#4709 Spatial whole transcriptome profiling of human normal liver uncovers unique insights into metabolic zonation

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Introduction

The liver is the largest internal organ in the human body. It is involved in three major vital functions: storage, detoxification and synthesis. The liver helps improve digestion, removes toxins and other impurities from the body, regulates blood clotting, removes bacteria from the blood and maintains the balance of hormones.

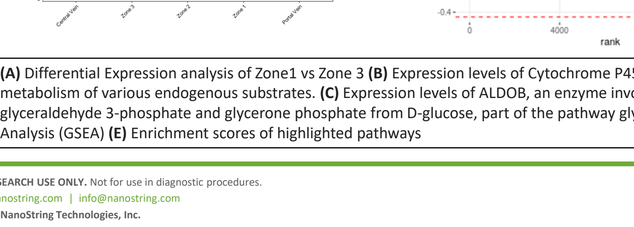
Understanding the physiology and functions of the liver and cancer requires knowing transcriptional patterns driving biological activities within the functional structures of the tissue, especially the zonated features of the liver metabolic networks.

Using the powerful and unique capabilities of GeoMx® Digital Spatial Profiler (DSP) with the Whole Transcriptome Atlas (WTA) panel to resolve functional units within FFPE tissues in situ, here we report the spatial analysis of whole transcriptomes across three micro-dissected zones (pericentral zone 3, intermediate zone 2 and periportal zone 1) of human normal liver. We also report the whole transcriptome expression data from Kupffer cells, portal traits and interlobular bile ducts from four normal liver samples.

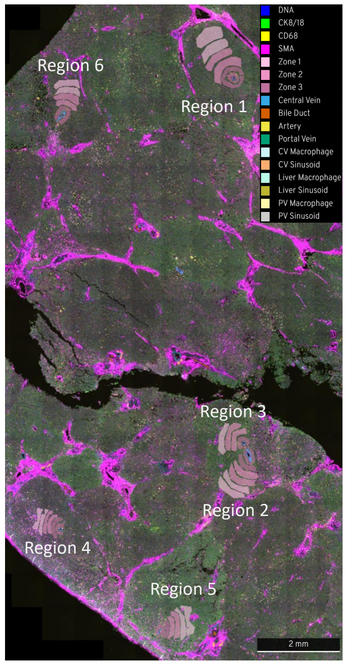
GeoMx® DSP enables direct in situ expression profiling

Tagged Oligonucleotide Chemistry

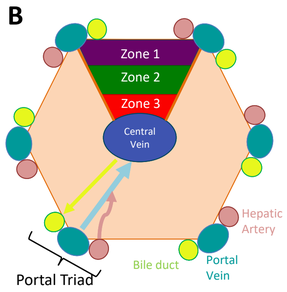
GeoMx Digital Spatial Profiler (DSP) uses oligonucleotides which hybridize to target mRNAs to quantitatively read out DNA tags which are selectively released *in situ* by specifically shining UV light into certain regions of the tissue

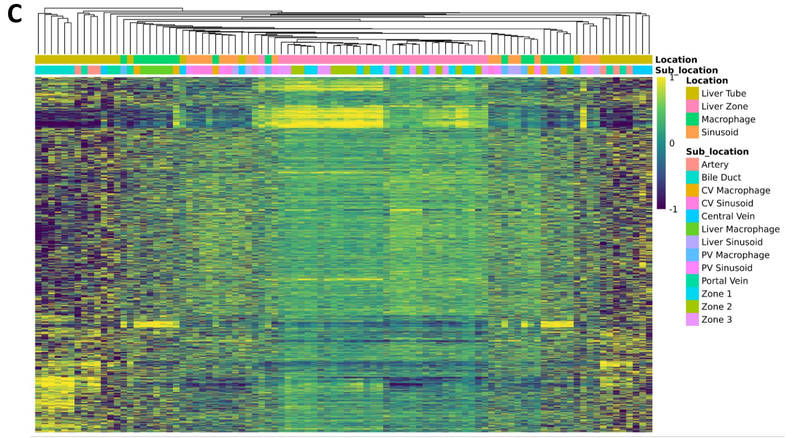


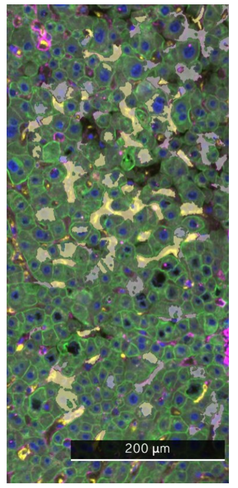
Liver Anatomy

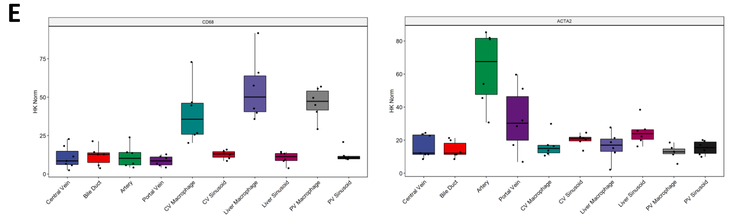


(A) Representative 5 µm Formalin Fixed Paraffin Embedded Normal Liver tissue; visualized with CK8/18 (hepatocytes), CD68 (Kupffer cells/macrophages), α-SMA (sinusoid and stellate cells) and SYTO 83 (nuclei). Sample was molecularly profiled with the GeoMx Human Whole Transcriptome Atlas (WTA) assay.
(B) The functional unit of the liver, lobules are divided into three zones. **Zone 1** is highly perfused and plays a large role in oxidative metabolism including beta-oxidation, gluconeogenesis, bile formation, cholesterol formation, and amino acid catabolism. **Zone 2** is the pericentral region between zones 2 and 3. **Zone 3** is least perfused and plays the largest role in detoxification, biotransformation of drugs, ketogenesis, glycolysis, lipogenesis, glycogen synthesis, and glutamine formation.



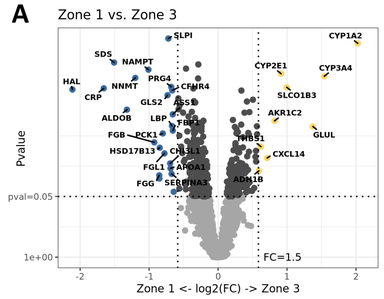


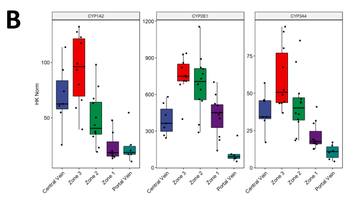


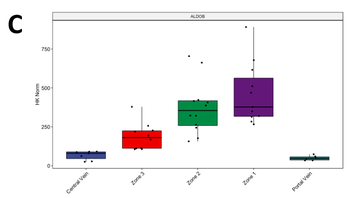


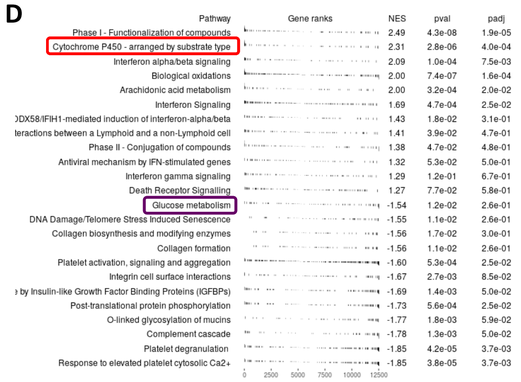
(C) Unsupervised clustering accurately classifies histological structures of the liver **(D)** CD68 and SMA expression levels **(E)** Closeup of PV macrophages (highlighted light yellow) and PV sinusoid (highlighted grey)

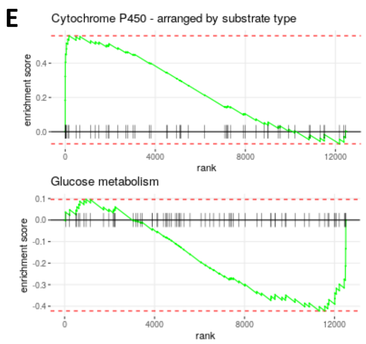
DE and GSEA results for Zone 1 vs. Zone 3 reveal metabolic zonation





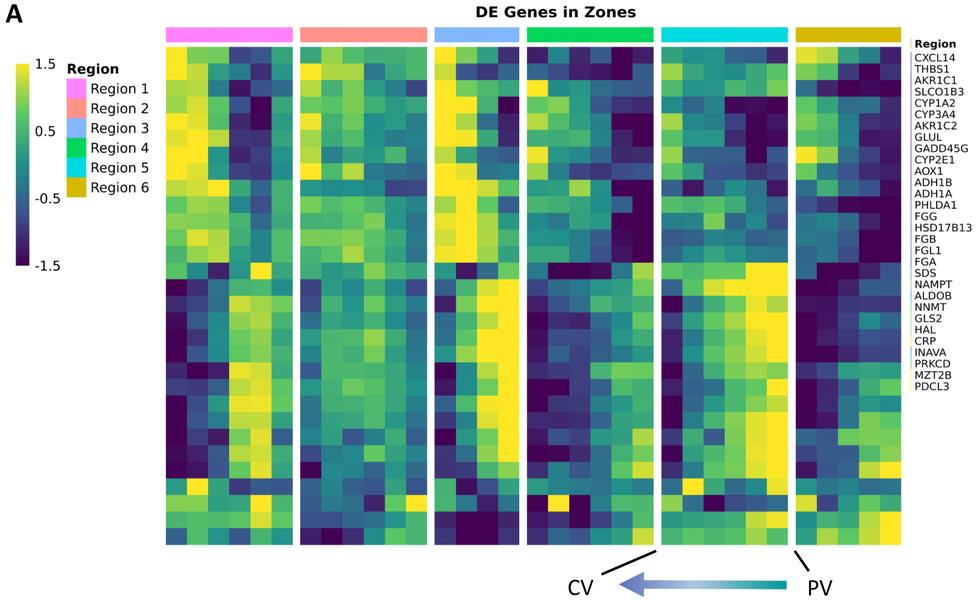




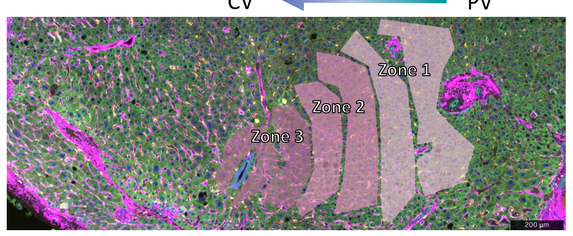


(A) Differential Expression analysis of Zone1 vs Zone 3 **(B)** Expression levels of Cytochrome P450 monooxygenase involved in the metabolism of various endogenous substrates. **(C)** Expression levels of ALDOB, an enzyme involved subpathway that synthesizes D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose, part of the pathway glycolysis **(D)** Gene Set Enrichment Analysis (GSEA) **(E)** Enrichment scores of highlighted pathways

Gradient pattern along the porto-central axis of top differentially expressed genes



(A) Differentially expressed genes in 6 lobules (Regions 1-6) follow a gradient expression pattern along the porto-central axis.



Conclusions

- Whole transcriptome profiles of zone 1 and zone 3 have revealed 32 differentially expressed targets (fold change > 1.5, p value < 0.05) which showed a gradient expression pattern along the porto-central axis.
- Important pathways involved in metabolisms including biological oxidations (CYP1A2, CYP2E1, CYP3A4, ADH1A, and ADH1B) showed high enrichment in zone 3 and decreased towards zone 1. In contrast, pathways including glucose metabolism (ALDOB and PCK1) and amino acids metabolism showed high enrichment in zone 1 and decreased towards zone 3.
- GeoMx technology with WTA data has revealed clear metabolic zonation in the liver along the porto-central axis and can be further utilized to study the whole transcriptomic differences in normal and diseased tissues.