Differential Expression of Liver Regulatory Genes with Embryonic PCB Exposure

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University of Maryland

The opinions expressed in this presentation are those of the authors; the research does not represent the official position of the funding agency.
Background

- General Electric plants on Hudson River (HR)
- PCBs deposited into HR from 1940s until 1977 (PCBs banned) – 209K – 1.3M lbs dumped (EPA estimate)
- Superfund site
Background

- PCBs entered river as mixtures (Aroclors)
- Our study – mixture based on congener profile found in spotted sandpiper
- Interested in sublethal endpoints – gene expression
  - Looking for affected pathways
  - Looking for novel biomarkers
  - Whole-genome approach – Microarray
- Model animal – Japanese quail
- Liver as organ of interest
Japanese Quail

- Not as sensitive as chickens to toxic effects
  - LD50s (Head et al., 2008)
  - AhR Sequence (Head et al., 2008)
- Good model of endocrine system
- Conserved homology with chickens
Study Design and Methods

- Egg Collection, Injection, and Incubation
  - Incubated for 3 days prior to injection
- Tissue Collection
  - Livers collected at hatch
- RNA Extraction

<table>
<thead>
<tr>
<th>PCB Concentration (ng PCB/g)</th>
<th>TEQs (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6,000</td>
<td>0.79</td>
</tr>
<tr>
<td>12,000</td>
<td>1.6</td>
</tr>
<tr>
<td>49,000</td>
<td>6.3</td>
</tr>
</tbody>
</table>
Study Design and Methods Cont’d

- Microarray Validation
  - Five species: Quail, American Kestrel, Tree Swallow, Domestic Turkey, and Domestic Duck

- Microarray Experiment
  - Quail females
  - N= 7 (Vehicle), 5 (Low), 2 (Medium), and 2 (High)
  - Statistics
  - Genes BLASTed against chicken genome
  - Cluster and Pathway Analyses

- qPCR
  - Males (n= 6 [untreated], 4, 2, 3, and 7)
  - Genes of interest from microarray experiment
Results - Microarray Validation

Del-Mar 14K Chicken Integrated System Microarray

- Neuroendocrine system: 5929
- Fat: 4800
- Liver: 2635
- Muscle: 2398
- Reproductive tract: 2008
- Quality control: 64

Data Generated by Tom E. Porter Lab, University of Maryland
Results - Microarray Validation

Species Comparison

Data Generated by Tom E. Porter Lab, University of Maryland
Results – Microarray Analysis

- SAS analysis of microarray data
  - One-way ANOVA for each spot
  - 285 spots on array were statistically significant
  - 159 spots had $\geq 8$ samples represented – submitted to cluster analysis

- Cluster analysis (GeneCluster)
  - Finding similar expression profiles by allowing genes to cluster into self-organizing maps (SOMs)
Results – Cluster Analysis
Results – Pathway Analysis

- Biorag (www.biorag.org)
- 124 spots submitted
- 40 spots (i.e. genes) retained
- Many pathways returned with only a few genes in them, but several genes fell into a number of pathways, so...
Study 1 Results – Pathway analysis continued

- 7 broad categories of pathways were created

<table>
<thead>
<tr>
<th>Pathway Category</th>
<th># Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Damage/Repair and Tumor Regulation</td>
<td>4</td>
</tr>
<tr>
<td>Replication, Mitosis, Protein Synthesis, Gene Regulation, and Cell Cycle</td>
<td>12</td>
</tr>
<tr>
<td>Xenobiotic Metabolism</td>
<td>4</td>
</tr>
<tr>
<td>Glycolysis, Gluconeogenesis, and Energy Balance</td>
<td>17</td>
</tr>
<tr>
<td>Protein Degradation and Proteosome Complex</td>
<td>7</td>
</tr>
<tr>
<td>Stress and Immunity</td>
<td>4</td>
</tr>
<tr>
<td>Cell Communication, Adhesion, Transport, and Signalling</td>
<td>13</td>
</tr>
</tbody>
</table>
Results – q PCR

Cytochrome P450 1A5

Normalized mRNA Levels

Untreated (n=5)  Vehicle (n=4)  Low (n=2)  Medium (n=2)  High (n=7)

PCB Treatment

A  A  AB  AB  B
Results - qPCR

Cytochrome B5

- Untreated (n=6)
- Vehicle (n=3)
- Low (n=2)
- Medium (n=2)
- High (n=5)

PCB Treatment

Normalized mRNA Levels

- A
- B
- C

(n=6) (n=3) (n=2) (n=2) (n=5)
Results - qPCR

Glutathione S Transferase

Normalized mRNA Levels

PCB Treatment

Untreated (n=5)  Vehicle (n=3)  Low (n=2)  Medium (n=3)  High (n=6)

A  A  ABC  B  C
Results - qPCR

**GAPDH**

<table>
<thead>
<tr>
<th>PCB Treatment</th>
<th>Normalized mRNA Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated (n=6)</td>
<td>AB</td>
</tr>
<tr>
<td>Vehicle (n=4)</td>
<td>A</td>
</tr>
<tr>
<td>Low (n=2)</td>
<td>AB</td>
</tr>
<tr>
<td>Medium (n=2)</td>
<td>AB</td>
</tr>
<tr>
<td>High (n=7)</td>
<td>B</td>
</tr>
</tbody>
</table>
Results - qPCR

Fructose Bisphosphate Aldolase B

Normalized mRNA Levels

PCB Treatment

Untreated (n=5)
Vehicle (n=4)
Low (n=2)
Medium (n=2)
High (n=7)
Results - qPCR

KDELR2

Normalized mRNA Levels

PCB Treatment

Untreated (n=5) Vehicle (n=4) Low (n=2) Medium (n=3) High (n=7)
Conclusions and Future Directions

- Genes can be detected by microarray and qPCR technologies
- Sex Difference?
- Many affected pathways
  - Can explore MOA and find new biomarkers
- Further studies:
  - Steroid metabolism and clearance
  - Other CYP450 genes
  - Endocrine disruption
  - Energy balance
Acknowledgements

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