NEW INSIGHTS INTO THE TETRODOTOXIN ACCUMULATION AND METABOLISM IN THE PUFFERFISH *Takifugu rubripes*

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Background

- Tetrodotoxin (TTX) is a well-known potent marine neurotoxin and it exists in most of pufferfishes.
- TTX plays a variety of physiological and ecological functions in pufferfish. TTX could be toxin or drug for humans depending on the dose.
- However, the underlying mechanism of TTX accumulation and metabolism in pufferfish is not clear yet.
- Here we investigate the potential regulatory genes involved in TTX accumulation, translocation and detoxification in tiger puffer *Takifugu rubripes*.

Experiment model

Fish sampling and TTX detection → Transcriptome sequencing and DEGs analysis → Functional enrichment analysis → Validation of gene expression data

Results

Variation in TTX concentrations

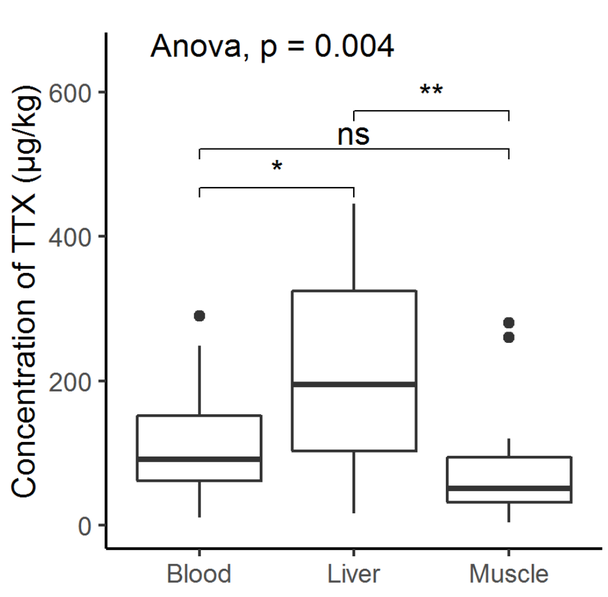

Figure 1 Boxplot of TTX concentration differences among blood, liver, and muscle tissues in the toxic *T. rubripes*. One-way ANOVA results among three tissues expressed as the P-value. The statistical significance of LSD test is indicated by asterisks (ns, p ≥ 0.05, *p < 0.05, **p < 0.01). Black dots indicate outliers.

Differentially expressed genes

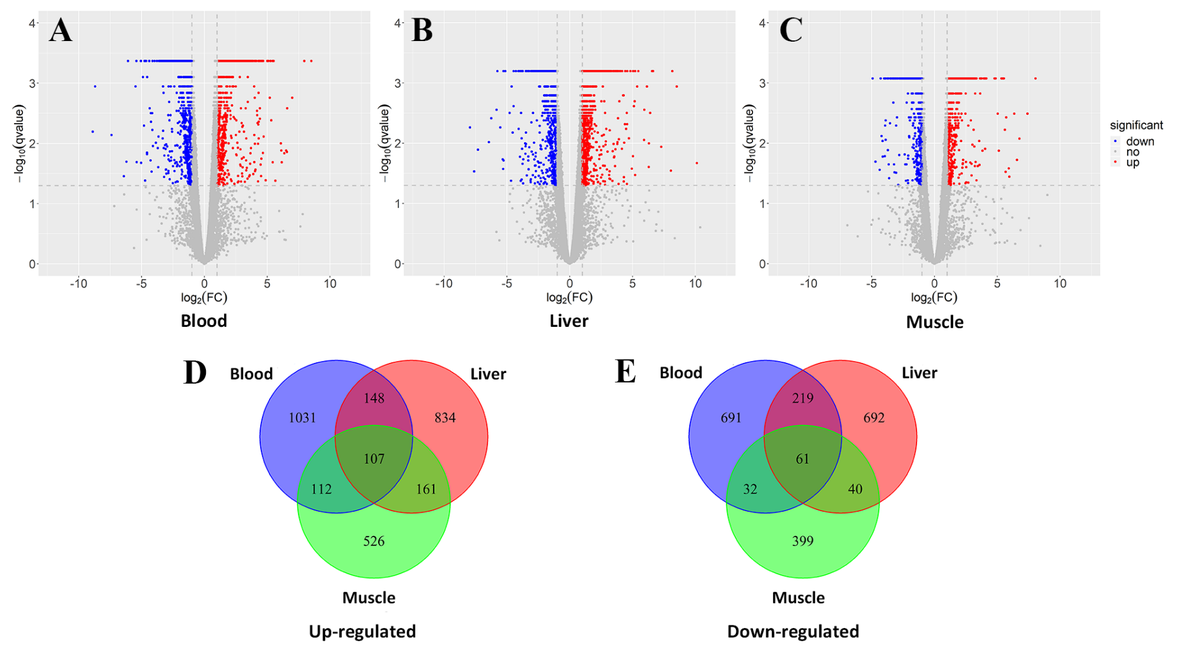

Figure 2 Differentially expressed genes. Volcano plots displaying up- and downregulated DEGs in (A) blood, (B) liver, and (C) muscle tissues between toxic and non-toxic *T. rubripes*. Red dots represent upregulated genes (*q*-value < 0.05, log2FC > 1), and blue dots represent downregulated genes (*q*-value < 0.05, log2FC < − 1). Venn diagrams depict the number of unique and shared DEGs with (D) upregulated and (E) downregulated expression among the different tissues.

Enrichment analysis and validation of TTX-related genes

Figure 3 Enrichment analysis of DEGs and TTX-related genes. Bubble plots of representative enriched GO terms and KEGG pathways based on significant DEGs in toxic versus non-toxic *T. rubripes* (A) blood, (B) liver, and (C) muscle tissues. Heatmaps represent the cluster analysis of TTX-related gene expression levels based on the FPKM of toxic and non-toxic specimens in *T. rubripes* (D) blood, (E) liver, and (F) muscle tissues. Pearson correlation was used as the clustering method.

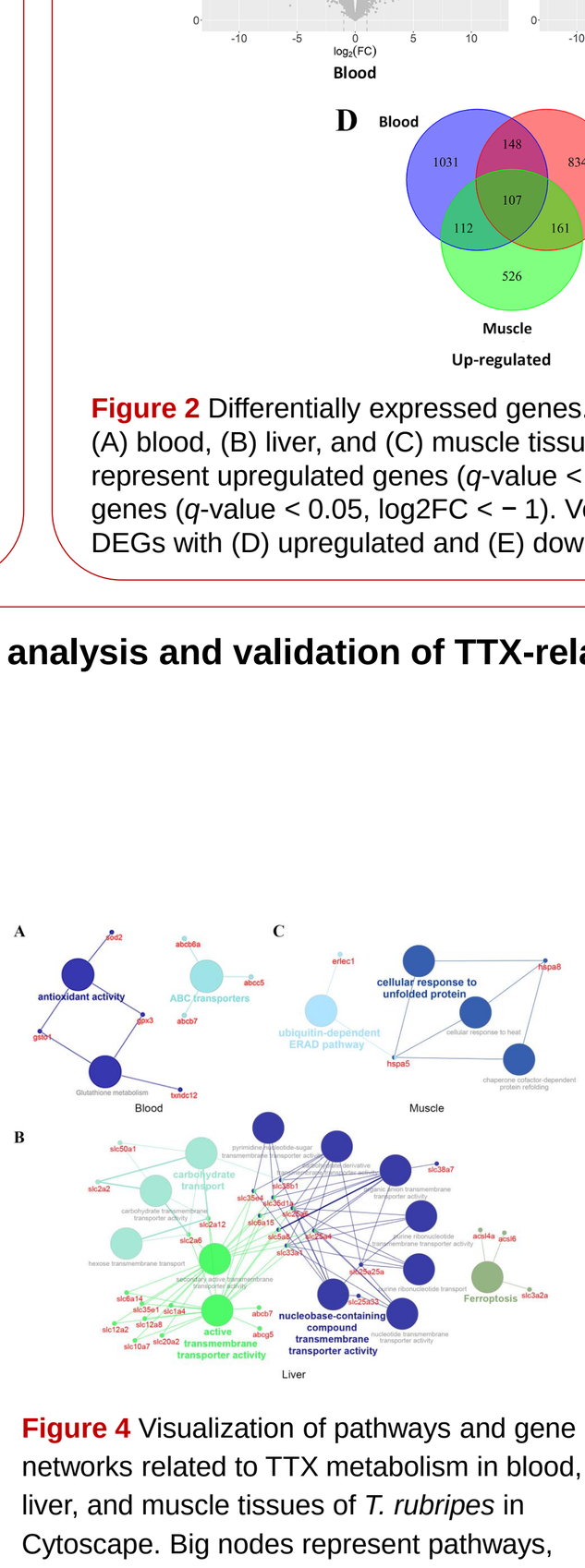

Figure 4 Visualization of pathways and gene networks related to TTX metabolism in blood, liver, and muscle tissues of *T. rubripes* in Cytoscape. Big nodes represent pathways, and small nodes represent genes.

Figure 5 Validation of TTX-related DEGs identified via RNA-seq using qRT-PCR. Bar plots show the pairwise comparison of the expression fold-changes in genes in toxic versus non-toxic specimens detected using qRT-PCR and RNA-seq in (A) blood, (B) liver, and (C) muscle tissues. The log2FC values were calculated using the $2^{-\Delta\Delta Ct}$ method and FPKM, respectively. Unfilled bars represent log2FC in qRT-PCR; grey bars represent log2FC in RNA-seq. Each bar represents mean ± SD (n = 6). (D) Scatter plot showing the correlations of TTX-related genes expression patterns between qRT-PCR and RNA-seq, as determined using the log2FC values. TTX-related genes in the blood, liver, and muscle are indicated in blue, yellow, and red dots, respectively, with the corresponding regression lines. Pearson correlation coefficients (R) and P-values were calculated and are shown on the upper left.

Highlights

- TTX content is organ- and environment-dependent in *Takifugu rubripes*.
- SOD, GST, ABC, GPx, TXN, IAP, SLC, and ER were reported to be involved in TTX metabolism in pufferfish for the first time.
- ABCs, ACSLs, SLCs, and APOs show potential influence on TTX accumulation and translocation in *T. rubripes*.
- SLCs play critical and diverse roles in TTX metabolism in *T. rubripes*.