The Children's Environmental Health & Disease Prevention Research Center at Dartmouth

Readily measurable epigenetic marks and significance

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Modes of Epigenetic Regulation

- DNA Methylation
- Histone post-translational modification
- Genomic Imprinting

Transcription Control

- RNA-mediated regulation

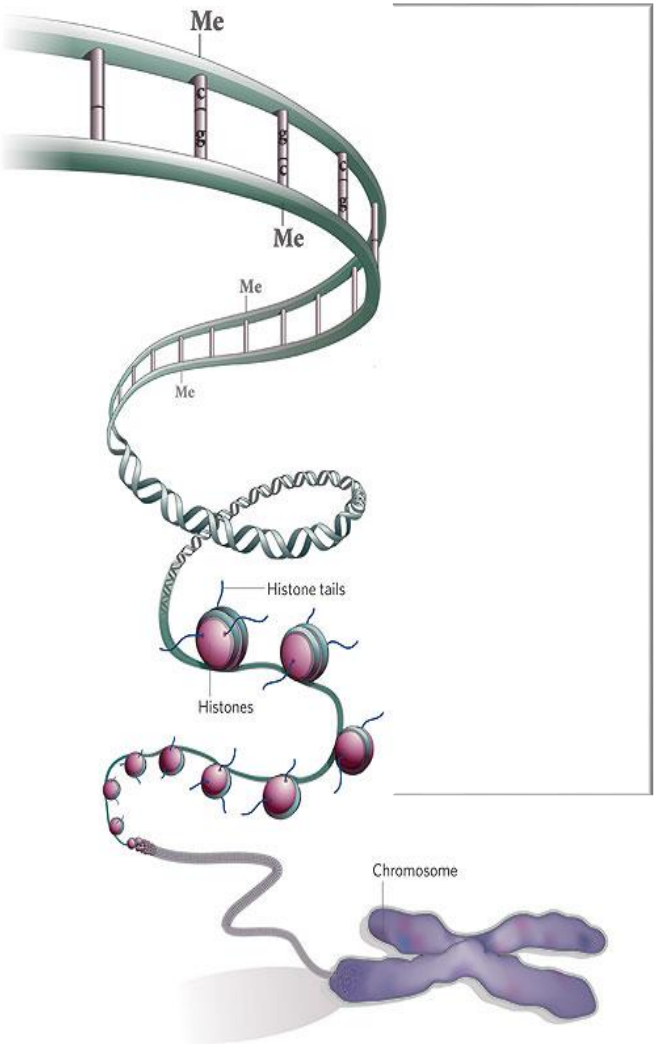
Post-transcription Control

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**

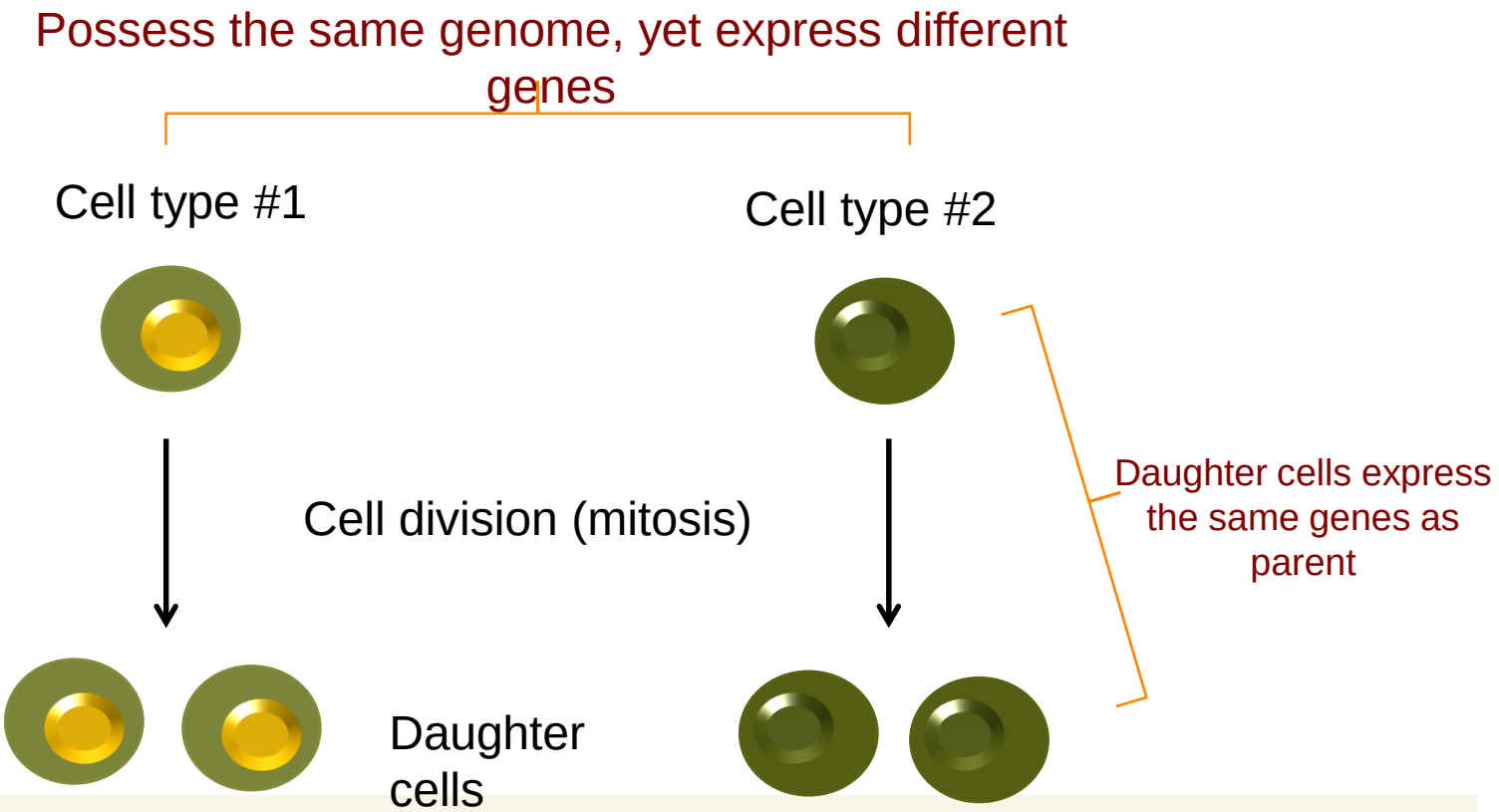
Possess the same genome, yet express different genes

Cell type #1

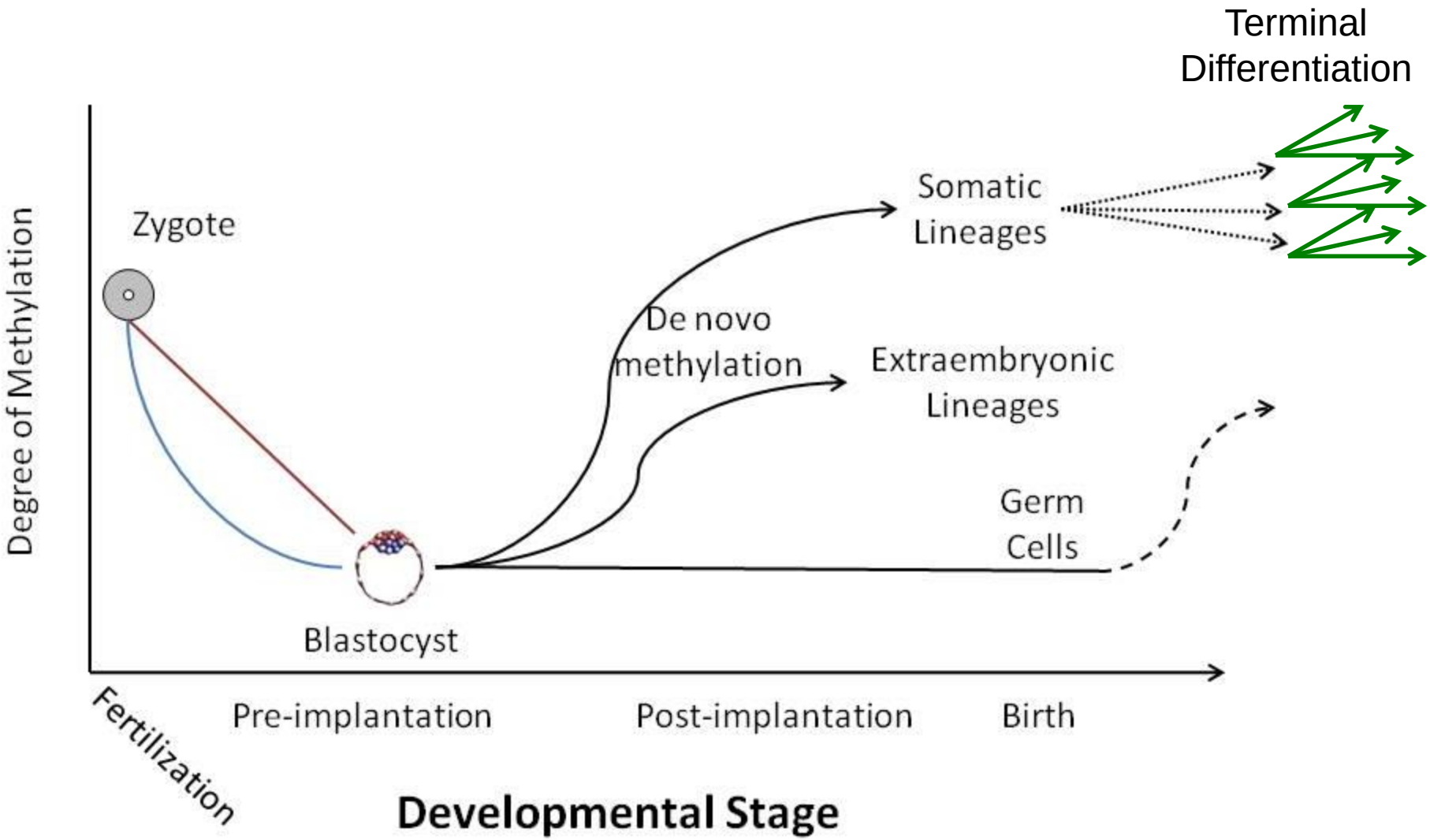
Cell type #2

Role for Epigenetic Mechanisms in "Normal" Tissue

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



Epigenetic Patterning Set in Development



Adapted from: Jaenisch. Trends Genetics 1997

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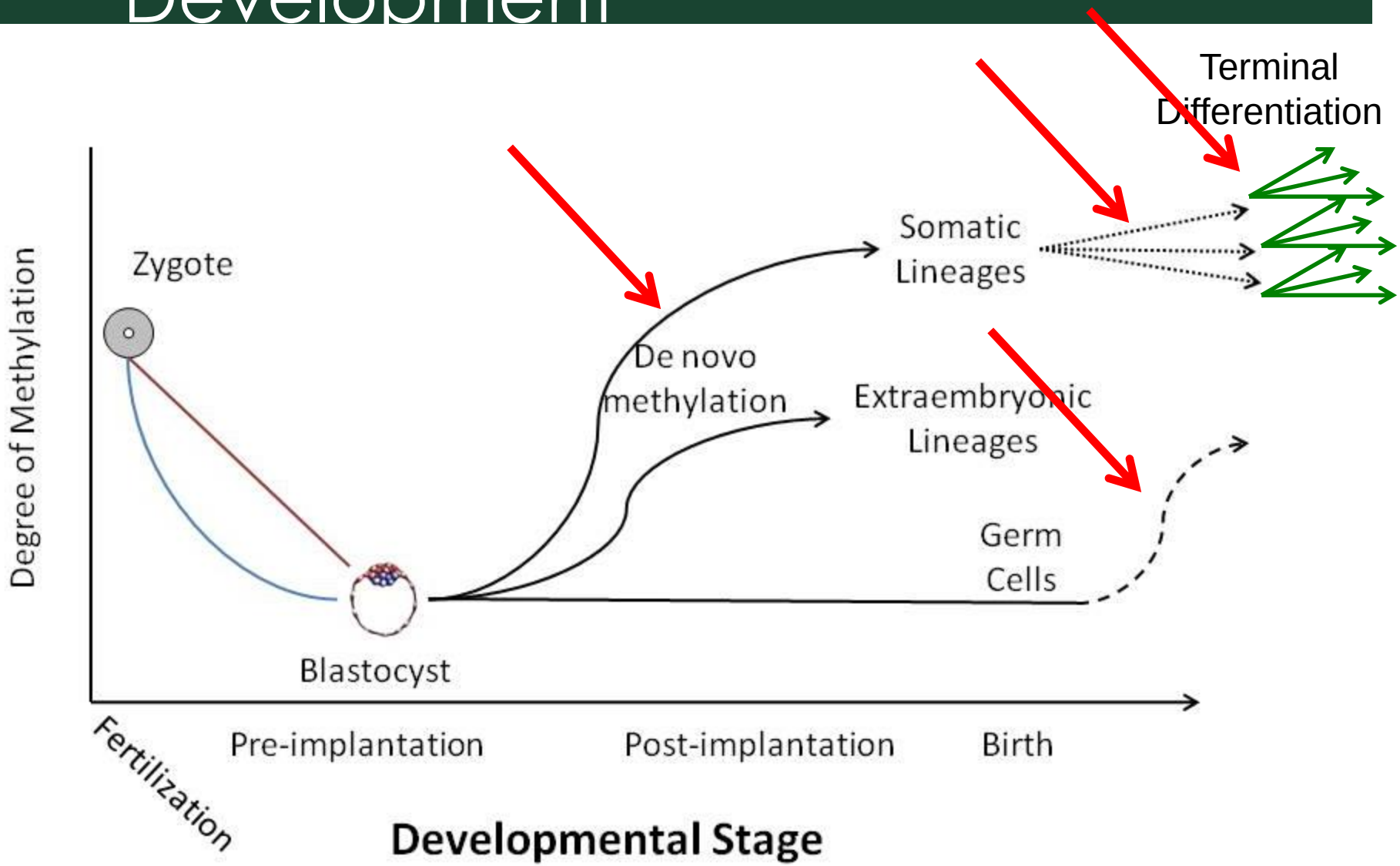

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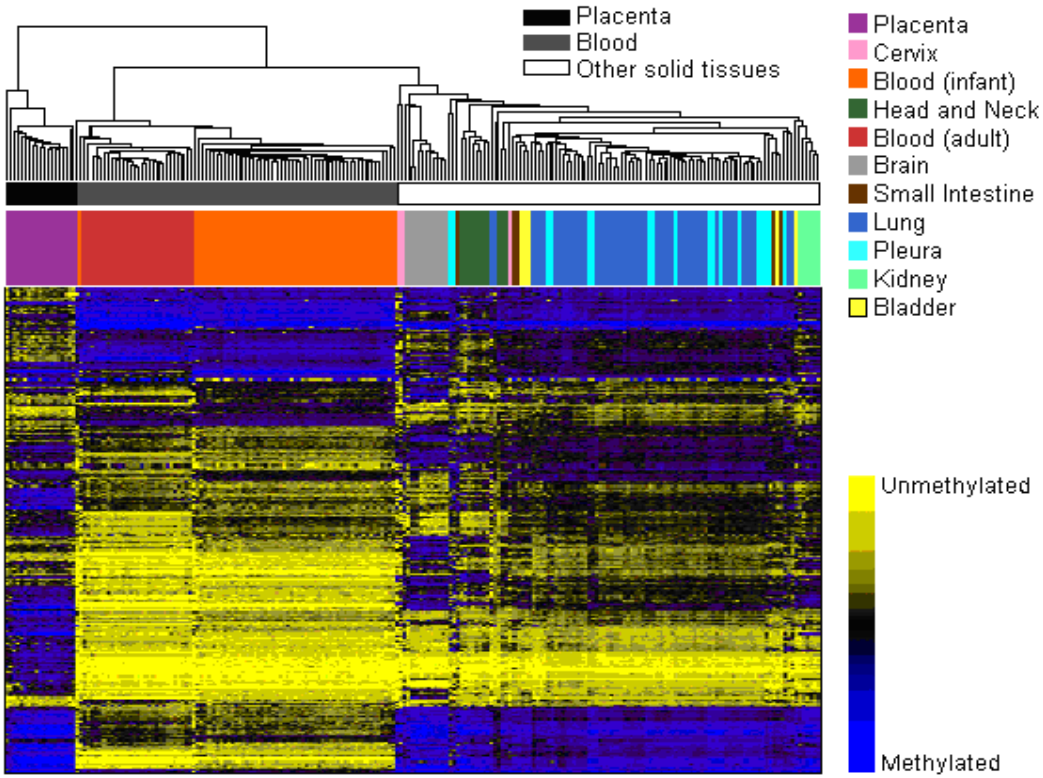
Epigenetic Mechanisms Differ by Tissue/Cell and Impart Distinct Functions



Christensen et al. PLoS Genetics 2009

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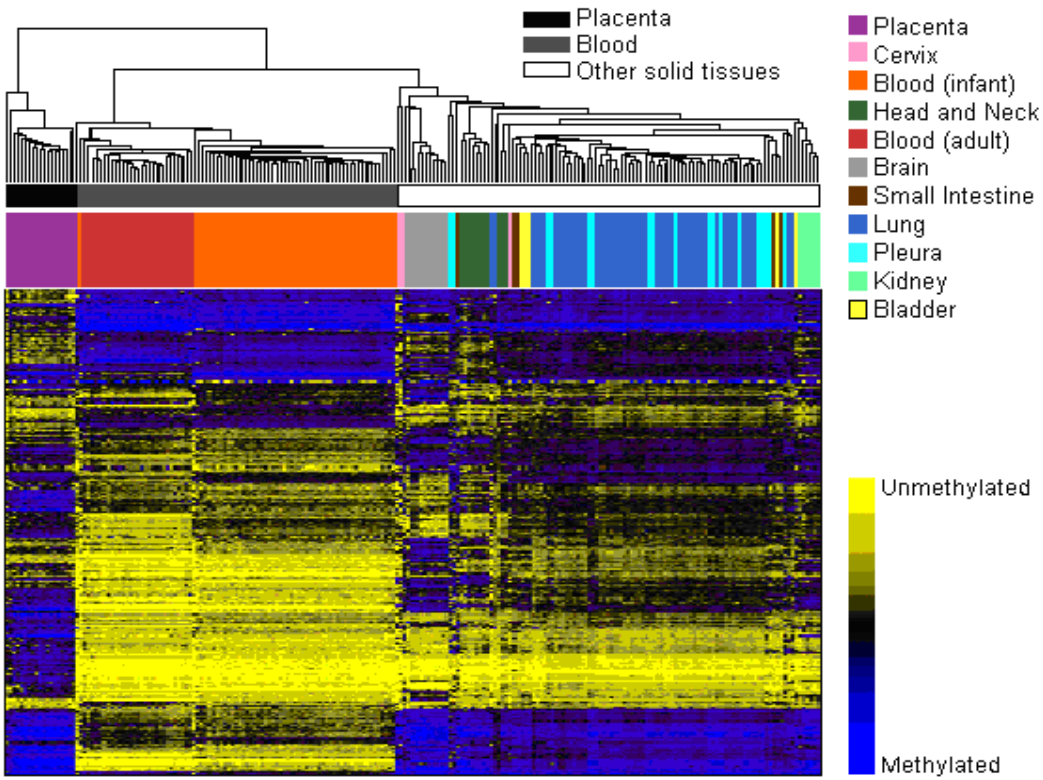

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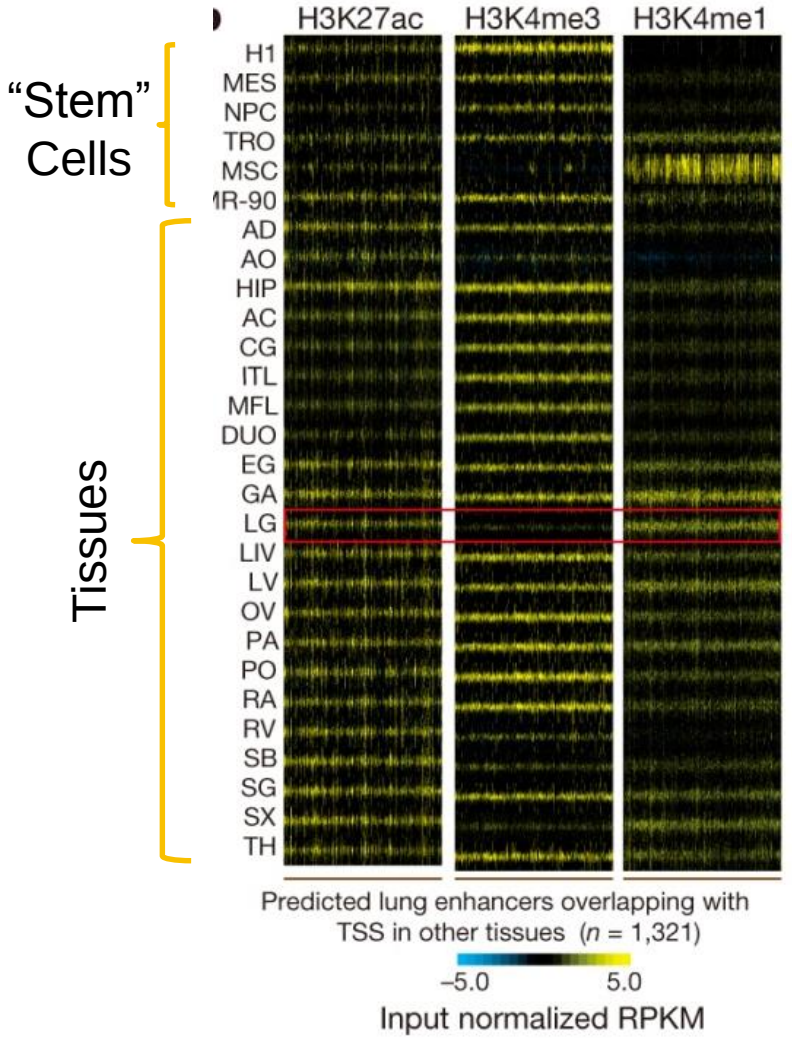

D Leung *et al. Nature* 2015

So….is all hope lost in examining epigenetic variation in risk?

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Must consider implications of epigenetic variation to the function of the cell/tissue of measurement

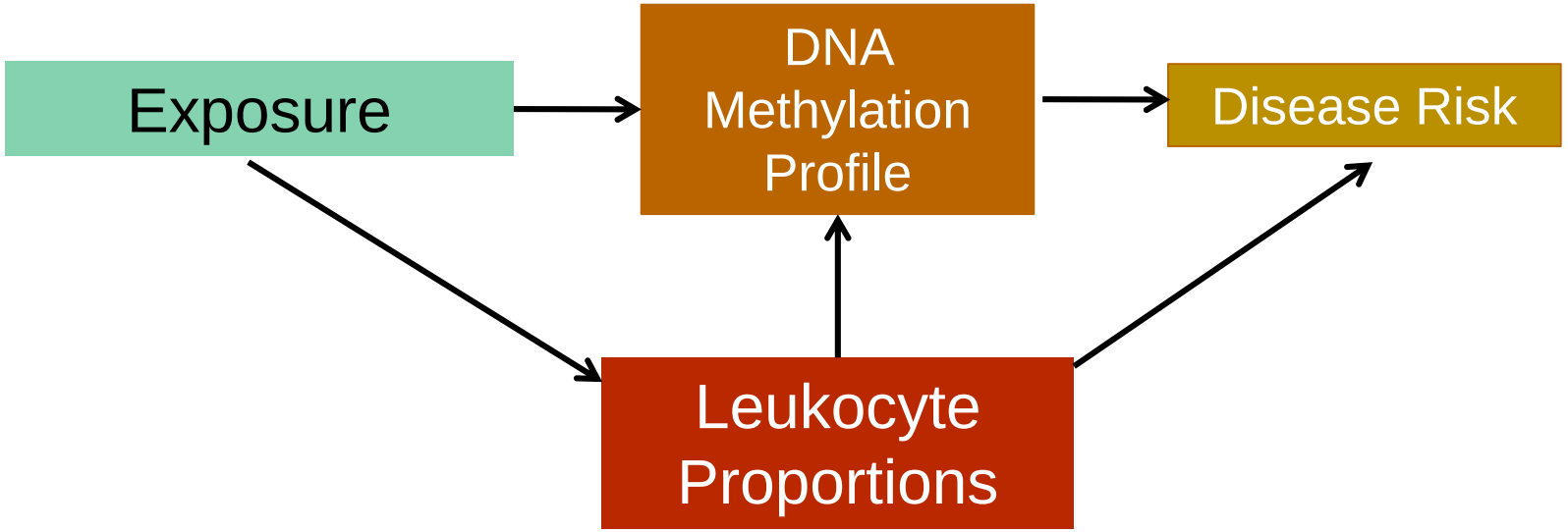
Accessible Tissues for Epigenetic Risk Markers

- 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
  - Hematopoetic 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



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

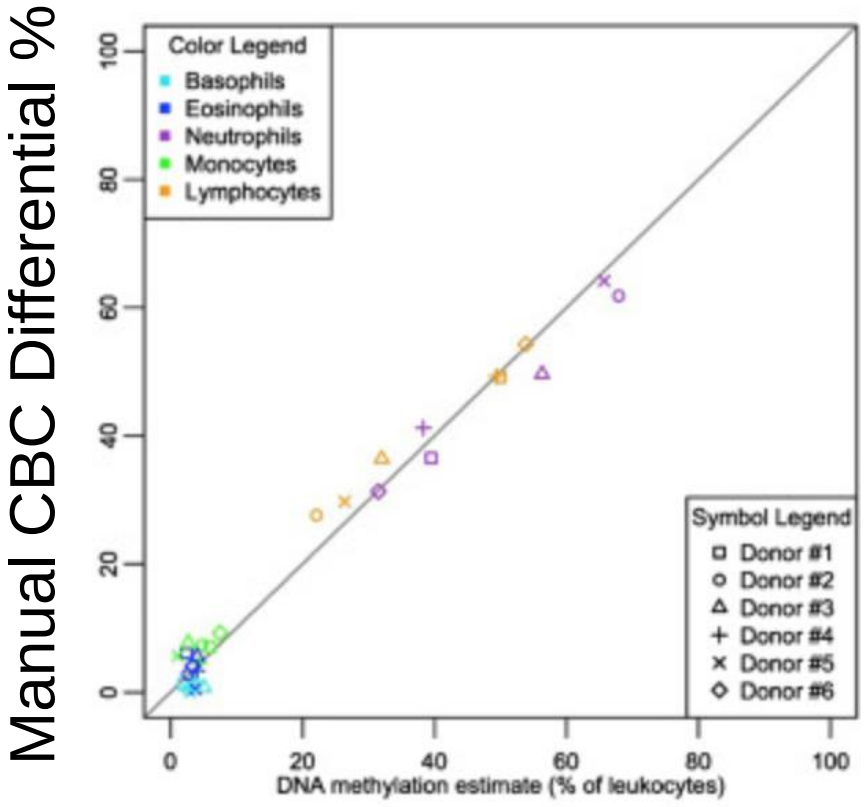
BMC Bioinformatics

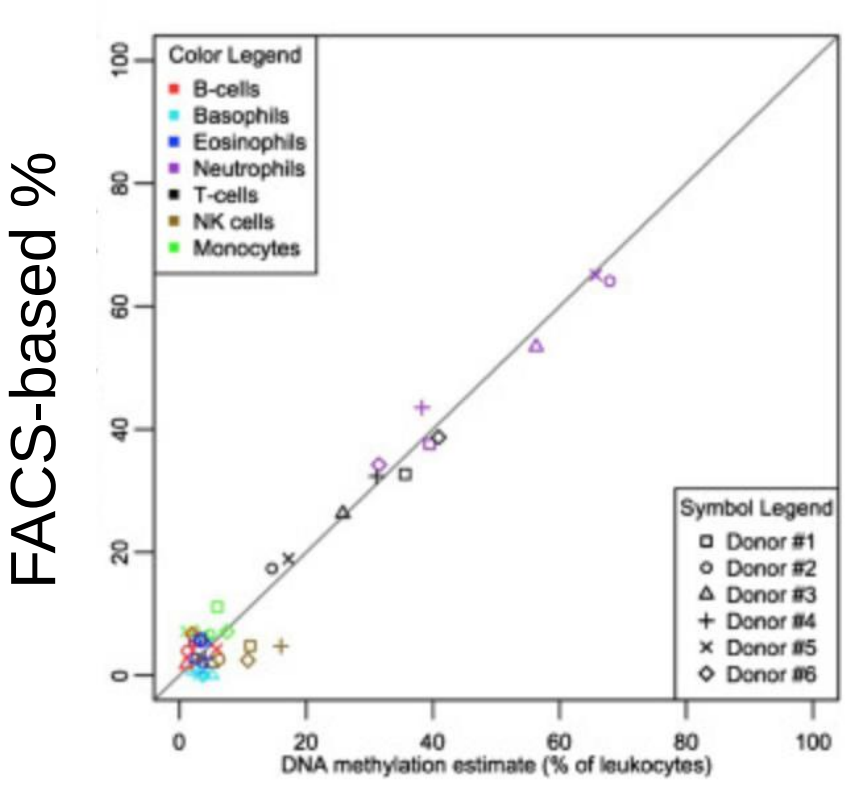
RESEARCH ARTICLE Open Access

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:





Methylation Array-based Estimate%

Arsenic Example

Koestler EHP 2013 (NH, urinary arsenic):

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

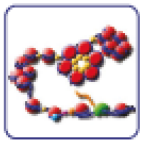
Kile Epigenetics 2014 (Bangladesh, Water As):

	Effect Estimate (Raw)[a] [% composition]	Effect Estimate (Bias-Adj)[b] [% composition]	SE[c]	*P* value[d]
B cell	-1.4	-1.4	0.71	0.056
Granulocyte	1.4	1.6	1.84	0.430
Monocyte	0.5	0.5	0.50	0.310
Natural Killer	-0.7	-0.9	0.73	0.317
T Cell (CD4+)	-7.4	-9.2	1.97	0.0002
T Cell (CD8+)	5.5	7.4	1.54	0.0004

Why cell composition is important

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

Bauer *et al. Clinical Epigenetics* (2015) 7:81
DOI 10.1186/s13148-015-0113-1



CLINICAL EPIGENETICS

RESEARCH Open Access

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

Acknowledgments

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  - Karl Kelsey – Brown
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