YBX1-cBAF subunit interactions and their effects on hepatic lipid metabolism in a setting of diet induced obesity

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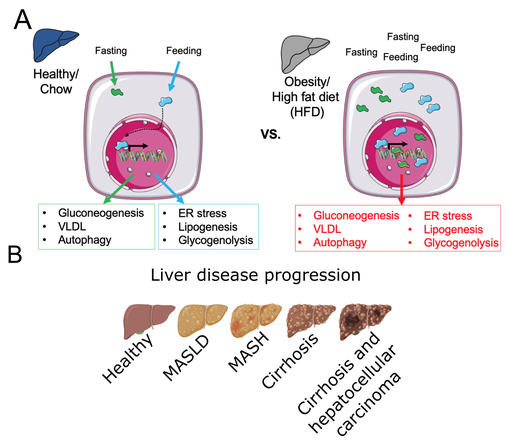
Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing global health challenge, driven by excessive hepatic lipid accumulation in the context of obesity and insulin resistance. With limited therapeutic options, identifying molecular drivers of MASLD progression is critical. Recent findings implicate Y-box binding protein 1 (Ybx1) as a maladaptive factor promoting MASLD in diet-induced obesity (Jordan et al., 2024), yet its exact role in hepatic lipid metabolism remains unclear. Here, we show that Ybx1 interacts with members of cBAF chromatin remodeling which suppress hepatic lipid accumulation. Contrary to initial expectations that Ybx1 would function in tandem with cBAF to promote adipocyte-like gene expression, our preliminary data suggests that knockdown of two cBAF subunits enhances lipid accumulation in hepatocyte-like cells, regardless of exogenous lipid exposure. These findings suggest that cBAF-mediated lipid oxidation is impaired by loss of key subunits, driving excess lipid storage. Moreover, we now suspect YBX1 binds cBAF and cBAF-target sites on adipogenic loci in a negative regulatory capacity. Ongoing studies aim to confirm and characterize this interplay between chromatin remodeling, gene expression, and metabolism and MASLD pathogenesis.

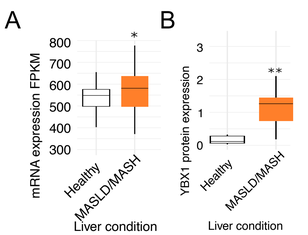
Background

1. Dysregulated lipid metabolism-related gene expression underlies pathogenesis of liver disease in a setting of diet-induced obesity (DIO)



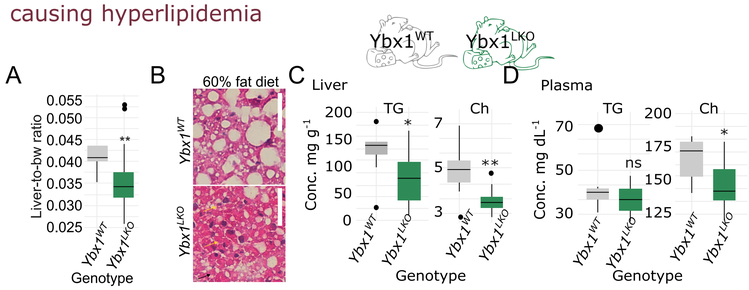
A, Dysregulation of hepatic transcription factors by chronic high-fat diet results in maladaptive lipid accumulation in hepatocytes. **B,** Excessive lipid accumulation results in immune response characteristic of metabolic-dysfunction associated steatohepatitis (MASH), which can then progress to advanced liver disease

2. YBX1 is upregulated in humans with liver disease



A, FPKM of YBX1 in patients with MASLD-MASH compared to healthy control liver (n=26 healthy, 31 MASLD-MASH; p < 0.05, Linear Mixed Effects Model). **B,** Densitometric quantification of YBX1 expression in healthy (n=6) and MASLD/MASH (n=9) livers of obese patients (36-60 BMI).

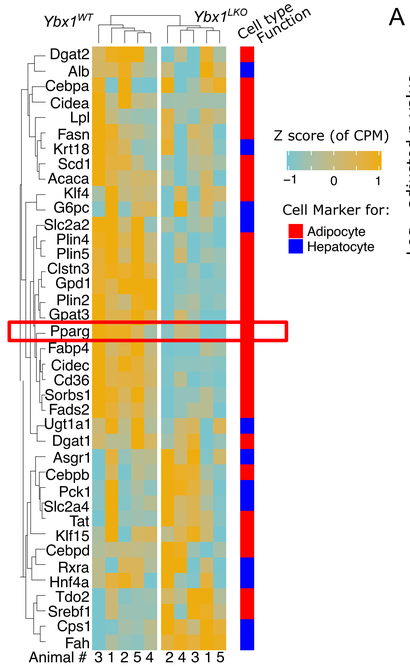
3. Ablation of hepatic Ybx1 suppresses hepatic steatosis without causing hyperlipidemia



A, Liver-to-body weight ratio in DIO mice of given genotype. **B,** Representative images of H&E-stained liver sections from DIO mice. **C,** Biochemical analysis of whole liver and **D,** plasma lipids from DIO mice. For A,C, and D, *P value <0.05, ** < 0.01, Student's t-test.

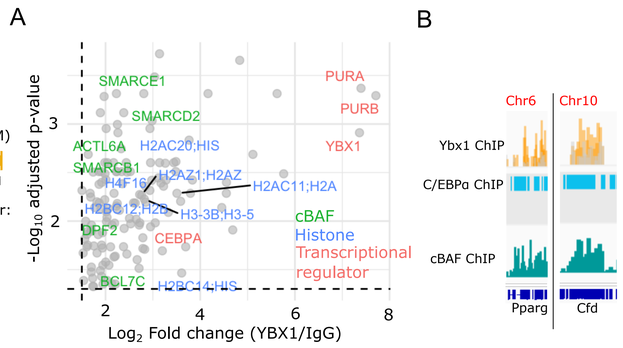
Background (continued)

5. Ybx1 promotes adipocyte-like gene expression in DIO liver



Heatmap of Z-score normalized counts per million reads of mRNA-seq from Ybx1LKO vs. Ybx1WT whole-liver lysates derived from DIO mice, of hepatocyte and adipocyte markers selected using Human Protein Atlas expression data.

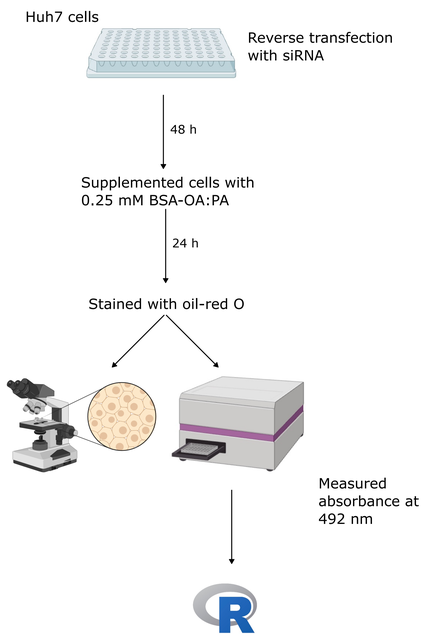
6. Nuclear YBX1 interacts with C/EBPα-cBAF and histones on adipogenic loci in DIO liver



A, Volcano plot of histones, cBAF complex members, and transcriptional regulators which coIP with YBX1 from the nuclei of DIO liver and quantified with LC-MS/MS. Fold change indicative of anti-YBX1 antibody vs. anti-IgG control antibody. **B,** IGV tracks of ChIP-seq analysis of liver from DIO Ybx1WT mice over input (yellow) and DIO Ybx1KO over input (gray); Cebpa chIP-seq profile from mouse liver (GSM427088), Brg1 (cBAF subunit) chIP-seq in differentiating mouse cells (GSM7770720) and 3t3 mouse epithelial cell ATAC-seq on day 1 of induced adipogenesis (GSM5174367) at the Pparg locus on chr 6 and Cfd locus on chr 10.

Hypothesis: cBAF is required for enhanced hepatocyte lipid accumulation in a setting of diet-induced obesity

Methods



Cell culture, siRNA, and fatty acid treatment: Huh-7 cells were cultured under standard laboratory conditions. Cells were reverse transfected with siRNA targeting candidate genes (or negative siRNA control) with LipoFectamine RNAiMAX transfection reagent. To model a high-fat diet, cell media (RPMI, 10% Fetal Bovine Serum) was supplemented with 0.25 mM oleic and palmitic acid conjugated to Bovine Serum Albumin (BSA) or BSA only control for 16 h.

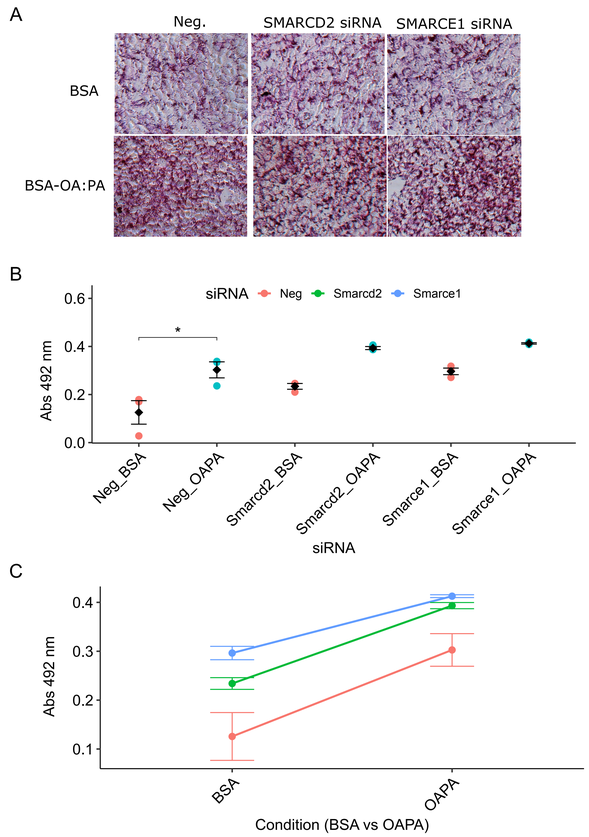
Oil-red O (ORO) staining: After treatment, cells were fixed in 4% paraformaldehyde solution for 30 m and then stained with freshly diluted and filtered ORO (dissolved in 2-propanol) for 30 m. Finally, cells were washed twice with ddH2O to remove unincorporated ORO.

Microscopy: Cells were imaged on a Revolution inverted microscope (Echo) at 10x and 20x magnification.

ORO extraction and 492 nm absorbance: ORO was extracted from cells using 2-propanol and the 492 nm absorbance was measured using a Accuris Instruments Smart 96T spectrophotometer.

Data analysis: Background corrected 492 nm absorbance readings were plotted with R using the ggplot2 package. Statistical analysis was done with R.

Results



A, Images of ORO-stained Huh7 cells. **B,** Dotplots of Abs492 nm of ORO extracted from Huh7 cells after 24 h BSA-OA:PA exposure. Each dot indicates a well. Each dot is one well of a 96-well plate. * P < 0.05, Student's t-test. **C,** Induction plot. No significant interactions were identified. Two-way analysis of variance test.

Conclusions

Preliminary results indicate siRNA against SMARCD2 and SMARCE1 did not suppress overall lipid accumulation as expected. Instead, our findings suggest knockdown of either cBAF subunit increases baseline lipid accumulation and has little to no effect on enhancing lipid accumulation when cells are subjected to exogenous fatty acid treatment.

Next steps

Wę will generate Ybx1 overexpression lentivirus to study Ybx1 x Smarcd2 interaction on hepatic lipid accumulation.

We will perform a DuoLink Proximity Ligation Assay in hepatocytes with and without BSA-OA:PA treatment to determine how SMARCD2, SMARCE1, CEBPA, and YBX1 interact in different nutrient scenarios.

References

Jordan, JM, Qiao, J, Zou, C, Stenseels, S, Haczeyni, F, Fraim, A, Mendoza, A, de Jong, YP, and Ersoy, BA. (2024). Ybx1 guides C/EBPα and cBAF chromatin-remodeling complex to promote adipogenic gene expression in steatotic hepatocytes. bioRxiv. doi: https://doi.org/10.1101/2024.10.25.620017. (Preprint)