Epigenetics
Epigenetics

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

The two main components of the epigenetic code:

1. Methylation Code
   - DNA methylation: Methyl marks added to certain DNA bases repress gene activity.

2. Histone Code
   - Histone modification: A combination of different molecules can attach to protein tails of proteins called histones. These modifications control gene expression.

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

Qiu NATURE 441: 143
DNA and destiny

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

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

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.

Cancer continuum

DCCPS covers cancer continuum

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

Early Detection
- Breast, cervical, colorectal cancer screening

Diagnosis
- Incidence, Stage at diagnosis

Treatment
- Trends in cancer treatment

Life After Cancer
- Financial burden of cancer care, Cancer survivorship

End of Life
- Mortality, Person – years of life lost

Prevention: restoring transcription, halting progression, or stopping metastasis

Cancer recurrence
Secondary cancer
Cancer development

Cancer Development is a Multi-step Process

Genetic alterations and the progression of colorectal cancer

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

<table>
<thead>
<tr>
<th>Age</th>
<th>40 years</th>
<th>50 years</th>
<th>60 years</th>
<th>70+ years</th>
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</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Normal or near normal</td>
<td>Atypical hyperplasia</td>
<td>Ductal carcinoma in situ</td>
<td>Invasive cancer</td>
</tr>
<tr>
<td>Cervix</td>
<td>Normal or near normal (CIN 1)</td>
<td>Dysplasia, CIN 2/3</td>
<td>Carcinoma in situ</td>
<td>Invasive cancer</td>
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<tr>
<td>Colon</td>
<td>Normal or near normal</td>
<td>Adenomatous polyps</td>
<td>Invasive cancer</td>
<td></td>
</tr>
<tr>
<td>Lung (smokers)</td>
<td>Normal or near normal</td>
<td>Pre cancer</td>
<td>Invasive cancer</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>Normal or near normal</td>
<td>Neoplasia</td>
<td>Invasive cancer</td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>Normal or near normal</td>
<td>Dysplasia (PIN)</td>
<td>Invasive cancer</td>
<td></td>
</tr>
</tbody>
</table>

[Diagram showing different stages of cancer progression]
Paradigm shift

### Paradigm shifts in genetics

<table>
<thead>
<tr>
<th>Period</th>
<th>Description</th>
<th>Key Concepts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1850-1900</td>
<td>Proto-genetics</td>
<td>Mendelian inheritance</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Darwin, natural selection</td>
</tr>
<tr>
<td>1900-1950</td>
<td>Age of genetics</td>
<td>gene concept, mutation, genotype-phenotype</td>
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<tr>
<td>1950-2000</td>
<td>Age of DNA</td>
<td>structure, genetic code, genome sequence</td>
</tr>
<tr>
<td>2000-</td>
<td>Age of epigenetics</td>
<td>epigenetic code, epigenome, epigenetic medicine</td>
</tr>
</tbody>
</table>
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)
Cancer genes
GWAS hits
Genome wide associations
There’s more to the genome than its sequence

- 4D Nucleome
  - Roadmap
  - Epigenomics, ENCODE, IHEC
- Human Genome Project
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


Core of Histone Molecules

 duplex DNA (blue) histone octamer (yellow)

chromatin fiber

Nucleosome

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

Unmethylated CpG → Hypermethylation → Inactivated Tumor Suppressors

Methylated CpG → Hypomethylation → Activated Oncogenes
Epigenetics

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

EPIGENETICS

Methylation of DNA
Modifications of histones
RNA-mediated modifications

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

Genomic imprinting

Acetylation
Methylation
Phosphorylation
Ubiquitination
Epigenome components
Methylation
Mechanism of DNA methylation
Chromatin modifications
Exercise

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

https://www.youtube.com/watch?v=JMT6oRYgkTk
Aldehyde and nitric oxide, present in cigarette smoke induce phosphorylation of histones resulting in decreased histone deacetylase 2 activity.
E-cigarette vapors can damage the vital immune system.
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

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.
Early life exposure

Cancer Medicine
REVIEW

Early-life exposures to infectious agents and later cancer development
Vidya Vedham1, Mukesh Verma1 & Somdat Mahabir2
1Methods and Technologies Branch, National Cancer Institute, National Institutes of Health (NIH) 9609 Medical Center Drive, Rockville, Maryland 20850
2Environmental Epidemiology Branch, Epidemiology and Genomics Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health (NIH) 9609 Medical Center Drive, Rockville, Maryland 20850

Keywords
Cancer, early life exposure, infectious agents, perinatal transmission

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E-mail: mahabir@mail.nih.gov

Cancer Med. 2015 Dec;4(12):1908-22
For further information, contact

Abstract
There is a growing understanding that several infectious agents are acquired in early life and this is the reason why available vaccines target the newborn, infants, and adolescents. Infectious agents are associated with cancer development and it is estimated that about 20% of the world’s cancer burden is attributed to infectious agents. There is a growing evidence that certain infectious agents acquired in early life can give rise to cancer development, but estimates of the cancer burden from this early-life acquisition is unknown. In this article, we have selected five cancers (cervical, liver, Burkitt’s lymphoma-leukemia, nasopharyngeal carcinoma, and adult T-cell leukemia-lymphoma) and examine their links to infectious agents (HPV, HBV, HCV, EBV, and HTLV-1) acquired in early life. For these agents, the acquisition in early life is from mother-to-child transmission, perinatal contact (with genital tract secretions, amniotic fluids, blood, and breast milk), saliva, sexual intercourse, and blood transfusion. We
Scientific goal

ECHO Scientific Goal

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

https://www.nih.gov/echo/pediatric-cohorts
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
The effects of maternal anxiety during pregnancy on IGF2/H19 methylation in cord blood.

Mansell T\textsuperscript{1,2}, Novakovic B\textsuperscript{1,2}, Meyer B\textsuperscript{1,2}, Rzehak P\textsuperscript{1,3}, Vuillermin P\textsuperscript{1,2,4,5}, Ponsoby AJ\textsuperscript{1,2}, Collie P\textsuperscript{3,5}, Burgner D\textsuperscript{1,2}, Saffery R\textsuperscript{1,2}, Ryan J\textsuperscript{1,2,6,7}; BIS investigator team.

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.”
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 epigenomic profiling and presents how this information can be used to understand vulnerabilities of high-risk populations.
Tumor alterations

Genotype → Epigenotype → Metabotype → Phenotype

Total alterations affecting protein-coding genes in selected tumors

Average number and types of genomic alterations per tumor, including single-base substitutions (SBS), small insertions and deletions (indels), amplifications, and homozygous deletions, as determined by genome-wide sequencing studies. For colorectal, breast, and pancreatic ductal cancer, and medulloblastomas, translocations are also included. The published data on which this figure is based are provided in table S1D.
Genetic mutations

Genetic mutations of epigenetic modifiers in cancer

DNA methylation

Nucleosome remodeling

Histones and variants

miRNAs

Histone-modifying enzymes

Histone PTM readers

The epigenetic machinery

Baylin and Jones (2016)
DNA methylation and carcinogenesis

DNA Methylation and Carcinogenesis

DNA Methylation

Abnormal Increases
- Tumor suppressor gene inactivation
  - Methylation of both alleles
  - Methylation of 1 allele and mutation or deletion of the other

Abnormal Decreases
- Proto-oncogene activation and up-regulation of other DNA sequences
  - For imprinted genes: hypomethylated allele replaced by mitotic recombination with hypermethylated allele or methylated de novo
- Latent viral activation or retroelement activation

No Changes
- Chemically induced mutations preferentially at m5C residue
- Poor repair of m5C
- Increased DNA Rearrangements and possibly aneuploidy
- Deamination—spontaneous conversion of m5C to T mutations in tumor suppressor genes
Integrin signaling
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
Histone acetylation

[Diagram of histone acetylation]
Micro RNA signatures

Mirco RNA Signatures in Human Cancers

Micro RNA Polymorphism to Identify High Risk Populations

Mir-31 inhibits metastasis in breast cancer
Micro RNA methylation

Lujambio and Esteller*, Cell Cycle 8: 377
Extracellular vesicles

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

ALTERATIONS OF HISTONE H3 MODIFICATIONS IN LIVER DURING METHYL DEFICIENCY

Interplay between H3K9me3, H3K9Ac, and H3S10ph
## Epigenetic regulation

<table>
<thead>
<tr>
<th>Modification</th>
<th>Methylation</th>
<th>Acetylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Mono-methylation</td>
<td>--</td>
</tr>
<tr>
<td>H3K4</td>
<td>Activation</td>
<td>--</td>
</tr>
<tr>
<td>H3K9</td>
<td>Activation</td>
<td>Repression</td>
</tr>
<tr>
<td>H3K27</td>
<td>Activation</td>
<td>Repression</td>
</tr>
<tr>
<td>H3K36</td>
<td>--</td>
<td>Repair</td>
</tr>
<tr>
<td>H3K79</td>
<td>Activation</td>
<td>Activation</td>
</tr>
<tr>
<td>H3R17</td>
<td>--</td>
<td>Activation</td>
</tr>
<tr>
<td>H4K5</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H4K8</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H4K12</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H4K16</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>H4K20</td>
<td>Activation</td>
<td>Activation</td>
</tr>
<tr>
<td>H4K16</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Single cell epigenomics

**SINGLE CELL EPIGENOMICS**

**Single cells isolated from**
- Blood
- Breast milk
- Exfoliated cells
- Hair
- Oral swab
- Pancreatic fluid
- Saliva
- Skin
- Tissue
- Urine

**Implications of single cell epigenomics**
- Risk Assessment to identify high-risk individuals
- Diagnosis
- Prognosis
- Screening
- Follow up treatment and co-morbidity

**Single Cell Epigenomics**
- Identify open and closed chromatin
- Identify cell-specific transcription factors
- Determine nucleosome position
- Identify active and repressive transcription state

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

20 Diagnosing Cancer Using Histone Modification Analysis

Mukesh Verma and Deepak Kumar

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Books

Books edited by Mukesh Verma
Epigenetic changes

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

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

Nature 483:637-639

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Epigenetic drugs

The evidence, plus the successful approval of a first generation of drugs intended to target epigenetic pathways, has convinced almost every major drug company to invest in cancer epigenetics, says Mukesh Verma, a programme officer at the NCI. For example, Novartis, a pharmaceutical firm with its headquarters in Basel, Switzerland, has more than 200 employees working in epigenetics, most of them in cancer, says El Li, head of China Novartis’s Institute for Biomedical Research, based in Shanghai. Last year, GlaxoSmithKline in London, in addition to funding its own epigenetics team, paid $20 million to partner with Epizyme in a deal in which Epizyme could ultimately receive as much as $800 million. "GISK’s group is partnering with us as it is also competing with us on other programmes," says Epizyme’s chief scientific officer, Robert Copeland. "It makes for an interesting dynamic."

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

There is also an intense demand for talent. In particular, epigenetics companies and individual labs need
Exfoliated cells are good sources of DNA to study epidemiology.
Histone enzymes
Methylation and acetylation enzymes
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 of epigenetic modulation with 5-azacytidine and valproic acid in patients with advanced cancers.

- The maximum tolerated dose was 75 mg/m² of 5-AZA in combination with valproic acid.
- Dose-limiting toxicities were neutropenic fever and thrombocytopenia, which occurred at a dose of 94 mg/m² of 5-AZA.
- Stable disease lasting 4 to 12 months (median, 6 months) was observed in 14 patients (25%).

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

The combination of 5-AZA and valproic acid is safe at doses up to 75 mg/m² for 5-AZA in patients with advanced malignancies.
Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-trans retinoic acid in acute myeloid leukemia and myelodysplastic syndrome.


- 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)

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

**Total: 84 studies**

Environmental damage
Epigenetic inhibitors

FDA Approved Epigenetic Inhibitors

5-Azacitidine
Decitabine
Valproic acid
SAHA
Approved epigenetic drugs

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

**HDAC Inhibitors**
- Vorinostat (approved in 2006)
  For Cutaneous T cell Lymphoma
- Romidepsin (approved in 2009)
  For Cutaneous T cell Lymphoma
- Panobezstat (approved in 2015)
  For Multiple Myeloma
- Belinostat (approved in 2014)
  For Peripheral T-cell Lymphoma
**Epigenetic drugs**

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>Epigenetic therapy</th>
<th>Drug combination</th>
<th>Patient selection</th>
<th>Response</th>
<th>Pharmacodynamic target validation?</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal stromal tumours</td>
<td>Panethromycin (pan-deacetylase inhibitor)</td>
<td>Paclitaxel and everolimus</td>
<td>Patients with refractory gastroentero-duodenal tumours refractory to imatinib and sunitinib</td>
<td>1 of 11 partial response; 7 of 11 stable disease; 3 of 11 progressive disease</td>
<td>Yes</td>
<td>87</td>
</tr>
<tr>
<td>Wild-type KEAP1-mutated colorectal cancer</td>
<td>Decitabine (demethylating agent)</td>
<td>Decitabine and panitumumab (monoclonal antibody against EGFR)</td>
<td>Patients with progressive disease on standard therapy and previously treated with cetuximab</td>
<td>2 of 20 partial response; 11 of 20 stable disease; 7 of 20 progressive disease</td>
<td>No</td>
<td>88</td>
</tr>
<tr>
<td>Advanced solid tumours</td>
<td>Azacytidine, (demethylating agent); Vismodegib (pan-deacetylase inhibitor)</td>
<td>Azacytidine, volutinc acid and carboplatin</td>
<td>Advanced cancer and progressive following standard therapy (platinum-based) or no standard effective therapy available</td>
<td>6 of 12 stable disease; 26 of 12 progressive disease</td>
<td>Yes</td>
<td>89</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Decitabine (demethylating agent)</td>
<td>Decitabine and carboplatin</td>
<td>Initial response by RECIST and/or CA125 criteria when progressing 6-12 months after previous platinum therapy</td>
<td>3 of 35 CA125 partial response; 1 of 35 RECIST partial response</td>
<td>Yes</td>
<td>88</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Decitabine (demethylating agent)</td>
<td>Decitabine and carboplatin</td>
<td>Progression or recurrence within 6 months of platinum-based compound</td>
<td>1 of 17 complete response; 5 of 17 partial response</td>
<td>Yes</td>
<td>77</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Azacytidine (demethylating agent)</td>
<td>Azacytidine, carboplatin and anti-endoglin</td>
<td>Progression or recurrence within 6 months of platinum-based compound</td>
<td>1 of 25 complete response; 2 of 25 partial response</td>
<td>Yes</td>
<td>90</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Azacytidine (demethylating agent)</td>
<td>Azacytidine, abiraterone and enzalutamide</td>
<td>Progression or recurrence on any combination of anti-androgens or after completion of enzalutamide for 1 year</td>
<td>19 of 34 PSADT &gt;36 months; 13 of 34 PSADT &gt;6 months; 9 of 34 PSADT &gt;12 months</td>
<td>Yes</td>
<td>91</td>
</tr>
<tr>
<td>ER- and PR-positive breast cancer</td>
<td>Vorinostat (pan-deacetylase inhibitor)</td>
<td>Vorinostat and taxol</td>
<td>Progression or response on any hormone inhibitor or completion of tamoxifen for 1 year</td>
<td>8 of 16 partial response</td>
<td>Yes</td>
<td>82</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Belinostat (pan-deacetylase inhibitor)</td>
<td>Belinostat and carboplatin</td>
<td>Progression or recurrence on platinum-based and taxol treatment</td>
<td>2 of 9 objective response</td>
<td>No</td>
<td>93</td>
</tr>
<tr>
<td>Epithelial ovarian cancer</td>
<td>Belinostat (pan-deacetylase inhibitor)</td>
<td>Belinostat and carboplatin</td>
<td>platinum-refractory or resistant disease</td>
<td>15 of 35 objective response</td>
<td>Yes</td>
<td>94</td>
</tr>
</tbody>
</table>
Combination therapy

### AML subtypes and combination therapy

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

Source: Leukemia & Lymphoma Society

Cancer letters 17 July 2018
Epigenetic Therapy for Colorectal Cancer
Vivek Vaish, Tripti Khare, Mukesh Verma, and Sharad Khare

Abstract
Aberrations in epigenome that include alterations in DNA methylation, histone acetylation, and miRNA (microRNA) expression may cause the progression of colorectal cancer (CRC). These aberrations change...
Combination epigenetic therapy has efficacy in patients with refractory advanced non-small cell lung cancer.


Department of Oncology, Johns Hopkins University, Baltimore, Maryland 21231, USA.

Abstract
Epigenetic alterations are strongly associated with the development of cancer. We conducted a phase III trial of combined epigenetic therapy with azacitidine and entinostat, inhibitors of DNA methylation and histone deacetylation, respectively, in extensively pretreated patients with recurrent metastatic non-small cell lung cancer. This therapy is well tolerated, and objective responses were observed, including a complete response and a partial response in a patient who remains alive and without disease progression approximately 2 years after completing protocol therapy. Median survival in the entire cohort was 6.4 months (95% CI: 3.5 to 9.2), competing favorably with existing therapeutic options. Demethylation of a set of 4 epigenetically silenced genes known to be associated with lung cancer was detectable in serial blood samples in these patients and was associated with improved progression-free survival (P = 0.034) and overall survival (P = 0.035). Four of 19 patients had major objective responses to subsequent anticancer therapies given immediately after epigenetic therapy. Significantly, this study demonstrates that combined epigenetic therapy with low-dose azacitidine and entinostat results in objective, durable responses in patients with solid tumors and defines a blood-based biomarker that correlates with clinical benefit.
Low doses of DNA-demethylating agents
Intervention

Potential Steps for Intervention

A Model for Colorectal Tumorigenesis

Modified from Jubb et al. J Path. 195: 111.
Microsatellite instability

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

Identification of tumor clusters.

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

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

Genes hypermethylated in individuals with smoking history:
CDKN2A, RSSF1A, ARH1, MGMT, RARbeta

Sputum from cancer survivors and cancer patients show different methylation pattern
Mesothelioma

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

ASBESTOS

MESOTHELIOMA

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

Epigenetic pattern

Epigenetic Patterns in the Progression of Esophageal Adenocarcinoma

Cancer Research
61:3410

Cancer Progression

Risk factors
- Gastroesophageal Reflex Disease (GERD)
- Smoking
- Higher Body Mass Index (BMI) or obesity
Esophageal cancer

Esophageal Cancer: Probability of Survival

Brock et al. Clinical Cancer Research, 9: 2912
### Pancreatic Cancer: Methylation of p14ARF and p16INK4a

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample Size</th>
<th>p16INK4a</th>
<th>p14ARF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic Carcinoma (PCA)</td>
<td>39</td>
<td>19/39</td>
<td></td>
</tr>
<tr>
<td>Chronic Pancreatitis (CP)</td>
<td>16</td>
<td>0/16</td>
<td></td>
</tr>
<tr>
<td>Normal Pancreatogram (NAD)</td>
<td>6</td>
<td>0/6</td>
<td></td>
</tr>
</tbody>
</table>

Sample: Pancreatic Fluid

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

Distinct chromatin states of human PDAC
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.
DNA methylation
DNA methylation has been proposed as a triage for women infected with HPV and may eventually directly complement or replace HPV screening as a one-step molecular diagnostic and prognostic test.

Elevated methylation in cervical cancers and high-grade CIN (CIN2 and CIN3), most prominently in Genes CADM1, EPB41L3, FAM19A4, MAL, miR-124, PAX1, and SOX1.

Elevated methylation of the HPV16 L1 and L2 open reading frames, in particular, is associated with CIN2, CIN3 and invasive cancer.
Methylated genes
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shin HJ et al.</td>
<td>Link STAT4 expression in human T cells is regulated by DNA methylation but not by promoter polymorphism.</td>
</tr>
<tr>
<td>Espinoza CR, Feeney AJ</td>
<td>The extent of histone acetylation correlates with the differential rearrangement frequency of individual VH genes in pro-B cells.</td>
</tr>
<tr>
<td>Gasche JA, Hoffmann J, Boland CR, Goel A</td>
<td>Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells.</td>
</tr>
<tr>
<td>Fujisawa T, Joshi BH, Puri RK</td>
<td>Histone modification enhances the effectiveness of IL-13 receptor targeted immunotoxin in murine models of human pancreatic cancer.</td>
</tr>
<tr>
<td>Tahara T et al.</td>
<td>Association between IL-17A, -17F and MIF polymorphisms predispose to CpG island hyper-methylation in gastric cancer.</td>
</tr>
</tbody>
</table>
Epigenomics Grants Predictive Biosciences Rights to Use a Biomarker in a Prostate Cancer Test

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

Quest Diagnostics Incorporated is a leading provider of diagnostic services.
Bladder cancer methylation

Bladder Cancer
Methylation of LAMC2 in Exfoliated Cells Isolated from Urine

A

METHYLATION INDEX (MI)

LOW MI (n = 71)

HIGH MI (n = 20)

P = 0.002

MONTHS AFTER DIAGNOSIS

0 10 20 30 40 50 60

B

SURVIVAL PROBABILITY

P = 0.04

MONTHS AFTER DIAGNOSIS

0 10 20 30 40 50 60

Another Study:
Schistosomes and Bladder Cancer

(Sathyanarayana et al. Can Res 64: 1425)
CpG island hypermethylation
Anticancer phytochemicals

ANTICANCER PHYTOCHEMICALS
(Representative chemopreventive phytochemicals and their dietary sources)
Key points

- Dietary factors with known epigenetic properties could be used to modulate the expression of cancer-related genes.
- As some epigenetic changes can be reversed chemically, epigenetics has tremendous implications for disease intervention and treatment.
- Epigenetic changes at specific loci are associated with differential disease risks and may be modified by nutritional interventions.
- Confounding variables in diet and nutrition-associated studies should be considered carefully in the research design.
- Epigenetic variations may be utilized in developing personalized nutritional recommendations for cancer control and prevention.
Carcinogenesis

**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, GST$\pi$-foci  | >54 weeks, GST$\pi$-tumor  | Liver tumor

*Igor et al. 2007 (personal communication)*
Nutritional Epigenetics and the Prevention of Hepatocellular Carcinoma with Bioactive Food Constituents.

Moreno FS1, Heidor R1, Poortvliet IP2.

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 non-nutrient diet bioactive components can reduce the occurrence and 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.
Epigenetic foods
Research opportunities

Research Opportunities and Challenges

Will inclusion of epigenetic markers help in identification of new risk factors (modifiable factors and host factors) in different races and ethnic groups?

Will epigenetic markers in cohort and case-control studies improve sensitivity and specificity of markers and help in identifying high-risk populations?

Are genetic and epigenetic events correlated during cancer development?

Are there race/ethnicity specific miRNAs and noncoding RNAs?

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

How can we overcome EWAS technical challenges?
How to address challenges

How are we addressing these challenges?
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.

- **Trans-NIH, all Institutes/Centers participate**
- **NIH Common Fund**
Roadmap
Epigenome consortium

http://ihec-epigenomes.org/
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.
Ongoing programs

- Clinical Centers (U01)
  - 6-7 awards, total costs: $43,000,000
- Consortium Coordinating Center (U24)
  - 1 award, total costs: $10,000,000
- 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

Other Ongoing Programs

1. Moonshot
2. All of US
3. PCGA
4. TCGA

Tissue Samples
- Human blood, muscle, adipose
- Animal heart, liver, lung, brain
NIH

NIH... Turning Discovery Into Health

Mukesh Verma, PhD
vermam@mail.nih.gov