Session 2:
Biomarkers of epigenetic changes and their applicability to genetic toxicology

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Workshop:
"Genetic Toxicology: Opportunities to Integrate New Approaches"

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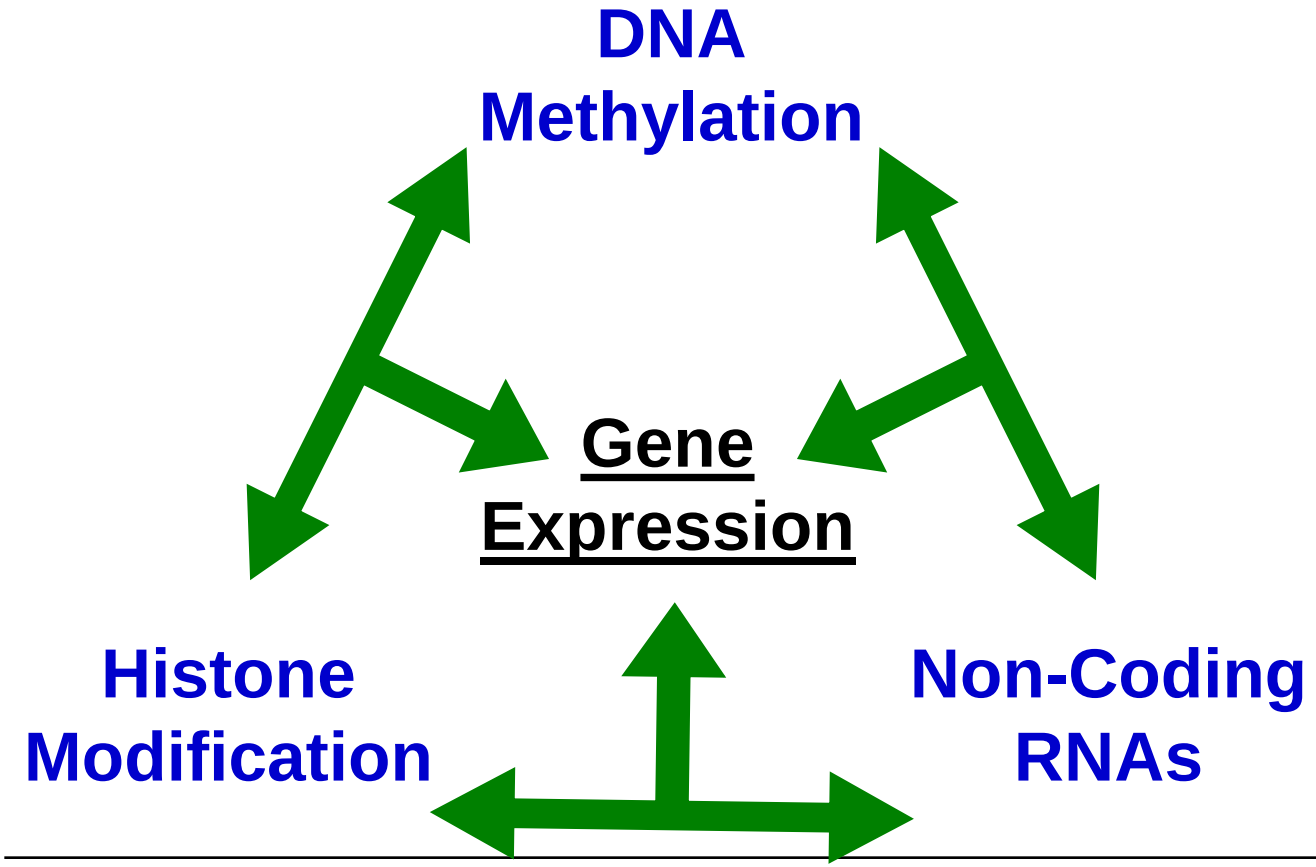
WHY A SESSION ON EPIGENETICS

- *Mutations*: heritable *c*hanges to the sequence of bases of the genome
- *Epimutations:* heritable non-sequence changes to the cellular genome which affect the capacity of its constituent genes to be expressed.
- Genetic toxicology tests are routinely used to predict mutagenic and carcinogenic potential of chemicals
  - Predictive performance good but not great!
- Should we be paying more attention of *epimutagenesis* in safety assessment studies ?

D. G. MacPhee (1998): Epigenetics and epimutagens: some new perspectives on cancer, germ line effects and endocrine disrupters, Mutation Research, 400, 369-379.

EPIGENETICS

Heritable modifications superimposed on DNA base sequence that regulate gene expression



DNA Methylation

- Normal and essential biological process
  - X-chromosome inactivation, development, cellular differentiation, chromatin structure, imprinting, genome stability
- Imprinting maternal vs. paternal expression
- Demethylation in post-fertilization zygote
- Remethylation during development

Hypermethylation = less transcription

Hypomethylation = more transcription

Histone Modifications

- Core histones H2, H2B, H3, H4 can be modified (e.g., methylated, acetylated, phosphorylated, sumolated, ubiquitinated)
- Some examples…

		Gene Expression			
		H3K4	**H3K9**	**H3K27**	**H2BK5**
Acetylation			↑		
Methylation	**mono-**	↑	↑	↑	↑
	di-		↓	↓	
	tri-	↑	↓	↓	↓

Non-coding RNA

Particular interest in microRNA

- Influence gene expression transcriptionally, post-transcriptionally (mRNA degradation and translational inhibition), and by chromatin conformation
- Increase genomic integrity by silencing transposons and stabilizing centromeres

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1:45PM A new paradigm for epigenetic control of cell phenotype: Dynamic reprogramming of tRNA modifications and ribosomes controls selective translation of stress response proteins

Dr. Peter Dedon (Massachusetts Institute of Technology)

2:15PM Epigenomics and impact for drug safety sciences

Dr. Jennifer Marlowe (Novartis)

2:45PM Epigenetic traits as biomarkers of carcinogenesis

Dr. Igor Pogribny (U.S. Food and Drug Administration, NCTR)

3:15PM MIR-34 prevents in vivo lung tumor initiation and progression in the therapeutically resistant *KRAS;TRP53* mouse model

Dr. Andrea Kasinski (Yale University)

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- Questions for the discussion
  - Can biomarkers of epigenetic changes improve/complement the safety assessment process?
  - Is there a need to design screening systems to identify “epimutations” with potential trans-generational inheritance?
  - Can new, less invasive trans-species biomarkers of exposure, susceptibility and effect be identified from accumulated knowledge on epigenetics phenomena?
  - What do we know about the dose-response relationship for epigenetic effects?

Can biomarkers of epigenetic changes improve/complement the safety assessment process?

- Current biomarkers are based on limited # of chemicals
  - Daunting to define baseline
- Are they sensitive enough to detect changes at low doses?

Is there a need to design screening systems to identify “epimutations” with potential trans-generational inheritance?

Can new, less invasive trans-species biomarkers of exposure, susceptibility and effect be identified from accumulated knowledge on epigenetics phenomena?

What do we know about the dose-response relationship for epigenetic effects?

Discussion

- How to differentiate between biomarkers of epigenetic changes – adverse/adaptive
  - Not a lot of mechanistic meaning yet in our understanding
  - Still need to mine current biomarkers data for meaningful correlations – need prospective human study
- Epigenetic markers useful for safety eval prospectively?
  - miRNAs? (precedence in cancer research)
  - Do we know enough to use any right now?
    - Use epigenetic tests as screening (?) tests for large numbers of chemicals (e.g. follow-up positives with 2-year bioassay)
    - Biomarkers for mechanistic insight vs. predictive risk (as long as measurement correlates well with outcome)
- How predictive are in vitro cell lines for in vivo situation?
- miRNAs downstream from p53 as biomarkers
- Human biomarkers preferably assessed from blood
  - Need better understanding of how biomarkers get in urine/blood
- Regulatory eval – based on dose; need better dose-response evaluation with biomarkers, histopath, other markers (AST), etc.
- Epigenetic changes in germ cells (transgenerational effects)

Recent Safety Assessment Reviews

LeBaron *et al*. (2010): Epigenetic and chemical safety assessment, Mutation Res., 705, 83-95.

Rasoulpour *et al*. (2011): Epigenetic screening in product safety assessment: are we there yet? Toxicolo. Mech. Methods, 21: 298-311.

Priestley *et al.* (2012): Epigenetics – relevance to drug safety science, Toxicology Research (In Press; DOI: 10.1039/c2tx00003b)