News Release

Title

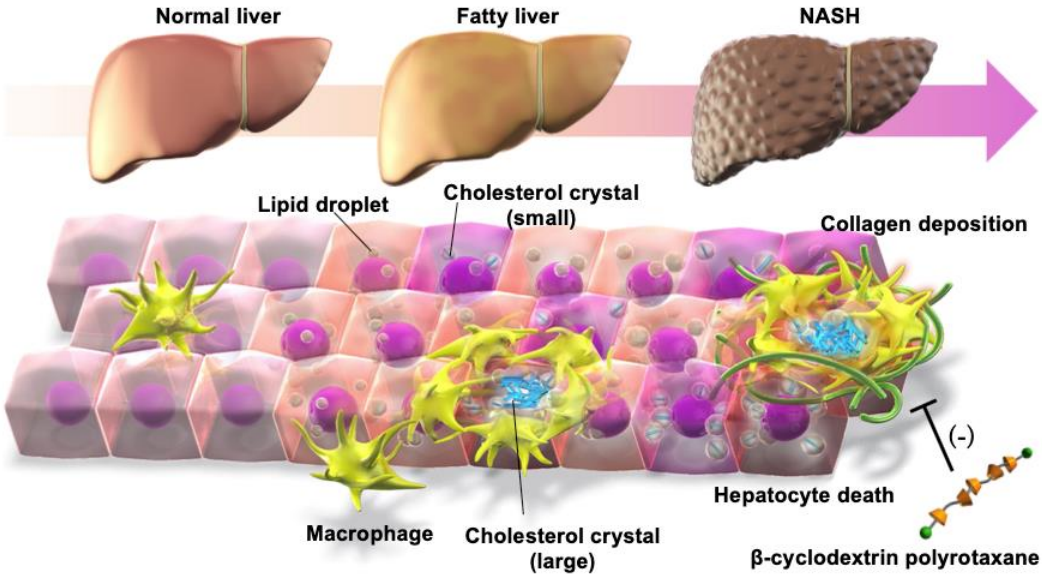
Lysosomal cholesterol overload in macrophages promotes liver fibrosis in a mouse model of NASH

Key Points

- Cholesterol crystals are formed in dead hepatocytes of NASH livers and cholesterols accumulate in macrophages that phagocytose them.
- Treatment of cholesterol crystals induces NASH-specific activation and profibrotic phenotypes in macrophages isolated from steatotic livers.
- Our unique supramolecule polyrotaxane effectively excreted cholesterol from macrophages and ameliorated liver fibrosis in NASH mouse models.

Summary

The research group has revealed a novel pathological mechanism that cholesterol accumulation in macrophages promotes liver fibrosis in the development of non-alcoholic steatohepatitis (NASH). Along with the global increase in obesity, one in four individuals develops fatty liver, and 10-30% of them progress to NASH characterized by chronic inflammation and fibrosis. Much attention has been paid to NASH because it is recognized as a leading cause of hepatocellular carcinoma, whereas there are no approved therapeutic strategies for NASH. The concept of 'lipotoxicity' has been pointed out that cytotoxic lipids, such as cholesterol, may directly cause cell death or act in a proinflammatory and profibrotic manner in the pathogenesis of NASH. This study demonstrated that cholesterol overload triggers phenotypic changes and profibrotic activation of macrophages interacting with dead hepatocytes. Furthermore, we successfully synthesized a novel supramolecular β-cyclodextrin polyrotaxane (βCD-PRX) that promotes excretion of lysosomal cholesterol within macrophages, and revealed that βCD-PRX effectively ameliorates in NASH mouse models (conceptual diagram).



Research Background

Fatty liver is now the most common chronic liver disease in the world, affecting approximately 30% of the Japanese population. Among those with fatty liver, about 10-30% progress to more severe condition known as non-alcoholic steatohepatitis (NASH), which is characterized by inflammation and fibrosis. As effective treatment for viral hepatitis has developed in recent years, NASH is predicted to become the leading cause of hepatocellular carcinoma in the near future. In contrast to simple steatosis with triglyceride accumulation, cytotoxic lipids such as cholesterol is thought to induce hepatocellular death and activate proinflammatory and profibrotic pathways in the pathogenesis of NASH. Although statins inhibit cholesterol synthesis in hepatocytes and widely used to treat hypercholesterolemia, their therapeutic effect on NASH has not been proved. Thus, it has not been fully elucidated how cholesterol accumulation in the liver promotes disease development.

Increased hepatocyte death has been considered as a prominent feature of NASH. In murine and human NASH, we have already reported unique histological structures in which dead hepatocytes are surrounded by macrophages, as a driving force of liver fibrosis in NASH. While these macrophages express CD11c on their cell surface and exhibit NASH-specific activation, the underlying mechanism by which macrophages undergo transformation remains unknown.

Research Results

1) By electron microscopy and polarized light microscopy analysis, cholesterol crystals were observed in the remnant lipids of dead hepatocytes. CD11c-positive macrophages that accumulate around dead hepatocytes showed lysosomal dysfunction defects increased cholesterol content. Similar

findings were observed in human NASH liver.
2) The cyclic oligosaccharide β-cyclodextrin (βCD) is known to foam the inclusion complex with free cholesterol. We synthesized a supramolecule polyrotaxane (PRX) by combining a number of βCDs and a linear polymer with acid-cleavable stoppers at the both ends. Under the acidic environment of the lysosomes, βCDs are released and promote cholesterol excretion.
3) Using NASH mouse models, 6-week βCD-PRX treatment attenuated liver fibrosis by reducing cholesterol content in CD11c-positive macrophages.
4) Treatment of cholesterol crystals induced lysosomal damage in cultured macrophages, which in turn upregulated profibrotic genes via activation of transcription factors, TFE3/TFEB and Egr1.

Research Summary and Future Perspective

In this study, we demonstrated that cholesterol derived from dead hepatocytes induces lysosomal dysfunction and NASH-specific activation in macrophages, thereby promoting liver fibrosis, and βCD-PRX ameliorates liver fibrosis through decreasing free cholesterol in macrophages. Recent advances in analytical techniques have led us to understand the characteristics of macrophages, which play crucial roles in the development of NASH. This study provides evidence that lysosomal cholesterol overload triggers macrophage phenotypic changes and promotes the development of NASH, which could be a novel therapeutic target for NASH.

Publication

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