RESEARCH HIGHLIGHT

Learning human liver biology in humanized mice

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A new study describes development of a chimeric mouse liver repopulated with nearly all human hepatic cell types albeit to varying extents. This model yielded novel information on unique human hepatocyte metabolic function which is regulated by local microenvironmental signals, specifically sinusoidal endothelial cell-secreted WNT2.

The human liver is a highly active and essential metabolic organ, performing up to 500 biochemical "tasks" every day. These enzymatic reactions are tightly spatially organized within the hepatic lobule forming distinct "zones" of metabolic gene expression. However, what regulates these zonated gene expression programs, whether intrinsic or extrinsic to the hepatocyte, and the mechanisms underlying dysfunction in human disease pathogenesis, remain poorly understood. Identifying these biological mechanisms may improve our overall understanding of chronic liver disease, which cause ~2 million deaths annually worldwide,[1] and aid in target identification and therapeutic development. In a new study in *Cell*, the authors developed a novel chimeric mouse liver, repopulated with human liver cells, to study human-specific liver pathophysiology.[2] They further demonstrated that human non-parenchymal cells (NPCs), specifically the liver sinusoidal endothelial cells (LSECs) in pericentral zone, control human hepatocyte cholesterol metabolism and bile acid production. They found that LSEC-derived WNT2 regulates hepatocyte cholesterol transport and detoxification to bile acids, thus highlighting a direct link between microenvironmental cues and zonal hepatocyte metabolic function.

In their study, Kaffe et al. first established a humanized mouse liver containing both human hepatocytes and NPCs, including immune cells, hepatic stellate cells (HSCs), and endothelial cells (ECs), the latter two cell types particularly important for regulating liver zonation, repair, and regeneration.[3] Prior to this study, humanized mouse livers were typically devoid of human NPCs, but populated with mouse NPCs, limiting the ability to study the influence of the human hepatic microenvironment on human hepatocyte function. Additionally, developing physiologically accurate and representative models of human liver diseases is important because murine models do not fully recapitulate the native human physiology of the liver, with many species-specific metabolic differences in how mice metabolize drugs, fats, and proteins.[4]

Building on their previous work developing a humanized mouse liver,[5] the authors utilized a genetically engineered mouse line, MISTRG6-*Fah*$^{-/-}$, which has 6 human immune genes integrated into the mouse genome: *M-CSF*, *IL-3*, *GM-CSF*, *SIRPa*, *TPO*, and *IL6*, in the *RAG2/IL2Rg* double knockout mouse, with the fumarylacetoacetate hydrolase (*Fah*) gene knocked out as well. This genetic combination allows for repopulation with human immune cells and hepatocytes, following human CD34$^+$ hematopoietic stem cell and hepatocyte transplantation and withdrawal of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, respectively. Additionally, as an orthogonal approach to validate their findings in an additional model, they utilized another mouse line, MISTRG6-RAF (retrorsine-APAP-anti-mouseFAS), which eliminates mouse hepatocytes through administration of retrorsine (inhibits mouse hepatocyte proliferation) and APAP (induces mouse hepatocyte death) prior to human hepatocyte and CD34$^+$ hematopoietic stem cell engraftment.

Given that CD34 is expressed during fetal liver development and not just in hematopoietic cells of origin, the authors hypothesized that CD34$^+$ fetal liver cell transplantation may also allow for successful humanization of all NPC types. Indeed, intrahepatic transplantation of both human CD34$^+$ fetal liver cells and hepatocytes resulted in co-humanization of both hepatocytes and NPCs with varying cell proportions, unique cell-specific and zonated gene signatures, and spatial architecture resembling human livers in mice. Cell type characterization techniques including single-cell RNA sequencing and immunostaining on the chimeric livers revealed cell type-specific markers and recapitulated normal spatial and zonal organization of hepatocytes, HSCs, ECs, cholangiocytes, and immune cells (Fig. 1a).

The authors next tested whether the humanized livers responded to various forms of hepato-biliary injury and hence recapitulated human disease pathophysiology (Fig. 1b). They utilized conventional models of liver disease including the western diet, 3,5-diethoxycarboncyl-1,4-dihydrocollidine (DDC) diet, or carbon tetrachloride (CCl_4) injections to induce either hepatocyte-, cholangiocyte-, or HSC-driven injury-repair responses. In response to each of these insults, the pathology observed in the humanized mouse livers mimicked that evident in patients (and mouse models) with diseases evoking human cell type-driven responses. Native mouse livers challenged with similar types of injury also demonstrate these hallmark pathologic features albeit at different extent and with somewhat distinct genetic or proteomic signatures. However, the human hepatocytes under the influence of human NPCs in these humanized mice displayed unique qualities such as enhanced susceptibility to western diet and exhibited enriched human-specific metabolic gene signatures for lipid and cholesterol biosynthesis and metabolism, differentiating this model from conventional mouse models to study liver injury in more closely recapitulated human pathologies. Thus, this humanized mouse model may serve as an improved system for understanding human liver disease mechanistic processes, and as a platform for investigating new targeted therapies with a precision medicine-based approach.

As an example of this paradigm shift in further understanding mechanisms of human-specific liver pathophysiology, the authors

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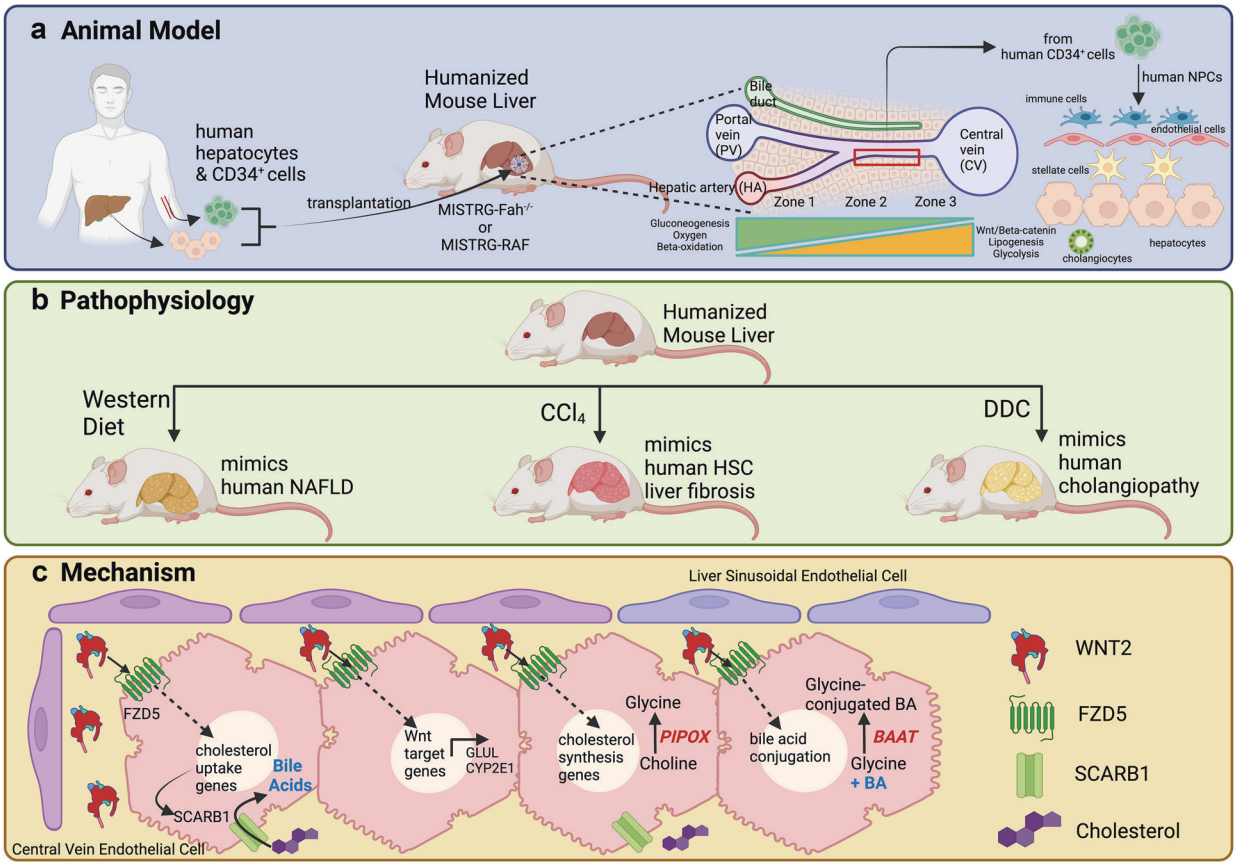

Fig. 1 Schematic illustration demonstrating the unique findings related to human hepatocyte metabolic function in humanized mouse livers. a A humanized mouse liver model is developed via engraftment of human hepatocytes and CD34^{+} fetal liver cells into mice on the MISTRG6-*Fah*$^{-/-}$ or MISTRG6-RAF background. Following mouse liver injury, repopulation of the liver leads to human hepatocytes along with human NPCs, including immune cells, cholangiocytes, HSCs, and ECs. **b** The humanized mouse liver can be used to study human liver cell type-specific responses following different forms of hepato-biliary injury (e.g., western diet, DDC diet, or CCl_4). **c** Bulk RNA sequencing on human hepatocytes repopulated with either mouse or human NPCs revealed that FZD5 is one of the few receptors upregulated in the presence of human NPCs and functions via paracrine signals delivered by LSEC-derived WNT2 to regulate human hepatocyte cholesterol transport and detoxification to bile acids.

then focused on how human NPCs could influence the metabolic fate of hepatocytes. They used bulk RNA sequencing to compare differentially expressed genes in human hepatocytes co-populated with either mouse or human NPCs to address species-specific and meaningful interactions, and focused on the frizzled receptor 5 (FZD5) for downstream analysis as this was one of the few genes selectively enriched in human but not mouse hepatocytes.[6] WNT2 and WNT10B are FZD5 ligands, both found to be expressed in human and mouse with WNT2 expressed predominantly in ECs in humans, while mostly in ECs in mouse along with other cells. We have previously described WNT2 to be secreted from LSECs in addition to central vein ECs in zone 3, along with their importance in liver zonation and regeneration.[7] Strikingly, treatment of cultured human hepatocytes with either human or mouse WNT2 resulted in different metabolic phenotypes, with human WNT2 shifting the balance toward more glycine-conjugated bile acids over taurine-conjugated bile acids. Also, human WNT2 treatment of human hepatocytes resulted in expression of cholesterol transport genes. The true strength of the paper was its ability to mechanistically demonstrate in vivo that human WNT2 secretion from LSECs influences human hepatocyte metabolism via FZD5. Specifically, using the MISTRG6-RAF model, they engrafted human hepatocytes with NPCs and functionally manipulated the expression of either WNT2 or FZD5 to demonstrate that WNT2 is secreted by human LSECs and signals via FZD5 to control hepatocyte cholesterol transport and bile acid conjugation (Fig. 1c).

Overall, these findings illustrate a unique advancement for modeling of human liver disease pathophysiology in vivo. They also validate an important and conserved role of the Wnt-Fzd-β-catenin axis in metabolic liver zonation and function, although the specific ligand and receptor interactions may vary from species to species. The role of Wnt-β-catenin signaling in cholesterol and bile acid metabolism has been shown in mice previously.[8,9]

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ADDITIONAL INFORMATION

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