Transcriptomic profiling of clobetasol propionate-induced immunosuppression in challenged zebrafish embryos

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Introduction

In ecotoxicological hazard assessment of chemicals, it is currently neglected to consider immunotoxicity, which is mainly due to the lack of standardized test methods. A major problem of the assessment of immunotoxicity is that the function of the immune system is based on an activation by pathogens. It follows that an impairment of the immune system can only be comprehensively assessed if suppression to an activated state is considered.

In this study, we present an approach, which applies transcriptomic analysis on a zebrafish embryo infection model, with and without suppression by clobetasol propionate (CP), in order to identify potential biomarkers for immunotoxicity.

Methods

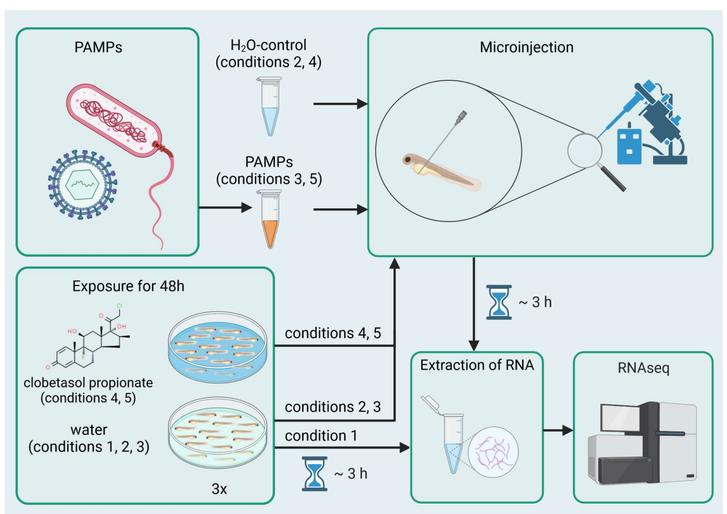

Figure 1: Experimental design. Created with Biorender.com

The following 5 combinations of treatments, which are also depicted in figure 1, were defined as conditions and controls in order to consider as many factors as possible: Untreated (1), water injection (2, control), PAMP injection (3, PAMP), water injection + CP exposure (4, CP), PAMP injection + CP exposure (5, PAMP_CP).

Freshly fertilized zebrafish embryos were exposed to 250 nM of CP or water for 48 hours. Then they were manually dechorinated and injected with a mixture of different pathogen associated molecular patterns (PAMPs) or water. Three hours post injection the embryos were introduced into extraction of total RNA, which was then sent to Illumina sequencing. Differentially expressed genes (DEGs), compared to the control, were identified by applying a cutoff of 0.01 on p-values corrected for multiple testing, as well as on the top 10% of absolute log2 fold-changes (LFC).

Results

Principle component analysis (figure 2A) revealed that 72.5% of the data's variance were explained by PC1 and PC2. The individual conditions clustered clearly and CP exposure accounted for a greater proportion of the variation than PAMP injection. Comparing the water injection control with the untreated control, which clustered close to each other, suggests that the injection process itself had only a minor contribution to gene expression changes. The venn diagram (figure 2B) includes the DEGs observed in conditions 3, 4 and 5, each compared to control condition 2.

The DEGs observed upon PAMP injection are shown in a scatter plot (figure 2C) comparing the LFC-values in PAMP injection and PAMP injection with CP exposure. Genes that were strongly regulated in PAMP but less strongly (LFC ratio PAMP_CP/PAMP ≥ 1.5) by PAMP_CP are colored in blue and are referred to as "hypo-responsive" genes.

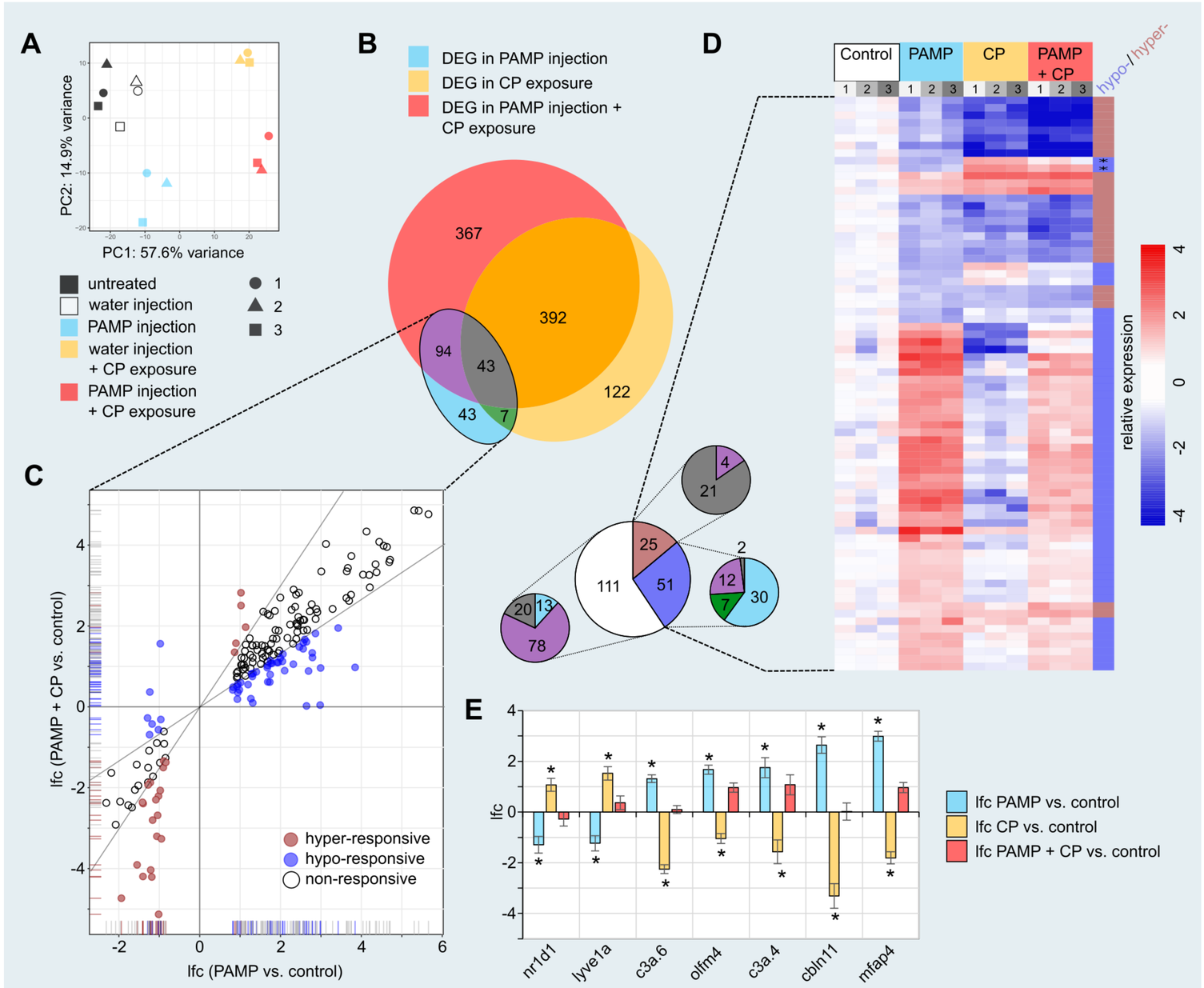

*Figure 2: **A** PCA of all conditions. **B** Venn diagram of DEGs found in conditions PAMP, CP and PAMP_CP. **C** Scatter plot of all DEGs found in PAMP including the classification into hyper-, hypo- or non-responsive genes. **D** Heatmap showing additive behavior of the majority of hypo- and hyper-responsive genes. **E** Barplot showing LFC of oppositely regulated genes, making them potential biomarkers of immunosuppression*

"Hyper-responsive" genes, colored in red showed an inversed relationship. All other genes with similar expression in both conditions were classified as "non-responsive". A more detailed view of al 74 hypo- and hyper-responsive genes (figure 2D) is given as heatmap showing relative expression of all conditions shown in the venn diagram, while the piecharts illustrate the composition of each gene set with respect to figure 2B. We observed that the LFC of almost every case in PAMP_CP was a result of additive effects of the individual PAMP and CP conditions. Interestingly, the 7 genes from the intersection of PAMP and CP (figure 2B and D colored in green) were all oppositely regulated in PAMP and CP, which makes them potential biomarkers of immunosuppression (figure 2E).

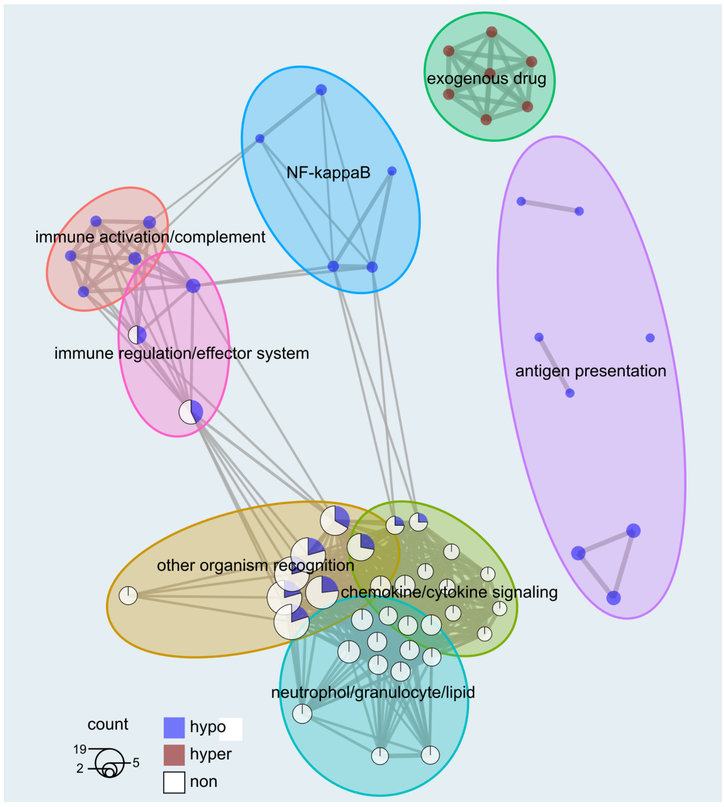

Figure 3: Network plot of over representation analysis of hypo-, hyper- and non-regulated DEGs

Network clustering of over-represented genes in biological processes (figure 3) revealed that processes associated with the hypo-responsive genes partially clustered separately from those associated with the non-responsive genes and were assigned to NF-κB, complement activation, and antigen presentation, whereas processes associated with the hypo-responsive genes completely clustered separately and were associated with response to exogenous drugs.

Conclusion

By applying this novel approach for the detection of immunotoxicity we were able to identify potential biomarkers which will help to increasingly address immunotoxicity in the environmental hazard assessment of substances.

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