Epigenomics

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Disclosures: Personalis, GenapSys, SensOmics, Qbio
Definition: Epigenomics

The study of the complete set of epigenetic modifications on the genome that can modify DNA instructions, turning on and off genes, or control the production of proteins.

These modifications occur when chemical compounds or proteins bind to DNA and “mark” the genome. They do not alter the sequence itself.
A Symphonic Example

DNA

Epigenetic changes

Phenotype
A Symphonic Example
Identical twins are not identical.

Factors outside our DNA influence of phenotypes and health.
Epigenomics: Two Major types

1) DNA methylation
2) Histone modification
The Epigenome – DNA Methylome

Often occurs at CpG

>28 million CpG sites in the human genome.

Human genome consists of vast oceans of DNA sequence containing few but heavily methylated CpG dinucleotides.

Punctuated by short regions with unmethylated CpGs occurring at higher density, forming “CpG islands” in the genome, often at gene promoters.

*HydroxyC methylation*: associated with gene regulation (not discussed)
Methylation in promoters: Gene expression turned off

Ac: Acetylation

Inactive

Active Chromatin

Inactive

Active Chromatin

Ac: Acetylation
The Human Epigenome

Methylation in promoters:
Gene expression turned off

Modifying enzymes

Dnmt: DNA methyltransferase

TET: “Demethylation”

HMT: Histone MethylTransferase

KDM: Histone Demethylase

HAT: Histone AcetylTransferase

HDAC: Histone DeACetylase

Maleszewska & Kaminska, Cancers, 2013
How to detect DNA methylation: Bisulfite sequencing

• Sodium bisulfite converts unmethylated Cytosine into Uridine
  Converts to Thymine after PCR amplification
• Does not effect on methylated Cytosine.
Whole-genome versus targeted bisulfite sequencing

- Whole-genome bisulfite sequencing is very expensive.

- WGBS is also inefficient, as about 65% of all 101-bp reads do not even contain any CpGs.

- To detect partial methylation at high sensitivity need a greater sequencing depth.

- → Targeted bisulfite sequencing instead

Lee et al (2013) Cancer Letters
DNA Methylation Associated with Environmental and Other Factors

• Nutrition
• Exercise
• Environmental exposure
• Aging
• Disease

Much of what we know comes from model organism
Nutrition and DNA Methylation

Honey Bees
Extensive differential methylation workers and queen

“Royal Jelly” silences Dnmt3 DNA Methyl transferase
You Can Buy This Stuff

Royal Jelly
2,000 mg
100% PURE
Ultra Mega Strength
Genuine, Freeze Dried
ONE CAPSULE
Equivalent To Fresh State
75 CAPSULES • Dietary Supplement
Nutrition and DNA Methylation

Agouti Mouse
- Paracrine signalling molecule that affect coat color
- Yellow, obese, diabetic unmethylated
- Brown healthy methylated
- Transposon
Nutrition and DNA Methylation

Agouti Mouse

- Affected by Nutrition and Environment
- Methyl rich diet (SAM, folate) by Mom Suppresses yellow
- BPA – (bisphenol A) polycarbonate plastic precursor
DNA Methylation Associated with Many Diseases and Traits

Asthma and Allergies (Nadeau)

Aging

Nutrition

Cancer

Exercise
DNA Methylation Correlates With Age

Measure Methylation at 353 CpG Sites “Clock CpGs”

Correlates Well with Age Across Many Tissues

Advanced Aging in AIDs Patients

19 HIV+ and 19 HIV- PBMCs; ~9 yr follow up

Identification of HIV infection-related DNA methylation sites and advanced epigenetic aging in HIV-positive, treatment-naive U.S. veterans.
Human DNA methylation at putative MEs is influenced by season of conception in the Gambia

Gambia – rainy season: hungry, dry season: lots of food

Waterland et al., PLoS Genet 2010
Human DNA methylation at putative MEs is influenced by season of conception in the Gambia

VTRNA2-1 gene is affected
- Affects immune system & Tumor suppressor

Exercise and DNA Methylation

- Many studies have linked exercise and DNA methylation differences are specific genes

Recent study Lindholm et al

23 people. Bike use one leg 4X/Week 45’ for 3 months; other leg is control

Measure gene expression and DNA methylation (arrays) beginning and end of training on each leg (muscle biopsy)

4919 sites differed in trained leg (5% FDR)

Epigenetic Age and Other Lifestyle Factors


Epigenetic clock analysis of diet, exercise, education, and lifestyle factors.

Quach A1, Levine ME1, Tanaka T2, Lu AT1, Chen BH2, Ferrucci L2, Ritz B3,4, Bandinelli S5, Neuhouser ML6, Beasley JM7, Snetselaar L8, Wallace RB8, Tsao PS9,10, Absher D11, Assimes TL9, Stewart JD12, Li Y13,14, Hou L15,16, Baccarelli AA17, Whitsel EA12,18, Horvath S1,19.

Author information

Abstract

Behavioral and lifestyle factors have been shown to relate to a number of health-related outcomes, yet there is a need for studies that examine their relationship to molecular aging rates. Toward this end, we use recent epigenetic biomarkers of age that have previously been shown to predict all-cause mortality, chronic conditions, and age-related functional decline. We analyze cross-sectional data from 4,173 postmenopausal female participants from the Women’s Health Initiative, as well as 402 male and female participants from the Italian cohort study, Invecchiare nel Chianti. Extrinsic epigenetic age acceleration (EEAA) exhibits significant associations with fish intake (p=0.02), moderate alcohol consumption (p=0.01), education (p=3x10-5), BMI (p=0.01), and blood carotenoid levels (p=1x10-5)-an indicator of fruit and vegetable consumption, whereas intrinsic epigenetic age acceleration (IEAA) is associated with poultry intake (p=0.03) and BMI (p=0.05). Both EEAA and IEAA were also found to relate to indicators of metabolic syndrome, which appear to mediate their associations with BMI. Metformin—the first-line medication for the treatment of type 2 diabetes—does not delay epigenetic aging in this observational study. Finally, longitudinal data suggests that an increase in BMI is associated with increase in both EEAA and IEAA. Overall, the epigenetic age analysis of blood confirms the conventional wisdom regarding the benefits of eating a high plant diet with lean meats, moderate alcohol consumption, physical activity, and education, as well as the health risks of obesity and metabolic syndrome.
Personal Omics Profile
94 months; >250 Timepoints; 11 Viral Infections

Chen et al., Cell 2012, unpublished
Extended Time Line

Glucose (mg/dL)

Normalized Range 70-99 mg/dL

Glycated HbA1c (%)

Normalized Range 3.8-5.7%

Time Course (Days)

-200 300 800 1300 1800

HRV

RSV

HRV

HRV

SkinRash/Itch

Adenovirus

Changed life style

Exercise
Transcriptome Changes Many Times Especially at Viral Infections
Methylome Changes Twice: At Glucose Misregulation Times

Glucose Homeostasis
Gene Inactivation by Mutation and Methylation: PDE4 involved in eosinophilia

PDE4 DIP Gene

Father

Mother

Inactivated by mutation

Inactivated by DNA Methylation

Lots of RNA

Few (3/47 reads) RNAs

Methylated CpGs
Epigenetics and Chromatin

- Electron micrograph of chromatin
- Nucleosome
- DNA
- Histones

K. Luger
Chromatin undergoes reversible chemical modifications, which have regulatory properties.
Two Common Epigenetics Marks
Lysine Acetylation and Methylation

- **K**
  - Acetylation: $\text{K} - \text{O} - \text{NH}$
  - Mass increase: +14 Da

- **Kme**
  - Methylation: $\text{K} - \text{O} - \text{NH}$
  - Mass increase: +42 Da

- **Kac**
  - Acetylation: $\text{K} - \text{O} - \text{NH}$
  - Mass increase: +42 Da

**DNA methylation**
- Methyl groups added to certain DNA bases repress gene activity.

**Histone modification**
- A combination of different molecules can attach to the "tails" of proteins called histones. These alter the activity of the DNA wrapped around them.
~350 New Histone Marks

<table>
<thead>
<tr>
<th>Me</th>
<th>Methylilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>Acetylation</td>
</tr>
<tr>
<td>Bu</td>
<td>Butyrylation</td>
</tr>
<tr>
<td>Fo</td>
<td>Formylation</td>
</tr>
<tr>
<td>OH</td>
<td>Hydroxylation</td>
</tr>
<tr>
<td>Ma</td>
<td>Malonylation</td>
</tr>
<tr>
<td>Su</td>
<td>Succinylation</td>
</tr>
<tr>
<td>Pr</td>
<td>Propionylation</td>
</tr>
<tr>
<td>Cr</td>
<td>Crotonylation</td>
</tr>
<tr>
<td>Hib</td>
<td>2-Hydroxyisobutrylation</td>
</tr>
<tr>
<td>Bhb</td>
<td>β-hydroxubutyrylation</td>
</tr>
</tbody>
</table>

**H1.2**

\[ \text{NH}_2-\ \text{Hib Hib Hib} \ \text{Su Cr Hib} \ \text{Su Hib Me Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Su Cr Hib} \ \text{Me Su Cr Hi} \ \text{Cr Hi} \ \text{Bu Hib} \ \text{Hib Hib} \ \text{Hib Hib} |
Additional chromatin modifications

Covalent modifications of histone proteins:
- Acetylation: $\text{AcK}$
- Lysine methylation: $\text{Me}_1\text{Me}_2\text{K}$
- Arginine methylation: $\text{Me}_1\text{Me}_2\text{R}$
- Phosphorylation: $\text{S/T}$
- Ubiquitinylation: $\text{UbK}$
- Sumoylation: $\text{SumoK}$

Non-covalent mechanisms: ATP-dependent chromatin remodeling
Writers, readers and erasers of histone modifications

MODIFICATION:
acetylation  acetyltransferases  deacetylases  bromodomains
methylation  methyltransferases  demethylases  chromodomains, PHD fingers, MBT
phosphoryl.  kinases  phosphatases  14-3-3, BRCT

From J. Wysoka
Histone Marks Representing a Variety of Functional Elements

Active Genes

Repressed Genes
Chromatin variation across individuals

Enhancer related marks are more variable than RNA

Kasowski et al 2013 Science
Epigenetics and Cancer

Cancer is a genetic disease

Cancer is also an epigenetic disease
Epigenetics and Cancer

1. Many epigenetic modifiers are mutated in cancer

2. Both global patterns and key targets are altered in methylation in cancer

3. Methylation patterns can guide therapeutics

4. Methylation can guide diagnostics and perhaps prognostics?
Epigenetic modifiers are frequent targets for mutation in cancer

Recent sequencing studies show that mutations in the classes of epigenetic modifiers are frequently observed in various types of cancers, highlighting the crosstalk between genetics and epigenetics:

- **DNMT3A** mutations in AML (next slide)
- **IDH1/2** mutations in glioblastoma
- **EZH2** mutations in breast, colon, pancreas, melanoma,…

**DNMT3A mutations in leukemia**

Mutations in *DNMT3A*, an enzyme involved in *de novo* methylation of CpGs, are among the most common somatic mutations, occurring in 25% of acute myeloid leukemia (AML).

*DNMT3A* mutations have also been found in myelodysplastic syndromes (MDS).

 Indicates that aberrant epigenetic modulation of the genome has a pathological role in leukemogenesis.
More Examples

Black: DNA Methylation
Red: Chromatin Modification

http://www.tumorportal.org/figure/2
The methylome and major changes that occur in cancer

The cancer methylome is characterized by widespread **Hypo-methylation** & focal CpG-island **Hyper-methylation**, often in promoters of tumor suppressor genes.

Stirzaker et al., Trends in Genetics, 2014
Methylation-based classification of colorectal cancer correlates with cancer gene mutation status

Hinoue, T et al. (2012). Genome research, 22(2), 271–82
BRCA DNA Methylation and Mutation in Ovarian Cancer

Genes can be inactivated by either Mutation or DNA Methylation
Familial Breast Cancer: Most Is Unexplained

From Jim Ford

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>FANCE</td>
<td>PMS2</td>
</tr>
<tr>
<td>ATM</td>
<td>FANCF</td>
<td>PRSS1</td>
</tr>
<tr>
<td>BLM</td>
<td>FANCG</td>
<td>PTCH1</td>
</tr>
<tr>
<td>BMPR1A</td>
<td>FANCI</td>
<td>PTEN</td>
</tr>
<tr>
<td>BRCA1</td>
<td>FANCL</td>
<td>RAD51C</td>
</tr>
<tr>
<td>BRCA2</td>
<td>LIG4</td>
<td>RET</td>
</tr>
<tr>
<td>BRIP1</td>
<td>MEN1</td>
<td>SLX4</td>
</tr>
<tr>
<td>CDH1</td>
<td>MET</td>
<td>SMAD4</td>
</tr>
<tr>
<td>CDK4</td>
<td>MLH1</td>
<td>SPINK1</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>MLH2</td>
<td>STK11</td>
</tr>
<tr>
<td>EPCAM</td>
<td>MSH6</td>
<td>TP53</td>
</tr>
<tr>
<td>FANCA</td>
<td>MUTYH</td>
<td>VHL</td>
</tr>
<tr>
<td>FANCB</td>
<td>NBN</td>
<td></td>
</tr>
<tr>
<td>FANCC</td>
<td>PALB2</td>
<td></td>
</tr>
<tr>
<td>FANCD2</td>
<td>PALLD</td>
<td></td>
</tr>
</tbody>
</table>
Cancer Genome-Epigenome Interaction:
Gene body methylation contributes to cancer-causing mutations

- Methylation of cytosine strongly increases the rate of C→T transition mutations.
- Due to “spontaneous deamination” of cytosine, which forms thymine.

- In somatic cells, gene body methylation is a major cause of cancer gene mutations in tumor suppressor genes, such as TP53.
The epigenome as a therapeutic target

The reversible nature of epigenetic modifications provides an exciting opportunity for the development of therapeutics.

Abnormal hyper-methylation often silences the expression of tumor suppressor genes. Drugs, such as Dacogen and Vidaza (5-azacytidine), may reverse tumor-associated gene silencing through their ability to disrupt DNA methylation.

The histone deacetylase inhibitors Zolinza and Istodax may reverse tumor-associated gene silencing through their ability to disrupt aberrant posttranslational histone alterations. (Subcutanteous T-cell lymphoma; CTCL)
Links Between DNA Methylation and Vitamin C

Vitamin C (ascorbic acid) is a cofactor for TET (as well as many other enzymes)

Stem cells treated with physiological Vitamin C demethylate their enhancers

TET mutations occur in leukemias and other cancers

Cancer patients often have low levels of Vitamin C
In mice Vitamin C helps reduce tumors.
Methylation of certain genes can influence sensitivity to chemotherapeutic drugs

Table 1. Stages toward the clinical application of DNA methylation markers for prediction of sensitivity to chemotherapeutic drugs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Cancer type</th>
<th>Stages in clinical application</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGMT</td>
<td>DNA repair</td>
<td>Glioma</td>
<td>Several clinical studies to define sensitivity to alkylating agents have been reported.</td>
<td>22, 28, 29</td>
</tr>
<tr>
<td>hMLH1</td>
<td>Mismatch repair</td>
<td>Colorectal cancer</td>
<td>Association with sensitivity to 5-fluorouracil has been reported.</td>
<td>30</td>
</tr>
<tr>
<td>WRN</td>
<td>DNA helicase</td>
<td>Colorectal cancer</td>
<td>Association with sensitivity to cisplatin has been reported.</td>
<td>33</td>
</tr>
<tr>
<td>FANCF</td>
<td>DNA repair</td>
<td>Ovarian cancer</td>
<td>Association with sensitivity to cisplatin has been reported.</td>
<td>34</td>
</tr>
<tr>
<td>CHFR</td>
<td>Mitotic checkpoint</td>
<td>Colorectal cancer</td>
<td>Several clinical trials to define sensitivity to microtubule inhibitors have been reported.</td>
<td>41, 42</td>
</tr>
<tr>
<td>14-3-3σ</td>
<td>G2-M checkpoint</td>
<td>Colorectal cancer</td>
<td>Association with sensitivity to DNA damaging agents has been shown in cell lines.</td>
<td>47</td>
</tr>
<tr>
<td>CDK10</td>
<td>G2-M checkpoint</td>
<td>Breast cancer</td>
<td>Association with sensitivity to tamoxifen has been reported.</td>
<td>51</td>
</tr>
<tr>
<td>p73</td>
<td>DNA damage checkpoint</td>
<td>Renal cancer</td>
<td>Association with sensitivity to cisplatin has been reported for cell lines.</td>
<td>52</td>
</tr>
</tbody>
</table>

CHFR, checkpoint with ring finger; FANCF, Fanconi anemia protein F; hMLH1, human mutL homolog 1; MGMT, O6-methylguanine-DNA-methyltransferase; WRN, Werner syndrome protein.

Toyota et al., Cancer Science, 2009
Best known example for promoter methylation influencing treatment: MGMT

- The O(6)-methylguanine-DNA methyltransferase (MGMT) encodes a DNA repair enzyme that can abrogate the effects of alkylating chemotherapy such as temozolamide.

- Alkylating chemotherapy damages DNA and kills tumor cells.

- However, if the MGMT gene is active (unmethylated promoter), the damage is rapidly repaired.

- Malignant gliomas may have the MGMT gene inactivated due to methylation of its promoter region.

- Thus, in gliomas, MGMT promoter methylation is a favorable prognostic marker in the setting of either radiation or chemotherapy.
DNA methylation of specific marker genes can be used as a biomarker for cancer detection and diagnosis.

Current available DNA methylation tests for colorectal carcinoma.

<table>
<thead>
<tr>
<th>Biomarker(s)</th>
<th>Application</th>
<th>Specimen</th>
<th>Test Name (Company)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Methylated SEPT9</td>
<td>Early Detection of CRC</td>
<td>PB</td>
<td>Epi proColon® 1.0 (Epigenomics)</td>
</tr>
<tr>
<td>- Methylated SEPT9</td>
<td>Aid in detection of CRC</td>
<td>PB</td>
<td>ColoVantage® (Quest Diagnostics)</td>
</tr>
<tr>
<td>- Methylated SEPT9</td>
<td>Detection of CRC</td>
<td>PB</td>
<td>Real Time mS9 (Abbott)</td>
</tr>
<tr>
<td>- Methylated BMP3 and</td>
<td>Early detection of advanced</td>
<td>Stool</td>
<td>sDNA-MT (Exact Sciences)*</td>
</tr>
<tr>
<td>NDRG4 - Mutant KRAS,</td>
<td>adenomatous polyps and CRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beta actin - Fecal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hemoglobin</td>
<td></td>
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</tbody>
</table>

PB = Peripheral Blood * = currently still investigational.

Simple performance in the medical practice

The Septin9 blood test allows you to offer your patients a new, reliable alternative for early detection of colorectal cancer. No intestinal preparation is required before performing the test. There are no restrictions with regard to food or drug intake. The patient can be tested at any time by a simple blood draw which can easily be included in a general health checkup.

Sampling for the test and communication and explanation of the test result is in the hand of the physician (Figure 5).

Summers et al., Journal of Cancer, 2013

http://www.epiprocolon.com
Conclusions

1. Epigenomic regulation is widespread

2. Occurs by multiple mechanisms

3. Is involved in many important biological processes
   Cancer, Aging, Nutrition

4. Cancer: Potentially for biomarkers for prognostics
   and diagnostics