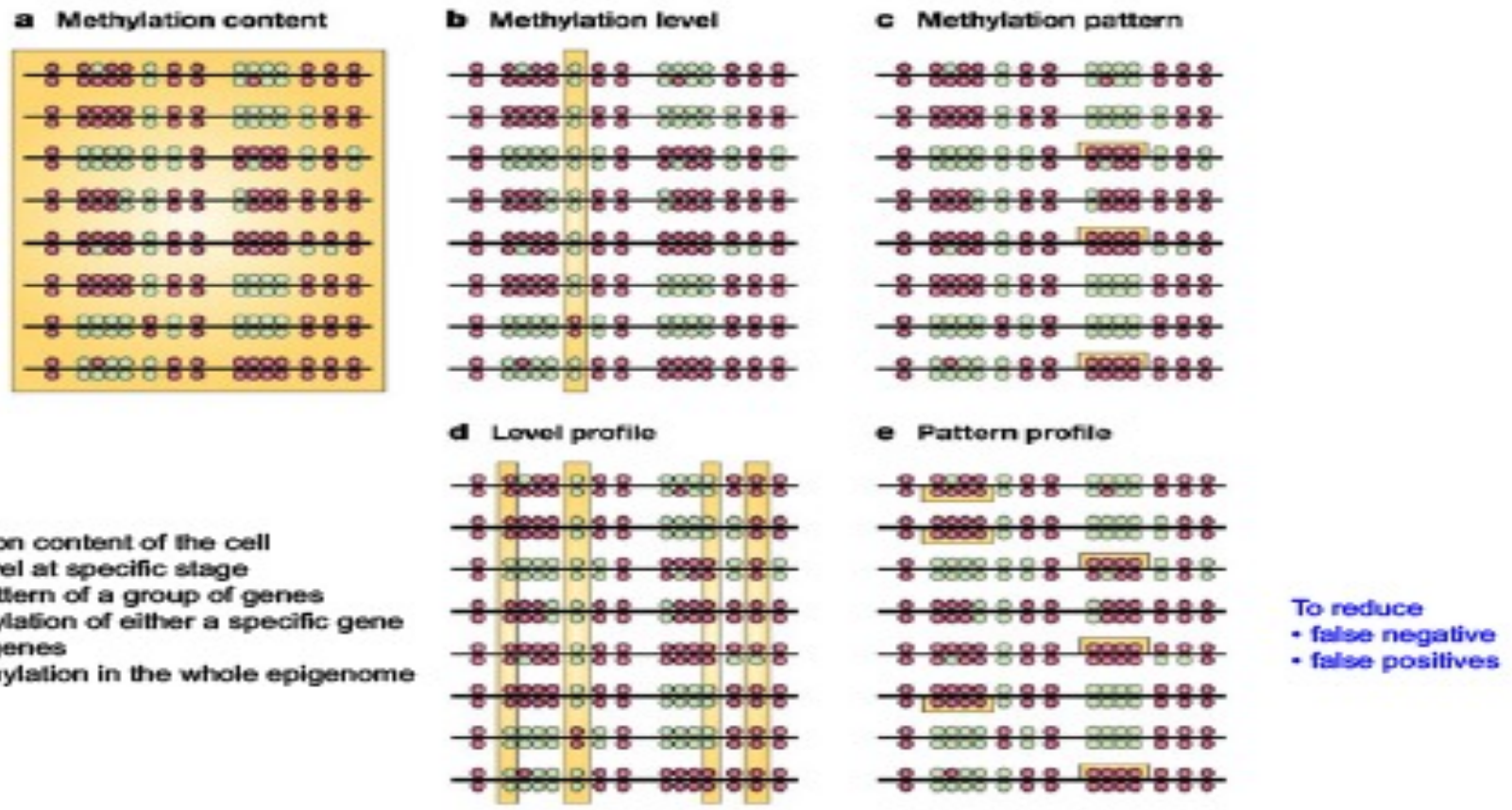


Integrin signaling



Deb et al. CMR. 2011 De c 8

Methylation

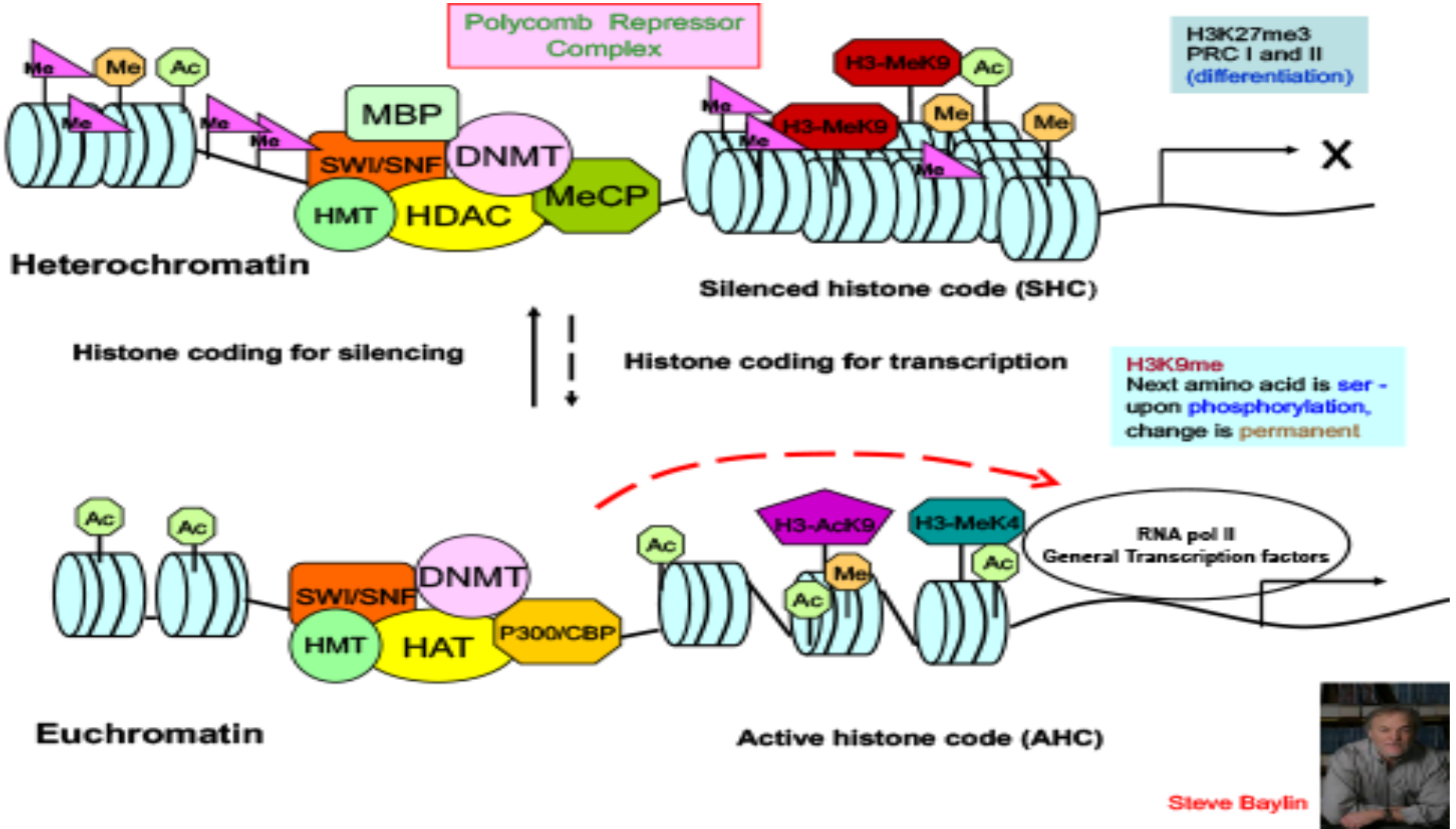


- Total methylation content of the cell
- methylation level at specific stage
- methylation pattern of a group of genes
- profile of methylation of either a specific gene or a number of genes
- pattern of methylation in the whole epigenome

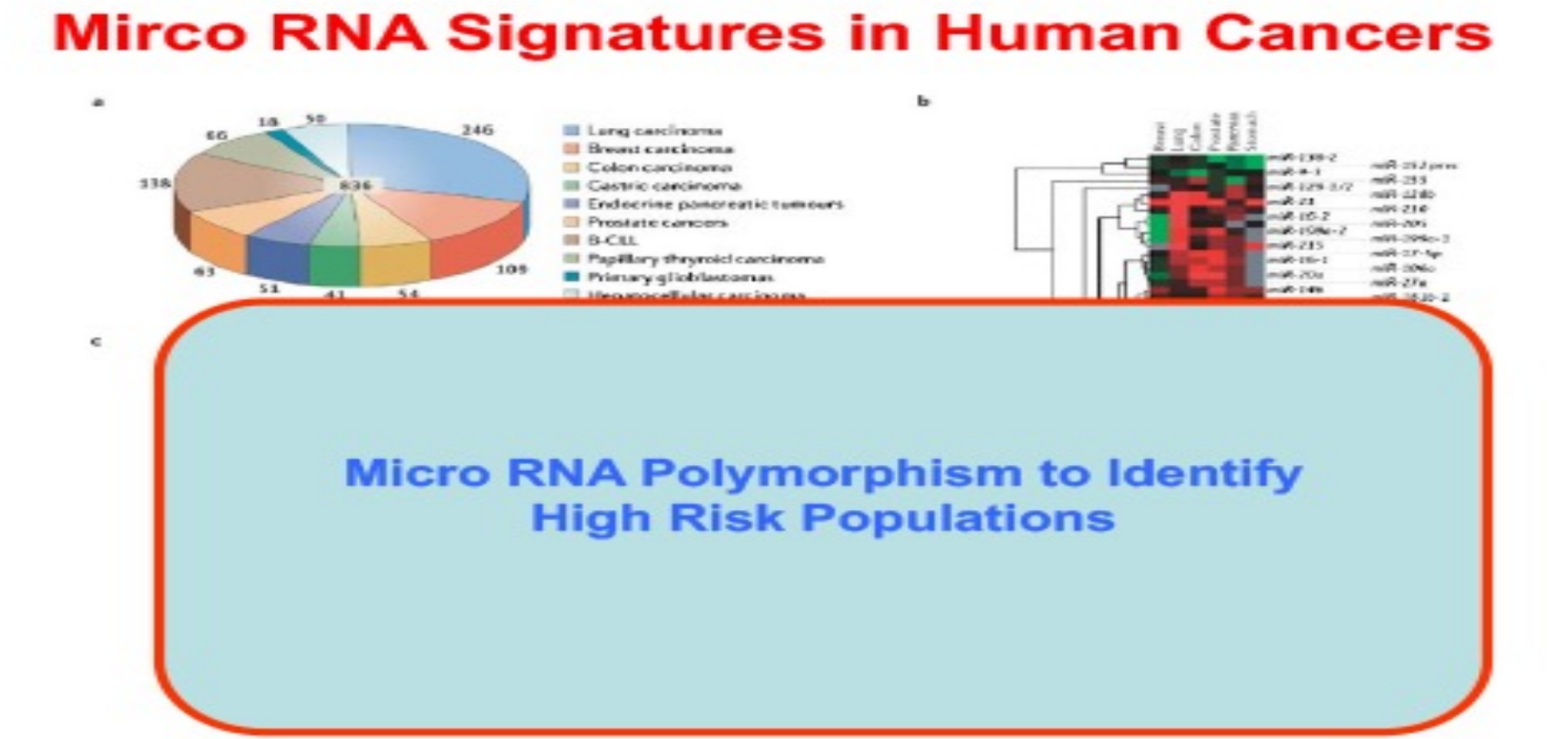
To reduce
- false negative
- false positives

Laird *Nat Rev Cancer* 3:253

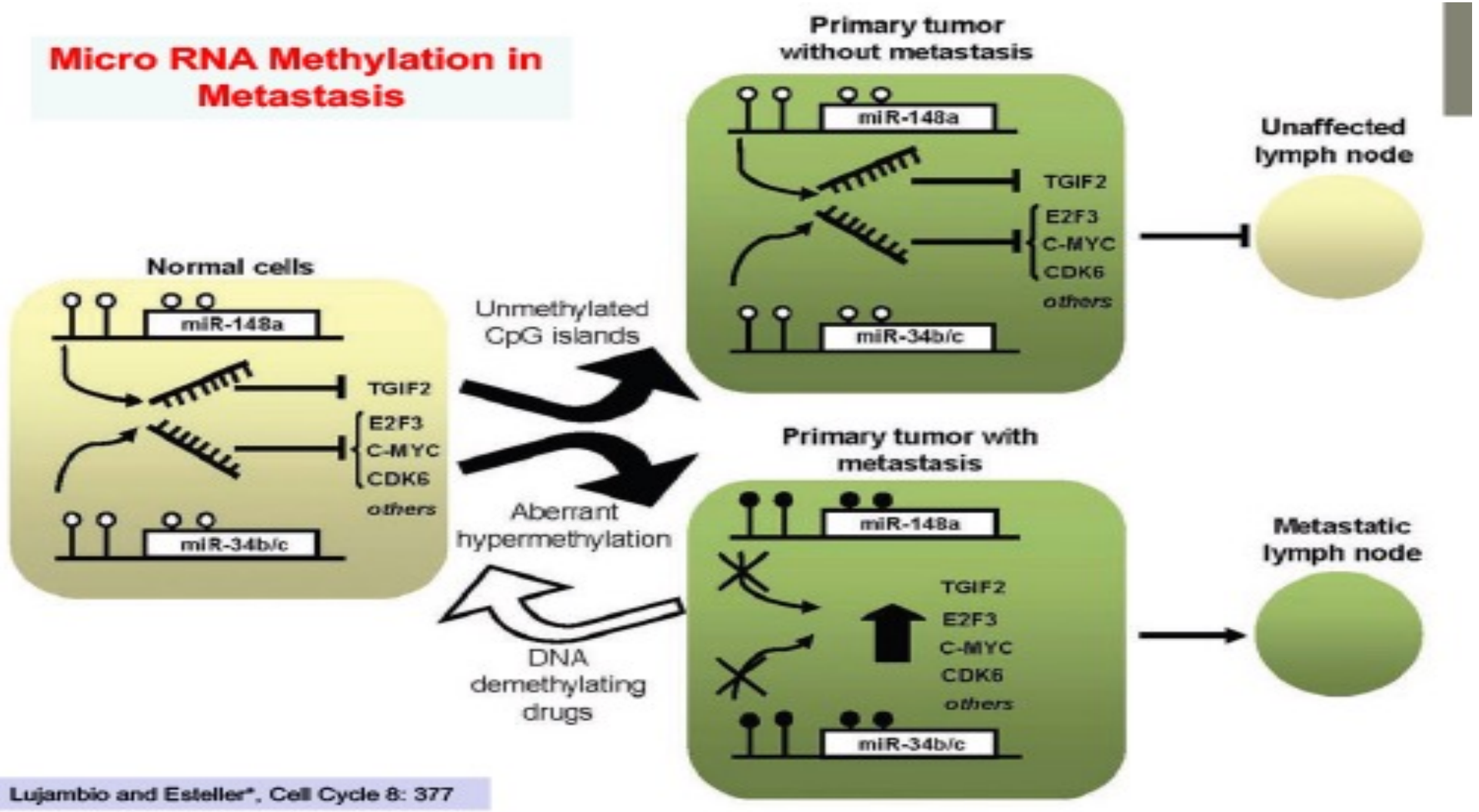
Histone acetylation



Micro RNA signatures



Micro RNA methylation



Methylation of microRNAs

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

Extracellular vesicles

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

BMC
Clinical Pathology

REVIEW Open Access

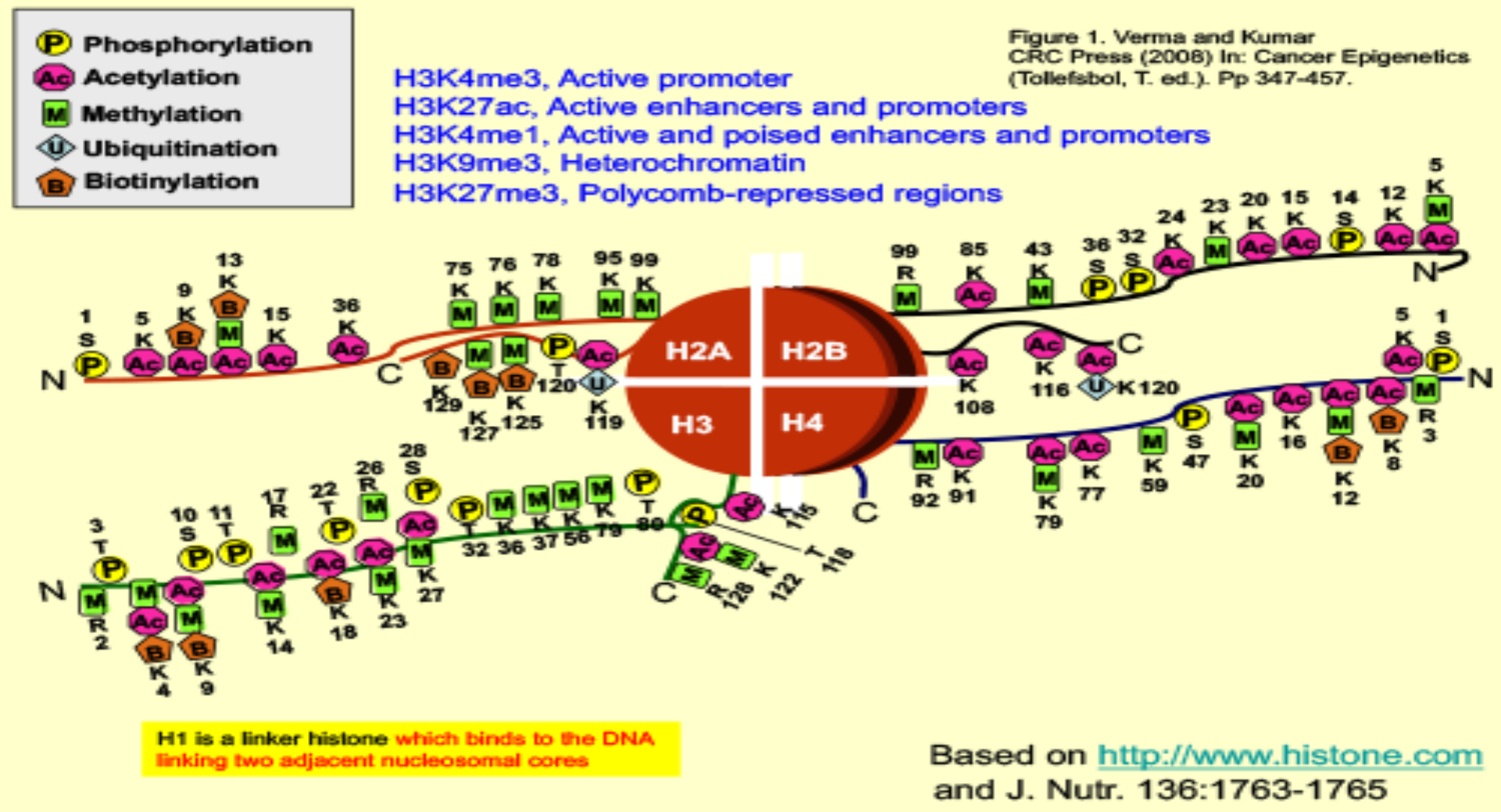
Extracellular vesicles: potential applications in cancer diagnosis, prognosis, and epidemiology

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

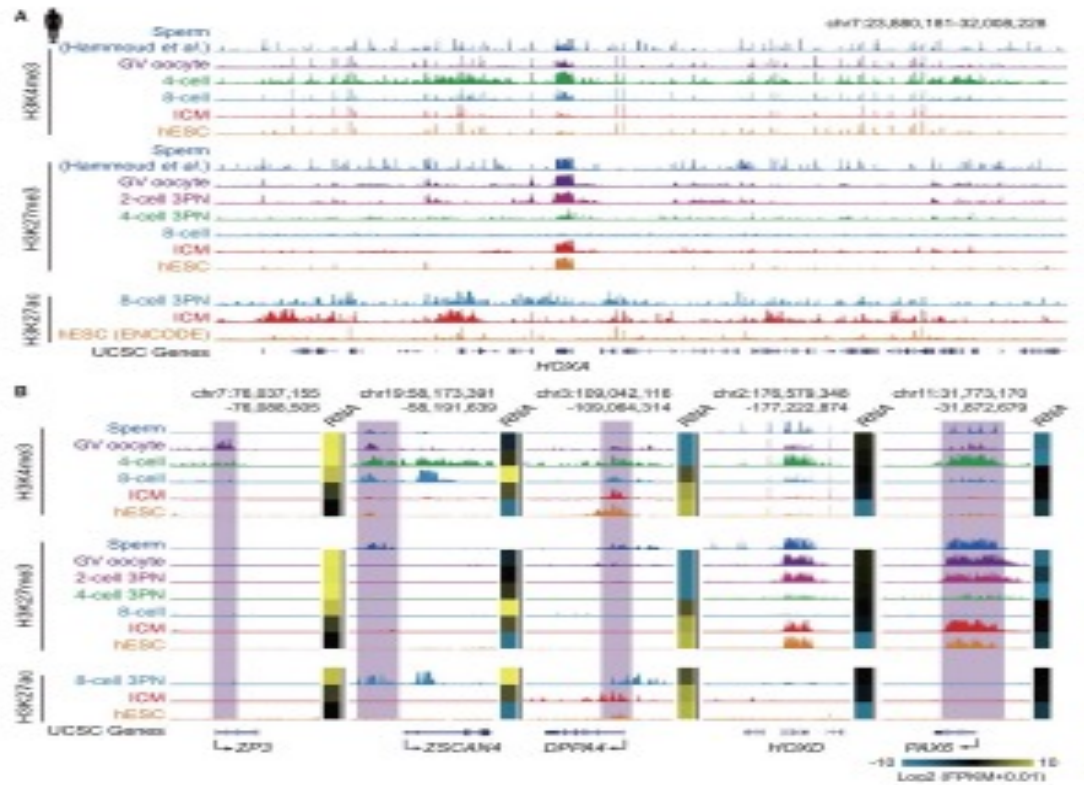
Activating: e.g. H3K4me3

Silencing: e.g. H3K9me3, H3K27me3

Histone modifications

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

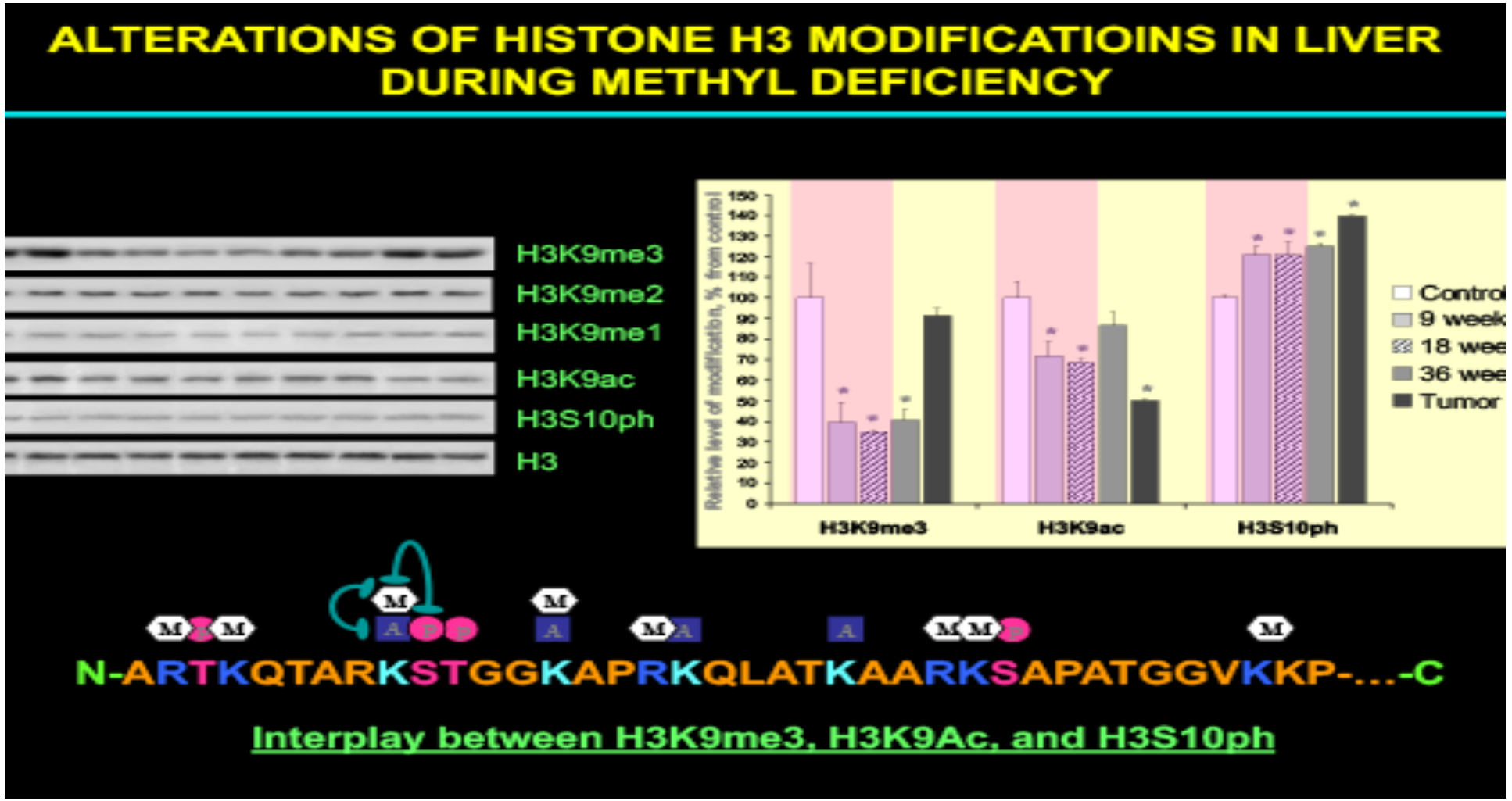
Histone mapping



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

Science
AAAS

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

Abnova

Histone modifications

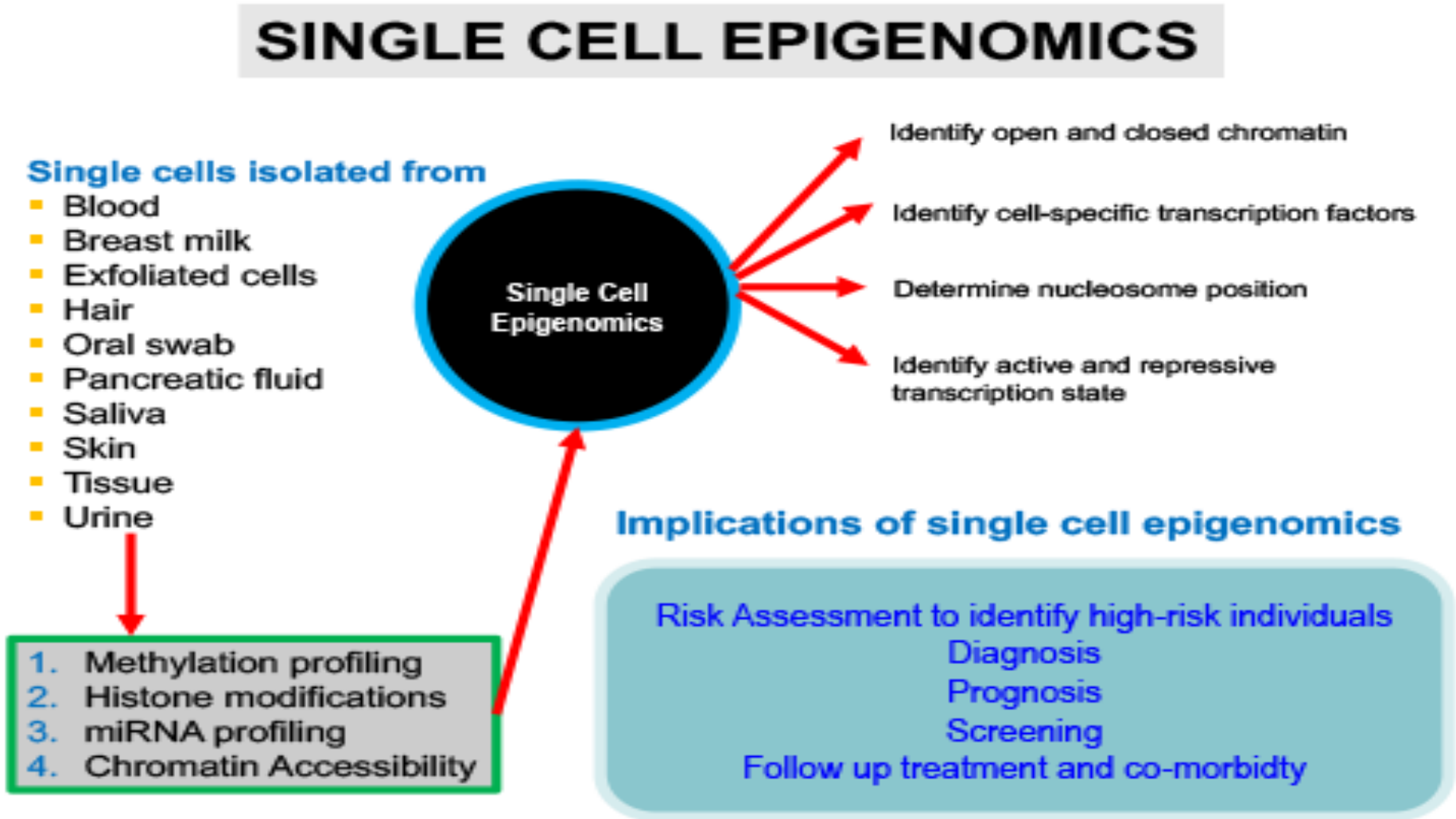
20 Diagnosing Cancer Using Histone Modification Analysis

Mukesh Verma and Deepak Kumar

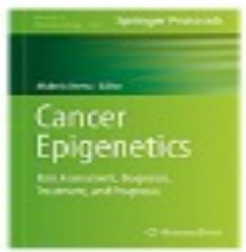
CONTENTS

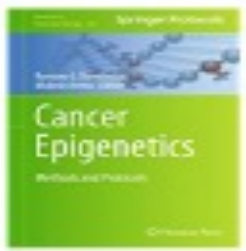
ISBN 9781420045796 - CAT# 45792

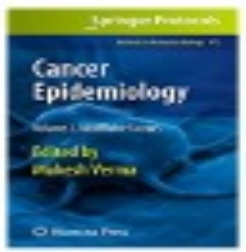
Single cell epigenomics

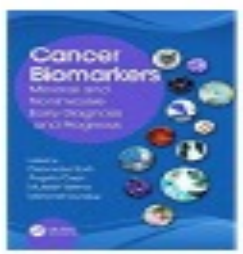


Books



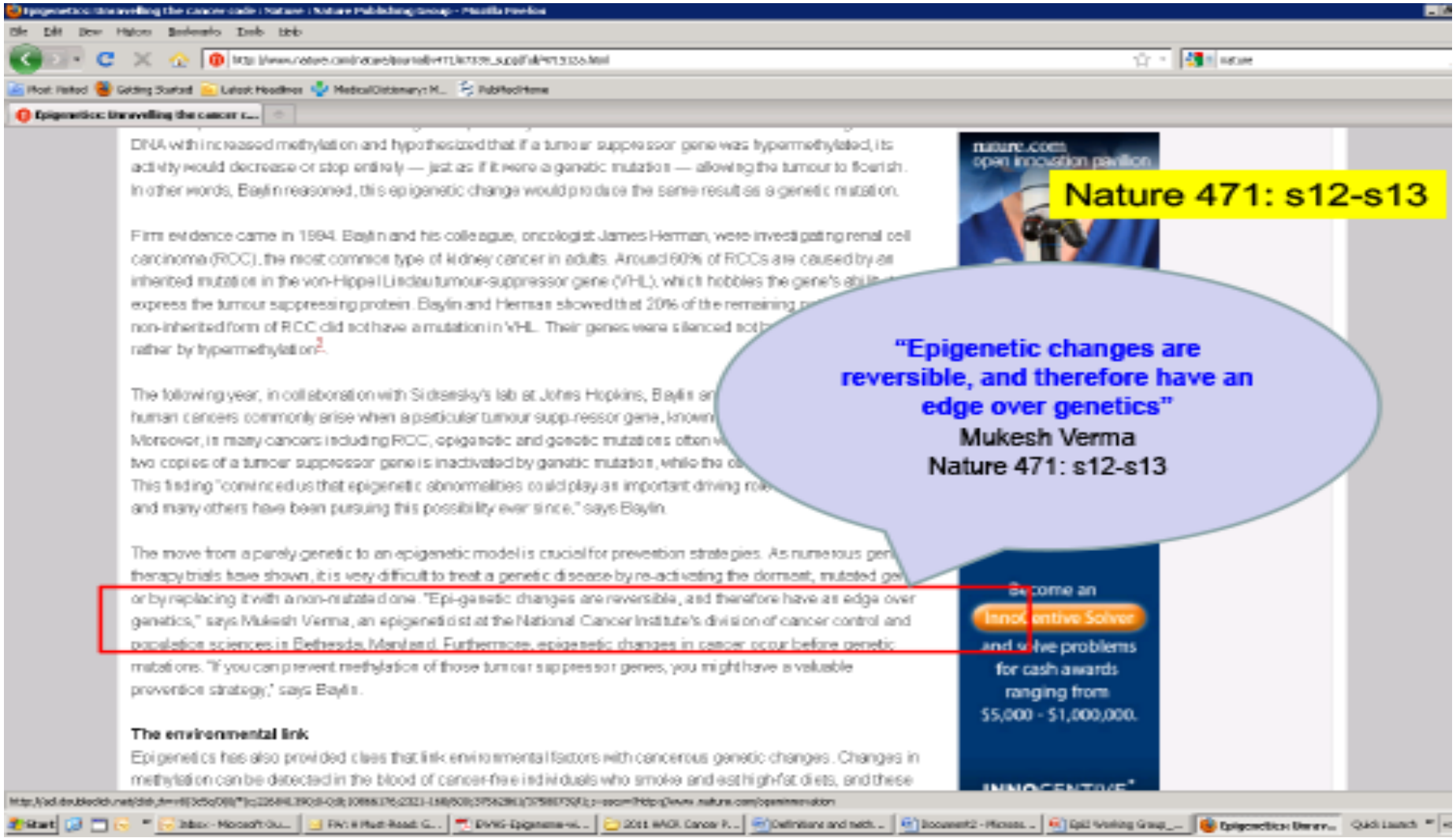




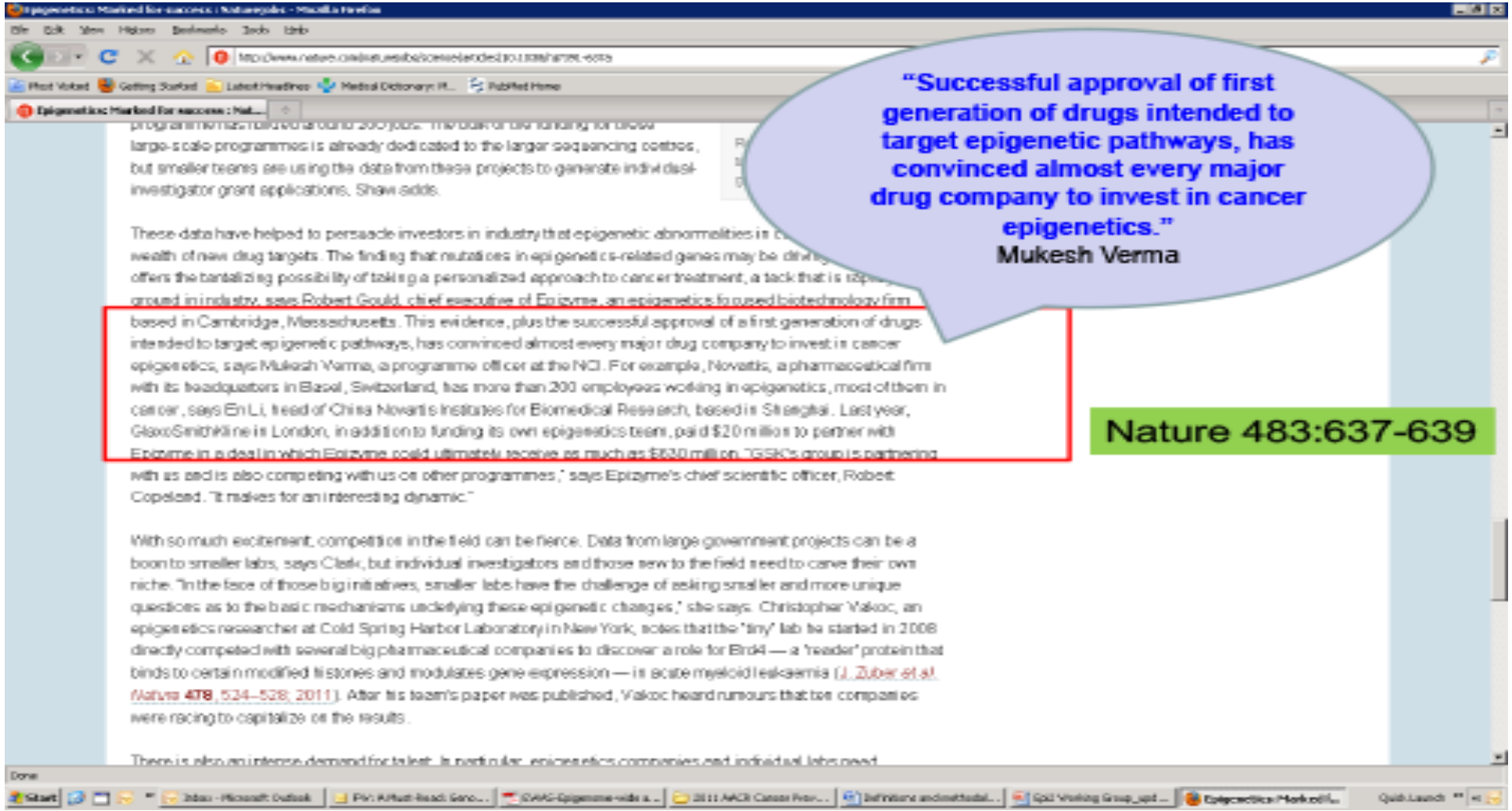


Books edited by Mukesh Verma

Epigenetic changes



Epigenetic drugs



Tumors and epigenetics

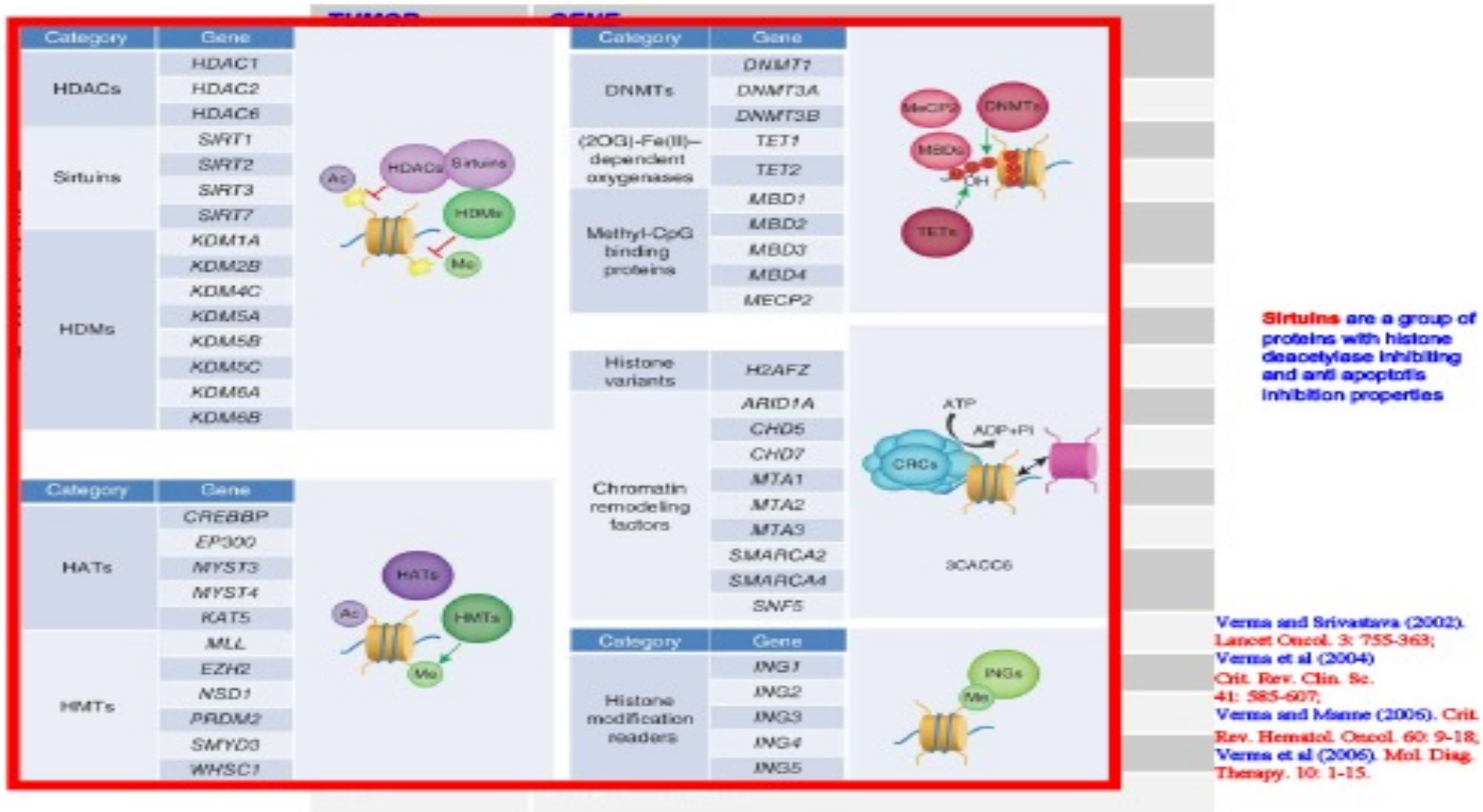
Tumor Types and Genes Regulated by Epigenetic Mechanism

TUMOR LOCATION	*GENE*
Breast	p16, BRCA1, GSTP1, DAPK, CDH1, TIMP-3
Brain	p16, p14^{ARF}, MGMT, TIMP-3
Bladder	p16, DAPK, APC
Colon	p16, p14^{ARF}, CREBP1, MGMT, hMLH1, DAPK, TIMP-3, APC
Endometrium	hMLH1
Esophagus	p16, p14^{ARF}, GSTP1, CDH1, APC
Head and Neck	p16, MGMT, DAPK
Kidney	p16, p14^{ARF}, MGMT, GSTP1, TIMP-3, APC
Leukemia	p15, MGMT, DAPK1, CDH1, p73
Liver	p16, CREBP1, GSTP1, APC
Lymphoma	p16, p15, CREBP1, MGMT, DAPK, p73
Lung	p16, p14^{ARF}, CREBP1, 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

Silencing of a group of promoters with Histone deacetylase inhibitors and demethylating inhibitor properties

Verma and Srivastava (2002) Lancet Oncol 3: 755-763
Verma et al (2004) Curr Rev Clin Sci 41: 585-607
Verma and Manne (2006) Curr Rev Hematol Oncol 06:9-18
Verma et al (2006) Mol Diag Therapy 10:1-15

Histone enzymes



Sirtuins are a group of proteins with histone deacetylase inhibiting and anti apoptotic 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

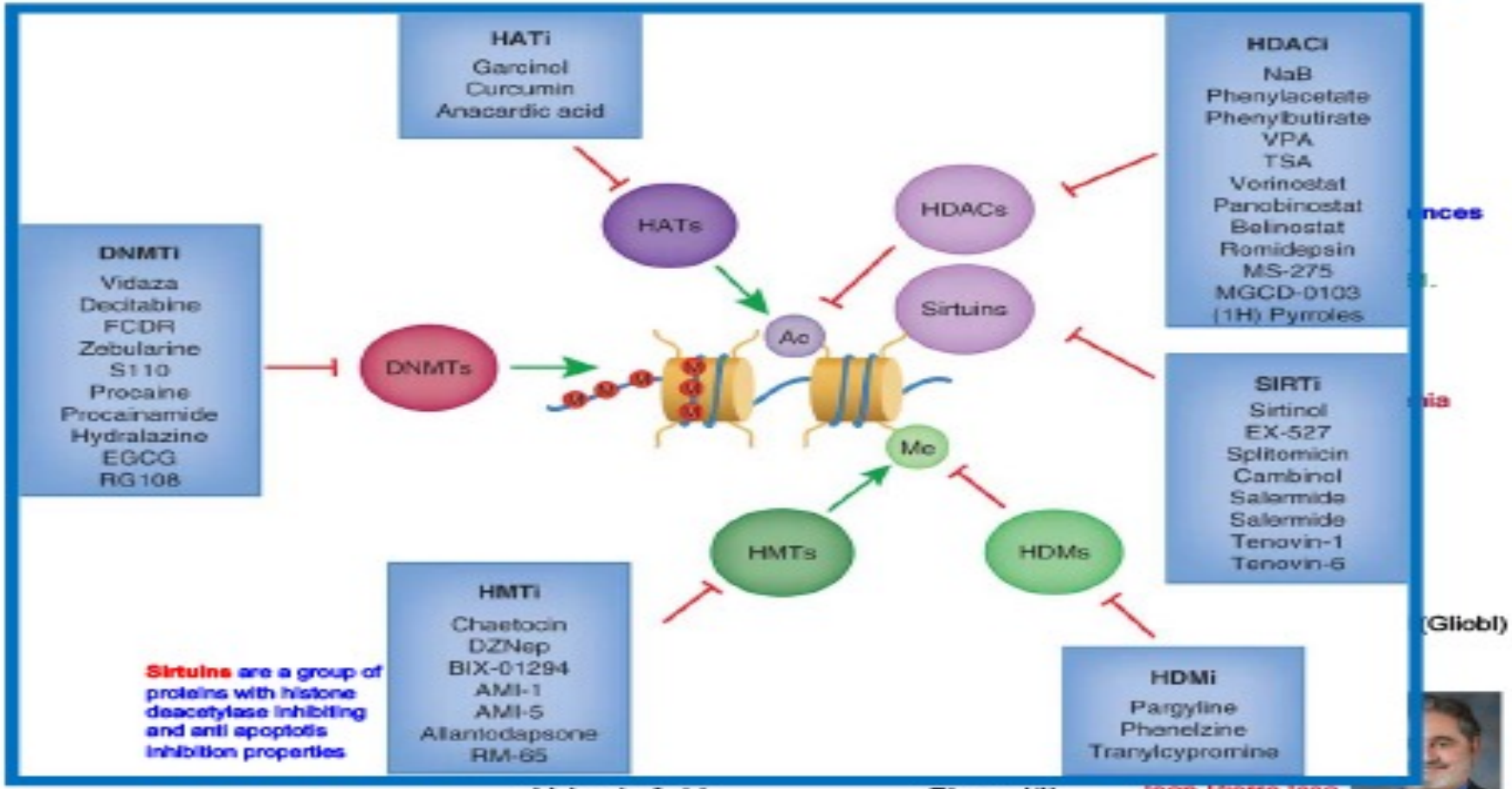
Target	Drug	Clinical Trial
DNA Methylation	5-Azacytidine	Phase I/II/III
	5-Aza-2'deoxycytidine	Phase I/II/III
	FCDR	
	Zebularine	
	Procainamide	
	EGCG	Phase I
	Psamaplin A	
	Antisense Oligomers	Phase I
Histone deacetylase	Phenylbutyric acid	Phase I/II
Sirtuins [illegible]	SAHA (Suberoylanilide hydroxamic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

Adverse Experiences
SAHA
[illegible] 2007.
[illegible] 109:31.

- [illegible]
- [illegible]
- [illegible]
- [illegible]
- [illegible]

[illegible]

Methylation and acetylation enzymes



Histone deacetylase inhibitors

Table 4. Classification of Histone Deacetylase Inhibitors

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

Current Medicinal Chemistry, 2006, *13*, 2909-2919

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

Phase I study

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

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)

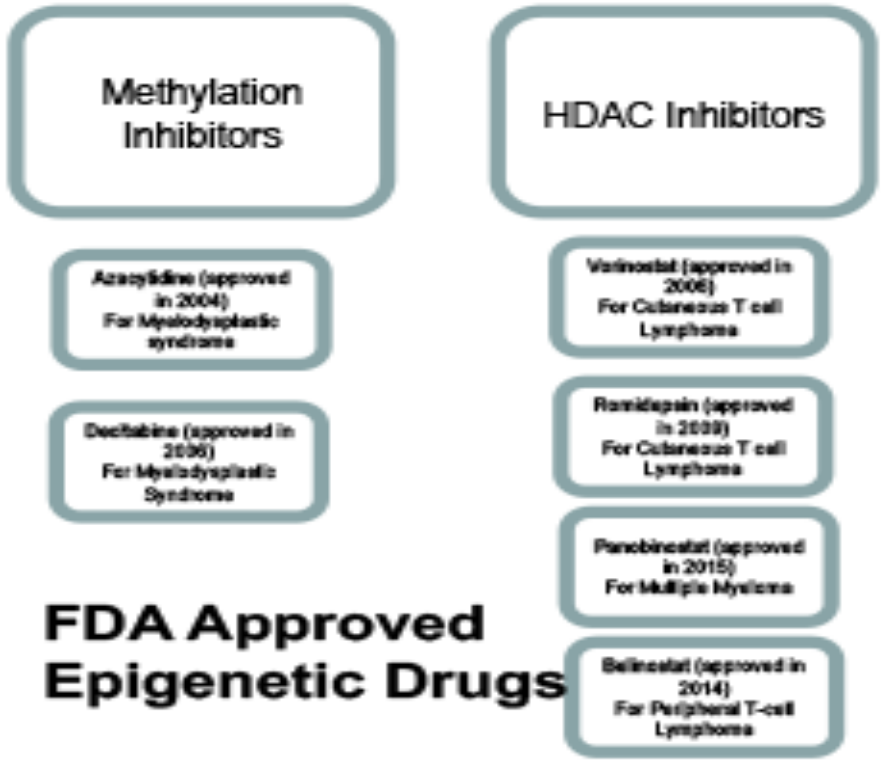
STATUS	STUDY
Completed	A Phase II Study of Epigenetic Therapy to Overcome Chemotherapy Resistance in Refractory Solid Tumors
Active Not Recruiting	Azacytidine and Valproic Acid in Patients With Advanced Cancers
Recruiting	Azacitidine With or Without MS-275 in Treating Patients With Myelodysplastic Syndromes,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

Total : 51 studies

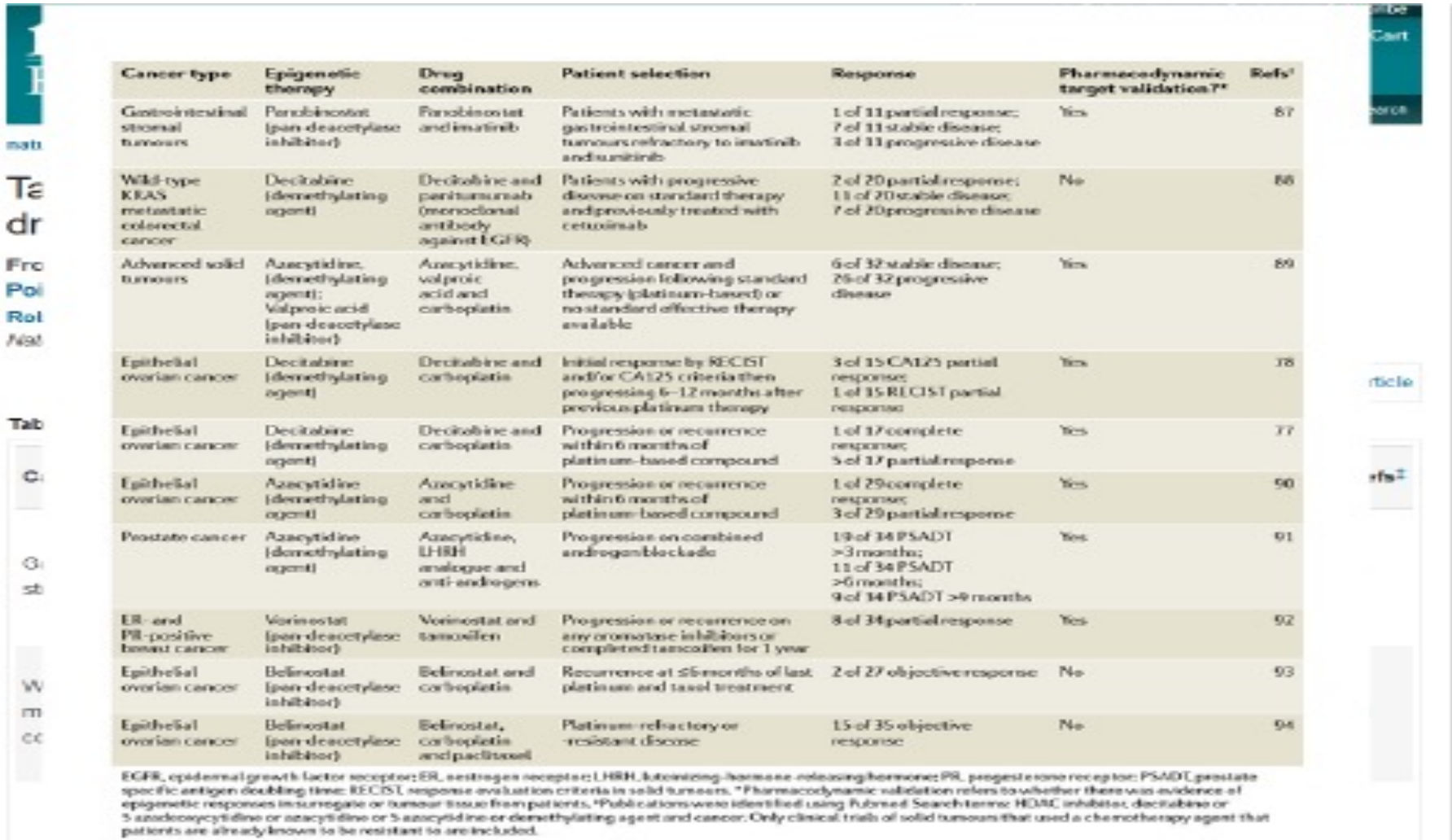
http://clinicaltrials.gov/ct2/results?term=methylation+inhibitors

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

Approved epigenetic drugs



Epigenetic drugs



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
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. ‡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

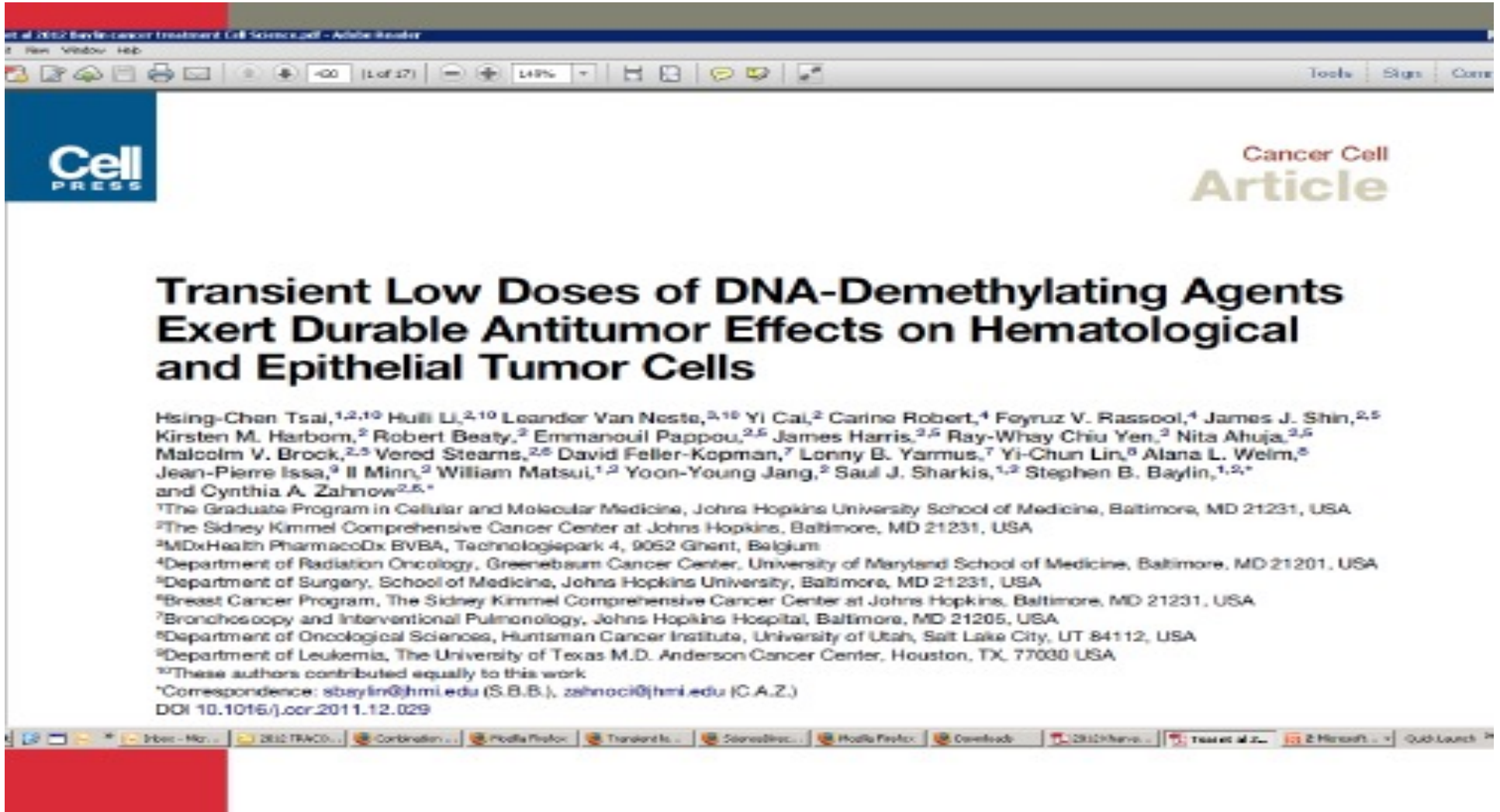
Pharmaceutical Participation

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] Hui 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[2,5,*]

[1]The Graduate Program in Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21231, USA
[2]The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231, USA
[3]MDxHealth PharmacoDx BVBA, Technologiepark 4, 9052 Ghent, Belgium
[4]Department of Radiation Oncology, Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA
[5]Department of Surgery, School of Medicine, Johns Hopkins University, Baltimore, MD 21231, USA
[6]Breast Cancer Program, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231, USA
[7]Bronchoscopy and Interventional Pulmonology, Johns Hopkins Hospital, Baltimore, MD 21205, USA
[8]Department of Oncological Sciences, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT 84112, USA
[9]Department of Leukemia, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030 USA
[10]These authors contributed equally to this work
*Correspondence: sbaylin@jhmi.edu (S.B.B.), zahnoci@jhmi.edu (C.A.Z.)
DOI 10.1016/j.ccr.2011.12.029

Reprograming and editing

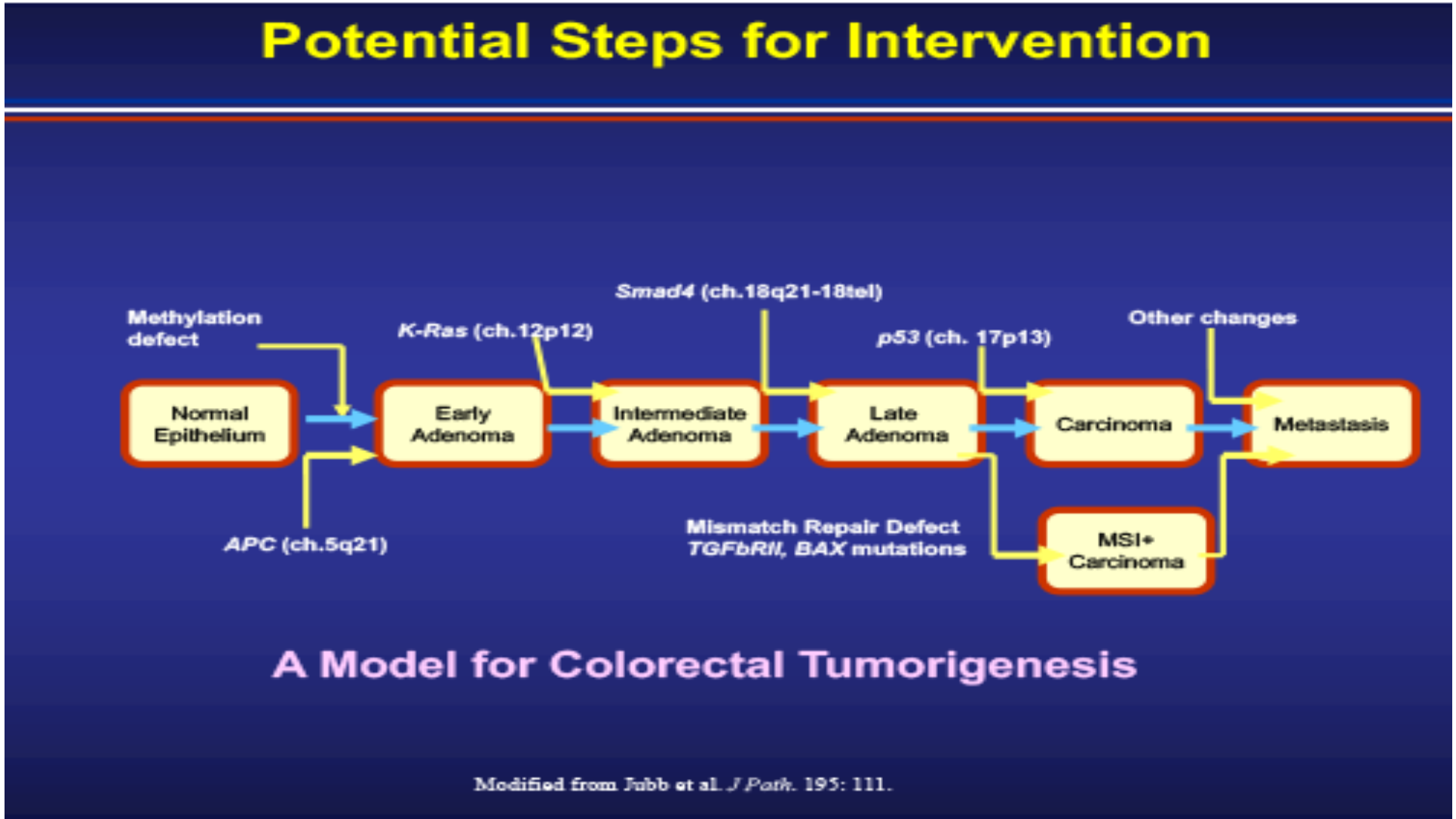
Epigenome Reprogramming and Epigenetic Editing

Table. Selected epigenomic editing companies

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

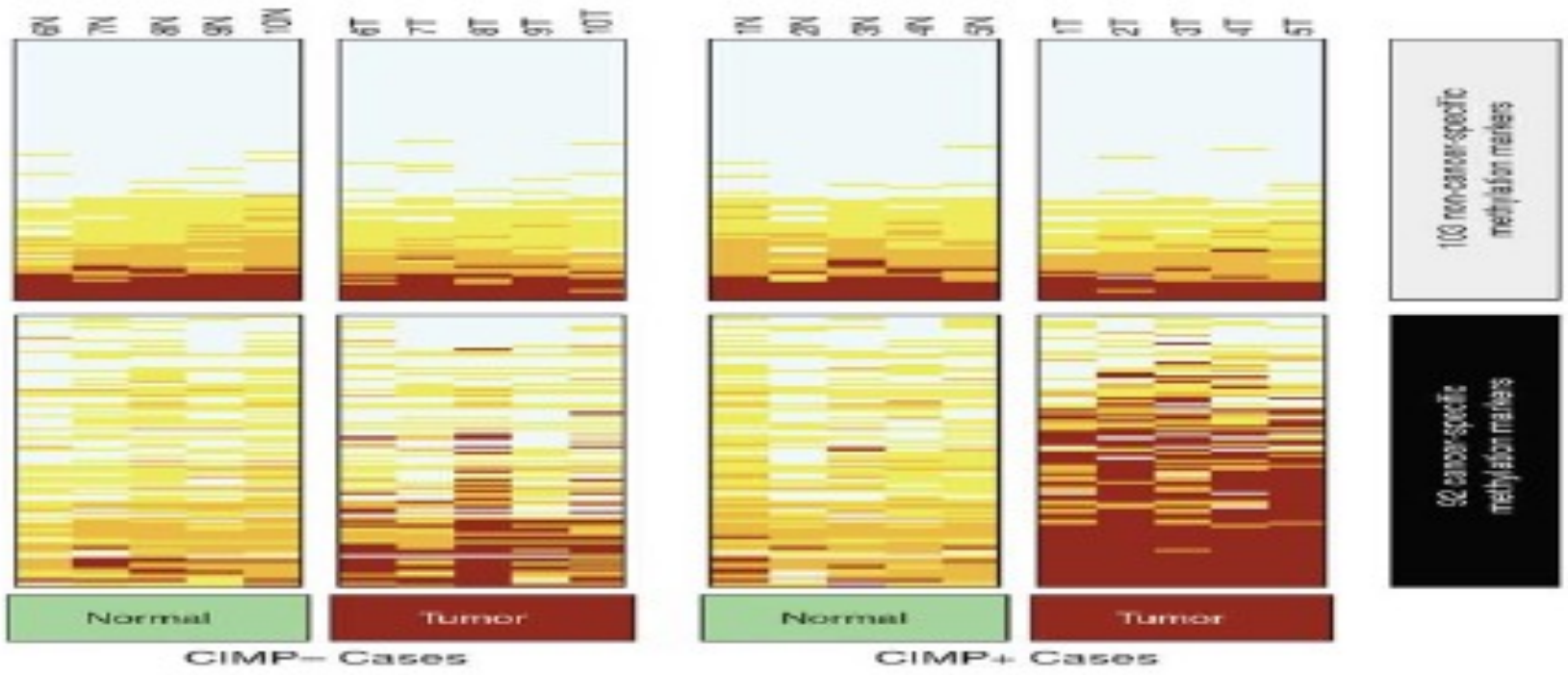
[a]Named EdiGene when founded in 2016 and renamed Modalis Therapeutics in 2019.
[b]Oral abstract session or scientific symposium.

Intervention



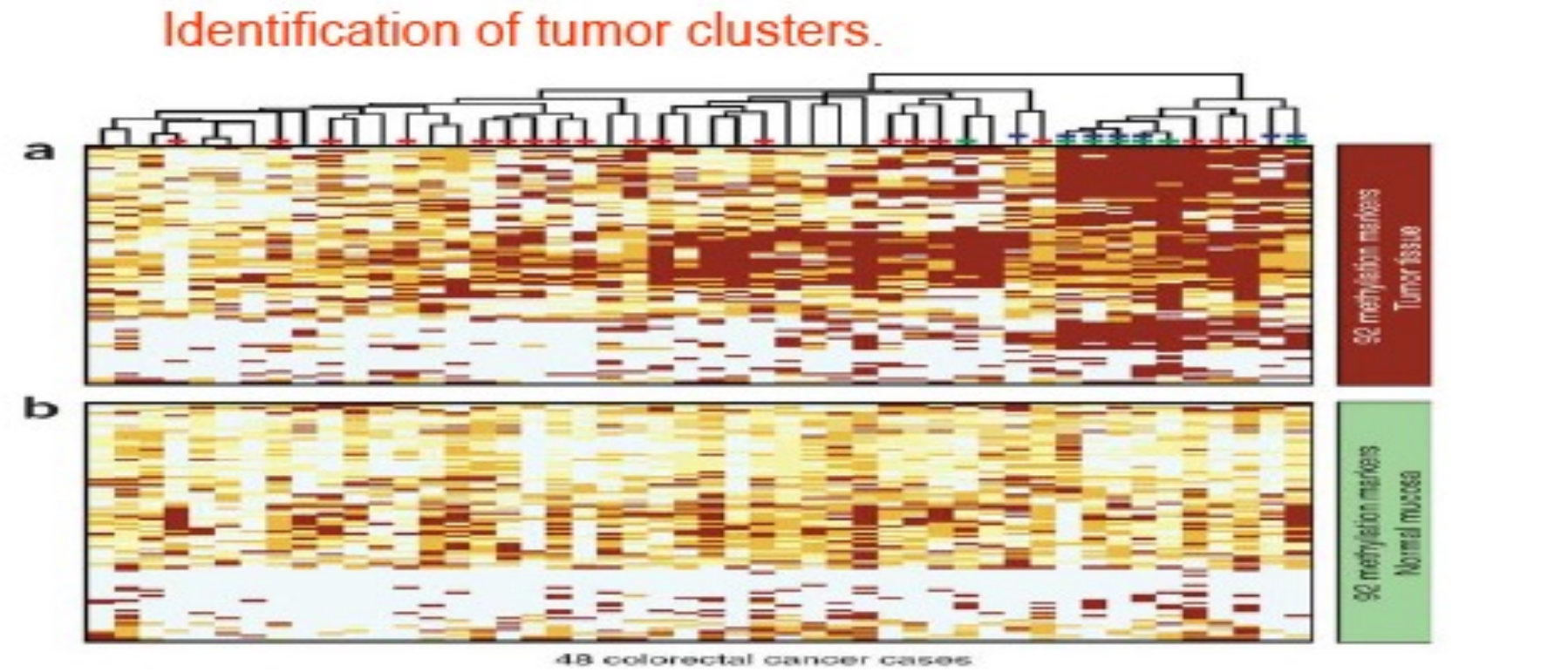
Microsatellite instability

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

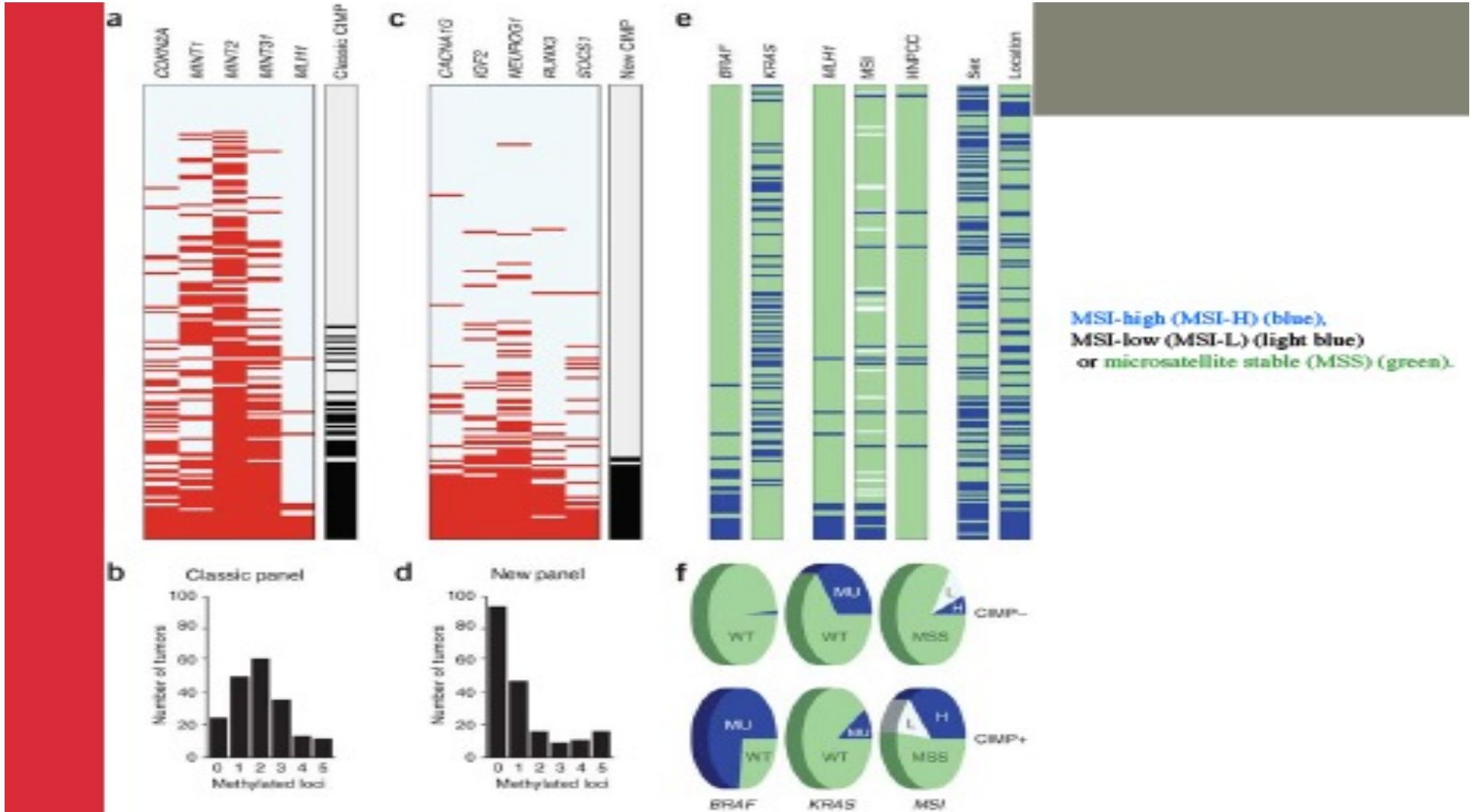


KRAS mutation indicated by a red rectangle overlaying the branch,
BRAF mutations indicated by a green rectangle
MSI-H cases designated with a blue rectangle.

48 Colorectal tumors

Nature Genetics **38**, 787 - 793

Genetic analysis



CRC markers

Validated CRC Markers in Clinical Trials

Table 1:

Validated DNA-methylation biomarkers for colorectal cancer

Clinical Use	Biomarkers	Available Commercial Assays	Study Design of Major Trials with Assay	Ref.
Stool-based CRC screening	mVIM,	ColoSure™	Case (N=42) control [N=241] study	109
	mBMP3 and mNDRG4	Cologuard® [detects mutant *KRAS*, and includes a FIT test]	Prospective cohort based clinical trial in screening population [N=9989]	110
blood-based diagnostic marker	mSEPT9	EpiproColon® 1.0, ColoVantage®; RealTime m99	Multiple trials. 1)Prospective cohort based clinical trial in screening population (N=7941) (Church);2) Case-Control study (N=269) (deVos); 3) Case-Control study [N=312] (Lofton-Day 2008)	125 135,136,139
	mSCATJ mWZFV	Colvera	Cross-sectional study (N=220)	138
Tissue-based prognostic markers	CIMP panel	NA	Multiple trials: 1. Case-Control study from 2 phase I/II clinical trials [N=31](Ogino 2007); 2. Case-Control study from phase 3 clinical trial [N=915] (Shovitz 2014);3) Observational cohort study [N=2060](Phipps 2015)	140, 142, 159, 188
Diagnostic tool to screen for Lynch syndrome	mMLAV	MLH1 Hypermethylation analysis	1. Cross sectional study [N=1066]	67

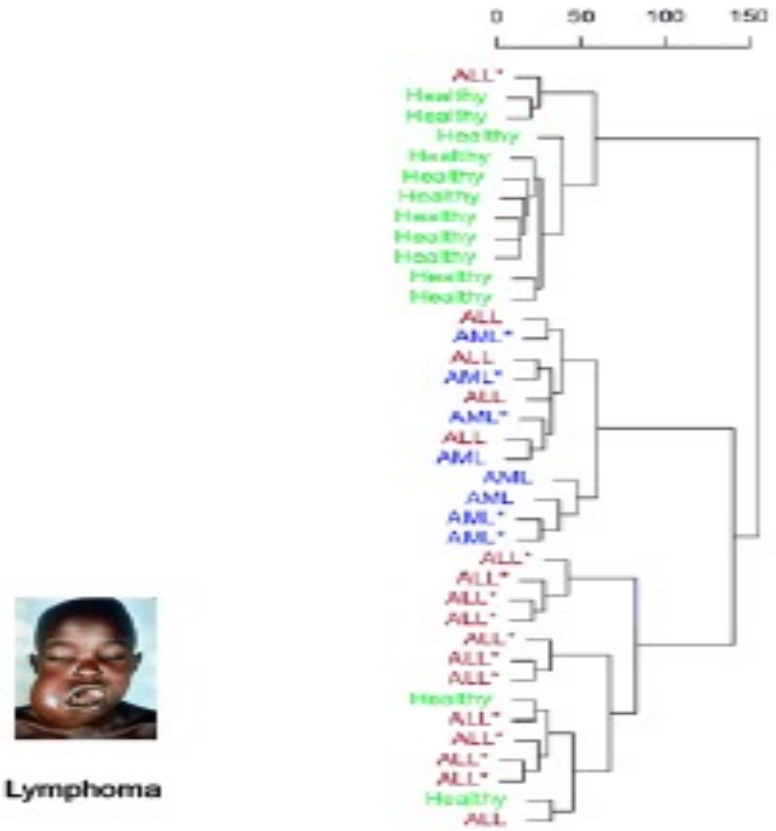
Gastroenterology. 2021 Feb; 160(3): 690–709.

Open in a separate window

Methylation analysis

National Cancer Institute

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

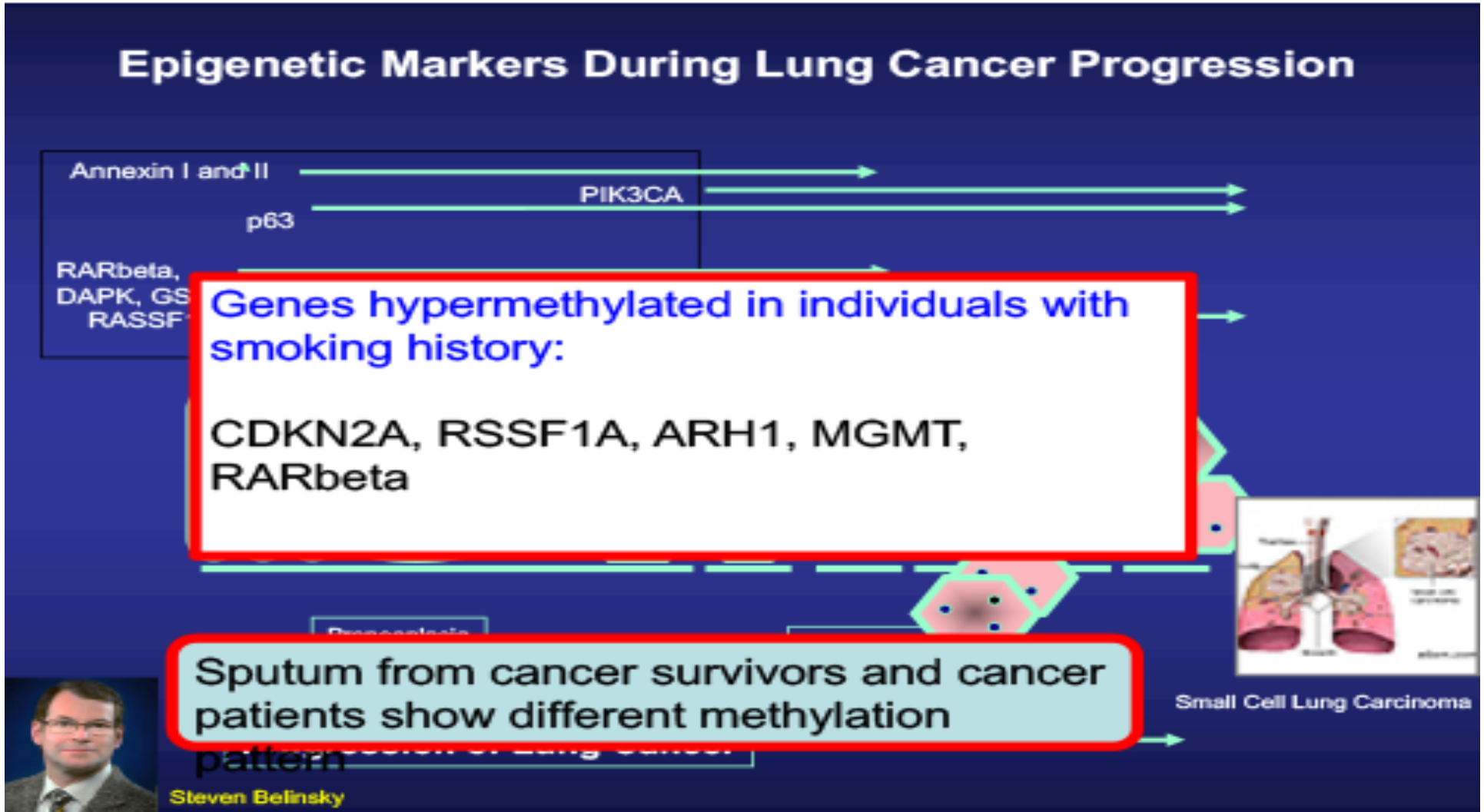


AML:Acute Myeloid Leukemia
ALL: Acute Lymphoblastic Leukemia

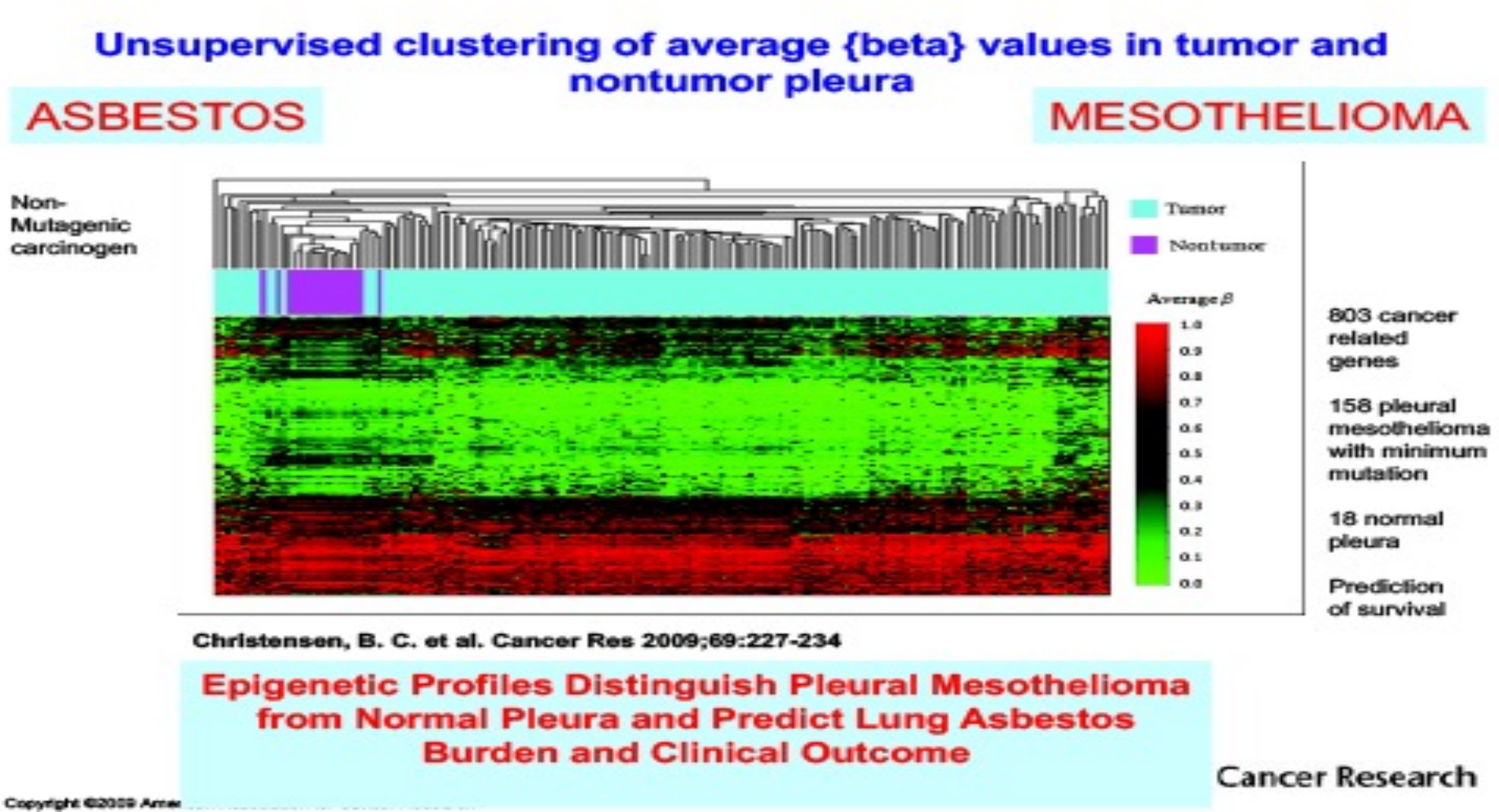
Lymphoma

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

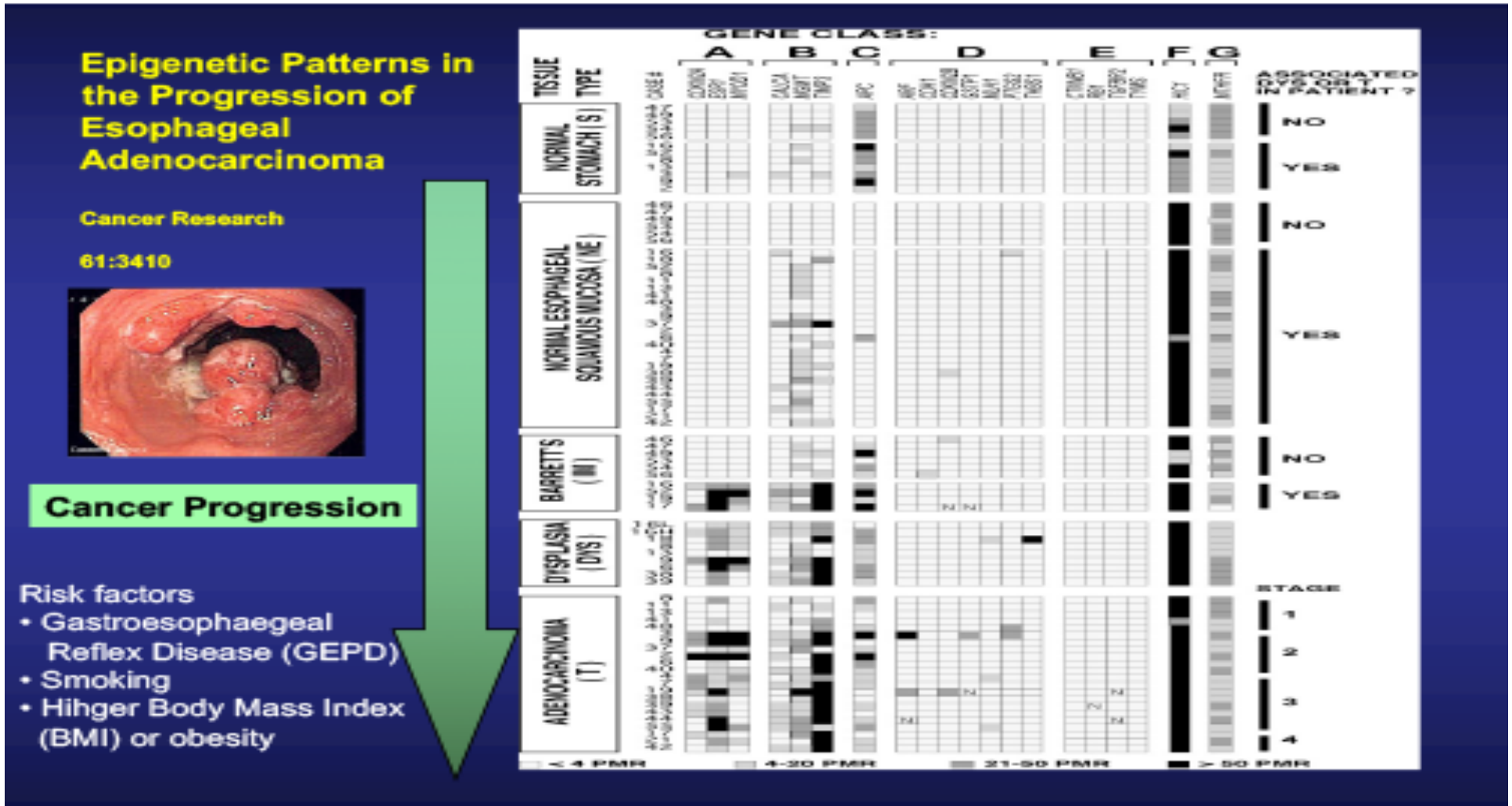
Epigenetic markers



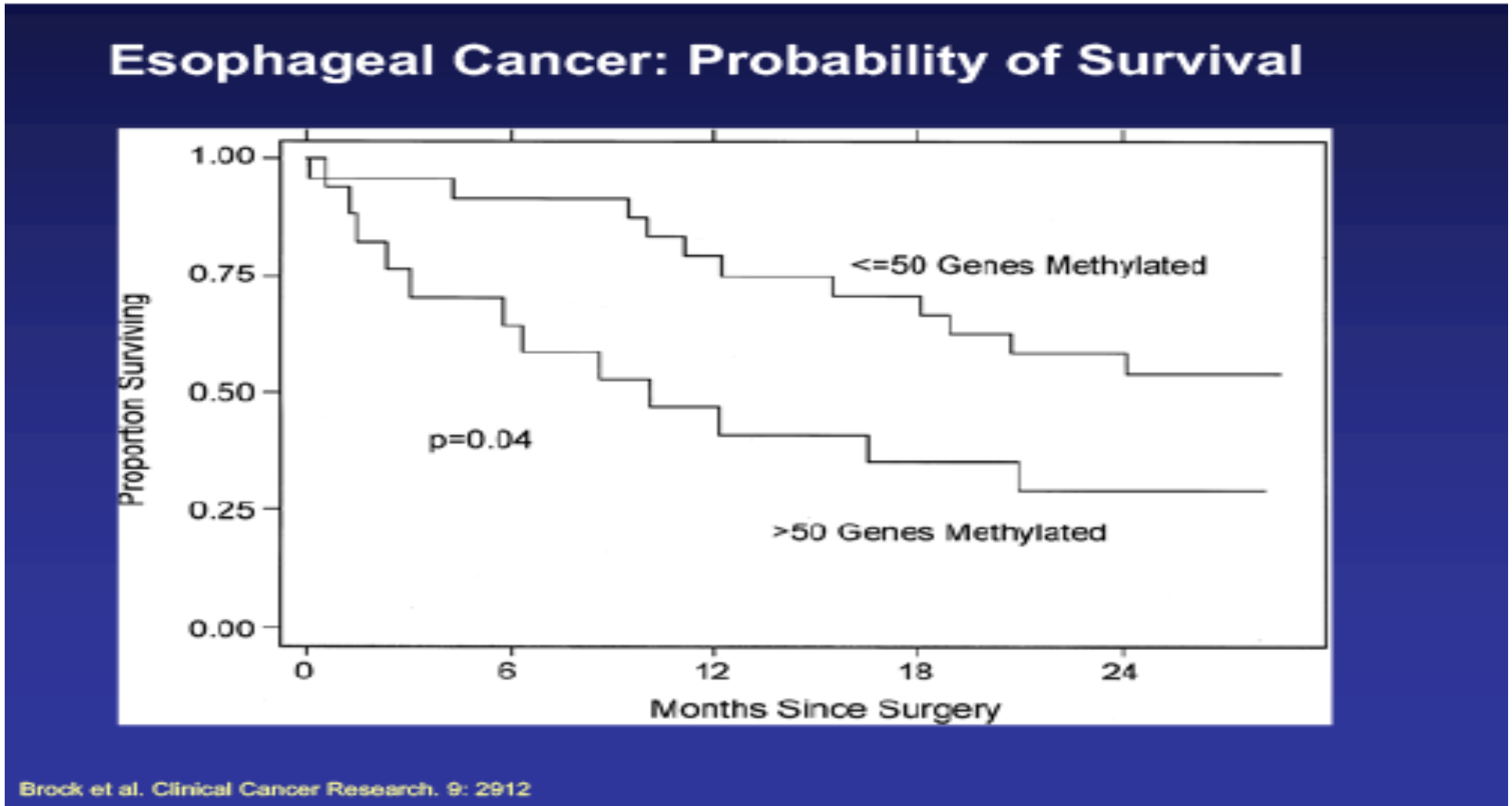
Mesothelioma



Epigenetic pattern



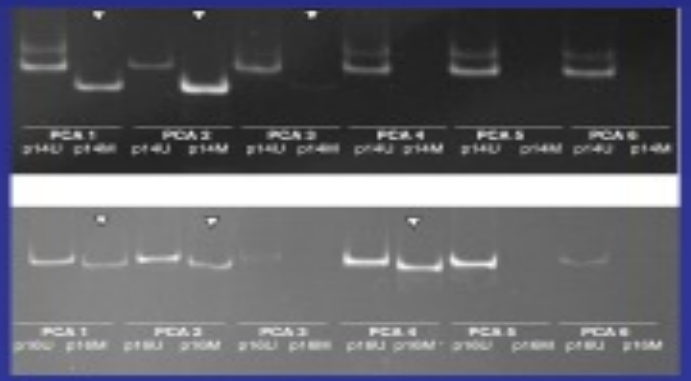
Esophageal cancer

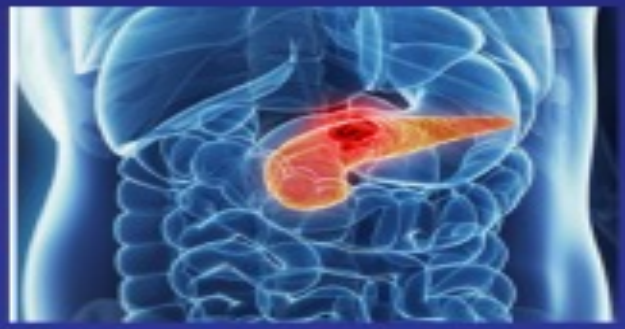


Pancreatic cancer

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



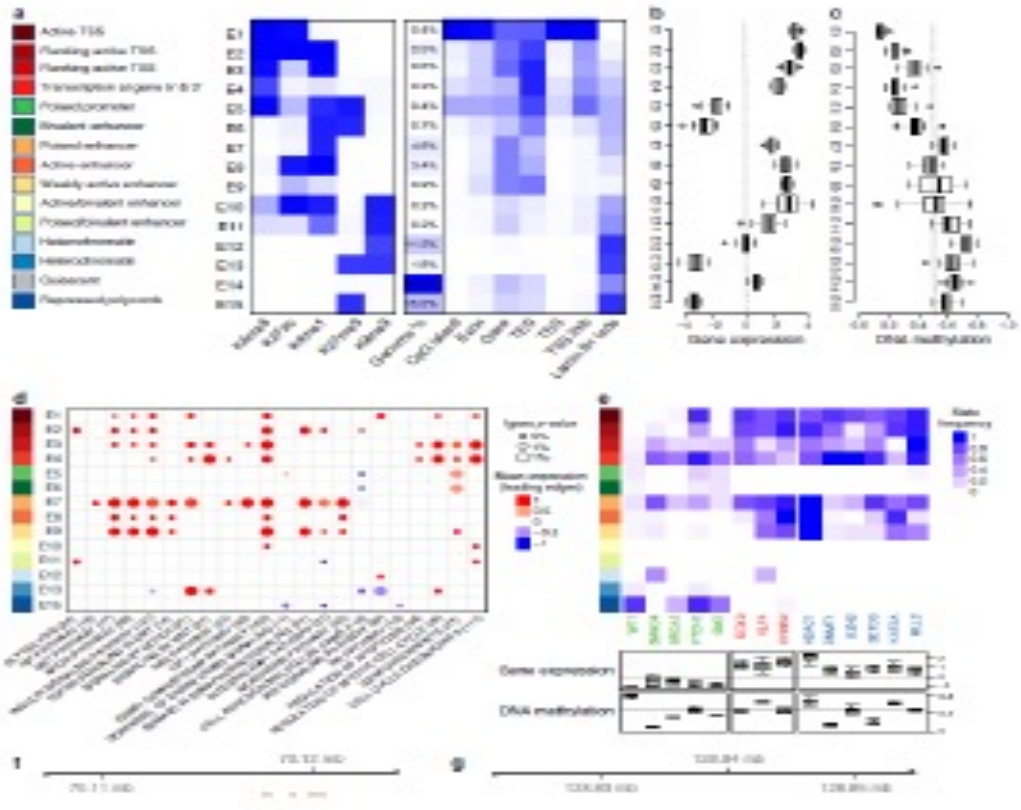


Sample: Pancreatic Fluid

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

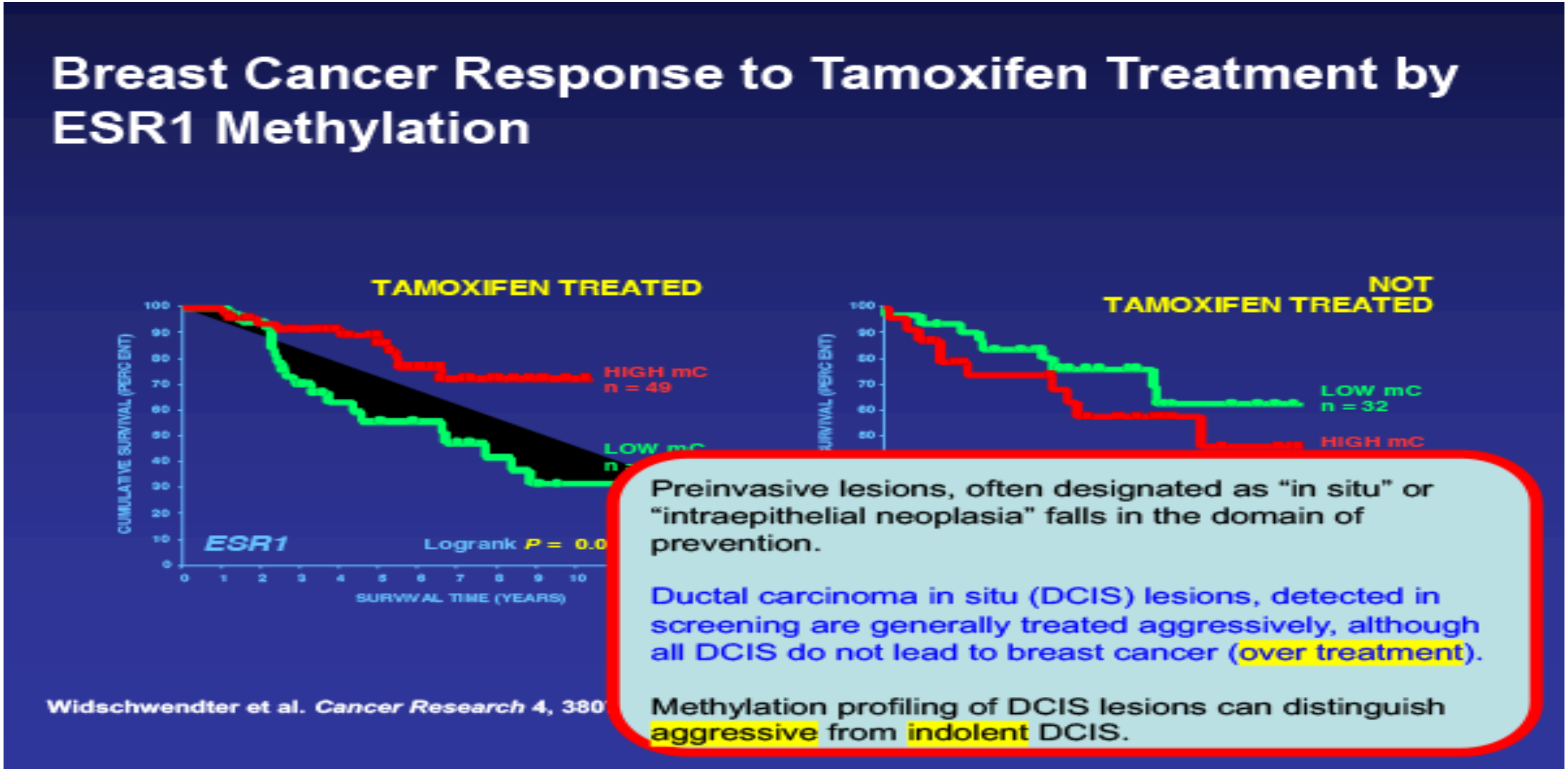
Chromatin states

Distinct chromatin states of human PDAC

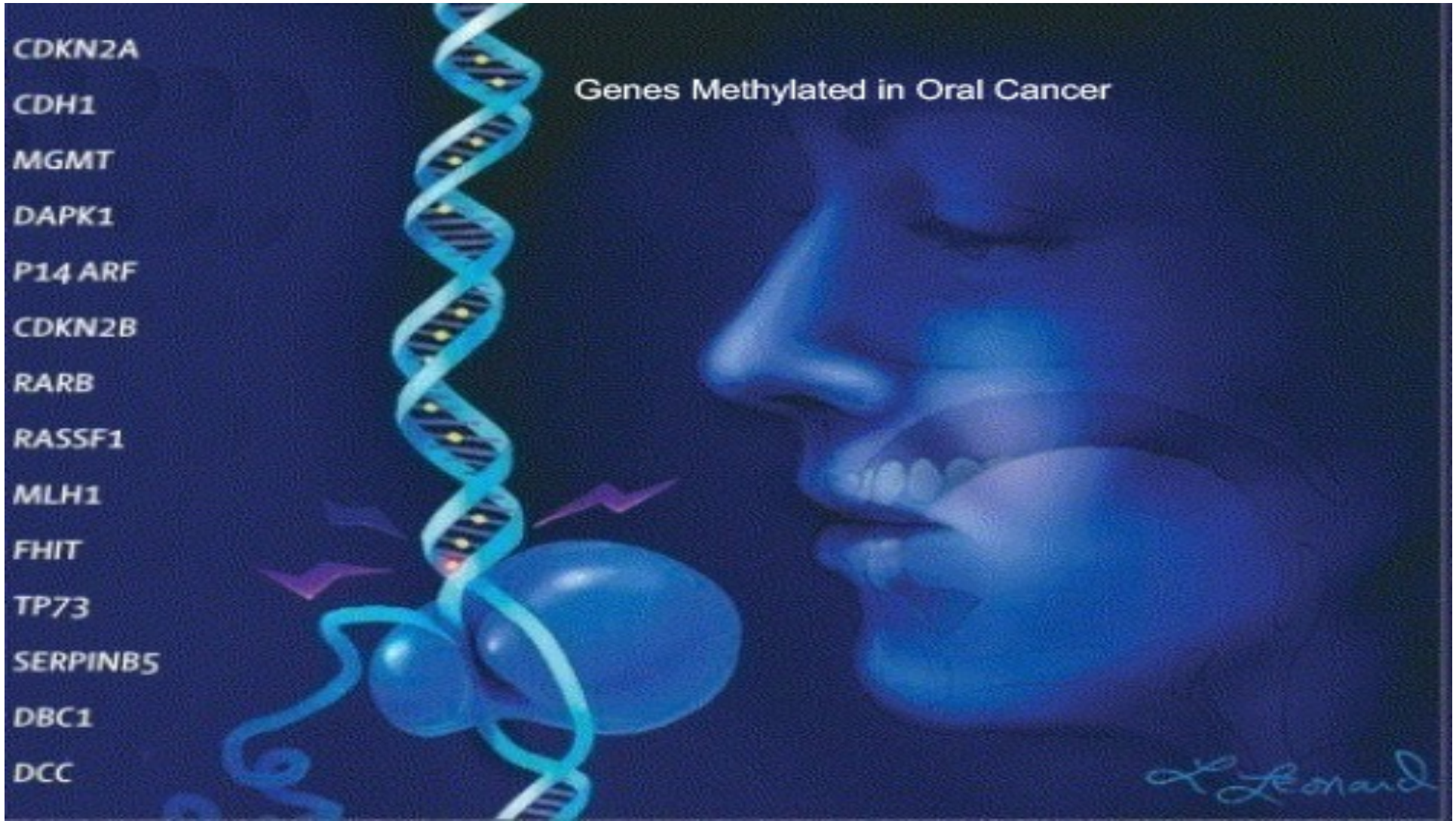


NATURE COMMUNICATIONS | (2018) 9:1978

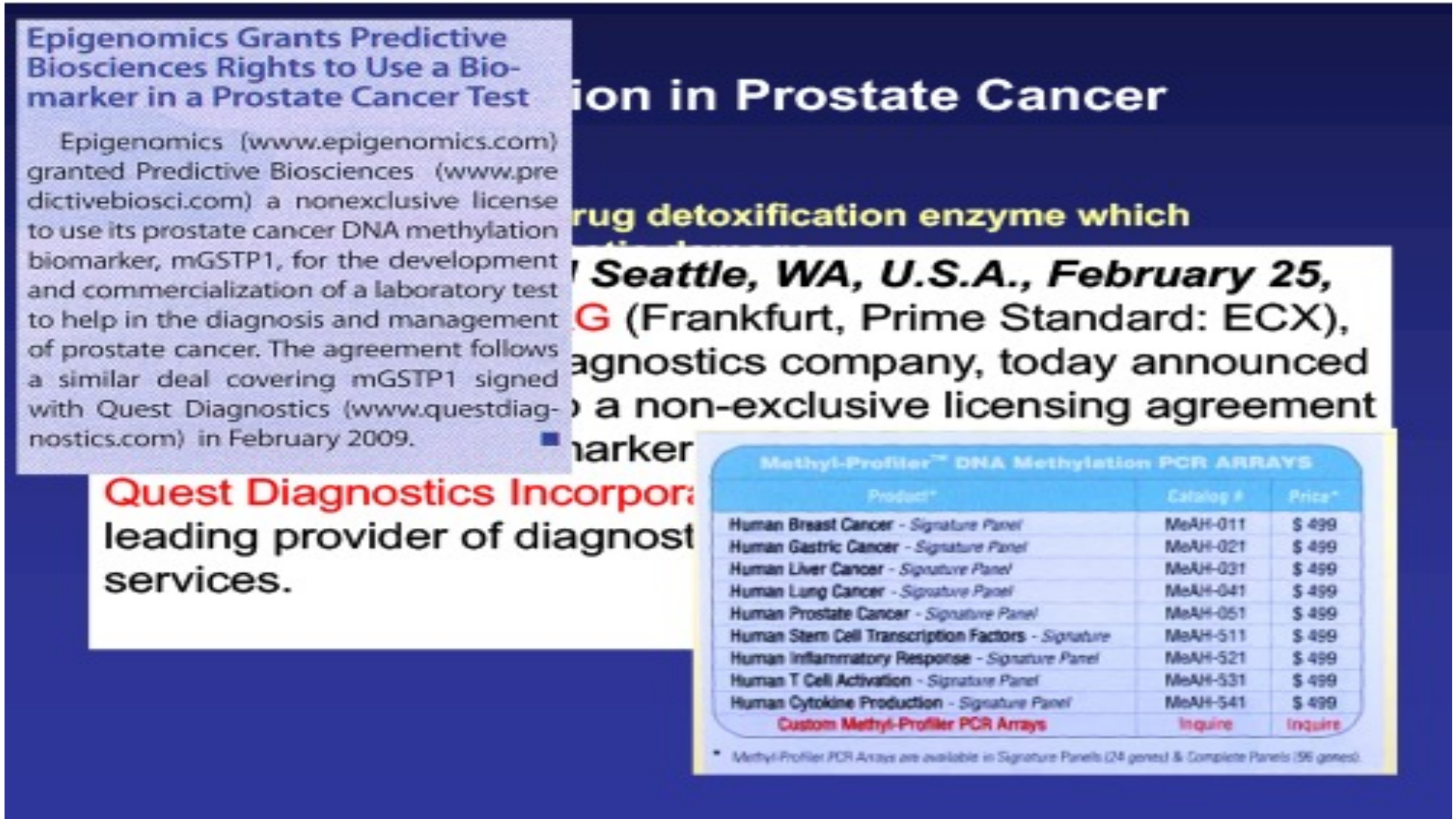
Breast cancer



Methylated genes

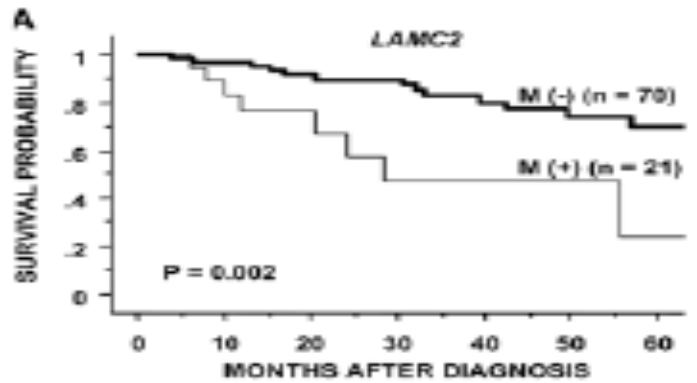


Biomarkers


Epigenomics Grants Predictive Biosciences Rights to Use a Bio-marker 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

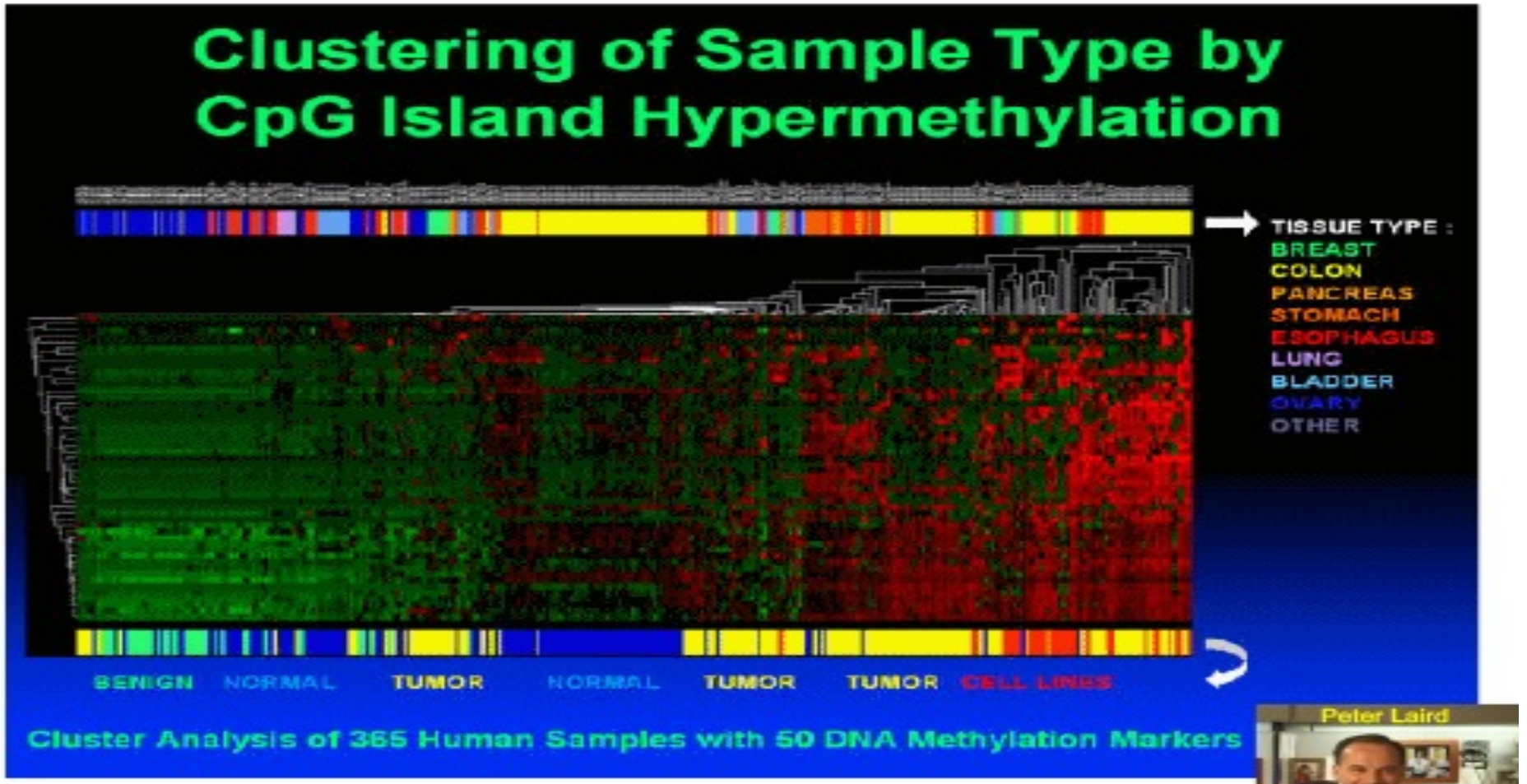


Another Study:
Schistosomes and Bladder Cancer

MI, Methylation Index

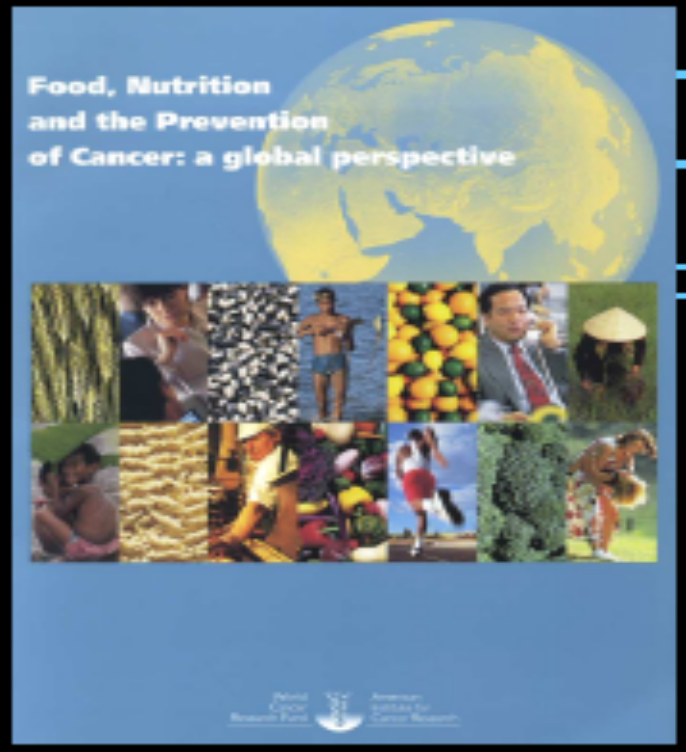
(Sathyanarayana et al. *Can Res* 64: 1425)

CpG island hypermethylation



Diet and cancer

DIET AND CANCER: FOCUS ON PREVENTION



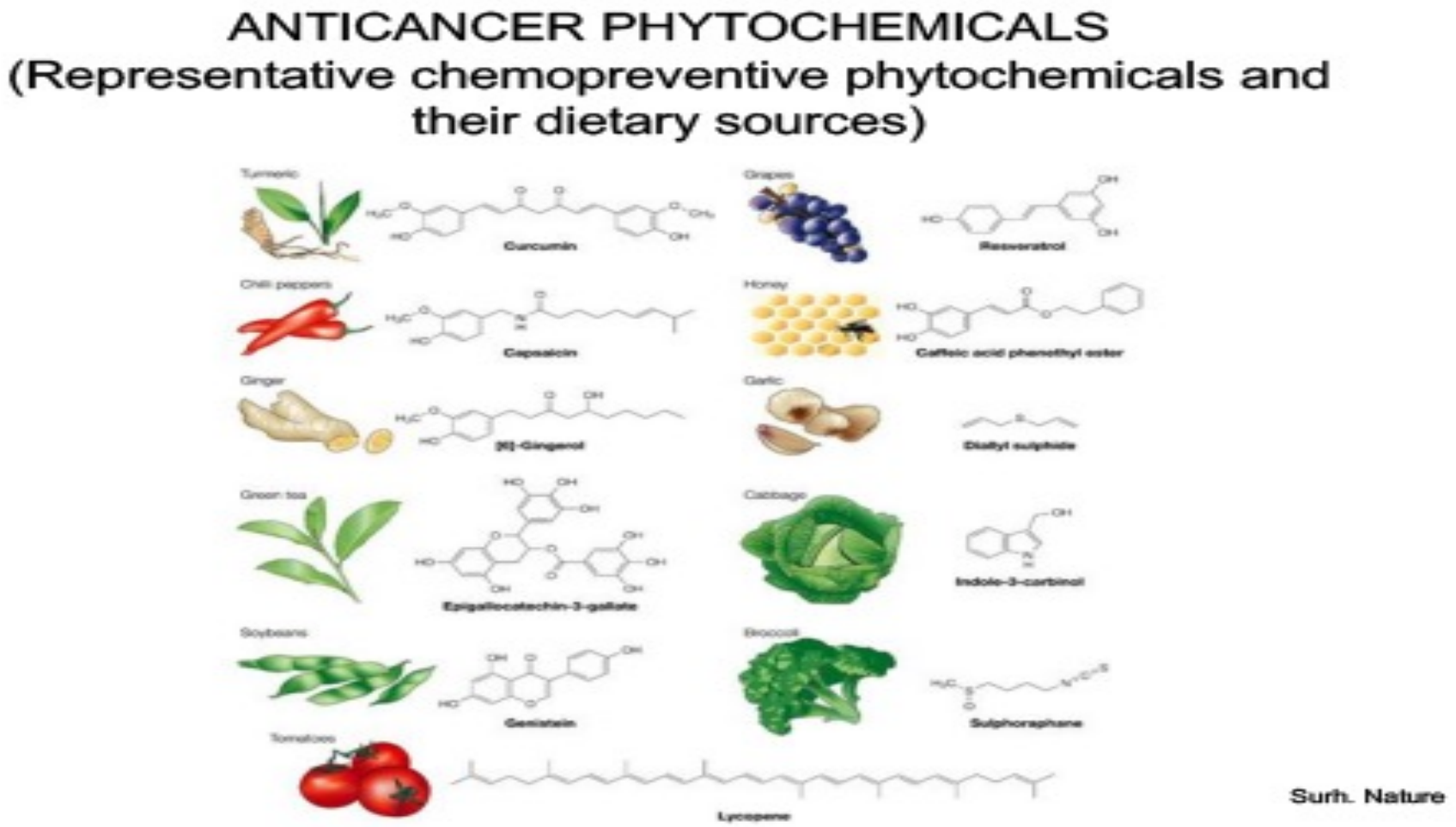
Cancer is principally caused by environmental factors, of which the most important are tobacco, diet and factors related to diet, including body mass and physical activity, and exposures in the workplace and elsewhere.

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

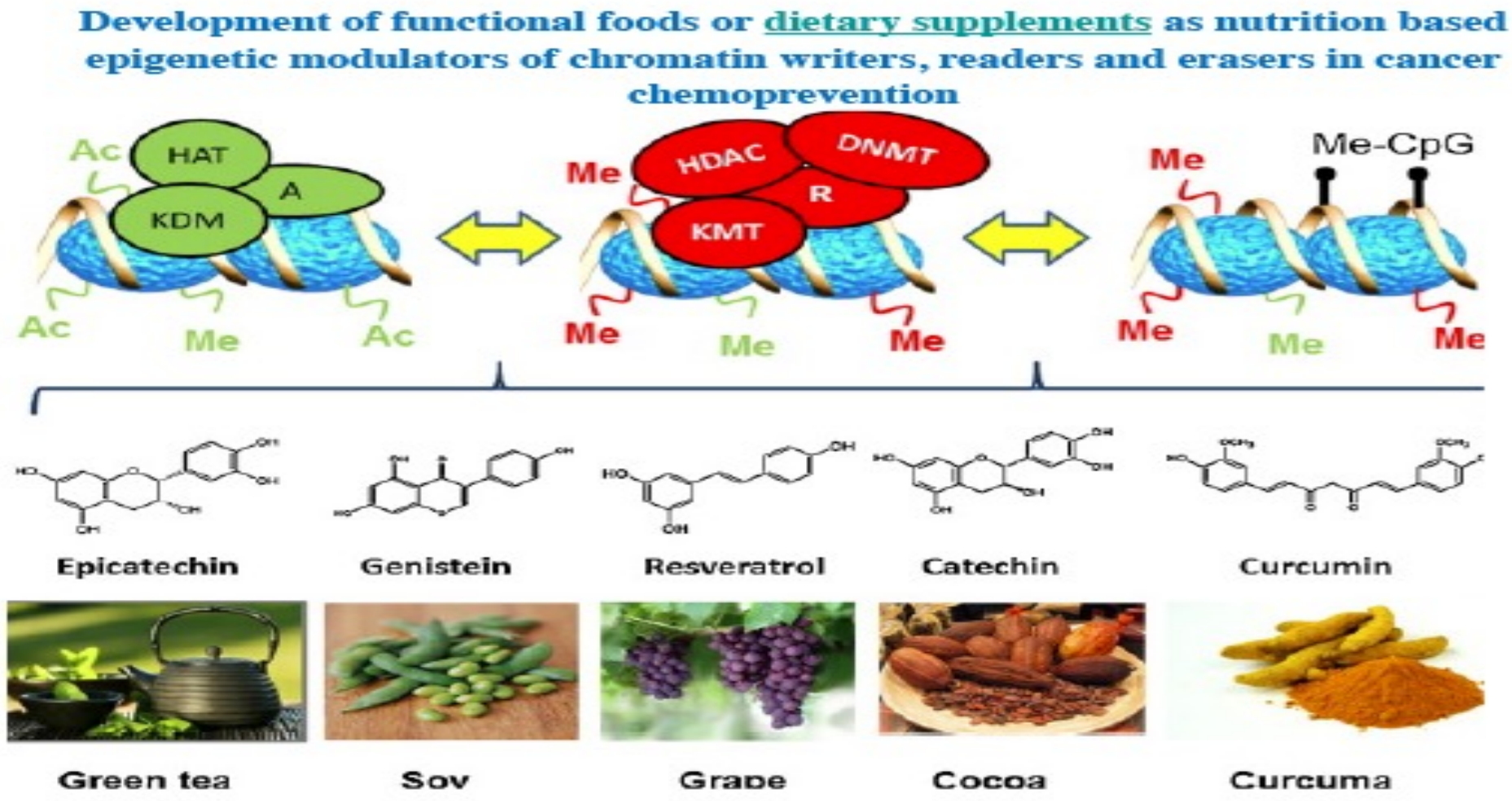
- Understanding the determinants of the earliest detectable phenotypes in initiated cells
- Uncovering the molecular mechanisms of action of dietary nutrients leading to cancer formation and prevention
- Defining effects of dietary compounds not only on cancer cells but on normal and precancerous cells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

Methyl deficiency

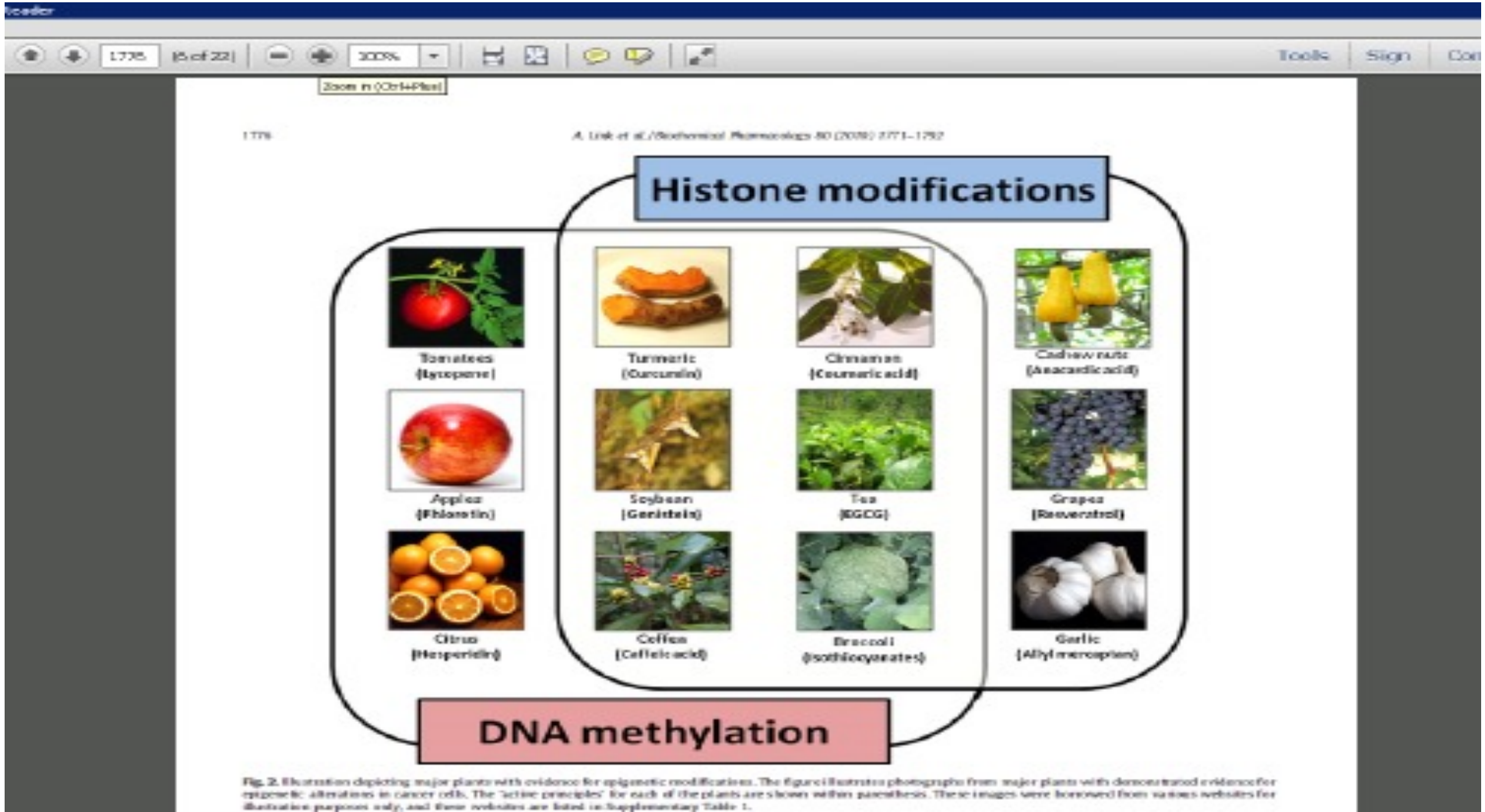
Anticancer phytochemicals



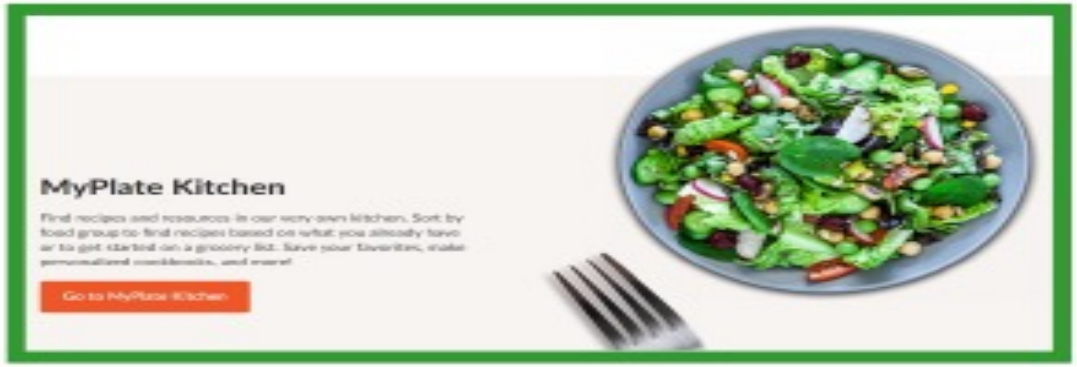
Dietary supplements



Epigenetic foods



Myplate



https://www.myplate.gov/

Mobile food record

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

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?

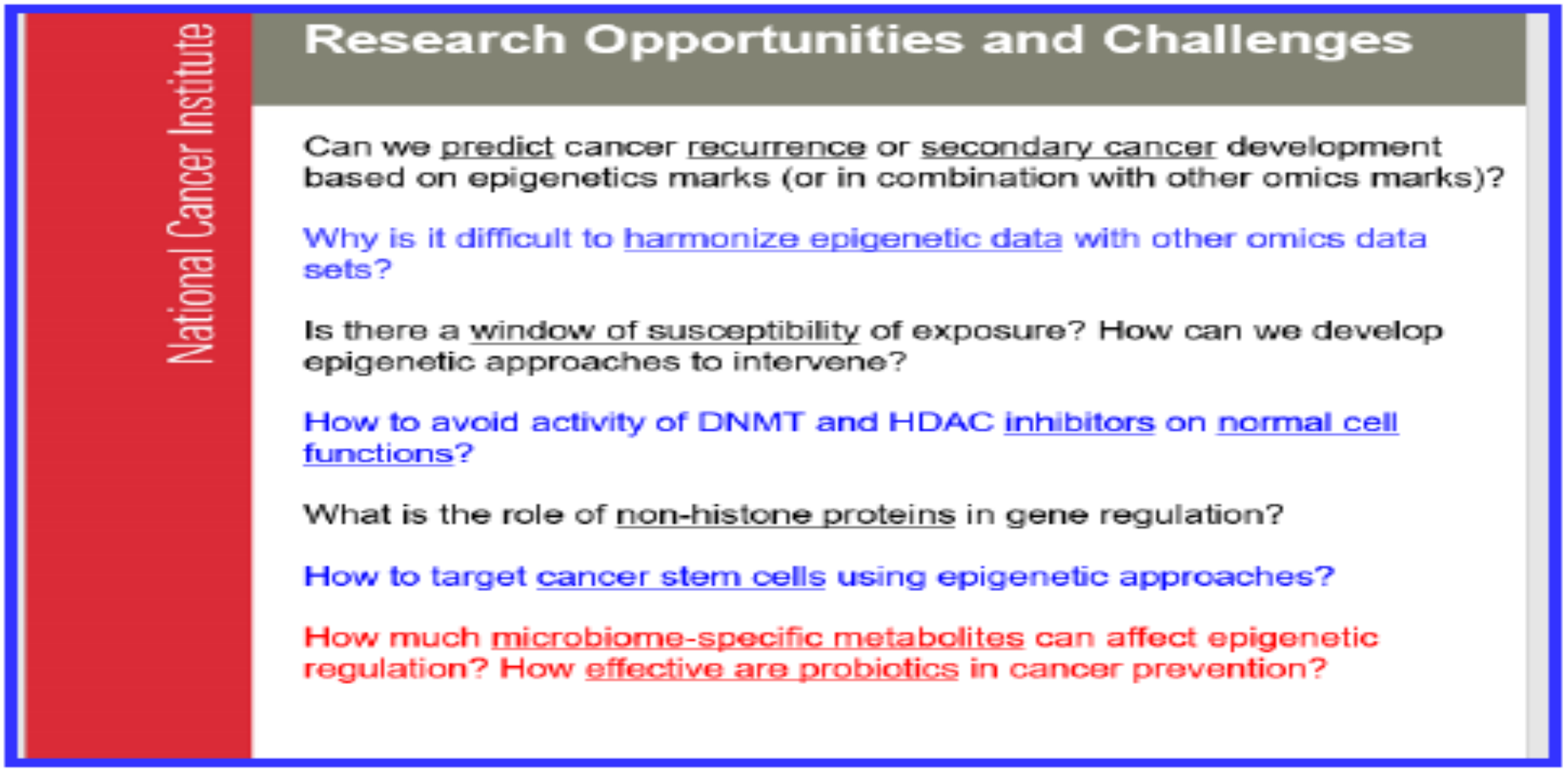
How can we overcome EWAS technical challenges?

Christopher Plass (Heidelberg)

Nancy Kiviat (Seattle)

Christine Ambrosone (Roswell Park, Buffalo)

Research challenges

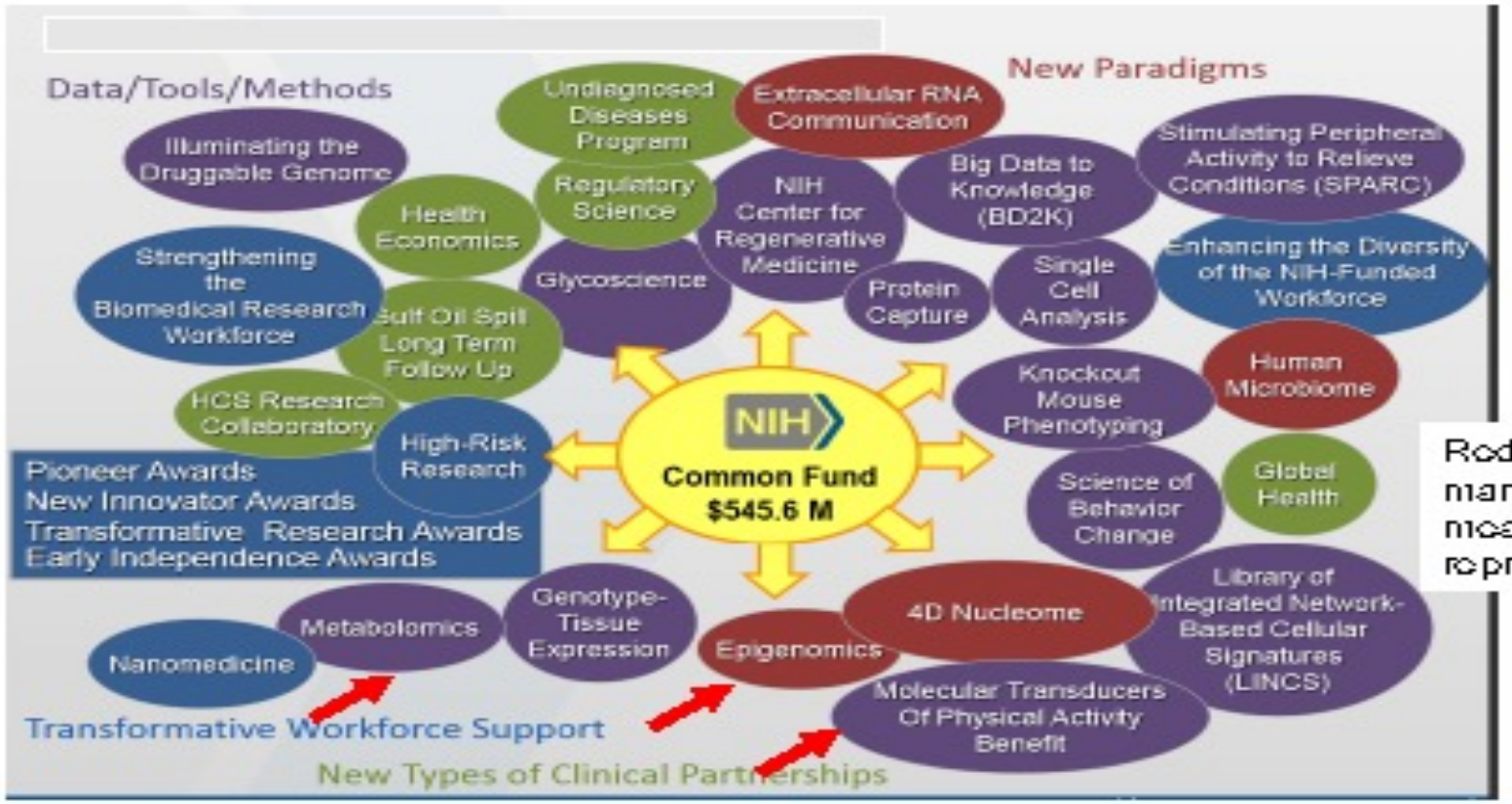


How to address challenges

National Cancer Institute

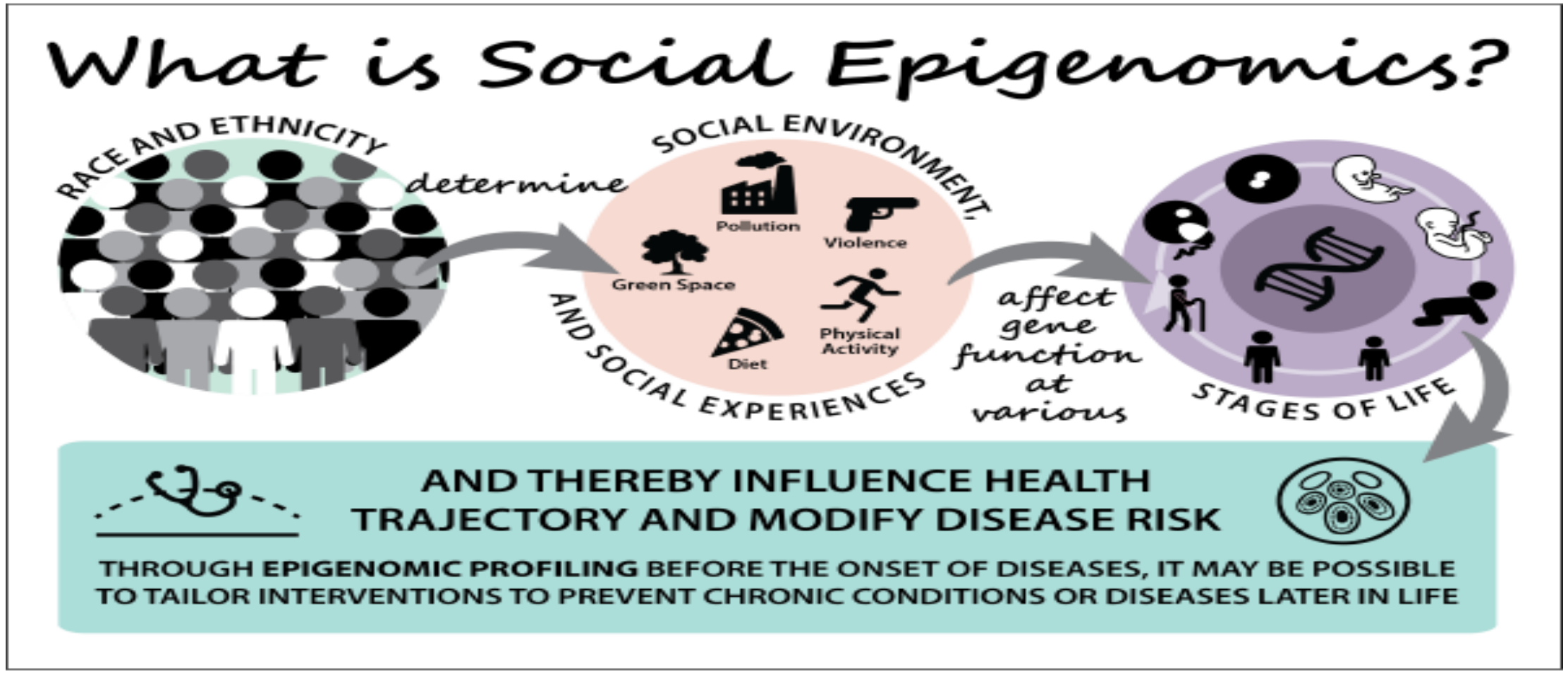
How are we addressing these challenges?

NIH common fund



Red arrow marks means that I represent NCI

Social epigenomics



Personalized medicine

Cancer Epidemiology, Biomarkers & Prevention

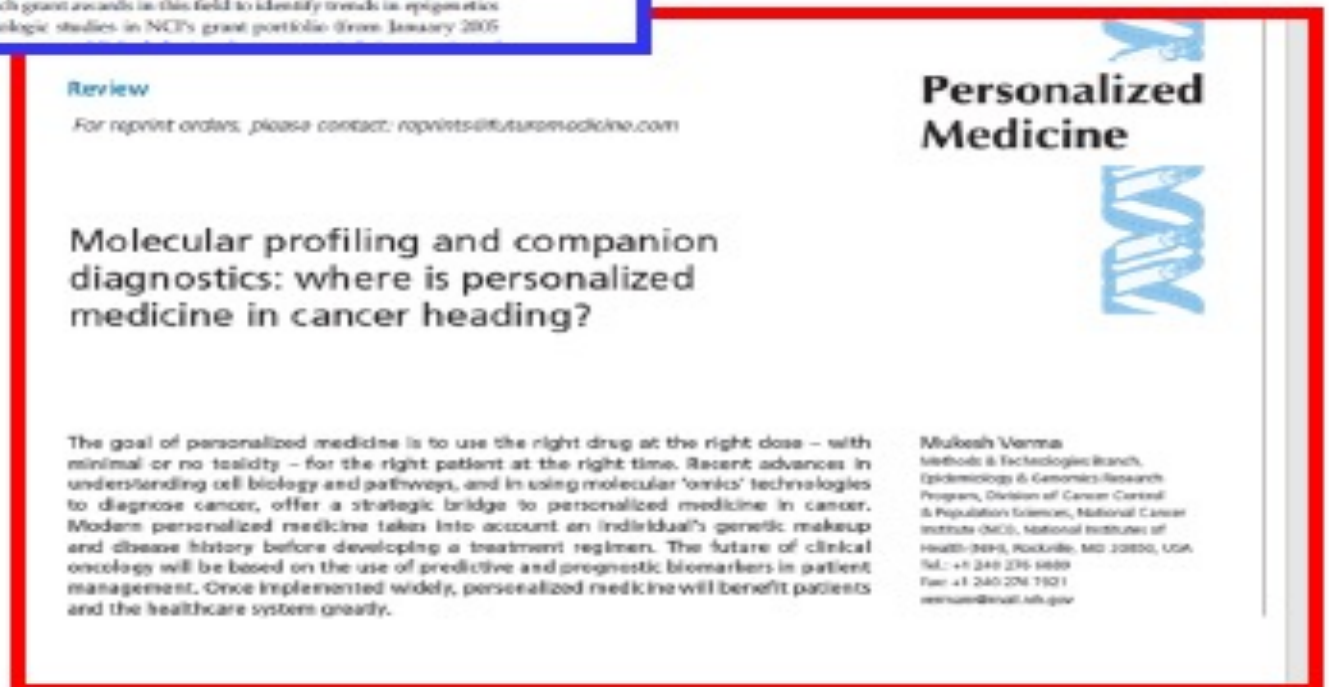
Minireview

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[3], 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 study 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

Personalized Medicine

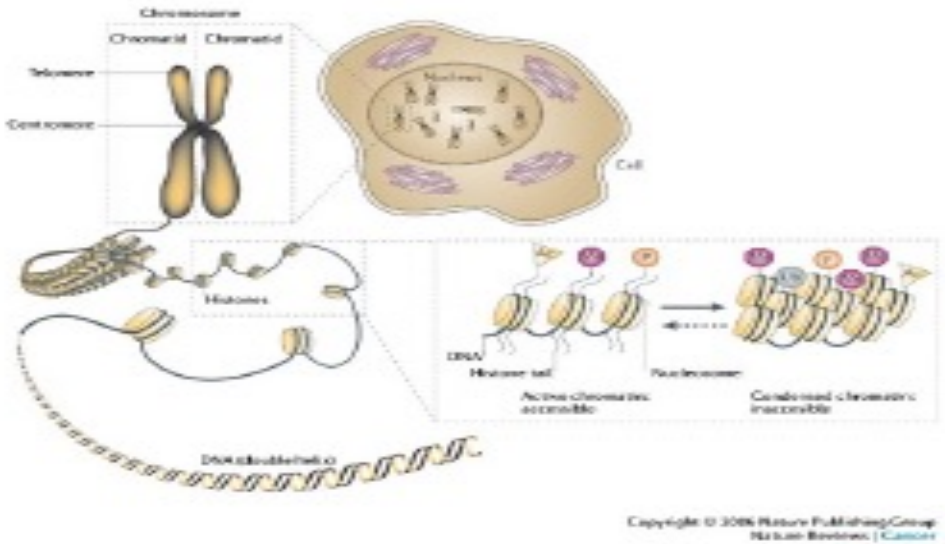
Molecular profiling and companion diagnostics: where is personalized medicine in cancer heading?

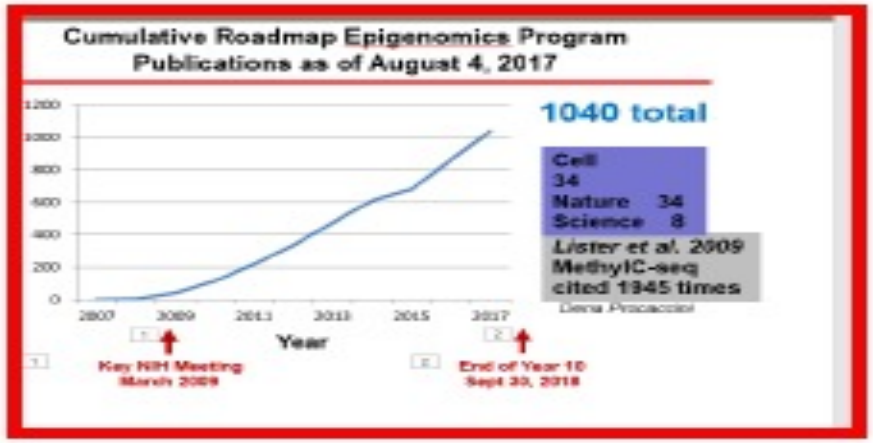
The goal of personalized medicine is to use the right drug at the right dose – with minimal or no toxicity – for the right patient at the right time. Recent advances in understanding cell biology and pathways, and in using molecular 'omics' technologies to diagnose cancer, offer a strategic bridge to 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 7821

Epigenetics roadmap

Epigenetics Roadmap



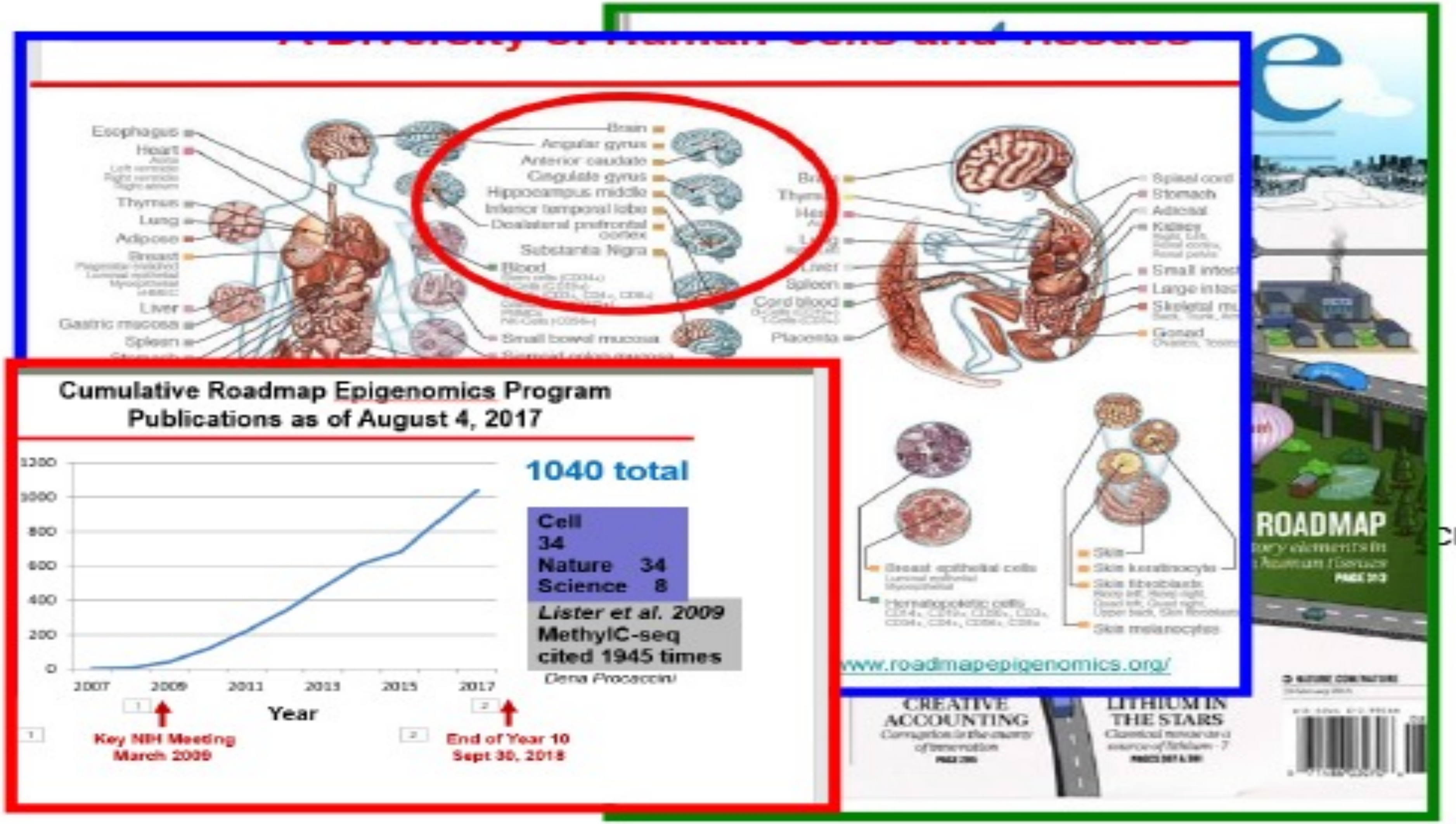


Epigenetically Regulated Diseases:
Several cancers, autoimmune disorders, reproductive disorders, and neurobehavioral and cognitive dysfunctions

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

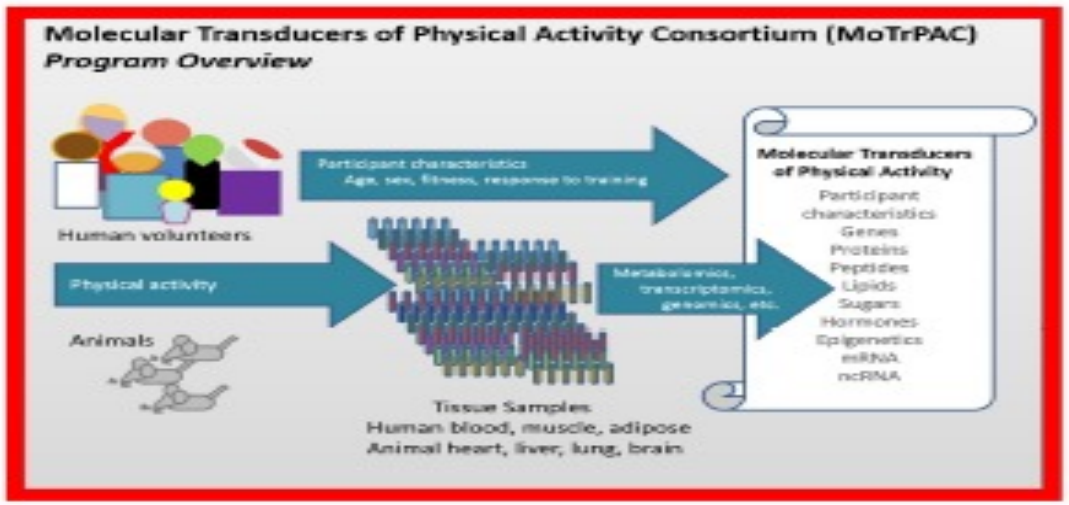


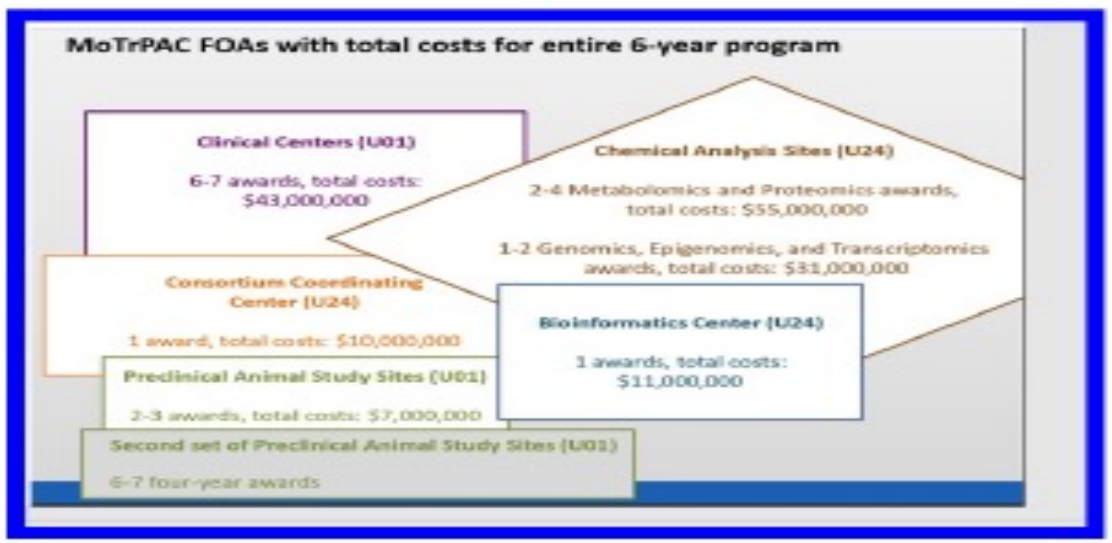
Cumulative Roadmap Epigenomics Program
Publications as of August 4, 2017
1040 total
Cell 34
Nature 34
Science 8
Lister et al. 2009
MethylC-seq
cited 1945 times
Year
Key NIH Meeting
March 2009
End of Year 10
Sept 30, 2018
www.roadmapepigenomics.org/
ROADMAP
CREATIVE ACCOUNTING
LITHIUM IN THE STARS

IHEC


IHEC
About
Research
Outcomes
Epigenomics
News+Events
Links
The International Human Epigenome Consortium (IHEC) unites scientists from all over the world working together to achieve one common goal:
Welcome to IHEC
Lectures
Research

Programs


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
miRNA
ncRNA

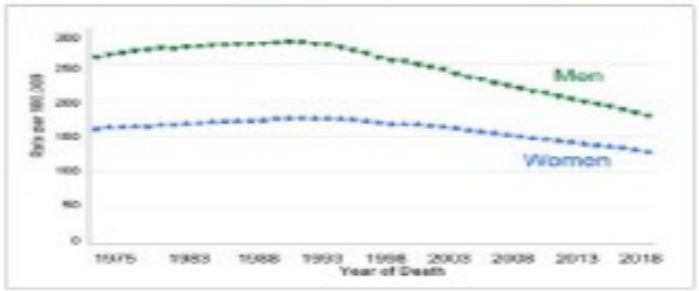

MoTrPAC FOAs with total costs for entire 6-year program
Clinical Centers (U01)
6-7 awards, total costs: $43,000,000
Chemical Analysis Sites (U24)
2-4 Metabolomics and Proteomics awards, total costs: $55,000,000
1-2 Genomics, Epigenomics, and Transcriptomics awards, total costs: $31,000,000
Consortium 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

Progress

Remarkable progress in cancer research

Declining cancer mortality, even in lung cancer and melanoma



AMONG U.S. MEN

DEATHS FROM THE MOST COMMON TYPE OF LUNG CANCER

FELL

↓ 3.2%

EACH YEAR FROM 2006 TO 2013

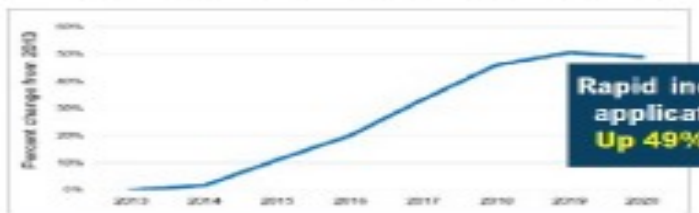
THEN FELL

↓ 6.3%

EACH YEAR FROM 2013 TO 2016

Average number of yearly FDA approvals 2006-2010: 13

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

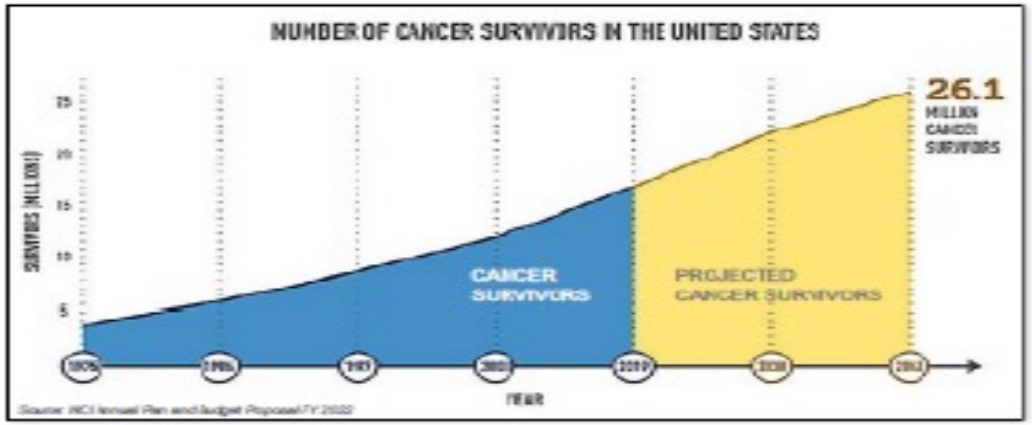


Rapid increase in R01 applications to NCI: Up 49% since 2013

Cancer Letters 5 November 2021

Cancer survivors

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

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.**