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USING A HUMAN LIVER-ON-A-CHIP MODEL TO STUDY ALCOHOL-ASSOCIATED LIVER DISEASE BY TARGETING LSEC AND ALDH2

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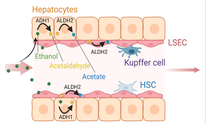
INTRODUCTION

The current experimental approach for studying alcohol-associated liver disease (ALD) has limitations due to insufficient expression and activity of alcohol-metabolizing enzymes in hepatocytes using standard cell culture systems and animal models. Even with a 3D in vitro organoid system, we experience non-physiological structure, inconsistent microenvironment, and uneven nutrient and oxygen supply. Therefore, we developed a Liver-on-a-Chip culture system using human liver cells, which express relevant levels of alcohol-metabolizing enzymes. There is an unmet need for a human-relevant in vitro 3D culture model to test new therapeutic approaches. In our initial study, we determined that ALDH2 is expressed in liver sinusoidal endothelial cells (LSEC) besides hepatocytes. Intriguingly, senescent LSECs showed reduced expression of ALDH2.

HYPOTHESIS

We hypothesize that reduced ALDH2 in diseased or unhealthy LSECs contributes to ALD development through accumulation of acetaldehyde in the liver. We used a human Liver-on-a-Chip model to test this hypothesis.



METHOD

- To address this unmet need, we employed human Liver-on-a-Chip culture system with human hepatocytes and LSECs. It maintains key characteristics of native liver function over long-term culture. We also utilized conventional collagen-coated plates for comparison and evaluated our Liver-on-a-Chip system.
- Three different conditions were set up in the experiment. (1) conventional collagen-coated plate with human hepatocytes. Chips were divided into two groups: (2) human hepatocytes only; (3) human hepatocytes with human LSECs. After day 3 of cell culture, they were further separated into two treatment groups: (1) non-treatment used for control; (2) 0.2% ethanol (~34mM) treatment to induce alcohol-associated hepatocyte injury.
- In the hepatocyte-LSEC co-culture, ALDH2 was silenced or pharmacologically activated by Alda-1 in LSECs.
- Culture medium was used to measure AST, ALT, acetaldehyde, and ethanol concentrations. Cells were used for RNA extraction and measurement using qPCR assay.
- Using single-cell RNA-seq data of human alcoholic cirrhosis and an in vitro model of senescent LSECs, ALDH2 expression and senescence markers were measured.

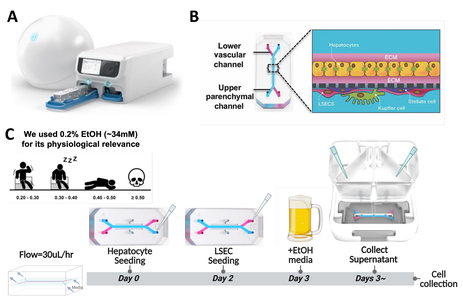


Figure 1. Flowchart of our human Liver-on-a-Chip experiments. (A) Image of organ-on-a-chip culture instruments. (B) Schematic image of human Liver-on-a-Chip. (C) The experiment protocol was used in this study.

RESULTS

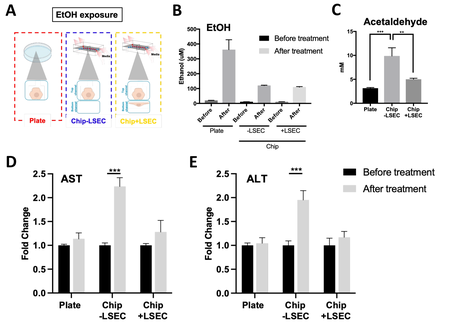


Figure 3. LSECs protect Hepatocytes against ethanol-induced injury. (A) Schematic image of this experiment. (B) Liver-on-a-Chip hepatocytes maintain more capacity of alcohol metabolism. (C) LSECs play a role for acetaldehyde metabolism. (D-E) AST and ALT elevation was suppressed by adding LSECs in Liver-on-a-Chip culture.

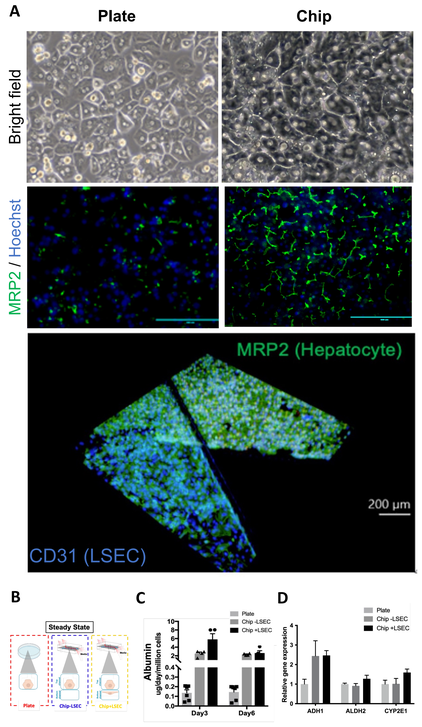


Figure 2. Liver-on-a-Chip mimics human liver physiology better than conventional plate. (A) Hepatocytes cultured on Liver-on-a-Chip maintain better biliary canaliculi shown in green color. (B) Schematic image of the experiment. (C) A Liver-on-a-Chip culture system had higher albumin concentrations than conventional 2D plate culture. (D) Cells cultured on the Liver-on-a-Chip system increased gene expression of ADH1, ALDH2, and CYP2E1.

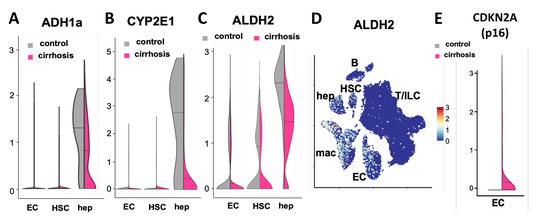


Figure 4. Expression of alcohol metabolizing enzymes in different liver cells. (A-C) ADH1a, CYP2E1, and ALDH2 expression in endothelial cells (ECs), Hepatic stellate cells (HSCs), and hepatocytes (hep) from control and patients with cirrhosis. (D) ALDH2 expression in UMAP of scRNA-seq from human liver cells. (E) CDKN2A expression in ECs.

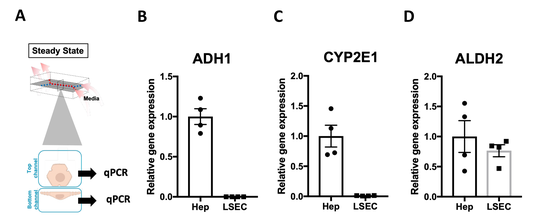


Figure 5. Alcohol metabolizing enzyme expression in human primary liver cells. (A) Schematic image of the experiment. (B-D) Human LSECs have high expression of ALDH2 but less expression of ADH1 and CYP2E1.

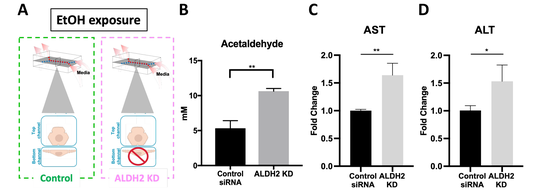


Figure 6. ALDH2 knockdown in LSECs results in more hepatocytes damage. (A) Schematic image of this experiment. (B) ALDH2 knockdown in LSECs chip induced increased acetaldehyde levels in culture media. (C-D) AST and ALT elevation was increased by knocking down LSEC ALDH2 in Liver-on-a-Chip culture.

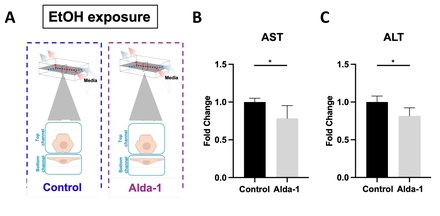


Figure 7. Alda-1, an ALDH2 activator, alleviated ethanol-induced hepatocyte injury. (A) Schematic image of the experiment. (B) The alda-1 treatment reduced AST and ALT levels in culture media.

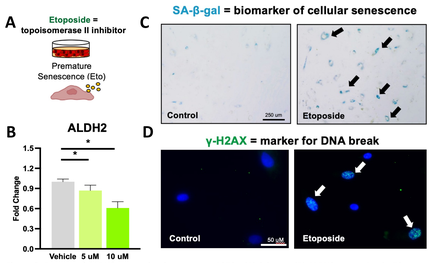


Figure 8. Decreased ALDH2 expression in senescent LSECs. (A) Schema. (B) ALDH2 expression in ECs treated with Etoposide. (C) SA-β-gal and (D) γ-H2AX staining in LSECs treated with Etoposide.

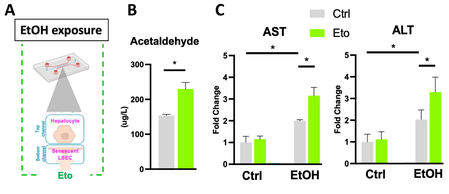


Figure 9. Senescent LSECs enhanced ethanol-induced liver injury. (A) Schema. (B) Chip with senescent LSECs (Eto) increased acetaldehyde levels in culture media. (C-D) AST and ALT were increased in Liver-on-a-Chip with senescent LSECs.

CONCLUSIONS

A human Liver-on-a-Chip system emulates alcohol-induced hepatocyte injury better than the conventional 2D plate culture. LSECs are protective against ethanol-induced hepatocyte injury. LSEC ALDH2 is critical in ALD because its expression contributes to the clearance of acetaldehyde. LSEC senescence could be a mechanism of developing severe alcoholic hepatitis in patients with cirrhosis. Overall, human liver-on-a-chip system is a useful tool to study ALD.

REFERENCES

1. **Nawroth JC, Petropolis DB, Seki E, Lu SC, Karalis KC, et al**. Modeling alcohol-associated liver disease in a human Liver-Chip. *Cell Reports 2021*
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