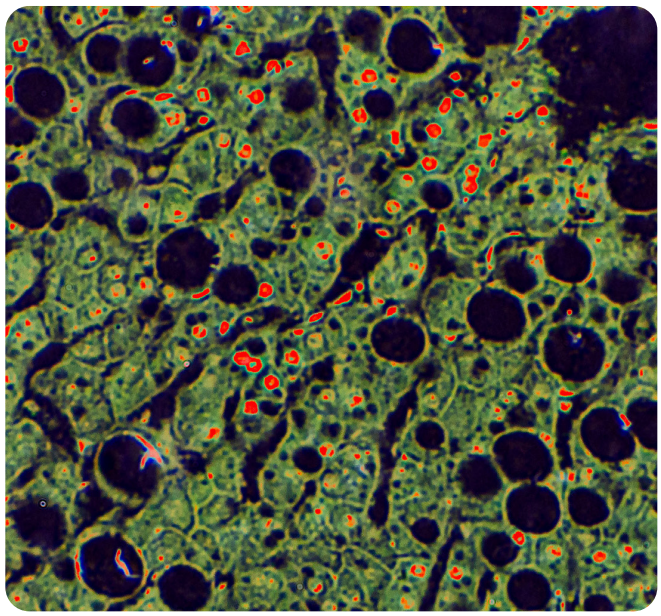
Bone morphogenetic protein 8B - A promising therapeutic avenue for NASH treatment

Introduction

The incidence and prevalence of non-alcoholic fatty liver disease (NAFLD), which is characterized by excessive accumulation of lipids within the liver, is high and progressively rising due to increasing obesity and metabolic syndrome prevalence. For some patients, NAFLD can progress to non-alcoholic steatohepatitis (NASH), which can lead to liver cirrhosis and hepatocellular carcinoma (HCC). The identification of new targetable pathways to ameliorate and/or reverse NASH is a global health priority. However, NASH pathophysiology is poorly understood and therapies to treat the disease are lacking.

Bone morphogenetic proteins (BMPs) are known to contribute to liver function and the regulation of iron and lipid metabolism, liver regeneration, and chronic liver disease progression. They are also members of the transforming growth factor beta (TGFβ)-BMP superfamily. TGFβ-BMP ligands exert counteracting effects on inflammation, proliferation, hepatic stellate cell (HSC) activation, fibrosis, and cell differentiation depending on their liver-cell-specific signaling pathways.

For the present study, Michele Vacca, from the University of Cambridge in the UK, and colleagues sought to investigate the impact of one member of the TGFβ-BMP superfamily, BMP8B, on the progression of NASH.[1] Their findings suggest that BMP8B is a major contributor to disease progression and they speculate that inhibition of BMP8B could be beneficial to prevent the shift from NAFLD to NASH. This paper highlights the key findings of their study.



Hepatic expression of BMP8B and SMAD signaling

To investigate whether BMP8B expression is upregulated in NASH, the researchers used quantitative real-time polymerase chain reaction (RTqPCR) to measure mRNA expression levels of BMP8B in two independent cohorts of NASH patients, and in mice. Their analysis revealed that BMP8B is overexpressed in human NASH and increases proportionally to disease stage. They also confirmed that BMP8B is expressed in both hepatocytes and HSCs. In contrast, BMP8B mRNA is almost undetectable in healthy livers of both humans and mice, which suggests that BMP8B signaling is a contributing factor in liver disease progression.

To study the signaling pathway of BMP8B, Vacca and colleagues used high content imaging and gene expression analysis to affirm the effect of BMP8B upregulation on TGFβ-BMP signaling *in vitro*. Previous research has found that most BMPs signal through one branch of the SMAD pathway: either via SMAD2/3, involving ALK receptor tyrosine kinase proteins ALK4, ALK5, and ALK7, or via SMAD1/5/9, involving ALK1, ALK2, ALK3, and ALK6. Interestingly, BMP8B signals via both branches of this pathway, but this information has never been confirmed in the liver.

In the present study, primary murine BMP8B-KO HSCs were treated with or without recombinant human BMP8 protein and/or an ALK1/2/3/6 or ALK4/5/7 inhibitor to study phosphorylation of SMAD2 or SMAD1/5/9. Images were acquired using the Opera Phenix® High Content Screening System (Revvity).

Imaging analysis revealed that BMP8 activated both SMAD2/3 and SMAD1/5/9 in an ALK-dependent manner and promoted the expression of SMAD targets in HSCs. Together, these findings confirm the unusual behavior of BMP8B to signal via both branches of the TGFβ-BMP pathway in HSCs, promoting their proinflammatory phenotype.

Confirming the role of *Bmp8b*

Given that HSCs are known to contribute to liver inflammation, the researchers proceeded to investigate the involvement of *Bmp8b* in HSC activation. Freshly isolated HSCs from WT and BMP8B-KO mice were cultured, and gene activation was studied using RTqPCR and immunofluorescence. The team found that the absence of *Bmp8b* was sufficient to attenuate HSC activation and their inflammatory phenotype. Furthermore, recombinant BMP8B rescued their pro-inflammatory and profibrotic phenotype. The absence of BMP8B also reduced the potential of the HSCs to modulate the behavior of inflammatory cells, which suggests that *Bmp8b* is a likely contributor to the early events mediating HSC activation, triggering proinflammatory and profibrotic responses.

3D *In Vitro* modeling

Finally, the researchers investigated whether BMP8B contributed to NASH pathophysiology and progression in human 3D *in vitro* NASH microtissues. The team used a novel *in vitro* microphysiological system (MPS) consisting of 3D perfused microtissues of primary human cells challenged with a mix of free fatty acids to mimic NASH. One week after the fat challenge, cells were treated with recombinant BMP8 with or without ALK inhibitors and TGFβ-BMPrelated gene expression changes were assessed by phosphoproteomic and transcriptomic analysis.

The team observed that BMP8 induced the phosphorylation of SMAD1 and SMAD3 and the transcription of their targets, and that these effects were prevented by ALK inhibitors. Comparative NGS analysis confirmed a comprehensive role for BMP8B in modulating TGFβ-BMP downstream effectors, pathways, and upstream regulators involved in inflammation, proliferation, HSC activation, and fibrosis.

Conclusion

Using high-content imaging and genetic analysis techniques, Vacca and colleagues have demonstrated that BMP8B is a major contributor to NASH progression in mouse and human models. Given that BMP8B is a secreted protein, not required for the normal function of the liver and selectively induced in liver disease, the team propose that preventing the induction of BMP8B and/or blocking its extracellular activity could be a safe strategy to limit the progression of NASH and, potentially, of other liver diseases. They note that more studies are needed to identify at which stage humans could benefit the most from this treatment and to develop an efficient *in vivo* inhibition that selectively targets BMP8B without interfering with the beneficial functions of the TGFβ-BMP system in liver pathophysiology.

Full paper

1. Vacca M, Leslie J, Virtue S, Lam B, Govaere O, Tiniakos D et al. Bone morphogenetic protein 8B promotes the progression of non-alcoholic steatohepatitis. Nature Metabolism. 2020;2(6):514-531.

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