Epigenomics: Resources, Obstacles, and Opportunities

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Interindividual Variability Meeting
September 30, 2015
Epigenomic Changes Have Been Implicated in a Wide Variety of Human Diseases

**Normal processes**
- Development
- Cell differentiation
- Aging

**External influences**
- Environmental exposures
- Nutrition
- Chemical toxins
- Metals
- Mediators of stress
- Infection
- Drugs of abuse

**Adverse health outcomes**
- Cancer
- Cardiopulmonary disease
- Autoimmune disease
- Obesity
- Diabetes
- AIDS
- Neurodevelopmental disorders
- Schizophrenia
- Depression
- Alzheimer’s Disease
- Addiction
Increasing Number of Publications in Neuroepigenetics

![Bar chart showing the increasing number of non-review publications in Neuroepigenetics from 2005 to 2013. The number of publications increases significantly from 2005 to 2013.](Image)
Why do we care about epigenetics?

- Molecular mechanisms of disease
- Biomarkers
- Identify new therapeutic targets
- Epigenetic therapeutics (druggable targets)
- Intergenerational effects
Outline

1  Environmental Epigenomics

2

3  Other Resources and Obstacles
Drugs of Abuse: Epigenetic Brain Changes

Cocaine
Nestler lab, Science, 2010
Nestler lab, PNAS 2011
Cowan lab, Neuron, 2012

Nicotine
Kandel lab, Sci Transl Med, 2011
Guidotti lab, PNAS 2008

Methamphetamine
Itzak lab, Mol Psychiatry, 2014
Cadet lab, PlosOne 2014
Grant lab, PlosOne 2014

Cannabinoids
Hurd lab, Biol Psych. 2012
Hurd lab, Neuropsychopharm 2014
Nagarkatti lab, JBC 2014

Opioids
Kreek lab, Neuropsychopharm. 2008
Loh and Wei lab, PNAS 2012

Alcohol
Goldman lab, PNAS 2011
Atkinson lab, PlosGenetics 2013
Lanfumey lab, Mol Psych 2014
Some Environmental Toxicants Associated With Epigenetic Changes

- Endocrine disruptors
- Pesticides, herbicides
- Heavy metals
- Organic pollutants
- Air pollutants
Histone acetylation controls chromatin structure and gene expression

Less histone acetylation
Decreased gene expression

More histone acetylation
Increased gene expression

COMPRESSED CHROMATIN

EXPANDED CHROMATIN

Histone Acetyltransferase (HAT, e.g. CBP)

Histone Deacetylase (HDAC)
HDAC3 inhibitor (RGFP966) enhances extinction of cocaine-seeking behavior

Single dose HDAC3 inhibitor:
- enhances extinction of cocaine conditioned place preference (CPP)
## Drugs of Abuse: Intergenerational Effects

<table>
<thead>
<tr>
<th>EXPOSURE</th>
<th>PHENOTYPE (generation)</th>
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</table>
| Morphine (i.p.) adolescent female rat | Increased morphine analgesia, male F1 progeny  
| THC (i.p.) adolescents rat       | Compulsive heroin seeking and altered striatal plasticity, male F1 progeny  
Szutorisz et al. Neuropsychopharm. 2014, 39: 1215-1323 |
| Cocaine self admin. adult male rat | Delayed acquisition of cocaine self-administration, male F1 progeny  
Vassoler et al. 2013 Nat. Neuro. 16: 42-47 |
What is the mechanism of cocaine-associated information transmission from father to son?

Increased BDNF promoter acetylation in sperm of cocaine-exposed fathers.

Cocaine can reprogram the sperm epigenome.

Vassoler et al. 2013 Nat. Neuro. 16: 42-47
Outline

1 Environmental Epigenomics

2 Roadmap Epigenomics Project

3 Other Resources and Obstacles
NIH Roadmap Epigenomics Program

- Mapping Centers
- Data Coord. Center
- dbGAP/GEO
- Computational Epigenomics
- Health and Disease
- Epigenetic Assay Improvement
- In vivo Epigenetic Imaging
- Functional Epigenomic Manipulation
- Novel Marks

88 grants R01 and R21
Epigenome Mapping Centers

**GOAL:** Generate comprehensive epigenomic maps for “normal” human cells and tissues

- Protocols, assay standards, analysis tools
- First human methylomes (*Nature* 2009)
- 111 comprehensive epigenome datasets
- Data publically accessible: [http://www.roadmapepigenomics.org/](http://www.roadmapepigenomics.org/)
A Diversity of Human Cells and Tissues

Answered how human cell types and tissues differ epigenetically.

Did not ask how humans vary epigenetically from one another for a particular cell or tissue.
IHEC Members:

**US: Roadmap Epigenomics**
- European Union
- Canada
- Germany
- Japan
- South Korea

**US: ENCODE**
- Singapore

**France**
- United Kingdom
- Australia

http://epigenomesportal.ca/ihec/grid.html
ASSAYS

CELLS/TISSUES

http://epigenomesportal.ca/ihec/grid.html
The Utility of Epigenomic Information

- Functional genomic prediction
- Environmental exposures
- Understanding development and differentiation
- Regenerative medicine (stem and iPS cells)
- Human disease
- Interpreting GWAS
- Biomarkers, diagnostics and therapies
- Exploring cross-talk between epigenomic mechanisms
Epigenomic Modifications Mark Functional Genomic Elements

- **Enhancers**
  - H3K4me1
  - H3K27ac
  - DNase

- **Promoters**
  - H3K4me3
  - H3K9ac
  - DNase

- **Transcribed**
  - H3K36me3
  - H3K79me2
  - H4K20me1
  - DNAmethyl

- **Repressed**
  - H3K9me3
  - H3K27me3

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Adapted from Brad Bernstein
Gene variants in human disease

Epigenomic data for many normal human cell/tissue types

77% of disease variants are in/near enhancer elements or promoters*

Variants are in regulatory regions NOT protein coding regions

Generate hypotheses about function

*DNAse I hypersensitive sites

Stamatoyannopoulos, Science 337:1190, 2012
Use Epigenomic Information for “Normal” Cells/Tissues to Identify Pathogenic Cell Types

Crohn’s disease

- immune cells (n=15)
- CD34+ (n=1)
- thymus (n=10)
- ES/primitive (n=9)
- intestine (n=28)
- other (n=268)

Identify cell types involved in disease

GWAS P-value threshold

Stamatoyannopoulos, Science 337:1190, 2012
GOAL: Computational analyses taking advantage of the publicly available reference epigenomic maps along with other data sets.

GRANTS: Funded 10 2-year R01s in September, 2014
Technology Development in Epigenetics

**GOAL:** Develop revolutionary technologies with the potential to significantly change epigenetics research.

- Histone dynamics *(Science, Henikoff)*
- Single molecule epigenomics *(PNAS, Soloway)*
- SXRT of epigenomic organization *(Cell, Larabell/Lomvardas)*
- PET imaging of histone deacetylases *(Neuroimage, Gelovani)*
  *(J Med Chem, Hooker)*

- 2008 General epigenetic technologies
- 2011 Epigenetic imaging

Jacob Hooker lab
**Functional Epigenomics RFA (R01)**

**GOAL:** Develop Tools and Technologies for Cell-type, Temporal, and/or Locus-specific Manipulation of the Epigenome

**GRANTS:** Funded 10 R01s in September, 2013

- Epigenome editing using TALE, ZNF, CRISPR
- Opto-epigenetic & chemo-epigenetic strategies
- Transgenic mouse resources
**GOAL:** Transform our understanding of the epigenomic basis of disease

- 2009 CF/IC split
- 2011 IC only

Total: 33 R01s, 12 ICs

**Cardiovascular**
**Environmental toxins**
**Asthma**
**Cancer**
**Insulin resistance**
**Autoimmune**
**Glaucoma**
**Alzheimer’s**
**Psychiatric**
**Autism**
**Substance abuse**

Altered epigenetic states associated with:

- Gestational age at birth *(Feinberg/Fallin)*
- Hepatocellular carcinoma *(Meltzer)*
- Superenhancers *(Young)*
- Schizophrenia and bipolar disorder *(Mill)*
- Alzheimer’s disease
Epigenome-Wide Association Studies (EWAS) for Alzheimer’s Disease

- AD postmortem brain case/control
- Genome-wide DNA methylation assay
- 71 sites associated with AD pathology
- Two papers, partially overlapping loci

Lunnon et al, Nat Neurosci. 2014 17:1164-1170
Lord and Cruchaga, Nat Neurosci. 2014 17:1138-1140
How Do Human Epigenomes Vary?

Considerations:

• Good measurement of exposure phenotype
• Disease mechanism or biomarker?
• Cell/tissue heterogeneity and selection
• Which epigenetic feature should be measured?
• Computational analysis
• Replication/causation

Recent Roadmap Epigenomics Program Publications

8 publications in Nature
- Integrative analysis 111 reference epigenomes
- Haplotypes
- 3D structure
- Autoimmune disease
- Neuronal differentiation
- Cancer cells of origin

15 additional publications, Nature-associated journals
- Alzheimer’s disease
- Epigenomic imputation
- Colon cancer
- Sexual dimorphism and fetal growth
- Stem cells
- Age-related epigenomic variation
- Breast cell epigenomes
- Roadmap Epigenome Browser
- Asthma susceptibility

Feb 19, 2015
Cumulative Epigenomics Program Publications as of September, 2015

680 total

Cell 27
Nature 28
Science 4

Lister et al. 2009 MethylC-seq cited 1514 times

1 Environmental Epigenomics

2 ROADMAP epigenomics PROJECT

3 Other Resources and Obstacles
Common Fund 4D Nucleome Program

4D Nucleome
(2015-2022?)

Next generation tools: to explore the relationship between genome organization and function

- Imaging (NIBIB)
- Nucleomics (NHLBI)
- Nuclear bodies (NIDA)

Reference maps: of the 4D organization of the genome in a variety of human cells/tissues and cell states

- Nuclear Org. & Function Centers (NIDDK)
- Data Coordination/Integration Center (NCI)
- Organizational Hub (NCI)
Epigenetic Imaging and Biomarkers

Improved *in vivo* imaging of epigenetic enzymes or changes

Epigenetic biomarkers:

- Blood cell types
- Olfactory neurons?
- Body fluids?
  - Common Fund exRNA Program
Perdurance and Prevention

How long do epigenetic changes due to environmental exposures last?

Reversibility?

Intergenerational inheritance

- Validate!
- Epigenetic or non-epigenetic transmission?
- Protecting epigenomes for future generations?
What is Extracellular RNA?

Extracellular RNA (exRNA):

• RNAs found outside original cell
• Important in cell-cell communication

Why not digested by extracellular RNases?

• Protected and exported by:
  o Extracellular vesicles (e.g. exosomes)
  o Packaged with lipids or proteins
How can exRNAs communicate?

(a) Transcription

(b) Extracellular space

(c) Docking

(d) mRNA translation

(e) mRNA degradation

(f) Epigenetic modification

(g) siRNA

(donor cell)

(target cell)

In Vivo Imaging Reveals Extracellular Vesicle-Mediated Phenocopying of Metastatic Behavior


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http://dx.doi.org/10.1016/j.cell.2015.04.042

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SUMMARY

Most cancer cells release heterogeneous populations of extracellular vesicles (EVs) containing proteins, lipids, and nucleic acids. In vitro experiments showed that EV uptake can lead to transfer of functional mRNA and altered cellular behavior. However, similar in vivo experiments remain challenging because cells that take up EVs cannot be discriminated from non-EV-receiving cells. Here, we used the Cre-LoxP system to directly identify tumor cells that take up EVs in vivo. We show that EVs released by malignant tumor cells are taken up by less malignant tumor cells located within the same and with distant tumors and that these EVs carry mRNA involved in migration and metastasis. By intravital imaging, we show that the less malignant tumor cells that take up EVs display enhanced migratory behavior and metastatic capacity. We postulate that the delivery of functional mRNA by EVs can be used to guide cell fate.

Neuronal Differentiation of Human Mesenchymal Stem Cells Using Exosomes Derived from Differentiating Neuronal Cells

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Abstract

Exosomes deliver functional proteins and genetic materials to neighboring cells, and have potential applications for tissue regeneration. One possible mechanism of exosome-promoted tissue regeneration is through the delivery of microRNA (miRNA). In this study, we hypothesized that exosomes derived from neuronal progenitor cells contain miRNAs that promote neuronal differentiation. We treated mesenchymal stem cells (MSCs) daily with exosomes derived from PC12 cells, a neuronal cell line, for 1 week. After the treatment with PC12-derived exosomes, MSCs developed neuron-like morphology, and gene and protein expressions of neuronal markers were upregulated. Microarray analysis showed that the expression of miR-125b, which is known to play a role in neuronal differentiation of stem cells, was much higher in PC12-derived exosomes than in exosomes from B16-F10 melanoma cells. These results suggest that the delivery of miRNAs contained in PC12-derived exosomes is a possible mechanism explaining the neuronal differentiation of MSCs.
Common Fund exRNA Program

http://exrna.org/
Timing of studies of phenotypic consequence

0y - Family studies of paternal exposures
40y - Natural experiments
100y - Long-lived families

Epigenomic variation

Gametogenesis
Conception
Blastocyst stage
Embryogenesis
Fetal development
Birth
Neonatal period
Infancy
Childhood
Adolescence
Adulthood
Ageing
Extreme longevity

Cumulative stochastic changes
Cumulative environmental changes