Application of spatial transcriptomics to investigate disruption of hepatic zonation in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin elicited progression of steatosis to steatohepatitis with fibrosis.

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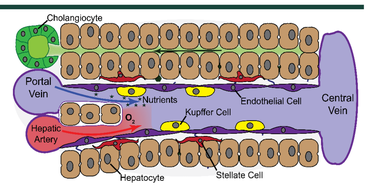
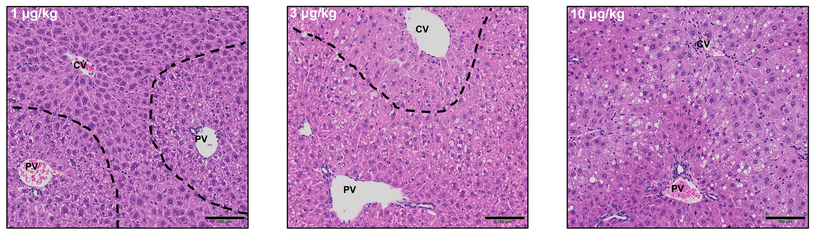
ABSTRACT

Cellular heterogeneity and structural organization are essential for normal liver homeostasis and its response to drugs and chemicals. The persistent environmental contaminant and potent aryl hydrocarbon receptor (AHR) agonist, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), elicits a distinct dose-dependent spatially resolved pattern of tissue damage. While histological assessment initially identified dose-dependent portal zone effects that expanded towards the central zone following oral gavage with TCDD, bulk and single-nuclei transcriptomic studies suggested initial responses occurred in the central zone leading to a loss of zonal identity that contributed to widespread tissue damage. To further investigate the dose-dependent spatially resolved transcriptomic effects of TCDD, Resolve Biosciences Molecular Cartography was used to characterize the spatial expression of 97 hepatic genes in male mice orally gavaged with sesame oil vehicle, 0.3, 3, or 30 µg/kg TCDD every 4 days for 28 days. Markers of portal (*Cyp2f2*) and central (*Glul*) hepatocytes showed expected zonation patterns at 0 – 3 µg/kg, that were markedly disrupted at 30 µg/kg. Centrally localized genes involved in carbohydrate metabolism (*Gulo*), antioxidant defense (*Gstm3*, *Gsta*), and lipid uptake (*Cd36*, *Vldlr*) co-localized with portal *Cyp2f2* expression following treatment with the highest dose. The dose-dependent central zone expression of *Cyp1a1*, *Fmo3*, *Nqo1*, and *Xdh* was consistent with higher *Ahr* expression levels in the central zone resulting in initial induction in the central zone but dose-dependently progressed to the portal zone. Furthermore, the emergence of a highly expressing *Gpnmb* and *Trem2* macrophage sub-population that also expressed *Adgre1* and *Cd5l* markers was consistent with non-alcoholic steatohepatitis (NASH) associated macrophage (NAMs) populations. At 30 µg/kg TCDD, *Tagln* expressing hepatic stellate cells (HSCs) co-localized with activated HSC marker *Acta2*, and the expression of collagens *Col1a1*, *Col1a2*, and *Col3a1* within periportal fibrotic tracts. Collectively, spatial transcriptomics indicated a global disruption of zonal identity within hepatocytes driven by increased expression of genes characteristic of the central zone in the portal regions. These results suggest the loss of zonal identity may contribute to the hepatotoxicity and progression of steatosis to steatohepatitis with fibrosis elicited by TCDD. *This study was supported by be the National Institutes of Health Science Superfund Research Program (NIEHS SRP P42 ES004911) and National Human Genome Research Institute (NHGRI RO1 HG010789).*

INTRODUCTION & BACKGROUND



- The liver is organized into lobules exhibiting cellular and spatial heterogeneity resulting from gradients of incoming (portal) blood to exiting (central) and the presence of diverse cell types with distinct functions.
- 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a persistent environmental contaminant and aryl hydrocarbon receptor (AHR) agonist that causes zonal hepatotoxicity, the accumulation of triacylglycerols in fat droplets, the infiltration of inflammatory cells, and the development of portal fibrosis.

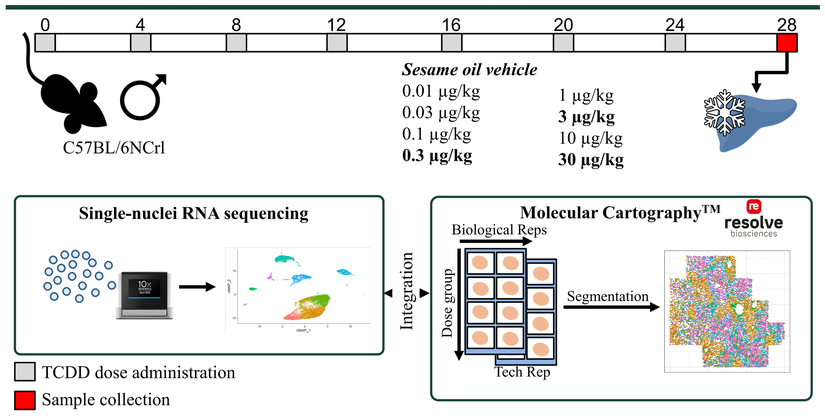


▲ FIGURE 1. Representative photomicrographs of dose dependent TCDD-elicited zonal hepatotoxicity. Representative micrographs of liver sections from mice gavaged with sesame oil vehicle or TCDD every 4 days for 28 days. The edge of tissue damage (dashed line) was drawn manually to illustrate the progression from periportal to panacinar hepatotoxicity. CV = Central Vein; PV = Portal Vein; scale bar = 100 µm.

OBJECTIVE

Evaluate the dose-dependent and spatially resolved changes in gene expression associated with TCDD-elicited NAFLD pathogenesis

STUDY DESIGN & EXPERIMENTAL APPROACH



▲ FIGURE 2. Overview of experimental approach for single cell transcriptomic analyses of TCDD treated mouse livers.

Male C57BL/6NCrl mice were gavaged every 4 days (grey boxes) for 28 days with sesame oil or 0.01 – 30 µg/kg TCDD. Liver samples were immediately frozen in liquid nitrogen and stored at -80ºC. Hepatic nuclei were isolated, stained with DAPI, and FACS sorted to obtain a single nuclei suspension. Nuclei (~3-6k/sample) were then processed using the 10X Genomics Chromium Controller to generate single cell libraries. Frozen liver sections were also sectioned for spatial transcriptomic analysis of 97 genes at subcellular resolution using the Resolve Molecular Cartography services.

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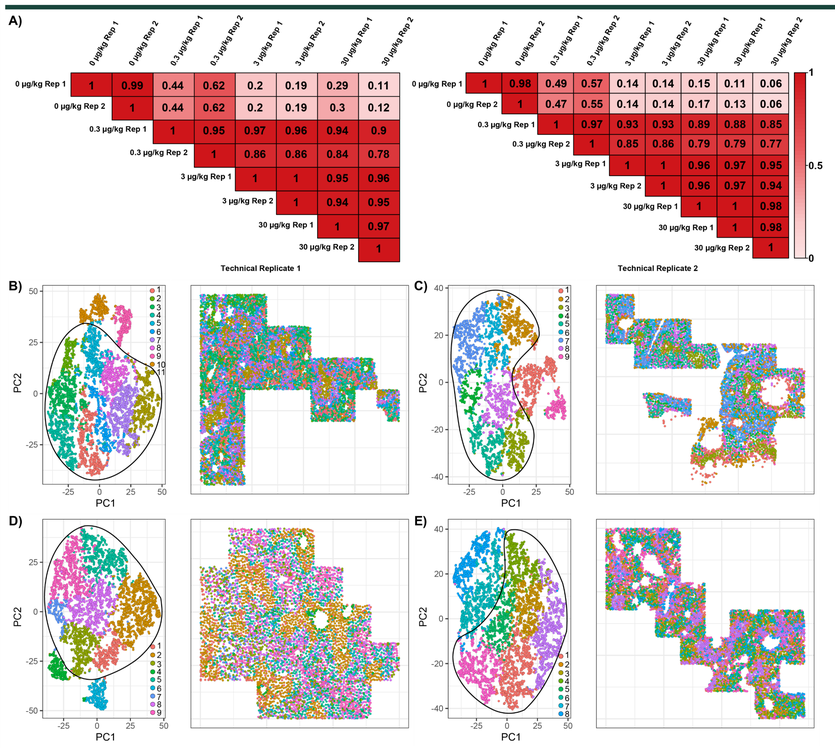
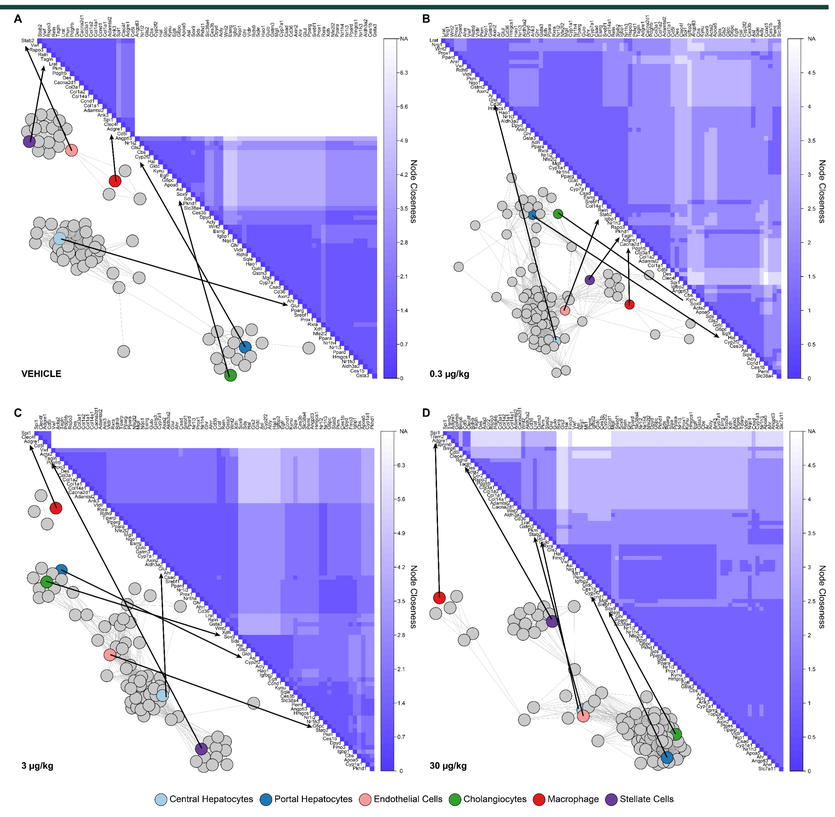
The authors have no conflicts of interest to declare

OVERVIEW OF SPATIAL TRANSCRIPTOMIC ANALYSIS



▲ FIGURE 3. Overview of biological and technical replications by spatial transcriptomic analyses.

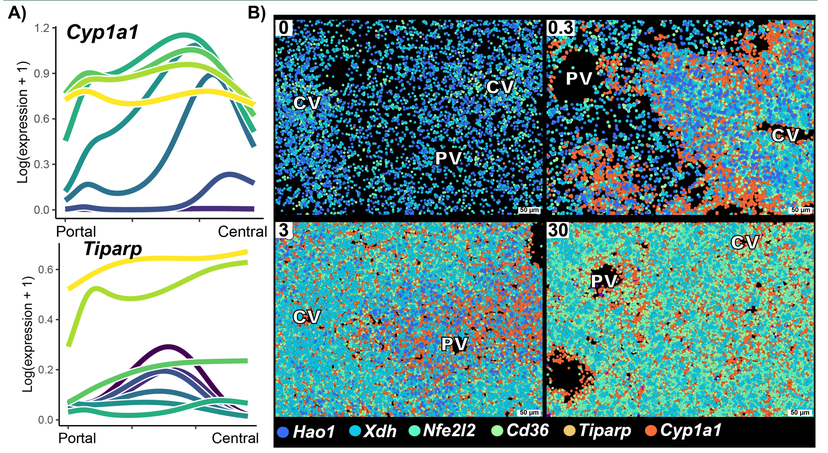
(A) Pearson correlation of transcript counts within regions of interest (ROI) between biological replicates across two technical replicates. ROIs were processed using Baysor and MERINGUE to identify individual cells and clustered according to similarity in gene expression. Representative PCA plots and color-coded ROIs are shown for liver sections of mice gavaged every 4 days for 28 days with (B) sesame oil vehicle control, (C) 0.3 µg/kg, (D) 3 µg/kg, or (E) 30 µg/kg TCDD. Each tissue section was processed independently (cluster numbers are not equivalent for each sample). Mapped ROIs show location of cells colored by cluster. Images are not equally scaled due to differences in ROI dimensions.



▲ FIGURE 4. Network analysis of spatially resolved gene co-expression.

Networks and connectivity plots are shown for (A) sesame oil vehicle, (B) 0.3, (C) 3, (D) 30 µg/kg TCDD. The distance between nodes (connectivity) is show in the heatmaps. Absence of connection between two genes is represented as white (highest value). Colored nodes in the network represent marker genes for each cell type.

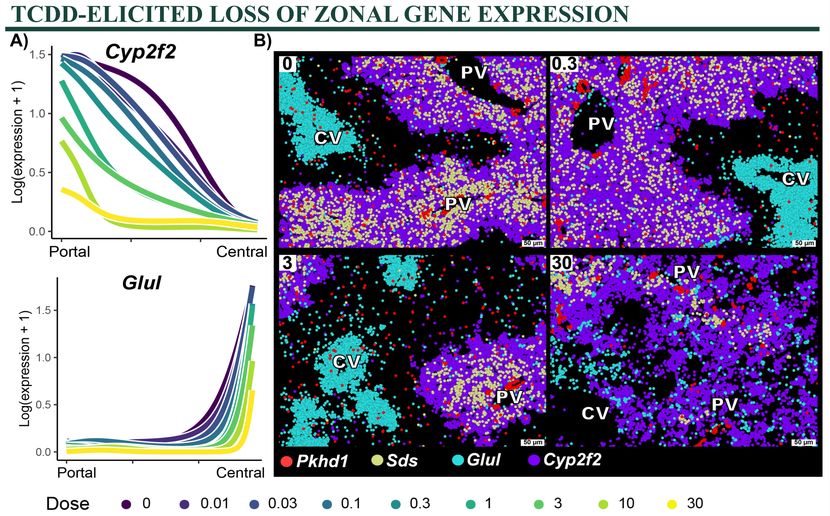
SPATIALLY RESOLVED EXPRESSION OF AHR TARGETS



▲ FIGURE 5. Spatially resolved expression of AHR target genes.

(A) Pseudospace ordered nuclei from snRNAseq data was examined for classical AHR targets *Cyp1a1* and *Tiparp* demonstrating dose-dependent zonal induction in mice gavaged following treatment with TCDD every 4 days for 28 days. (B) Molecular cartography was used to visualize dose-dependent spatial expression of AHR target genes *Hao1*, *Xdh*, *Nfe2l2*, *Tiparp*, and *Cyp1a1*. Point colors are ordered as layers (top to bottom) to facilitate visualization genes expressed at lower levels. Scale bar represents 50 µm.

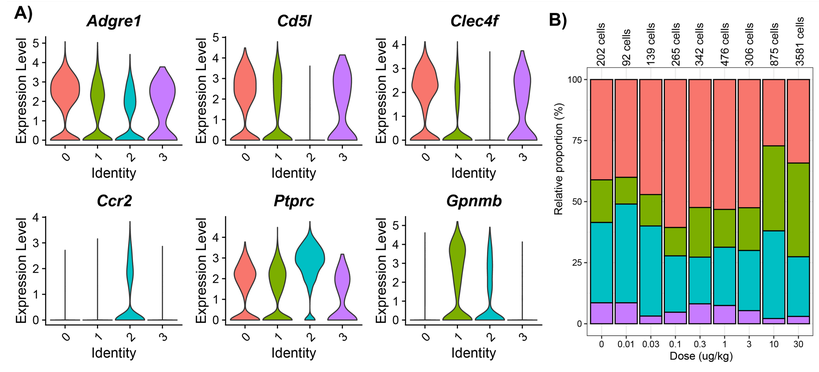
TCDD-ELICITED LOSS OF ZONAL GENE EXPRESSION



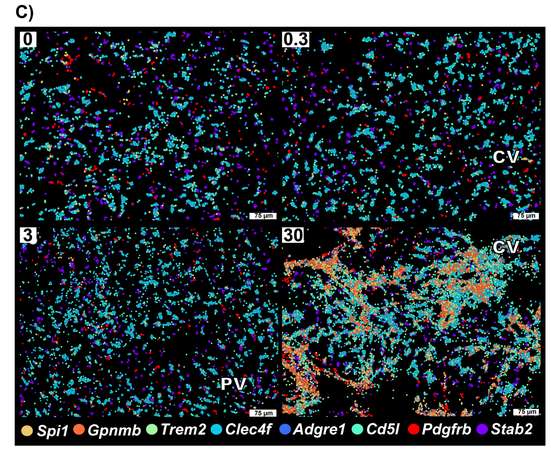
▲ FIGURE 6. Spatially resolved expression of zonal marker genes.

(A) Pseudospace ordered nuclei from snRNAseq data was examined for expression of the portal (*Cyp2f2*) and central (*Glul*) hepatocyte markers. (B) Molecular cartography was used to visualize dose-dependent spatial expression of hepatocyte zone marker genes. Point colors are ordered as layers (top to bottom) to facilitate visualization of genes expressed at lower levels. Scale bar represents 50 µm.

EMERGENCE OF NASH-ASSOCIATED MACROPHAGE



C)



◄ FIGURE 7. Characterization of macrophage subpopulations.

(A) Macrophage were subset and re-clustered from snRNAseq dataset identifying 4 subpopulations. (B) Relative proportions of each subpopulation is shown for each dose group. (C) Molecular cartography was used to visualize dose-dependent spatial expression of macrophage genes *Spi1*, *Gpnmb*, *Trem2*, *Clec4f*, *Adgre1*, and *Cd5l*. Marker genes for stellate cells (*Pdgfrb*) endothelial cells (*Stab2*). Point colors are ordered as layers (top to bottom) to facilitate visualization of genes expressed at lower levels. Scale bar represents 75 µm.

SUMMARY

- Spatial transcriptomics was used to complement snRNAseq data and characterize dose-dependent zonal gene expression. Lobular zonation was clearly distinguishable at doses 0 – 3 µg/kg TCDD whereas at 30 µg/kg TCDD, gene expression was disrupted such that *Cyp2f2* and *Glul* no longer served as reliable markers of portal and central zonation, respectively.

DOSE

Vehicle	0.3 µg/kg	3 µg/kg	30 µg/kg
• Normal liver and expression	• Normal zonation • *Cyp1a1* induction in the central zone	• Normal zonation • *Tiparp* induction in the central zone • Panacinar induction of *Cyp1a1*	• Loss of zonal expression patterns • Panacinar differential expression of AHR target genes. • Emergence of *Gpnmb*/*Trem2* positive macrophage (NAMs) co-localizing with activated stellate cells.

- TCDD-elicited induction of AHR battery genes is dose- and cellular location (zone)-dependent, but not consistent with the emergence of tissue damage.