A Multi-Omic Approach to Elucidate Contaminant Modes-of-Action in the Zebrafish Larvae

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Challenges in Environmental Monitoring

• Assessment/monitoring contaminants are difficult
• Environmental samples are complex
• Sensitivity issues with whole animal standard toxicity assays
• Demands for a high throughput, sensitive and reliable evaluation method
Omics Technology

- Increase detection sensitivity
- Early indicator of environmental impact
- Multi-omics approach → insights towards important molecular interactions

Preventative action

Early indicators

Adverse biological outcomes
Zebrafish Larvae (*Danio rerio*)

- A broadly applicable model species
- Established strains
- Alternative and equivalent testing version to the adult
- A whole organism *in vitro* assay
Objectives of the Study

1. Capture unique molecular signatures in response to chemical exposure
   - Metabolomics
   - Transcriptomics

2. Identify interactions and establish biological modes-of-actions
# Zebrafish Larvae Exposure Design

<table>
<thead>
<tr>
<th>Age</th>
<th>Untreated</th>
<th>VEH</th>
<th>3 Conc.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>96 hpf</td>
<td>Embryo Media</td>
<td>0.1% EtOH</td>
<td>PPCPs, metals, petroleum derivatives, endocrine disruptors, mixtures, effluent, etc</td>
</tr>
</tbody>
</table>

*Environmentally relevant concentrations

- **Contaminants**
  - 5 X
  - AB/Tub strain
  - 24h exposure
  - Metabolomics
    - 80 larvae/replicate
  - Transcriptomics
    - 8 larvae/replicate
Omics Tools – Targeted Metabolomics

**Targets of Interest**
- Primary metabolites
- 216 targets → 8 classes

**Instrumental Analysis**
- LC- or FI-MS/MS
- Quantification by isotope dilution or internal standard approaches
Omic Tools – Targeted Transcriptomics

• ZF qPCR Toolbox
  – 5 normalizers
  – 33 target genes harvested from literature and vetted

• Biological System Populated
  – Energy/beta oxidation, xenobiotic, lipid/fatty acid metabolism
  – Glutamate, GABA synthesis/metabolism
  – Ion transportation/metabolism
  – Peroxisome proliferator
  – Endocrine response
  – DNA damage, oxidative stress, proteolysis
  – Immunoregulation
  – Transcription, cell signaling
  – Neuron regulation/development
Omics Tools – Populated Biological Pathways

- response to copper ion
- response to cadmium ion
- response to metal ion
- response to inorganic substance
- response to transition metal ion transport
- neuron-neuron synaptic transmission
- ionotropic glutamate receptor signaling pathway
- synaptic transmission, glutamatergic
- glutamate receptor signaling pathway
- # of genes
- superoxide metabolic process
- cellular response to reactive oxygen species
- removal of superoxide radical
- reactive oxygen species metabolic process
- Perioxidases
- cellular response to oxygen radical
- cellular response to superoxide
- anatomical structure homeostasis
- Steroid hormone biosynthesis
- response to xenobiotic stimulus
- NOD-like receptor signaling pathway
- Arginine biosynthesis
- small molecule biosynthetic process
- regulation of growth
- regulation of neuron apoptotic process
- neuron apoptotic process
- cell-type specific apoptotic process
- negative regulation of neuron death
- regulation of neuron death
- neuron death
- # of genes
- g:Profiler
- Cytoscape V3.0
RESULTS – DIPHENHYDRAMINE
An anti-histamine compound
• Summary of the observations
• Separation of vehicle control (CC) and treatment groups
• Effect observable in low dose
• Cross Validated:
  • Q2>0.6
  • Permutation test:p <0.05
Transcript Abundance

- 6 genes were affected
- A general reduction in transcript abundance
- Effects observed in the low dose group

Increasing concentrations (2, 200, 2000 µl DPH/L)
Integration of Omics

- Cross Validated: Q2<0.5; permutation P-value<0.05
Integration of Omics

Metabolomics Networks

Transcript Affected

Relative Fold Change

- VEH
- DPH
Integration of Omics

Metabolite Change

- Normalized Concentration
  - VEH     DPH
  - Glu
  - Gln

Transcript Change

- Relative Fold Change
  - VEH     DPH
  - gria2b
  - slc2a9l2

VIP scores

AXYS ENVIRIO
Summary

- DPH at environmentally relevant level induced significant changes
- Metabolite and transcript changes can be used in network/pathway analysis
- Gene expression complements metabolomic analysis as indicator of metabolic effect at the transcript level
- This approach may provide a more robust identification of environmental impacts
Acknowledgements

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AXYS: Targeted Metabolomics

qPCR training and analysis

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