Epigenetics: Basic Principals and role in health and disease

Cambridge Masterclass

Workshop on Epigenetics in GI Health and Disease

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Overview

• Basic principals of Epigenetics
  • Molecular biology
  • Concept and main mechanisms

• Epigenetics in health and disease
  • Cellular differentiation
  • Cell type specific gene expression
  • Genomic imprinting
  • Epigenetics and cancer
  • Multifactorial “complex” disease

• Summary
Molecular biology: back to basics

DNA → Transcription/Translation → Protein

Genotype → Environment/ageing/disease → Phenotype
Background: DNA and chromatin

H2A, H2B, H3, H4
Background: transcription and translation
Background: gene structure

Upstream

Promoter Region
CpG Island

Downstream

`5'` `5` `3`

- untranslated region (UTR)
- open reading frame (ORF)
- intron
From Genotype to Phenotype

- Each cell contains exactly the same copy of DNA
  - i.e. DNA sequence
- Gene expression/Phenotype vary between cell type
  - e.g. brain cell versus liver cell
- What are the mechanisms regulating cell/tissue type specific gene expression
  - What determines the phenotype?
Epigenetics: 1942

Conrad Hal Waddington (1905–1975)

• Developmental biologist

• Epigenetics formed as a combination of genetics and epigenesis: “Epigenetic landscape”

• Conceptual model of how genes might interact with their surroundings to produce a phenotype
Epigenetics today: Definition

The study of heritable changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence.

Epigenetic mechanisms include:

- Histone modification (e.g. acetylation, methylation)
- DNA methylation
- Expression of regulatory RNAs (e.g. miRNAs, tiRNAs)
Epigenetics: Histone modifications
Epigenetics: Histone modifications

Post translational modifications:

- **Ac**: acetylation
- **Me**: methylation
- **Ub**: ubiquination
- **SU**: sumoylation
- **P**: phosphorylation
Epigenetics: Histone modifications

(b) Transcription possible

Gene “switched on”
- Active (open) chromatin
- Unmethylated cytosines (white circles)
- Acetylated histones

Gene “switched off”
- Silent (condensed) chromatin
- Methylated cytosines (red circles)
- Deacetylated histones

Euchromatin

Heterochromatin

Transcription impeded

Jenke and Zilbauer, Current Opin Gastroenterol 2012
Epigenetics: DNA methylation
Epigenetics: DNA methylation

- occurs on 5’ Position of Cytosine in the context of the dinucleotide sequence CpG

- majority (75%) of all CpG dinucleotides in the mammalian genome are methylated

- Exception: CpG Islands in promoter regions
DNA methylation and gene transcription

In principal:

- Methylation of CpG (e.g. within promoter region)
  - Increase: reduced transcriptional activity
  - Decrease: increased transcriptional activity
Epigenetics: regulatory RNAs
Epigenetics: regulatory RNAs

- micro RNAs (miRNAs)
- small: 20-30 nucleotides
- “non-coding”
- \(~5\%\) of human genome codes for miRNAs (and others)
- Regulate gene expression
- Major focus of research:
  - e.g. disease biomarkers
  - long intervening non-coding RNAs (lincRNAs)
miRNA: principal mechanism
Epigenetics: two important facts

- **Influenced by environment**
  - Diet
  - Drugs/Pharmaceuticals
  - Development
  - Ageing

- **Heritable**
  - During mitosis and possibly miosis
  - Resetting during gametogenesis (incomplete)
  - Possibly trans-generational
    - Leading to acquired phenotype
Epigenetics: role in health and disease

- Cell differentiation
- Tissue/cell specific gene expression
- X-chromosome inactivation
- Genomic imprinting
- Silencing of repetitive (transposable) elements
- Malignancy, cancer development
- Emerging role in complex traits and immune mediated diseases
Epigenetics: Cellular differentiation
Epigenetics: Cellular differentiation

- “As per definition of Epigenetics”
  - Each cell type contains the same copy of DNA
  - During cellular differentiation specific phenotypes develop
    - Without changing the underlying DNA sequence
  - Most studied model: haematopoiesis
Epigenetics: Haematopoiesis

Hematopoiesis

CD19^+ B Cell
CD4/8^+ T Cell
Orthochromatic erythroblast
Reticulocyte
Erythrocyte (Red Blood Cells)

CD14^+ Monocyte
CD16^+ Neutrophil

Pluripotent Stem Cell
Pluripotent Stem Cell multiplication

Lymphoid Stem Cell

Basophilic myelocyte
Monocyte/granulocyte progenitor

Megakaryocyte
Thrombocytes (Platelets)

Eosinophilic myelocyte
Basophilic myelocyte

Eosinophil
Basophil
A comprehensive methylome map of lineage commitment from hematopoietic progenitors

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Epigenetics: Haematopoiesis

Abstract

Epigenetic modifications must underlie lineage-specific differentiation as terminally differentiated cells express tissue-specific genes, but their DNA sequence is unchanged. Hematopoiesis provides a well-defined model to study epigenetic modifications during cell-fate decisions, as multipotent progenitors (MPPs) differentiate into progressively restricted myeloid or lymphoid progenitors.
Epigenetics: Haematopoiesis

Ji et al.
Figure 1. Examples of known lineage-related genes showing differential DNA methylation between lymphoid and myeloid progenitors
Figure 1. Examples of known lineage-related genes showing differential DNA methylation between lymphoid and myeloid progenitors
Epigenetics: Regulating cell type specific gene expression
DNA methylation analysis in human lymphocyte subsets

- Analysis of 6 healthy individuals
- PBMCs extracted
- Isolation of 5 cell subsets:
  - CD14 (Monocytes)
  - CD16 (Neutrophils)
  - CD19 (B-Cells)
  - CD8 and CD4 (T-cells)

Diagram:
- CD19^+ B Cell
- CD4/8^+ T Cell
- Erythrocyte (Red Blood Cells)
- Reticulocyte
- Orthochromatric erythroblast
- Megakaryocyte
- Eosinophilic myelocyte
- Basophilic myelocyte
- Myeloblast/monoblast
- Eosinophilic myelocyte
- Monocyte/granulocyte progenitor
- Neutrophil
- Basophil
- Megakaryocyte
- Thrombocytes (Platelets)
- CD14^+ Monocyte
- CD16^+ Neutrophil

Hematopoiesis

Pluripotent Stem Cell

Multiplication
Study design/Materials & Methods

Cells/cell subsets

RNA → mRNA

flow through

RNA → gDNA

flow through

mRNA → methylation analysis

Requirement:
MeDIP-seq: 3µg
Illumina array: 1.5µg

expression profile

Requirement:
~500ng
Unsupervised clustering analysis

Sample relations based on 485836 loci

Cell subset specific DNA methylation profile

Zilbauer and Rayner et. al, under review
Unsupervised clustering analysis

Sample relation based on 485836 loci

Cell subset specific DNA methylation profile
Identification of “regulatory” DMRs

- Correlation of DMRs (differentially hypomethylated regions) with gene expression
- Cell type specific hypomethylated regions
  - Hypomethylation is associated with gene expression
Regulatory Hypomethylated Regions (rHMRs)

Cell type specific HMRs that correlate with gene expression
Summary and learning points

• Epigenetic mechanisms regulate cellular differentiation

• Epigenetic mechanisms regulate gene expression
  - Hence, all epigenetic mechanisms/signatures are highly cell type specific

• Investigating epigenetic mechanisms requires purification of individual cell subsets

• Correlation of epigenetic signatures (e.g. DNA methylation) with gene expression allows identification of potentially regulatory elements
Genomic imprinting
Genomic imprinting: Definition

- Genomic imprinting is an epigenetic phenomenon by which certain genes can be expressed in a parent-of-origin-specific manner.
Genomic imprinting: In principal

- Diploid organisms contain two copies of the genome in each somatic cell
  - 2 alleles – each inherited from one parent
  - Most genes are expressed from both alleles simultaneously

- Genomic imprinting in mammals
  - <1% of genes
  - Only one allele is expressed
  - Expression depends on parental origin
  - Example: Insulin like growth factor 2 (IGF2/igf2)
    - Expressed only by paternal allele
Genomic imprinting: In principal
Genomic imprinting: Disease

- **Prader-Willi and Angelman syndrome**
- Genetic mutation (e.g. deletion) of an Imprinted region
  - chromosomal region 15q11-13
  - PWACR – Prader Willi Angelman Critical Region
Genomic imprinting: Disease

- Prader-Willi Syndrome
  - Paternal deletion of region
  - Maternal copy is imprinted (i.e. silenced)
  - Symptoms: hypotonia, obesity, and hypogonadism

- Angleman syndrome
  - Maternal deletion of region
  - Paternal copy is imprinted (i.e. silenced)
  - Symptoms: epilepsy, tremors, smiling facial expression
Epigenetics: Role in Cancer development
Role of DNA methylation in health and disease

- Role in Cancer development

Epigenetics: Unravelling the cancer code
Vicki Brower
*Published online 23 March 2011*
Epigenetics: Role in “complex” disease
IBD – Disease Pathogenesis

- Luminal microbial antigens and adjuvants
- Immune response
- Environmental triggers
- Genetic susceptibility

IBD
Epigenetics: Impact of maternal diet on disease susceptibility

Maternal methyl-donor supplementation induces prolonged murine offspring colitis susceptibility in association with mucosal epigenetic and microbiomic changes

Tiffany D. Schaible¹, R. Alan Harris², Scot E. Dowd³, C. Wayne Smith¹ and Richard Kellermayer¹,*
Epigenetics and the intestinal microbiota

Epigenetic Regulation of TLR4 Gene Expression in Intestinal Epithelial Cells for the Maintenance of Intestinal Homeostasis

Kyoko Takahashi, Yutaka Sugi, Akira Hosono and Shuichi Kaminogawa

*J. Immunol.* 2009;183;6522-6529; originally published online Oct 21, 2009; doi:10.4049/jimmunol.0901271

http://www.jimmunol.org/cgi/content/full/183/10/6522
IBD- Facts and open questions

• Genetic predisposition explain 15-20% (at best)
  • What causes the remaining 80 to 85%?

• Rapid increase in IBD incidence in recent decades
  • In the absence of major changes to the human genome

• Major impact of environmental factors
  • E.g. western diet
Epigenetics in IBD

A novel framework to provide a plausible explanation for some of the main missing links
Revised model of IBD pathogenesis

Jenke and Zilbauer, Current Opin Gastroenterol 2012
Summary and learning points

- Epigenetics is the study of heritable changes of phenotype/gene expression that are due to mechanisms other than changes to the DNA sequence
  - Histone modifications
  - DNA methylation
  - Expression of non-coding RNAs (e.g. miRNAs)
Summary and learning points

- Epigenetic mechanisms operate in a highly cell-type specific manner (as per definition)
- Isolation / purification of cell subsets is a prerequisite prior to investigating the potential role of epigenetic mechanisms in health and disease
- Epigenetics has the potential to provide a novel framework for explaining some crucial missing links in immune mediated diseases including IBD
- Potential for future studies exciting but challenging
  - E.g. whole genome approach
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