ROLE OF EPIGENETIC EVENTS IN RESPONSES TO TOXIC AGENTS

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**GLOSSARY**

**CpG sites**: Regions of DNA where a cytosine nucleotide occurs next to a guanine nucleotide in the linear sequence of bases along its length. "CpG" stands for cytosine and guanine separated by a phosphate, which links the two nucleosides together in DNA. The "CpG" notation is used to distinguish a cytosine followed by guanine from a cytosine base paired to a guanine.

**DNA methylation**: Methylation of cytosine residue at CpG sites.

**Hemimethylated CpG site**: -CG-

**Unmethylated CpG site**: -CG-

**Histone methylation**: The modification of certain lysine or arginine residues in a histone protein by the addition of one, two, or three methyl groups.

**Histone acetylation**: The modification of certain lysine residues in a histone protein by the addition of the acetyl group.
EPIGENETICS – heritable changes in gene expression mediated by methylation of DNA, modifications of histone proteins, nucleosome positioning along DNA, or by non-coding RNAs that are not due to any alteration in the DNA sequence.

Waddington C., PNAS, 1939
GENETICS VERSUS EPIGENETICS

GENETICS
Heritable transmission of information based on differences in DNA sequence

SNP

MTHFR, C677T
GCC→GTC

EPIGENETICS
Heritable transmission of information in the absence of changes in DNA sequence

Epigenetic changes

Allis CD et al., In: Epigenetics, 2007
EPIGENETIC MODIFICATIONS IN NORMAL CELLS
Cytosine DNA methylation is a covalent modification of DNA, in which a methyl group is transferred from S-adenosylmethionine (SAM) to the 5 position of cytosine by a family of cytosine (DNA-5)-methyltransferases.
### Distribution of CpG sites in the human genome

<table>
<thead>
<tr>
<th>Sequence compartment</th>
<th>CpG</th>
<th>G + C (%)</th>
<th>CpG Obs/Exp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome</td>
<td>29,848,753</td>
<td>41</td>
<td>24</td>
</tr>
<tr>
<td>Promoter</td>
<td>1,876,802</td>
<td>62</td>
<td>89</td>
</tr>
<tr>
<td>First Exon</td>
<td>508,553</td>
<td>56</td>
<td>65</td>
</tr>
<tr>
<td>Other Exons</td>
<td>1,337,271</td>
<td>48</td>
<td>40</td>
</tr>
<tr>
<td>DNA Transposons</td>
<td>565,601</td>
<td>29</td>
<td>23</td>
</tr>
<tr>
<td>Line Transposons</td>
<td>3,242,225</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>LTR Transposons</td>
<td>1,958,798</td>
<td>37</td>
<td>19</td>
</tr>
<tr>
<td>SINE Transposons</td>
<td>7,479,682</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>Alpha Satellite</td>
<td>~766,000</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Classical Satellite</td>
<td>~1,140,000</td>
<td>34</td>
<td>67</td>
</tr>
<tr>
<td>Other</td>
<td>8,358,888</td>
<td>42</td>
<td>15</td>
</tr>
</tbody>
</table>

#### Methylation domains (repeated DNA sequences)

- **Simple tandem repeat**
  - SR SR SR SR

- **DNA transposon**
  - IR Transposon IR

- **LTR – endogenous retrovirus**
  - LTR gag pol env 3'LTR

- **Non-LTR autonomous retrotransposon: LINE**
  - TSDR MSC P ORF1 ORF2 A/T Rich Site TSDR

- **Non-LTR non-autonomous retrotransposon: SINE**
  - DR SVA Element Poly(A) DR TTTT

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Rollins RA et al., Genome Res, 2006

Wilson AS et al., BBA, 2007
**POST-TRANSLATIONAL HISTONE MODIFICATIONS**

**CHROMATIN**

**NUCLEOSOME**

**H2A**

**H3**

**H4**

**H2B**

**POST-TRANSLATIONAL HISTONE MODIFICATIONS**

**TYPES AND ROLES OF HISTONE MODIFICATIONS**

<table>
<thead>
<tr>
<th>Histone modifications</th>
<th>Role in transcription</th>
<th>Histone-modified sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>activation</td>
<td>H3 (K9, K14, K18, K56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4 (K5, K8, K12, K16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2B (K6, K7, K16, K17)</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>activation</td>
<td>H3 (S10)</td>
</tr>
<tr>
<td>Methylation</td>
<td>activation</td>
<td>H3 (K4, K36, K79)</td>
</tr>
<tr>
<td></td>
<td>repression</td>
<td>H3 (K9, K27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4 (K20)</td>
</tr>
<tr>
<td>Ubiquitination</td>
<td>activation</td>
<td>H2B (K123)</td>
</tr>
<tr>
<td></td>
<td>repression</td>
<td>H2A (K119)</td>
</tr>
<tr>
<td>Sumoylation</td>
<td>repression</td>
<td>H3 (?)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H4 (K5, K8, K12, K16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2A (K126)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H2B (K6, K7, K16, K17)</td>
</tr>
</tbody>
</table>
miRNA BIOGENESIS AND FUNCTION

Zhang et al., AJP, 2007
**ROLE OF EPIGENEIC MODIFICATIONS IN NORMAL CELLS**

- **X-CHROMOSOME INACTIVATION** – balances gene expression between males and females
- **GENOMIC IMPRINTING** – ensures parental-specific gene expression in diploid cells essential for development
- **SILENCING OF REPETITIVE ELEMENTS** – ensures maintenance of proper chromatin order
- **TISSUE-SPECIFIC DNA METHYLATION** – controls gene- and tissue-specific epigenetic patterns
- **CHROMATIN ORGANIZATION** – controls proper expression of genetic information
TECHNOLOGICAL APPROACHES TO EPEIGENETIC ANALYSES
**APPROACHES TO DETECTING DNA METHYLATION**

**Global DNA methylation**
- High performance liquid chromatography (HPLC)
- Immunocytochemistry for 5-methylcytosine
- High performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC-ES-MS/MS)
- Methyl-acceptance assay
- Cytosine extension assay
- Analysis of LINE-1 and AluI methylation

**Gene-specific methylation**
- Methylation-sensitive PCR
- Bisulfite genomic sequencing
- Methylation-specific PCR (MCP)
- Methylation-sensitive single-nucleotide primer extension (Ms-SNuPe)
- Combined bisulfite restriction analysis (COBRA)
- CpG island microarray
- Methylated DNA immunoprecipitation
- Pyrosequencing

**Search for new hypermethylated genes**
- Demethylating drugs and expression microarrays
- Amplification of intermethylated sites (AIMS)
- Methyl-PCR arbitrary techniques
- Restriction landmark genomic scanning (RLGS)
APPROACHES TO DETECTING DNA METHYLATION: METHYLATED DNA IMMUNOPRECIPITATION AND PYROSEQUENCING

Genomic DNA

DNA fragmentation

<table>
<thead>
<tr>
<th>DNA modification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bis-DNA modification</td>
</tr>
</tbody>
</table>

5-methylcytosine (5mC) Antibodies

Immunoprecipitation

Enriched Me-DNA (IP fraction)

Sample preparation

Sequenced on machine

Alignment software

Reads aligned to a reference genome

Methylation analysis

B. High-throughput Sequencing

Genome-wide profiling (MeDIP)

Methylation status of the gene

Correlation with gene expression

Methylation and Gene expression

Martel et al., Toxicol., 2010

Locus-specific profiling (Pyrosequencing)

GGTCAGTGAC/mCG

Bisulfite conversion

GGTUAGTGAMCG

PCR amplification

GGTTAGTGAT/CG

Pyrosequencing analysis

Bis-DNA modification

Genomic DNA

DNA fragmentation

Input DNA (Input)

Cy5(red)  Cy3 (green)

Denature

High density microarray

Methylation analysis

A. Array Hybridization

Comprehensive view of genome function & activity
APPROACHES TO DETECTING HISTONE MODIFICATIONS

- Liquid chromatography-electrospray mass spectrometry
- Immunohistochemistry
- Chromatin immunoprecipitation analysis
- Western immunoblotting
HOW DO ENVIRONMENTAL EXPOSURES ALTER DNA METHYLATION AND HISTONE MODIFICATION PATTERNS?
MECHANISMS LINKING ENVIRONMENTAL EXPOSURES TO EPIGENETIC ALTERATIONS

Exposure

- Oxidative stress
- Methyl-group availability
- Methyltransferases activity

DNA methylation
Histone code
miRNA

Altered gene expression and chromatine condensation

Disease risk

- Inflammation
- Endocrine disruption
- Clonal selection
ALTERATIONS OF DNA METHYLATION INDUCED BY ENVIRONMENTAL FACTORS

Baylin SB, Jones PA., In: Epigenetics, 2007
CYTOSINE DNA METHYLATION

DNA alterations

Cytosine (C) → SAM → DNMT1, DNMT3a, DNMT3b → SAH → 5-methylcytosine (m^5C)
CYTOSINE DNA METHYLATION

Cytosine (C) → SAM → SAH → 5-methylcytosine (m^5C)
OVERVIEW OF ONE-CARBON METABOLISM

Davis CD and Uthus EO, Exp Biol Med, 2004
FACTORS THAT ALTER DNA METHYLATION STATUS:
ONE-CARBON METABOLISM

Pogribny IP et al., Mutat Res, 2004
CYTOSINE DNA METHYLATION

DNA alterations

Cytosine (C) → 5-methylcytosine (m5C)

SAM → SAH

DNMT1, DNMT3a, DNMT3b
FACTORS THAT ALTER DNA METHYLATION PATTERNS: DNA METHYLTRANSFERASES

GENOMIC HYPO METHYLATION IN DNMT1 HYPO MORPHIC MICE

Gaudet et al., Science, 2003
CYTOSINE DNA METHYLATION

DNA alterations

Cytosine (C) → 5-methylcytosine (m^5C)

DNMT1
DNMT3a
DNMT3b

SAM → SAH
FACTORS THAT ALTER DNA METHYLATION STATUS:
DNA INTEGRITY

Valinluck V et al., Cancer Res, 2007
EXAMPLES OF EPIGENETIC CHANGES INDUCED BY ENVIRONMENTAL FACTORS
TOBACCO SMOKE

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans

VOLUME 83
Tobacco Smoke and Involuntary Smoking

LYON, FRANCE 2004
TOBACCO SMOKE AND EPIGENETICS: CARCINOGEN-INDUCED MUTATIONS

B[a]P

Denissenko MF et al., Science, 1996

ACROLEIN

Denissenko MF et al., PNAS, 1997

Feng Z et al., PNAS, 2006
**TOBACCO SMOKE AND DNA METHYLATION CHANGES**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Digest</th>
<th>HYPOM RAMs</th>
<th>HYPERM RAMs</th>
<th>NewM RAMs</th>
<th>Total</th>
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<td>DMBA: sham (vs. untreated)</td>
<td>Hpal</td>
<td>28</td>
<td>9</td>
<td>14</td>
<td>51</td>
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<tr>
<td></td>
<td>MspI</td>
<td>22</td>
<td>2</td>
<td>4</td>
<td>28</td>
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<tr>
<td></td>
<td>BssHII</td>
<td>18</td>
<td>3</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>68</td>
<td>14</td>
<td>30</td>
<td>112</td>
</tr>
<tr>
<td>0.48 mg: CS (vs. sham)</td>
<td>Hpal</td>
<td>22</td>
<td>8</td>
<td>6</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>MspI</td>
<td>23</td>
<td>15</td>
<td>28</td>
<td>66</td>
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<tr>
<td></td>
<td>BssHII</td>
<td>38</td>
<td>0</td>
<td>3</td>
<td>41</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>83</td>
<td>23</td>
<td>37</td>
<td>143</td>
</tr>
</tbody>
</table>

Phillips JM, Goodman JL, Toxicology, 2009

**Frequency of p16\(^{INK4A}\) methylation in bronchial epithelial cells**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sample type</th>
<th>Methylation frequency (%)</th>
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</thead>
<tbody>
<tr>
<td>Never</td>
<td>BEC</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>Smokers</td>
<td>BEC</td>
<td>25/137 (18)</td>
</tr>
</tbody>
</table>

Belinsky SA, Carcinogenesis, 2005

- **CDH1** \(P = 0.01\)
- **GSTP1** \(P = 0.2\)
- **RASSF1A** \(P < 0.01\)
- **CDKN2A** \(P < 0.01\)
- **MTHFR** \(P < 0.01\)

Vaisiere T et al., Cancer Res, 2009
MECHANISM OF ARSENIC-INDUCED EPIGENETIC ALTERATIONS

Environment → iAs Methylation → Competition for SAM → DNA-MT → Aberrant DNA Methylation → Altered DNA methylation, Altered Histone Modifications, Aberrant Gene Expression → Toxicity → Arsenicosis, Liver Cancer

- Aberrant Gene Expression
- Toxity
- Altered DNA methylation
- Altered Histone Modifications
- Competition for SAM
ARSENIC-INDUCED EPIGENETIC ALTERATIONS

**In vitro studies**

- Genomic DNA hypomethylation:
  - Arsenic Concentration (μM):
    - Control
    - 0.125
    - 0.250
    - 0.500
  - Time of Arsenic Treatment:
    - Control
    - 12 wks
    - 18 wks
    - 22 wks

- DNA Methylation (% control):
  - Control
  - 0.2 μM As Treatment
  - 1 μM 5-AzadeC

**In vivo studies**

- Percentage of control (%):
  - 5 MdC content (% of total cytosine)
  - Unmethylated cytosine

- Cpg Site Methylation (%):
  - Control
  - Arsenic

Zhao CQ et al., 1997; Chen H et al., Carcinogenesis, 2004; Reichard JF et al., BBRC, 2007.
EPIGENETIC ALTERATIONS INDUCED BY LOW-DOSE BENZENE EXPOSURE

Bollati V et al., Cancer Res, 2007

LINE-1

AluI

p15

MAGE-1

Zhang L. et al., Chemico-Biological Interactions, 2010

Bollati V et al., Cancer Res, 2007
EPIGENETIC ALTERATIONS INDUCED BY PRENATAL EXPOSURE TO BISPHENOL A

Dolinoy DC et al., PNAS, 2007
• Chronic obstructive pulmonary disease (COPD) is a leading cause of morbidity and mortality worldwide. 25-45% of patients with COPD have never smoked.

• 3 billion people, half of the world population, are exposed to smoke from different sorts of fuel (i.e., biomass fuel, transportation-related fuel, etc.).

• This suggests that exposure to fuel smoke is the biggest risk factor for COPD globally.
<table>
<thead>
<tr>
<th>Agent</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Global DNA hypomethylation</td>
</tr>
<tr>
<td></td>
<td>Gene-specific hypomethylation <em>(Er-α, cyclin D1)</em></td>
</tr>
<tr>
<td></td>
<td>Gene-specific hypermethylation <em>(p53, p16^{INK4A}, RASSF1A)</em></td>
</tr>
<tr>
<td></td>
<td>Inhibition of DNMT1 and DNMT3a expression</td>
</tr>
<tr>
<td></td>
<td>↓Histone H3K27 trimethylation</td>
</tr>
<tr>
<td></td>
<td>↑Histone H3K4 trimethylation</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Global DNA hypomethylation</td>
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<td>Inhibition of DNMT activity</td>
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<td>Gene-specific hypermethylation <em>(p16^{INK4A}, RASSF1A)</em></td>
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<td>Hydrazine</td>
<td>Global DNA hypomethylation</td>
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<td>Gene-specific hypomethylation <em>(p53, c-myc, HMG CoA reductase)</em></td>
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<td>Benzo[a]pyrene</td>
<td>Global DNA hypomethylation</td>
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<tr>
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<td>Gene-specific hypermethylation <em>(CYP1A1)</em></td>
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<tr>
<td></td>
<td>Promoter-specific histone H3 lysine 9 hypo- and hyperacetylation</td>
</tr>
<tr>
<td></td>
<td>CpG-methylation-associated mutations <em>(p53)</em></td>
</tr>
<tr>
<td>Aflatoxin B1</td>
<td>Gene-specific hypermethylation <em>(GSTP, MGMT, RASSF1A, p16^{INK4A})</em></td>
</tr>
<tr>
<td>2-Acetylaminofluorene</td>
<td>Gene-specific hypermethylation <em>(p16^{INK4A})</em></td>
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<tr>
<td></td>
<td>Increased DNMT1 expression</td>
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<td>↓Histone H4K20 trimethylation</td>
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<tr>
<td>Bisphenol A</td>
<td>Agouti gene, IAP element</td>
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<td>Gene-specific hypomethylation <em>(PDE4D4)</em></td>
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<td>Benzene</td>
<td>Global DNA hypomethylation</td>
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<tr>
<td></td>
<td>Gene-specific hypermethylation <em>(p15)</em></td>
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<tr>
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<td>Gene-specific hypomethylation <em>(MAGE-1)</em></td>
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<tr>
<td>Dibromoacetic acid</td>
<td>Global DNA hypomethylation</td>
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</tbody>
</table>
UNIFYING MECHANISM OF DNA HYPMETHYLATION CAUSED BY EXPOSURE TO CHEMICALS

Lee D. et al., Environmental Health Perspectives, 2009
EPIGENETIC CHANGES IN CANCER
**EPIGENETIC ALTERATIONS IN CANCER CELLS**

- Global DNA hypomethylation
- Gene-specific DNA hypermethylation
- Gene-specific hypomethylation
- Altered histone modifications
- Altered nucleosome positioning along DNA
- Altered expression of non-coding RNAs, e.g. microRNAs
EPIGENETIC GENE-SPECIFIC ALTERATIONS IN CANCER

NORMAL CELL

CANCER CELL

DNA METHYLATION CHANGES DURING SKIN CARCINOGENESIS

Status of global DNA methylation

CpG island methylation status of selected genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>NS</th>
<th>MCA3D</th>
<th>PB</th>
<th>MSCP6</th>
<th>PDV</th>
<th>PAM212</th>
<th>MSCB1 19</th>
<th>MSC11A5</th>
<th>HaCa4</th>
<th>Car B</th>
<th>Car C</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
<td>U</td>
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<td>U</td>
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<td>U</td>
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<tr>
<td>MLH1</td>
<td>U</td>
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<td>U</td>
<td>M</td>
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<td>M</td>
<td>M</td>
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</tr>
<tr>
<td>MGMT</td>
<td>U</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<td>M</td>
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</tr>
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<td>CDH1</td>
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<td>U</td>
<td>U</td>
<td>M</td>
<td>U</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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</tr>
<tr>
<td>Snail</td>
<td>U</td>
<td>M</td>
<td>M</td>
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<td>U</td>
<td>U</td>
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<td>U</td>
</tr>
<tr>
<td>MLT1</td>
<td>U</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
</tbody>
</table>

Abbreviations: NS - normal skin; M - methylated; U - unmethylated

Fraga MF et al., Cancer Res, 2004
PREDICTIVE POWER OF GENE METHYLATION FOR EARLY DETECTION OF HEPATOCOCELLULAR CARCINOMA

A

- p16: 44% methylated, 56% not methylated
- p15: 24% methylated, 76% not methylated
- RASSF1A: 30% methylated, 70% not methylated

B

- Any One Methylated Gene: 12% methylated, 88% not methylated
- Any Two Methylated Genes: 24% methylated, 76% not methylated
- All Three Methylated Genes: 12% methylated, 88% not methylated

Rivenbark AG, Coleman WB, Clin Cancer Res, 2007
### FREQUENCY OF RASSF1A METHYLATION IN BREAST CANCER CASES AND CONTROLS


<table>
<thead>
<tr>
<th>Sites</th>
<th>Subjects</th>
<th>$n$</th>
<th>$n$ positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All cases</td>
<td>61</td>
<td>11 (18)</td>
</tr>
<tr>
<td>New York</td>
<td>Cases</td>
<td>28</td>
<td>7 (25)</td>
</tr>
<tr>
<td>Family</td>
<td>Sibling controls*</td>
<td>10</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Ontario</td>
<td>Cases</td>
<td>33</td>
<td>4 (12)</td>
</tr>
<tr>
<td></td>
<td>Population-based controls#</td>
<td>29</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

* - Unaffected siblings from high-risk families. # - Population based healthy controls (age and race matched).
DO EPIGENETIC CHANGES PLAY A ROLE IN CARCINOGENESIS?
POSSIBLE MECHANISMS OF CHEMICAL CARCINOGENESIS

Miller, Cancer Res., 1970
CARCINOGEN-DNA ADDUCTS AS MARKERS OF EXPOSURE AND CANCER RISK

EXPOSURE → REACTIVE METABOLITES → CARCINOGEN-DNA ADDUCTS → CANCER

Environmental Exposure → Reactive Metabolites → Carcinogen-DNA Adducts → Cancer

Normal Cells → Initiated Cells → Endogenous

Endogenous

ACQUISITION OF ADDITIONAL RANDOM MUTATIONS

Clonal selection and expression of initiated cells → Mutator phenotype cells → Cancer cells

Benzo[a]pyrene

Benzo[a]pyrene-diol-epoxide (BPDE)

BPDE-DNA ADDUCTS

Cancer
METABOLIC ACTIVATION OF BD TO REACT ELECTROPHILES AND FORMATION OF DNA ADDUCTS

Goggin M et al., Cancer Res 2009
THB-Gua-BD ADDUCTS IN HEPATIC DNA FROM MICE EXPOSED TO 1,3-BUTADIENE

![Graph showing amount of THB-Gua adducts/10^7 ntds for Total, Racemic, and Meso categories.](image-url)
EFFECTS OF 1,3-BUTADIENE EXPOSURE ON EPIGENETIC ALTERATIONS IN THE LIVERS

**DNA methylation**

- [H]dCTP incorporation, %
  - Control 0 ppm
  - 1,3-butadiene 6.25 ppm
  - 1,3-butadiene 625 ppm

- Control: 100%
- 1,3-butadiene 6.25 ppm: *p < 0.05
- 1,3-butadiene 625 ppm: *p < 0.05

**LINE-1 methylation**

- Fold change
  - Control 0 ppm
  - 1,3-butadiene 6.25 ppm
  - 1,3-butadiene 625 ppm

- Control: 1.0
- 1,3-butadiene 6.25 ppm: *p < 0.05
- 1,3-butadiene 625 ppm: *p < 0.05

**Histone methylation**

- H3K9me3
- H4K20me3
- H3K27me3

- Control
- 1,3-butadiene 6.25 ppm
- 1,3-butadiene 625 ppm

- Loading:
  - H3K9me3
  - H4K20me3
  - H3K27me3
GENETIC AND EPIGENETIC MODELS OF CANCER INITIATION

ACQUISITION OF ADDITIONAL RANDOM MUTATIONS

Clonal selection and expression of initiated cells

Mutator phenotype cells

Cancer cells

ALTERATIONS IN CELLULAR EPIGENOME

Epigenetically reprogrammed cells

Mutator phenotype cells

Cancer cells
ADVANTAGES OF EPIGENETIC BIOMARKERS FOR CANCER RISK ASSESSMENT

- EARLY APPEARANCE OF CARCINOGEN-INDUCED EPIGENETIC ALTERATIONS
- STABILITY OF EPIGENETIC CHANGES
- INHERITANCE OF CARCINOGEN-INDUCED EPIGENETIC CHANGES THROUGH TRANSMISSION FROM ONE CELL GENERATION TO ANOTHER
- DETECTION AT RELATIVELY LOW COSTS
- SPECIFICITY FOR GENOTOXIC AND NON-GENOTOXIC CARCINOGENS
- GREATER NUMBER OF EPIGENETIC CHANGES OVER GENETIC ALTERATIONS AFTER EXPOSURE
CONCLUSIONS

EPGENETIC ALTERATIONS are a major component and driving force in development of pathological states associated with exposures to harmful personal lifestyle factors and environmental physical and chemical agents. Understanding and manipulating the epigenome holds enormous promise for preventing these pathological states. Specifically, epigenetics holds substantial potential for developing biological markers to predict which exposures would put exposed individuals at risk and, more importantly, which individuals will be more susceptible to developing a disease.