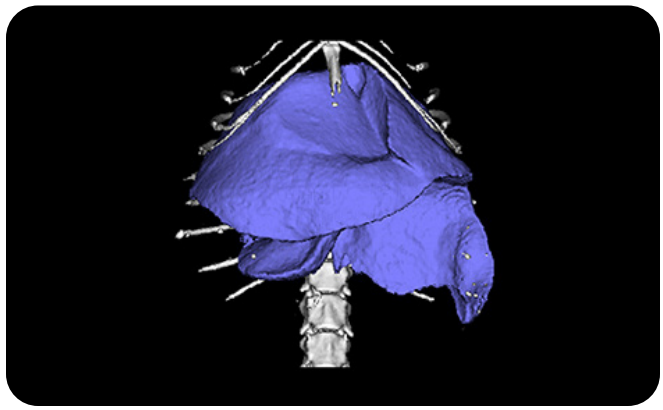
WHITE PAPER

Murine NASH model could provide insights into NASH development and progression

Nonalcoholic fatty liver disease (NAFLD) comprises a spectrum of disorders caused by a buildup of fat in the liver. Approximately one billion individuals worldwide are affected by NAFLD and various stages of disease are present in 70-80% of type II diabetic patients. These stages include simple fatty liver (steatosis), nonalcoholic steatohepatitis (NASH), fibrosis/cirrhosis, and NASH-associated hepatocellular carcinoma (NASH-HCC).

Simple fatty liver is generally reversible with diet control and exercise, whereas NASH requires medical attention, and may progress to end-stage liver disease (ESLD) from which HCC may develop. Approximately 15-25% of patients with simple fatty liver progress to NASH, and 10-20% of NASH patients may advance to fibrosis, and further to ESLD. NASH-associated ESLD has already become the major indication for liver transplantation in the US.

The underlying mechanisms for the development and progression of NAFLD are complex and multifactorial, and disease etiology and pathogenesis are not well understood. According to Professor Jian Wu, who is based at Fudan University Shanghai Medical College in China, the "multiple-hit hypothesis" is the primary theory for explaining the initiation and progression of NAFLD. However, this theory reduces the feasibility of identifying pharmacologic candidates that act on multiple targets in a synchronized way. "Moreover, chronic progression from simple fatty liver to NASH, and further to hepatic fibrosis, requires a prolonged intervention with minimal adverse effects," he said.



Characterizing a murine NASH model

Currently, there are no FDA-approved therapies for the treatment of NASH. A major barrier to the development of therapeutics is the lack of pre-clinical models of disease that are appropriately validated to represent the biology and outcomes of human disease. Various murine NASH models are available with features of fatty liver. However, Professor Wu noted that nutritional composition, feeding duration, and supplementation of fructose and/or glucose varies dramatically across studies, resulting in huge variances in the features of NASH. "It is challenging to estimate the efficacy of pharmacotherapeutic candidates in a pre-clinical setting when no standardized murine NASH model is available," he said. To address this, Professor Wu and colleagues have established a murine model of NASH, which provides a "means of standardization in end-point variables and offers a cross-board comparison of pharmacologic candidates in effectiveness and adverse effects."

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For the study,[1] NASH was induced by feeding mice a standard high-fat/calorie diet (HFCD), high fructose/glucose (HF/G) in drinking water, or a combination of both (HFCD-HF/G). After 16 weeks, the major endpoints of NASH were compared by measurable parameters in liver cell injury, hepatic fat content, insulin resistance, and fibrosis. The researchers found that HFCD-HF/G feeding resulted in a remarkable increase in body weight, subcutaneous and visceral adipose tissue, and macrosteatosis, accompanied with marked hepatocellular injury, inflammatory responses, fibrosis, and insulin resistance. HFCD-HF/G feeding also presented as typical NASH in histopathology, metabolic, and adipokine profiles in a progressive manner.

Commenting on their work, Professor Wu said: "We have established a murine NASH model which presents with significant steatohepatitis, inflammation, insulin resistance, and fibrotic progression in a scalable fashion. It has been proven to be reproducible and reliable for assessing efficacy of any intervention and for pathophysiologic exploration."[2]

In Vitro model systems

In addition to animal models, Professor Wu also utilizes *in vitro* models for liver disease research, including NASH, liver cancer, as well as regenerative medicine. "We often use primary cells isolated from normal or model animals," he said, adding that they also utilize tumor spheres or organoids for cancer research and liver buds for regenerative development as 3D platforms for various experimental designs. "These 3D organoids often include epithelial cells (hepatocytes, enterocytes or malignant cells, mesenchymal cells, and endothelial cells), and they are formed on extracellular matrix substratum. 3D models show prolonged preservation of morphology and function, and better mimic the microenvironment and metabolic pathways."

When working with 3D models, Professor Wu noted that it can be challenging to generate tissue organoids in a "size-controllable, proportion of cell component-desirable fashion". They also need to be structurally similar to real tissue. He added that tools for tracing phenotypes (morphology and function) in spatially situated cells is equally as important. "The imaging of different cell types in organoids is always challenging for a piece of living tissue. To trace special cell fate during differentiation, development, or invasion of tumor cells and to investigate the effects of microenvironmental manipulation and therapeutic intervention, we utilize the multichannel imaging options offered by the Operetta CLS®, with an adjustable focus distance."

Conclusion

Considering the multiple-hit hypothesis and the lack of understanding of NASH pathogenesis, Professor Wu believes it is important to take a multidisciplinary approach to address the challenges associated with fatty liver diseases. "Our approaches are multidisciplinary, including molecular, subcellular and cellular levels, animal models, and clinical observation," he said. "We focus on fatty acid synthesis and oxidation, lipotoxicity, energy balance and metabolism, as well as the gut-liver axis and circadian rhythm." He added that the common approaches used in his studies include omics at multiple levels, such as transcriptome (RNA-Seq), NGS sequencing, metabolomics, proteomics or lipidomics. "We use traditional approaches, such as pathophysiologic and pharmacologic assays, with cutting-edging omics at the genomic, transcription, and epigenetic levels."

Professor Wu concluded that it is difficult to predict how close research is to finding a treatment for NASH. "However, with more pharmacologic candidates, such as Resmetirom (agonist for thyroid hormone β-receptor), Semaglutide (GLP-1 analogue) or Lanifibranor (pan-PPAR agonist) in phase III trials and many others in the pipeline, it is anticipated that a novel treatment for NASH will be available in the near feature. A combination of therapeutics may be needed to address multiple hits better than a single drug candidate."

References

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