SUBSTANCE-SPECIFIC FINGERPRINTS OF THYROID DISRUPTION IN ZEBRAFISH EMBRYOS

Speaker: Steve Ayobahan
Eco’n’OMICs ATTRACT
Steve.ayobahan@ime.fraunhofer.de
www.ime.fraunhofer.de

Steve Ayobahan¹, Hannes Reinwald¹,³, Azora König¹, Julia Alvincz¹, Levente Sipos¹, Gisela Böhle¹, Bernd Göckener¹, Orr Shomroni², Gabriela Salinas², Henner Hollert³, Christoph Schäfers¹, Elke Eilebrecht¹ and Sebastian Eilebrecht¹

¹ Fraunhofer Institute for Molecular Biology and Applied Ecology, Applied Ecology and Bioresources Division, Schmallenberg, Germany
² NGS - Integrative Genomics Core Unit, Department of Human Genetics, University Medical Center, Göttingen, Germany
³ Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt, Frankfurt am Main, Germany
Endocrine disrupting chemicals (EDCs)

EDCs are chemicals that interfere with the endocrine system & trigger an adverse development, reproductive and immune effects to exposed organism or its descendant population.

OECD TGS

• Estrogen
• Androgen
• Thyroid hormones
• Steroidogenesis

Many organic compounds in the environment can interfere with and disrupt the thyroid system.

functions
• normal physiology
• development
• growth
• energy metabolism

Fish studies - an adverse outcome pathway (AOP) was recently proposed linking thyroid disruption to decreased swim bladder inflation and impaired swimming performance in fish.
Background

Current assessment of endocrine substances targeting thyroid hormone-related physiological processes, focuses on:

- Xenopus Embryo Thyroid Signaling Assay (XETA) (OECD, 2019)
- Amphibian Metamorphosis Assay (AMA) (OECD, 2009)

The required number of amphibians needed for these tests strongly contrasts the 3R principle of:

- reduction
- replacement and
- refinement of animal experiments
Test Strategy
Test strategy

Objective

- To demonstrate the sensitivity of -omics based approaches in assessing thyroid disruption in zebrafish embryo

Experimental design

- Test strategy
- Experimental design
- Biological replicates:
  - Controls
  - Low exposure (LE) ~NOEC
  - High exposure (HE) ~EC10

  - Semi-static incubation for 96 hr
  - 3 biological replicates
  - 15 embryos per well
### Test substances

**Thyroid hormone**

- 3,3',5-triiodothyronine (T3)
- **Exposure duration**: 4 days

<table>
<thead>
<tr>
<th>Control</th>
<th>Low exposure</th>
<th>High exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.14</td>
<td>186.60 µg/L</td>
</tr>
</tbody>
</table>

**Thyroid peroxidase inhibitor**

- 6-propyl-2-thiouracil (6PTU)
- **Exposure duration**: 4 days

<table>
<thead>
<tr>
<th>Control</th>
<th>Low exposure</th>
<th>High exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>108.81 mg/L</td>
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### Water analysis

<table>
<thead>
<tr>
<th>T3 (µg/L)</th>
<th>6-PTU (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nominal</td>
<td>Ø measured (n = 3x3)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.0</td>
<td>0.23</td>
</tr>
<tr>
<td>3.3</td>
<td>1.14</td>
</tr>
<tr>
<td>10.0</td>
<td>2.35</td>
</tr>
<tr>
<td>330.0</td>
<td>186.60</td>
</tr>
</tbody>
</table>

Transcriptome & proteome expression profile
Transcriptomics and Proteomics workflow

Control  
thyroid disruption

10 embryos

RNA-Seq Analysis
RNA extraction  
mRNA isolation  
library preparation

Illumina sequencing
fastq files
Filtering and Trimming
Alignment
Counting
DESeq2

MS-based proteomics
Protein extraction
Tryptic digestion
TMT labelling

LC-MS/MS analysis
RAW files
Database search
Protein Identification & Quantification
MSstatTMT

Test strategy
Result
Physiological responses

Physiological effects

**T3**
- Control: 0.23 µg/L
- 0.23 µg/L
- 1.14 µg/L
- 2.35 µg/L

**6-PTU**
- Control: 0.06 mg/L
- 0.06 mg/L
- 1.00 mg/L
- 8.39 mg/L

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**Results**
Transcriptomics & Proteomics Result
Normalization - Transcriptomics data

Differentially Expressed Genes (DEGs)

Results

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Overlap of transcriptome and proteome data

Common subset of both methods

Results
Gene Set Enrichment Analysis
Gene Set Enrichment Analysis

Results

Transcriptome

Proteome

6PTU-exposure

- immune response
- defense response
- sensory perception of smell
- sensory perception of chemical stimulus
- xenobiotic metabolic process
- reactive oxygen species biosynthetic process
- regulation of hormone levels
- response to xenobiotic stimulus
- innate immune response
- hormone metabolic process
- response to cytokine
- nervous system process
- neutrophil migration
- regulation of cell cycle
- inflammatory response
- ion homeostasis
- regulation of circadian rhythm
- negative regulation of cell cycle
- cardiac myofibril assembly
- muscle tissue development

- DNA conformation change
- skeletal system development
- connective tissue development
- cartilage development
- transmembrane transport
- striated muscle contraction
- sensory system development
- muscle system process
- muscle contraction
- sensory organ development
- ion transmembrane transport
Gene Set Enrichment Analysis

T3 - exposure

Transcriptome

- cellular response to DNA damage stimulus
- immune response
- defense response
- regulation of response to stress
- innate immune response
- inflammatory response
- response to cytokine
- glycolytic process
- developmental pigmentation
- detection of stimulus
- pigmentation
- visual perception
- sensory perception of light stimulus
- negative regulation of cellular component organization
- calcium ion transport
- sensory perception
- cellular response to tumor necrosis factor
- vasculature development
- skeletal system development
- muscle system development
- hormone biosynthetic process
- muscle contraction

Proteome

- visual perception
- sensory perception of light stimulus
- sensory perception
- lens development in camera-type eye
- nervous system process
- camera-type eye development
- visual system development
- system process
- eye development
- sensory organ development
- sensory system development
- ATP metabolic process
- pigmentation
- transmembrane transport
- cellular response to stimulus
- ion transmembrane transport
- growth
- developmental growth
- glycolytic process
- retina development in camera-type eye
- negative regulation of cellular process
- vasculature development

Results
To Conclude

Thyroidal AOP refinement

Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos

Hannes Reinwald a,b,1, Azora König a,1, Steve U. Ayobahan a, Julia Alvincz a, Levente Sipos a, Bernd Göckner c, Gisela Böhle d, Orr Shomroni e, Henner Hollert b, Gabriela Salinas e, Christoph Schäfers d, Elke Eilebrecht d, Sebastian Eilebrecht a,s

1 Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany
2 Department of Environmental Toxicology, University of Göttingen, Göttingen, Germany
3 Department of Environmental Research, Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany
4 Institute of Environmental and Food Analysis, Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany
5 NGS-Services for Integrative Genomics, University of Göttingen, Göttingen, Germany
Take home message

• The **identified transcriptome and proteome fingerprints** can be utilized for understanding thyroid disruption in early zebrafish development.

• Our study demonstrates that **omics-methodology can significantly improve the identification of endocrine disrupting substances targeting thyroid hormone-related physiological processes.**

• Overall, **our findings support the AOP development** for thyroidal ED assessment
Thank you for your attention

The ATTRACT Eco’n’OMICs Group

Dr. Sebastian Eilebrecht
Hannes Reinwald (MSc.)
Julia Alvincz
Fabian Essfeld (MSc.)
Fatma Marghany (MSc.)
Levente Sipos
Azora König (MSc.)
Dr. Steve Ayobahan

Contact - steve.ayobahan@ime.fraunhofer.de


