Epigenetics and Toxicology

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Division of Biochemical Toxicology
National Center for Toxicology Research
U.S.-Food and Drug Administration

The views expressed in this presentation do not necessarily represent those of the U.S. Food and Drug Administration
Updates in Toxicology

Hispanic Organization of Toxicologists - HOT

http://www.toxicology.org/groups/sig/hot/about.asp
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1. Epigenetics

2. Epigenetics and toxicology

3. Epigenetics and carcinogenesis

4. Epigenomic alterations in furan-induced hepatobiliary pathologies

5. Conclusions
Epigenetics

Inheritance of gene expression patterns without altering the DNA sequencing but by adapting the chromatin.
Inheritance of gene expression patterns without altering the DNA sequencing but by adapting the chromatin.

- **DNA methylation**
- **Post-translational histones modifications**
- **Non-coding RNAs**
Epigenetics

Topologically associated domains
3-dimensional space
Enhancer-promoter communication

Epigenetic marks interaction
MeCP2, HDAC, Sin3A
Transcriptional repressor

Polycomb silencing
PRC1 and PRC2 - H3K27me3

Histones variants
H3.3
H2A.X

Bivalent chromatin
H3K4me3/H3K27me3

Epigenomic signatures
All instructive chromatin alterations
H3K4me1/H3K27ac - enhancer region
H3K4me3 – promoter region
H3K27me3 – Polycomb repressed region

Adapted from Allis and Jenuwein, Nature Reviews, 2016
The epigenetic code

Genomic location of DNA methylation

Histone code

Methylation
Acetylation
Phosphorylation

Transcribed  Active
Repressed  HP1
DNA methylation

Nucleus
Epigenetic regulation

- **Writers**: DNMTs, HATs, HKMTs
- **Erasers**: TETs, HDACs, KDMs
- **Readers**: MBPs, Proteins with bromodomains or chromodomains

Adapted from Falkenberg and Jonhstone, Nature Reviews, 2014
Individual susceptibility to a given exposure is likely to depend on the epigenetic make-up that dictates an individual response and adaptation mechanism.

*Herceg et al., Carcinogenesis, 2013*
Epigenetics application in toxicology

- Identify chemicals with potential to cause adverse effects
- Elucidate mechanisms of action and affect weight of evidence conclusions
- Categorize compounds by mechanistic class
- Rank by relative potency and epigenetic signature
- Assist in identifying susceptible populations and life stages
- Use biomarkers of exposure and/or effects
- Help identify cumulative risk factors
- Better understand uncertainties of cross tissue or species paradigms

Adapted from Cote et al., COTOX, 2017
Epigenetics application in toxicology

- Mechanistic studies
- Biomarkers
- Risk assessment
Epigenetics application in toxicology

Mechanistic studies
Epigenetics application in toxicology

Mechanistic studies

Editor's Highlight: Organ-Specific Epigenetic Changes Induced by the Nongenotoxic Liver Carcinogen Methapyrilene in Fischer 344 Rats.

Shpyleva S¹, Dreval K¹, de Conti A³, Kindrat J¹, Melnyk S², Yan J³, Chen T³, Beland FA¹, Pogribny IP¹.

Gene-specific H3K9ac

Gene expression

Graph showing % of input with control and Methapyrilene, 40 mg/kg bw/day treatments. Bars for Prox1, Hnf1a, and Ppara with fold change comparisons for CTL and 40 treatments.
Epigenetics application in toxicology

Mechanistic studies

Biomarkers
Epigenetics application in toxicology

Clinical implementation of DNA methylation based cancer biomarkers

Mechanistic studies

Biomarkers

14,743 published papers describing DNA methylation bases biomarkers

Koch et al., Nature Reviews, July, 2018

1,800 markers

14
Epigenetics application in toxicology

Clinical implementation of DNA methylation based cancer biomarkers

Mechanistic studies

Biomarkers

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14 commercially available

GSTP1
APC
RASSF1
NDRG4
BMP3
SEPT9
SHOX2
TWIST1
OTX1
ONECUT2
MGMT
BCAT1
IKZF1

Koch et al., Nature Reviews, July, 2018
Epigenetics application in toxicology

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Clinical implementation of DNA methylation based cancer biomarkers

Koch et al., Nature Reviews, July, 2018
Epigenetics application in toxicology

Clinical implementation of DNA methylation based cancer biomarkers

Mechanistic studies

Biomarkers

Genomic location of DNA methylation

GSTP1 promoter

14,743 published papers describing DNA methylation bases biomarkers

1,800 markers

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FDA approved

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MGMT
BCAT1
IKZF1

97.1% Distinguish HCC x Normal 60%

Jain et al., PLoS ONE, 2012

Koch et al., Nature Reviews, July, 2018
Epigenetics application in toxicology

- Mechanistic studies
- Biomarkers
- Risk assessment

Carcinogen identification

International Agency for Research on Cancer

Volume 112 - 2015
Monographs Programme incorporates a formal search for studies on epigenetic effects in all evaluations.
Carcinogenicity testing – challenges

90,000 chemicals are currently used in commerce

< 2% exposure x human health

Cote et al., 2017

>700 chemicals tested in two-years bioassays

50% are carcinogens

50% Genotoxic carcinogens
- DNA adducts
- Mutation assays
- Chromosome alterations

50% Non-genotoxic carcinogens
- Epigenetic mechanisms
- Receptor-mediated pathways (dioxin)
Epigenetic carcinogens

- Drugs
  - DES
  - Prostaglandin E2
  - Hormone therapy
  - Cyclophosphamide

- Biological agents
  - HBV
  - HCV
  - Human papillomaviruses
  - Epstein-Barr virus
  - Helicobacter pylori

- Environmental
  - Arsenic
  - Cadmium
  - Nickel
  - Beryllium
  - Asbestos
  - X-radiation
  - Gamma-radiation
  - Smoky coal emissions

- Life style and diet
  - Tobacco smoking
  - Alcohol consumption
  - Obesity

Herceg et al., Carcinogenesis, 2013
Furan: food contaminant

Volatile compound widely used in the chemical manufacturing industry also found in a variety of heat-produced foods.

- Thermal degradation of sugars
- Oxidation of polyunsaturated fatty acids
- Decomposition of vitamin C

Mean exposure to furan for the U.S. population is **0.25 μg/kg** body weight (bw)/day which approximately one half is provided by coffee canned and jarred foods (4.9–48.5 ng/mL) orange juice (0.59 - 27.39 ng/mL) soy sauce (44.32–178 ng/mL) coffee (67.8–1476 ng/mL)
Furan as Environmental Hepatocarcinogen

“reasonably anticipated to be a human carcinogen”

2-year bioassay by the NTP (1993)

“possible human carcinogen (Group 2B)”

- Lifetime exposure of rats and mice to furan resulted in the development of:
  - cholangiocarcinoma;
  - hepatocellular adenoma;
  - hepatocellular carcinoma;
  - mononuclear cell leukemia.
Metabolic activation of Furan

Lack of evidence for genotoxicity of furan in vivo.
Furan: Experimental protocols

Protocol 1: dose and time effects

Protocol 2: stop exposure effects

Protocol 3: long term exposure effects
Dose- and time-dependent epigenetic changes in the livers of Fisher 344 rats exposed to furan.
Conti Ad¹, Kobets T, Escudero-Lourdes C, Montgomery B, Tryndyak V, Beland FA, Doerge DR, Pogribny IP.

**Protocol 1**

Male Fisher 344

- 90 days
- 180 days
- 360 days

5 days per week

- Control group
- Furan 0.92 mg/kg bw/day
- Furan 2.0 mg/kg bw/day
- Furan 4.4 mg/kg bw/day

**Protocol 2**

Male Fisher 344

- 0 days
- 90 days
- 180 days
- 360 days

90 days

- Control group
- Furan 8 mg/kg bw/day

Persistence of furan-induced epigenetic aberrations in the livers of F344 rats.
de Conti A¹, Kobets T¹, Tryndyak V¹, Burnett SD¹, Han T¹, Fuscoe JC¹, Beland FA¹, Doerge DR¹, Pogribny IP².
Dose- and time-dependent epigenetic changes in the livers of Fisher 344 rats exposed to furan.

**Protocol 1**

- Male Fisher 344
- 90 180 360 days
- 5 days per week

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**H3K9ac**

- Change from control, %
- 90 days 180 days 360 days

**H3K56ac**

- Change from control, %
- 90 days 180 days 360 days

**H3K9me3**

- Change from control, %
- 90 days 180 days 360 days

**Global DNA methylation**

- Change from control, %
- 90 days 180 days 360 days

Persistence of furan-induced epigenetic aberrations in the livers of F344 rats.

**Protocol 2**

- Male Fisher 344
- 0 90 180 360 days

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**Chromatin structure based on DNA methylation accessibility**

- Change from control, %
- 0 90 180 360 days

**Transcription factors**

- Open chromatin
- Furan
- Compact chromatin

**Gene expression**

- Upregulation
- Downregulation
Furan-induced transcriptomic and gene-specific DNA methylation changes in the livers of Fischer 344 rats in a 2-year carcinogenicity study.

Global transcriptomic analysis

Common differentially expressed genes (DEG)

Next Generation Bisulfite Sequencing – DNA methylation

1001 DEG
109 DMR-containing and inversely correlated with DEG
42 DMR/CpG island-containing and inversely correlated with DEG

Relevance to carcinogenesis
Furan-induced transcriptomic and gene-specific DNA methylation changes in the livers of Fischer 344 rats in a 2-year carcinogenicity study.

Tryndyk V1, de Conti A1, Dorgan JF1, Olson GR2, Beland FA1, Poqibiny IP3.

### Protocol 3

**Male Fischer 344**

- 728 days
- 5 days per week

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**Gene-specific methylation**

- **Areg**
  - Fold change
  - % of methylation
  - Fold change

**Gene-specific histones modifications**

- **Jag1**
  - Fold change
  - % of methylation
  - Fold change

- **Foxe1**
  - Fold change
  - % of methylation
  - Fold change
Summary:

- The exposure of F344 rats to furan resulted in dose- and time-dependent epigenetic changes consisting of:
  - Some of these changes persist, even when exposure to furan was discontinued.
  - Our findings illustrate that gene-specific DNA methylation changes have functional consequences including alterations in the genes *Areg*, *Jag1*, and *Foxe1* that are involved in key pathways associated with different aspects of liver pathology and may be an important component of furan hepatotoxicity and hepatocarcinogenicity.
Irreversible down-regulation of miR-375 in the livers of Fischer 344 rats after chronic furan exposure.

de Conti A¹, Tryndyak V¹, Doerge DR¹, Beland FA¹, Pogribny IP².

Expression of selected miRNAs involved in liver carcinogenesis

miR-34a, miR-93, miR-96, miR-122, miR-200a, miR-200b, miR-224, miR-375
miR-375 targets the oncogene YAP1

Cells transfected with miR-375

miR-375 - tumor suppressor miRNA

Hippo pathway

YAP

Genes related to proliferation, differentiation, EMT, fibrosis...
Protocol 3 YAP1 expression and miR-375 epigenetic dysregulation
New insights into the molecular mechanisms of chemical carcinogenesis: In vivo adduction of histone H2B by a reactive metabolite of the chemical carcinogen furan.

Nunes J¹, Martins IL¹, Charneira C¹, Pogribny IP², de Conti A², Beland FA², Marques MM¹, Jacob CC¹, Antunes AMM³.
New insights into the molecular mechanisms of chemical carcinogenesis: In vivo adduction of histone H2B by a reactive metabolite of the chemical carcinogen furan.

Nunes J¹, Martins IL¹, Charneira C¹, Pogribny IP², de Conti A², Beland FA², Marques MM¹, Jacob CC¹, Antunes AMM³.
Conclusions

➢ The results of these studies provide support to:

- the hypothesis of a non-genotoxic mode of action of furan;
- the importance of epigenetic alterations in the mechanism of furan hepatotoxicity.

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Acknowledgments

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National Center for Toxicological Research

Igor Pogribny, MD, PhD
Volodymyr Tryndyak, PhD
Kostantyan Dreval, PhD
Marta Pogribna, MD
Iryna Kindrat, MS
Svitlana Shpyleva, PhD

Frederick Beland, PhD