Readily measurable epigenetic marks and significance

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Modes of Epigenetic Regulation

- DNA Methylation
- Histone post-translational modification
- Genomic Imprinting
- RNA-mediated regulation

Transcription Control

Post-transcription Control
Role for Epigenetic Mechanisms in “Normal” Tissue

- Controls Gene Expression Potential
Role for Epigenetic Mechanisms in “Normal” Tissue

- Controls Gene Expression Potential
  - Cellular Response to Signals
    - Growth signals
    - Stressors
    - Damage Signals
  - By Altering Conformation & Packaging
  - Highly Dynamic – ATP dependent process
Role for Epigenetic Mechanisms in “Normal” Tissue

- Controls Gene Expression Potential
  - Cellular Response to Signals
  - Differentiation and Cellular Fate

Possess the same genome, yet express different genes

Cell type #1

Cell type #2
Role for Epigenetic Mechanisms in “Normal” Tissue

- Controls Gene Expression Potential
- Cellular Response to Signals
- Responsible for Differentiation and Cellular Fate

Possess the same genome, yet express different genes

Cell type #1

Cell division (mitosis)

Daughter cells

Cell type #2

Daughter cells express the same genes as parent
Epigenetic Patterning Set in Development

Adapted from: Jaenisch. Trends Genetics 1997
Epigenetic Patterning Set in Development

Adapted from: Jaenisch. Trends Genetics 1997
Epigenetic Mechanisms Differ by Tissue/Cell and Impart Distinct Functions

Christensen et al. PLoS Genetics 2009
Epigenetic Mechanisms Differ by Tissue/Cell and Impart Distinct Functions

Tissues

“Stem” Cells

Christensen et al. PLoS Genetics 2009

D Leung et al. Nature 2015
So….is all hope lost in examining epigenetic variation in risk?
So….is all hope lost in examining epigenetic variation in risk?

Must consider implications of epigenetic variation to the function of the cell/tissue of measurement
Accessible Tissues for Epigenetic Risk Markers

- Peripheral Blood
  - Implications in immune function/inflammation
  - Keep in mind immune system can have systemic impacts

- Buccal Cells/Saliva
  - Potential Route of exposure – biomarkers of exposure
  - Oral epithelia/immune function
  - Ectodermal derivation – early embryonic effects that may be similar to central nervous system

- Pathologic Specimens
  - Can be useful in context of case-only studies
  - Establish etiologic contributors based on molecular subcharacterization of disease
  - Not only cancer but other surgical procedures (biopsies, reduction surgeries, gastric bypass, etc)

- Cord Blood
  - Immune Function
  - Hematopoietic stem cells

- Placenta
  - Functional organ during development
  - Transport, metabolic, endocrine, immunologic functions

- Fetal membranes/Residual tissues
  - Amnion/Chorion – markers of developmental exposures/risk & functional effects
  - Umbilical cord artery or vein – similarities to cardiovascular tissues?

- Other accessible biofluids
  - Breast Milk
  - Urine
  - Ejaculate
The Cellular Heterogeneity Problem

- Tissues are made up of a variety of types of cells
- Epigenetic mechanisms define cellular specificity
- Even with a specific cell type there may be clonal variation
  - May play important functional implications
  - Can also represent additional differentiation events
    - E.g. NK cell activation
    - Even specific isolation (FACS, microdissection may not be enough)
- In general, we sample tissues not individual cells
  - We are measuring aggregate markers across a population of cells within a sample
- Blood is the poster child
  - All tissue samples are affected in greater or lesser ways
Handling the challenge of heterogeneity

Exposure → DNA Methylation Profile → Disease Risk

DNA Methylation Profile → Leukocyte Proportions

Houseman et al. BMC Bioinformatics 2012, 13:86
http://www.biomedcentral.com/1471-2105/13/86

RESEARCH ARTICLE  Open Access

DNA methylation arrays as surrogate measures of cell mixture distribution
Not only controls confounding but also **UNDERSTAND EFFECT**

- When reference is known...can use methylation array data to estimate cell proportions

- Accomando Genome Biol 2014:

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**Color Legend**
- Basophils
- Eosinophils
- Neutrophils
- Monocytes
- Lymphocytes

**Symbol Legend**
- Donor #1
- Donor #2
- Donor #3
- Donor #4
- Donor #5
- Donor #6

**FACS-based %**

**Methylation Array-based Estimate %**
Arsenic Example

Koestler EHP 2013 (NH, urinary arsenic):

<table>
<thead>
<tr>
<th></th>
<th>CD8⁺ T</th>
<th>CD4⁺ T</th>
<th>NK cells</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>iAs (per μg/L)</td>
<td>1.18 (0.12, 2.23)*</td>
<td>-1.24 (-3.15, 0.68)</td>
<td>-0.11 (-1.83, 1.62)</td>
<td>-0.78 (-1.91, 0.36)</td>
</tr>
<tr>
<td>MMA⁺ (per μg/L)</td>
<td>0.93 (-0.30, 2.15)</td>
<td>-0.24 (-2.62, 2.14)</td>
<td>-0.48 (-2.59, 1.62)</td>
<td>-0.68 (-1.88, 0.52)</td>
</tr>
<tr>
<td>DMA⁺ (per μg/L)</td>
<td>0.42 (-0.80, 1.64)</td>
<td>-0.10 (-2.40, 2.20)</td>
<td>-0.37 (-2.14, 1.41)</td>
<td>-0.22 (-1.46, 1.01)</td>
</tr>
<tr>
<td>iAs/(iAs + MMA⁺ + DMA⁺)</td>
<td>9.11 (0.44, 17.79)*</td>
<td>-11.82 (-27.66, 4.02)</td>
<td>-2.16 (-14.58, 10.27)</td>
<td>-6.05 (-16.4, 4.27)</td>
</tr>
</tbody>
</table>

Kile Epigenetics 2014 (Bangladesh, Water As):

<table>
<thead>
<tr>
<th></th>
<th>Effect Estimate (Raw) [ % composition ]</th>
<th>Effect Estimate (Bias-Adj) [ % composition ]</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cell</td>
<td>-1.4</td>
<td>-1.4</td>
<td>0.71</td>
<td>0.056</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>1.4</td>
<td>1.6</td>
<td>1.84</td>
<td>0.430</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.5</td>
<td>0.5</td>
<td>0.50</td>
<td>0.310</td>
</tr>
<tr>
<td>Natural Killer</td>
<td>-0.7</td>
<td>-0.9</td>
<td>0.73</td>
<td>0.317</td>
</tr>
<tr>
<td>T Cell (CD4+)</td>
<td>-7.4</td>
<td>-9.2</td>
<td>1.97</td>
<td>0.0002</td>
</tr>
<tr>
<td>T Cell (CD8+)</td>
<td>5.5</td>
<td>7.4</td>
<td>1.54</td>
<td>0.0004</td>
</tr>
</tbody>
</table>
Why cell composition is important

- Likely reflects the “effect” of the variation in epigenetic mark
- Example – GPR15 hypomethylation associated with smoking

DOI 10.1186/s13148-015-0113-1

A varying T cell subtype explains apparent tobacco smoking induced single CpG hypomethylation in whole blood
Challenges and Opportunities for Epigenetic Biomarkers

- Major questions remain about interpretation
  - Cell composition effect
  - Must be placed in context of tissue studied
    - Unlikely to be a reliable surrogate marker

- Consideration/Incorporation of various types of epigenetic mechanisms
  - DNA Methylation
  - Genomic Imprinting
  - Chromatin Modification
  - Small non-coding RNA (miRNA, rRNA, etc)
  - Long non-coding RNA (lncRNA)
  - Alternative splicing

- Other marks suffer from same challenges as well as more
  - Technological challenges – might be overcome with novel methods

- Potentially useful risk, clinical, interventional biomarkers
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