Transcriptomic analysis of the adult zebrafish liver in response to exposure to plasticizers and synthetic, steroidal estrogen.

Matthew Huff1,2, E. Starr Hazard1,3, Sean M Courtney1, Willian da Silveira1, Ludivine Renaud4, Gary Hardiman4

1MUSC Bioinformatics, Center for Genomics Medicine, 2MS in Biomedical Sciences Program, 3Library Science and Informatics, 4Department of Medicine, Medical University of South Carolina (MUSC), Charleston SC

Background

• Many industrial chemicals are widely dispersed in the environment. In recent years these endocrine disruptors (ED) have been labeled ‘contaminants of emerging concern’ (CERC), as they have potential to cause adverse effects on wildlife and human health (1-5).

• Two examples of important CERCs are nonylphenol (NP), a non-ionic surfactant, and DEHP, a 2-ethylhexyl phthalate (DEHP), both of which are termed xenosterogens (XE) as they can bind to the estrogen receptor (6,7) and disrupt estrogen physiology in mammals and other vertebrates.

Methodology

In vivo exposure

• Male zebrafish were obtained from a commercial fish farm (Euroaquarum Spa Bologna, Italy). The zebrafish were exposed for three weeks in 80 L tanks, housing 40 fish per tank, after being separated into two experimental periods:

  • The first group were exposed to 1000 nM of NP, with 1000 nM of E2 as a positive control.

  • The second group were exposed to 5.8 nM DEHP and 0.65 EE2.

• Following this exposure period, their livers were removed and frozen for molecular biology analysis. RNA was extracted with TRIzol reagent (Invitrogen) and purified on a QiaGen RNeasy column. RNA integrity was verified using RNA 6000 Nano Assay chips run in Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

RNA sequencing (RNA-seq)

• High throughput sequencing (HTS) was performed using an Illumina HiSeq2000 with each sample sequence to a minimum depth of ~50 million reads.

• RNA sequencing data was analyzed using the OnRamp Bioinformatics Genomics Research Platform RNAseq pipeline (11). This workflow utilizes hadoop software with automated data protection to seamlessly scale a 240 TB, 10-node server and storage infrastructure.

Results

• Transcript count data from DESeq2 analysis were sorted according to their adjusted p-value or q-value, which is the smallest false discovery rate (pDR) at which a transcript is called significant. FDR was calculated using the Benjamini-Hochberg multiple testing adjustment procedure.

Venn diagram highlighting the overlap of differentially expressed transcripts by XE exposure in zebrafish livers.

• Zebrafish are a popular model organism for their defined life cycle, and their genome’s similarity to humans, about 70% of human genes have a functional zebrafish homolog (9).

• The liver is the main site of metabolism of foreign chemicals, including XEs. Exposure to XEs has been associated with the development of adverse outcomes in the liver.

• XEs act via multiple toxicity pathways to induce adverse health outcomes. The adverse outcome pathways (AOP) framework is a new strategy that organizes mechanistic and/or predictive relationships between initial-chemical biological interactions, pathways and networks, and adverse phenotype outcomes (10).

Conclusions

• Exposure to the Xenosterogens NP, E2, and DEHP induces changes in gene expression in the livers of zebrafish.

• Each Xenosterogen is associated with unique changes in gene expression, while also sharing a pool of differentially regulated transcripts.

• Pathway analysis of significantly DE genes indicate a connection to NAFLD with the plasticizers DEHP and NP.

Bibliography

[References]

• Transcripts were considered to be differentially expressed when the p-value was <0.05 and the q-value was <0.05.

• Pathway analysis of significantly DE genes indicate a connection to NAFLD with the plasticizers DEHP and NP.