Different Epigenetic Marks and Significance for Risk Assessment

Epigenetics and Cumulative Risk Workshop
September 2-3, 2015

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Three categories of epigenetic marks

- DNA Methylation
- Histone Modification
- Noncoding RNA

Considerations for risk assessment

- Demonstrated causative relationship or biologically plausible association with adverse outcome
- Demonstrated dose-response relationship
- Mechanistic understanding desirable (AOP)
- Reproducible, cost-effective, facile measurement

Based on above considerations, DNA methylation and noncoding RNA mechanisms most amenable to risk assessment today.
Epigenetic Marks

- DNA Methylation
  - Occurs at 5’ position of C residues in CpG dinucleotides
  - 60 to 70% of CpG sites methylated in mammals
  - Generally associated with gene silencing
  - Catalyzed by DNA methyltransferase (DNMT) using SAM as cofactor
  - Five mammalian DNMT (1, 2, 3A, 3B, 3L)
    - DNMT1 catalyzes maintenance methylation
    - DNMT3A & 3B catalyze *de novo* methylation
  - Direct effect by excluding transcription factor binding
  - Indirect effect by recruiting methyl-CpG binding domain proteins, and in turn, ATP-dependent chromatin remodeling proteins and HDACs
- CpG Islands (CGI)
  - ≥200 bp & ≥ 50% GC content
  - Present in ~40% mammalian promoters
  - Often hypomethylated
- X chromosome Inactivation and Imprinting
• Methyl binding domain proteins
• ATP-Dependent Chromatin Remodeling
• Histone deacetylase
Example of DNA Methylation

- Study reported by Tang *et al.*

- Neonatal exposures

- Methylation-sensitive restriction endonuclease fingerprinting performed to identify differentially methylated DNA sequences

- Of fourteen (14) candidate genes identified, *Nsbp1* selected for further study because of important role in chromosome remodeling
  - Downregulation of *Nsbp1* results in suppression of tumor growth and metastatic phenotype

- CpG island (CGI) within *Nsbp1* promoter minimally methylated in immature, control mice, but increasing methylation and gene silencing with age

- Effect of DES or GEN treatment?
Example of DNA Methylation

Example of DNA Methylation

**Epigenetic Marks**

- **Non-coding RNA (ncRNA)**
  - piwi-interacting RNA (piRNA): 26-31 nt molecules that interact with piwi proteins to silence retrotransposons in germ line cells
  - long ncRNA: > 200 nt, involved in regulating gene transcription, splicing, and translation
  - microRNA (miRNA): 21 to 22 nt
    - Mammalian genomes encode ~800 conserved miRNA
    - Expression highly cell-type specific
    - Function
      - Promote mRNA degradation
      - Destabilize mRNA through shortening of polyA tail
      - Inhibit translation
    - Algorithms predict hundreds of targets for each miRNA
      - Current models suggest fine-tuning gene expression, typically no more than a 2-fold change
      - Coordinated regulation of a suite of transcripts results in a more robust phenotype
miRNA Synthesis

5’ Cap

Pri-miRNA

Drosha
DGCR8

Pre-miRNA

Exportin5

Cytoplasm

miRNA duplex

Argonaut proteins

RISC Complex

miRNA/Ago mRNA complex

NUCLEUS
miRNA Function

- **Direct Signal Mediation**
  - Signal → miRNA → Response

- **Signal Modulation**
  - Signal → Mediator → Response

- **Negative Feedback**
  - Positive Regulator → Signal → miRNA → Response

- **Positive Feedback**
  - Negative Regulator → Signal → miRNA → Response
miRNA as Toxicity Biomarkers

- Changes in miRNA expression prior to adverse phenotype and in some cases, appearance in various biological matrices
- Appearance in biological matrices linked to specific cellular responses, including death, proliferation, metabolism, and inflammation
- Advantages of miRNA vs. protein biomarkers
  - Greater stability due to lipoprotein complexes (exosomes)
  - Cell-type specific expression allows linkage of appearance in biological matrix to specific tissues/cells
  - Evolving, high throughput and sensitive detection methods
    - Global sequencing
    - Multiplexed arrays for specific miRNA panels
miRNA Toxicity Biomarker Example

- Study reported by Yang et al.

- Blood and urine samples from 3 cohorts
  - Healthy children with no APAP exposure (N=10)
  - Hospitalized children on standardized APAP therapy (N=10)
  - Children hospitalized due to APAP overdose (N=8) (dose = 59 to 559 mg/kg)

- miRNA quantified using small RNA global sequencing (serum) or PCR-based arrays (urine) with verification by qPCR

- Cluster analysis based on patterns of altered miRNA levels

- Comparison to serum ALT and APAP-protein adducts
miRNA Toxicity Biomarker

Correlation with APAP-protein adducts, but not serum ALT ($r=0.70$, $p<0.05$)

Correlation with APAP-protein adducts, but not serum ALT ($r=0.94$, $p<0.001$)

• Of 3 major categories of epigenetic marks, DNA methylation and ncRNA (miRNA) offer the greatest immediate promise for environmental health risk assessment

• Gaps still needing attention
  • Sensitivity and specificity as biomarkers for specific adverse outcomes
    • Incorporation into AOP framework?
  • Better understanding of MOA
  • Better understanding of interindividual variability, what is “normal,” and adaptive vs adverse response
  • Focus on non-cancer end-points
  • Increased focus on environmental chemical exposures at low doses