Human Liver Organoids as a Model for Metabolic Dysfunction-Associated Steatotic Liver Disease

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by hepatic triglyceride accumulation without secondary causes such as alcohol consumption or viral hepatitis. Affecting an estimated 24–45% of adults globally, MASLD has emerged as the most common chronic liver condition worldwide, closely associated with obesity, insulin resistance, and metabolic syndrome. Despite its prevalence and potential to progress to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, or hepatocellular carcinoma, there remains a lack of physiologically relevant human models for mechanistic studies and therapeutic testing. To address this, we established a three-dimensional liver organoid model of MASLD using induced pluripotent stem cells (iPSC)-derived human hepatocyte progenitor cells, which were differentiated into functional hepatocytes through defined growth factor protocols. These cells self-organized into organoids that recapitulate key features of hepatic architecture and enable more accurate modeling of liver function compared to traditional two-dimensional cultures. We optimized cell seeding density for reproducibility and viability and found that 500 cells per Matrigel drop yielded organoids with robust growth and consistent differentiation. To model steatosis, organoids were treated with increasing concentrations of a 1:1 mixture of oleate and palmitic acids, a standard approach for mimicking lipotoxic conditions observed in MASLD. Lipid accumulation was assessed using Nile Red staining, which selectively labels intracellular lipid droplets, and DAPI counterstaining to quantify nuclear content. Image-based quantitative analysis revealed a dose-dependent increase in the lipid-to-cell area ratio, validating the steatotic phenotype. This organoid-based model recapitulates hallmark features of MASLD and offers a scalable and reproducible platform for studying disease mechanisms. Future directions include integrating liver organoids into a microfluidic organ-on-a-chip platform to support multicellular tri-culture with stellate cells and liver sinusoidal endothelial cells, and real-time monitoring of hepatocellular injury, fibrosis, and inflammation. This approach will enable more comprehensive modeling of MASLD progression and facilitate preclinical drug screening using human cell-based models.