Investigating pathways of polycyclic aromatic hydrocarbon developmental toxicity in zebrafish with a comparative systems approach

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Mechanisms of polycyclic aromatic hydrocarbon-induced developmental toxicity

- PAHs are ubiquitous in the environment
  - Fossil fuels, combustion

- PAH exposures occur primarily via inhalation and ingestion

- Some are human carcinogens

- PAHs are measured in placental tissue

- Recent concern about developmental effects
Mechanisms of toxicity for most PAHs are unknown

- Air particulate matter can contain over 100 PAHs
- Toxicity data is scarce for substituted PAHs

Challenge: how can we efficiently determine developmental toxicity of these compounds and indentify mechanisms of action?
Assessing Developmental Toxicity of PAHs in Zebrafish

- 6 hpf: Expose to chemical
- 24 hpf: Evaluate
- 120 hpf: Evaluate for malformations, Immunohistochemistry

A large adult colony is required to support screening.

1 Embryo/well

Individual test compounds or mixtures dissolved in embryo media.
Differential response profiles induced by PAHs

A. Knecht Poster Presentation- OPAH toxicity screen
Screening biological effects of Portland Harbor passive sampler extracts

Water Passive Sampling

- Bioavailable fraction
- Before and after remediation

- Allan, et al; Bridging environmental mixtures and toxic effects. ET&C 2012
Identifying PAH toxicity pathways

- PAH toxicity is differentially dependent on AHR activation
  

<table>
<thead>
<tr>
<th>PAH</th>
<th>AHR Dependent</th>
<th>AHR Independent</th>
<th>CYP1A Dependent</th>
<th>DMSO Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAA</td>
<td>25 uM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DBT</td>
<td>25 uM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PYR</td>
<td>25 uM</td>
<td></td>
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</tbody>
</table>
Transcriptional responses precede malformations

Objectives:
- Define molecular pathways of PAH toxicity
- Identify biomarkers of PAH toxicity

6 hpf
- Expose to 25 μM BAA, DBT, PYR or Control (4 replicates)

24 hpf
- Collect RNA

48 hpf
- Microarray analysis of RNA expression
  (Agilent zebrafish V2 microarray, University of Wisconsin McArld Microarray Facility)

120 hpf
Correlating gene expression changes with PAH body burden

- **6 hpf**: Expose to 1, 5, 10, 25 uM PAH or Control (5 replicates)
- **24 hpf**: Extract with ethyl acetate
- **48 hpf**: Determine PAH body burden with gas chromatography-mass spectrometry (GC-MS, OSU SRP Core D)

**120 hpf**
Transcriptional profiles are PAH- and time-dependent

**OSU SRP Core C**

p < 0.05, ANOVA with 5% FDR
BAA induces a distinct expression profile

24hr 24hr 24hr 48hr 48hr 48hr

BAA

24h 38 genes

21

17

48h 107 genes

>2 FC

DBT 24h
PYR 24h
DBT 48h
PYR 48h
BAA 24h
BAA 48h

ZF Gene Symbol/ Probe ID
ahrra
ctrf2b
cyp1a
cyp1a
cyp1a
cyp1a
cyp1b1
cyp1b1
cyp1b1
cyp1b1
cyp1c1
cyp1c1
cyp1c1
cyp1c2
cyp1c2
tox1
sult6b1
DBT and PYR expression profiles

DBT
- 24 hpf: 357 genes
- 48 hpf: 561 genes

24 hr
- 262 genes
- 95 genes

48 hr
- 48 hpf: 656 genes
- 186 genes

PYR
- 24 hpf: 67 genes
- 48 hpf: 191 genes

24 hr
- 5 genes
- 62 genes

48 hr
- 186 genes
Comparing expression between PAHs at 24 hpf: unique profiles?

Unique to BAA

Unique to DBT

Unique to PYR

BAA 26 5

DBT 79 237

PYR 15 10
Differential PAH body burdens

<table>
<thead>
<tr>
<th></th>
<th>BAA</th>
<th>DBT</th>
<th>PYR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hpf</td>
<td>0.1</td>
<td>3.4</td>
<td>1.0</td>
</tr>
<tr>
<td>48 hpf</td>
<td>0.2</td>
<td>5.3</td>
<td>2.9</td>
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</table>
**DBT-PYR conserved transcriptional response**

A. 24 hpf

- \( r^2 = 0.771, P < 0.001 \)
- \( Y = 0.0682 + (0.622 \cdot X) \)

B. 48 hpf

- \( r^2 = 0.647, P < 0.001 \)
- \( Y = -0.0653 + (0.572 \cdot X) \)

**Direct comparison filter to identify genes differentially expressed by DBT and PYR (P < 0.05)**
<table>
<thead>
<tr>
<th>Biological Process</th>
<th>Downregulated genes</th>
<th>Upregulated genes</th>
<th>%</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hormone metabolic process</td>
<td>cyp1a, cyp1b1, cyp1c1, cyp1c2, si:dkey-94e7.2</td>
<td>farm, krt1, nefm, tpi1b, tnni2b.2</td>
<td>15.79</td>
<td>5.12E-03</td>
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<tr>
<td>tissue development</td>
<td>mstnb</td>
<td>foxq1l, ptn, si:ch211-173b8.2</td>
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<td>fatty acid biosynthetic process</td>
<td>elovl6, fads2, ptgds, si:ch73-131e21.5, tpi1b</td>
<td>ch25h, elovl7a</td>
<td>3.05</td>
<td>6.10E-04</td>
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<tr>
<td>ion transport</td>
<td>atp2a1l, ctp1b, gabra1, grn1b, KCNAB1, kcnip1b, kcnip3, LOC10000427, rhbg, sfxn4, si:ch211-195b13.1, si:ch211-221p4.4, slc24a5, zgc:101827, zgc:113361, zgc:158296</td>
<td>LOC571584, si:ch211-244h7.4, slc22a18, slc31a1, tmem38b, zgc:162356, zgc:162495</td>
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<td>skeletal muscle contraction</td>
<td>homrole, mb, si:rp71-17i16.4, tnni2b.2</td>
<td>ch25h, dhcr7</td>
<td>1.51</td>
<td>1.10E-03</td>
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<tr>
<td>steroid biosynthetic process</td>
<td>cyp17a1, hmgcs1, hsd17b7, lss, ndshl, rdh8l</td>
<td>ch25h, dhcr7</td>
<td>3.02</td>
<td>9.43E-04</td>
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<td>oxoacid metabolic process</td>
<td>acsf3, ctp1b, elovl6, fabp11b, fads2, ghra, hibadbhb, mdh1b, ptgds, rbp1a, rnp, si:ch73-131e21.5, tpi1b, tyrp1b, zgc:101827, zgc:113361, zgc:158296</td>
<td>ch25h, elovl7a, mthfd1</td>
<td>7.17</td>
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<tr>
<td>intermediate filament organization</td>
<td>dnajb6b, krt1-19d, krt23, nefm</td>
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<td>1.13</td>
<td>6.71E-03</td>
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<tr>
<td>negative regulation of cell</td>
<td>bdnf, cd9a, cx43, smad3b, tnfrsf9a, wfdc1, zgc:114127, zgc:158296</td>
<td>agt, msxe, notch2, tbx16, tnfb</td>
<td>4.91</td>
<td>1.67E-02</td>
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<tr>
<td>proliferation</td>
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<td>muscle cell development</td>
<td>homrole, LOC796577, myoz1a, zgc:158296</td>
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<td>sterol biosynthetic process</td>
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<td>ch25h, dhcr7</td>
<td>1.89</td>
<td>5.49E-03</td>
</tr>
</tbody>
</table>
Advancing PAH biomarkers and toxicity pathways

RNA-seq to expand and refine pathways with OPAHs and environmental mixtures

- Benzanthrone
- Benz(a)anthracene-7,12-dione
- 9,10-phenanthrene-quinone
- Portland harbor mixture samples

Investigating expression of biomarker genes in a wide range of samples

- OPAHs with differential toxicity profiles
- Environmental mixtures
Conclusions

PAHs induce developmental toxicity through diverse mechanisms

- Body burden data is important for discerning mechanistic vs. uptake differences and relating to other models

Associating toxicity pathways with PAH structures is essential for predicting toxicity

- Applying molecular toxicology to risk assessment

By comparing transcriptional changes induced by a diverse group of PAHs, we hope to identify translatable biomarker genes associated with PAH toxicity
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Questions?