**MULTI-OMICS ANALYSIS OF 2D & 3D CULTURES OF HUMAN HEPARG CELLS EXPOSED TO DEHP & AMIODARONE TO REVEAL METABOLIC DISORDERS SIGNATURES**

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**ABSTRACT**

This study aimed to assess metabolomic responses of 2D and 3D hepatocyte cell culture samples exposed to amiodarone and DEHP. We performed global untargeted metabolomics analysis using an Agilent 6540 QTOF instrument, two different analytical columns (C18 - RP and HILIC), and two different ionisation modes (positive and negative) to increase the coverage of the detected metabolites to the maximum. Data pre-processing and processing, which included data cleaning, log transformation, normalisation, and batch effects correction, was performed using the R - based packages e.g. XCMS. An R package, called xMSannotator, was used for Network-Based annotation retrieving information from HMDB, Metlin, and Lipid Maps. The significantly differential metabolites were determined by ANOVA unequal variance test followed by the Benjamini-Hochberg false discovery rate (FDR) correction. We also performed multi-omics pathway analysis using the differential expressed genes and metabolites. Multi-omics pathway analysis is a powerful approach enabling interpretation of omics data at a higher level than individual biomarkers, thus providing mechanistic insight into biomarkers dysregulation, which is causative or indicative of metabolic disorders. We followed two different methods for the integrated analysis of transcriptomic and metabolomic data that exploits the fact that genes and metabolites are linked through biochemical reactions, over-representation analysis (ORA) and enrichment analysis (EA). The joint p-value was calculated as described by Cavill et al. (2011) [1] based on the Fisher’s method was used to assess the joint expression/concentration difference of all the entities in each detected pathway. We assumed the independence of the pathway associations from the different data sets. Q-values were calculated with the false discovery rate method. The majority of the annotated statistically significant belong to the lipids, more precisely to the class of glycerophospholipids (49 out of the 87 metabolites), followed by the fatty acyls (10/87). Multi-omics analysis resulted in 142 significantly dysregulated pathways pathways related to oxidative stress and lipids metabolism. In conclusion, the multi-omics analysis allowed the identification of potential biomarkers related to toxicity mechanisms of investigated pollutants.

**KEYWORDS:** HepaRG, untargeted metabolomics, multi-omics analysis, Endocrine-Disrupting Chemicals, metabolic disorders

**REFERENCES**

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