Post-transcriptional regulation of hepatic lipid metabolism by Y-box binding protein 1

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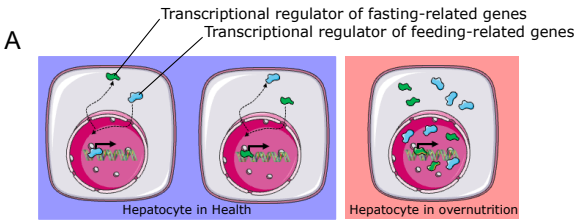
Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing global health challenge characterized by excessive hepatic lipid accumulation, frequently arising in the context of obesity and insulin resistance. Current therapeutic options remain limited, highlighting the need to elucidate the molecular drivers of disease progression. Y-box binding protein 1 (Ybx1) is a single-stranded nucleic acid binding protein that has emerged as a maladaptive factor that promotes MASLD in a setting of diet-induced obesity (Jordan et al., bioRxiv, 2024). Yet, its mechanistic role in hepatic lipid metabolism has not been fully elucidated. Here, we performed an integrated multi-omic analysis and identified ten genes directly bound by Ybx1 that displayed altered expression at both the mRNA and protein levels. We hypothesized that these genes would contribute to lipid accumulation in hepatocytes. To test this, we knocked down seven of the ten genes in hepatocyte-like Huh7 cells by reverse transfecting the cells with siRNA. Next, the cells were supplemented with oleic and palmitic acid (OAPA) for 24 h to simulate a high-fat diet. Cells were then stained with Oil Red O (ORO) and imaged for subsequent analysis in ImageJ. Finally, ORO was extracted from intracellular lipid droplets, and the absorbance of each sample at 492 nm was determined using a spectrophotometer. Our preliminary results suggest that among the seven genes tested Ybx1, siRNA against two—Vanin 1 (Vnn1), and Abhydrolase domain containing 2 (Abhd2)—mitigated OAPA-induced lipid accumulation. Conversely, when we knocked down Carboxylesterase 3 (Ces3), which appears to be post-transcriptionally negatively regulated by Ybx1, lipid accumulation increased. Overall, these findings suggest that Ybx1 can both positively and negatively regulate the stability of transcripts that lead to enhanced lipid storage in liver cells and reveal an additional layer upon which Ybx1 operates to reprogram liver cells. Future studies will confirm the phenotypic effects of Ybx1 targets by overexpressing these factors, confirming our multi-omic with ChIP-qPCR, and directly testing the effect of Ybx1 on mRNA stability. Together, these findings strengthen our understanding of early liver disease pathogenesis and point to novel therapeutic targets for the treatment of MASLD and other metabolic diseases.

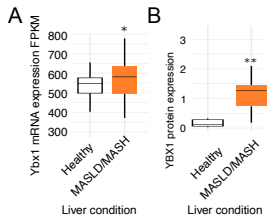
Background

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



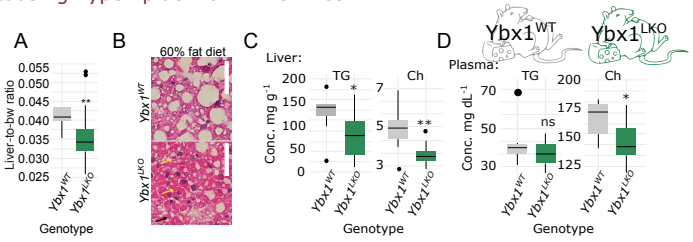
A, Dysregulation of hepatic transcription factors by chronic high-fat diet results in maladaptive lipid accumulation in hepatocytes. **B,** Excessive lipid accumulation results in immune response characteristic of metabolic-dysfunction associated steatohepatitis (MASH), which can then progress to advanced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

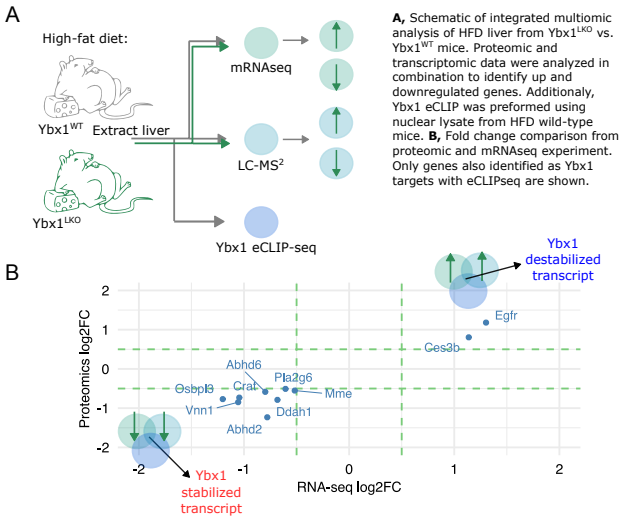
3. Ablation of hepatic Ybx1 suppresses hepatic steatosis without causing hyperlipidemia in DIO mice



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)

4. Identification of functional Ybx1 post-transcriptional regulatory targets



A, Schematic of integrated multiomic analysis of HFD liver from $Ybx1^{LKO}$ vs. $Ybx1^{WT}$ mice. Proteomic and transcriptomic data were analyzed in combination to identify up and downregulated genes. Additionaly, Ybx1 eCLIP was preformed using nuclear lysate from HFD wild-type mice. **B,** Fold change comparison from proteomic and mRNAseq experiment. Only genes also identified as Ybx1 targets with eCLIPseq are shown.

5. Genes with evidence of Ybx1 posttranscriptional regulation that have potential roles in hepatic lipid accumulation:

DDAH1: Dimethylarginine Dimethylaminohydrolase 1
Function: Metabolizes asymmetric dimethylarginine (ADMA) to regulate nitric oxide production and modulate inflammatory and metabolic signaling.

VNN1: Vanin 1
Function: Exhibits pantetheinase activity, contributing to coenzyme A metabolism and regulating oxidative stress and inflammatory responses.

CRAT: Carnitine O-Acetyltransferase
Function: Catalyzes the reversible transfer of acetyl groups to carnitine, playing a key role in fatty acid oxidation and energy metabolism.

CES3: Carboxylesterase 3
Function: Involved in the hydrolysis of ester-containing compounds, contributing to xenobiotic detoxification and lipid processing.

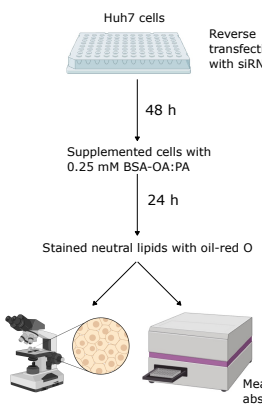
OSBPL3: Oxysterol Binding Protein-Like 3
Function: Participates in intracellular lipid transport and cholesterol homeostasis, influencing lipid signaling and distribution in hepatocytes.

ABHD2: Abhydrolase Domain Containing 2Function: Implicated in lipid hydrolysis and signaling.

ADHD6: Arylacetamide Deacetylase-like 6
Function: Potentially involved in detoxification pathways and lipid metabolism; its function is not fully characterized.

Hypothesis: Ybx1 post-transcriptional regulatory targets affect lipid accumulation in liver cells

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 ddH_2O to remove unincorporated ORO.

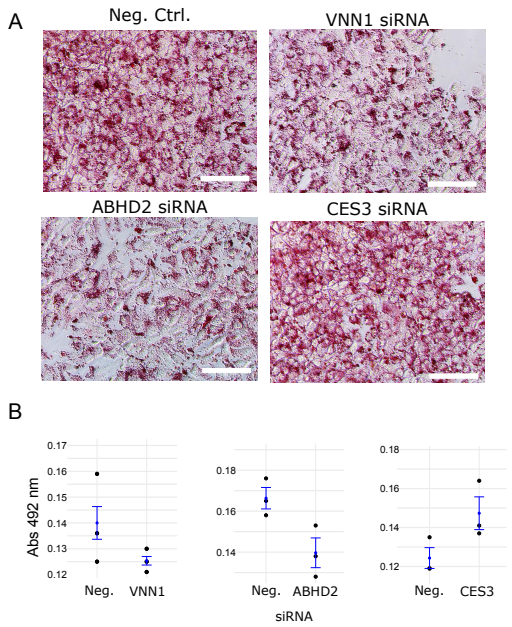
Microscopy:
Cells were imaged on a Revolution inverted microscope (Echo) at 20x magnification.

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

Three out of 7 siRNAs tested had marked effects on lipid accumulation in fatty acid-exposed Huh7 cells consistent with predicted direction based on multiomic analysis



A, Representative micrographs of oil-red O-stained Huh7 hepatocyte-like cells subjected to 16 h 0.25 mM BSA-OA:PA treatment after being reverse transfected with siRNA 48 h prior. Neg. = Negative control (no known complimentary endogenous target). **B,** Absorbance values at 492 nm wavelength from extraction of oil-red O-stained Huh7 cells. Each black dot represents the measurment from a well of a single representative trial. n=3/wells per genotype. Blue dots indicate mean; error bars indicate SEM.

Conclusions

These findings suggest a dual post-transcriptional regulatory role for Ybx1 in hepatic lipid metabolism and identify potential targets for MASLD therapy.

Next steps

1. Validate these targets using overexpression
2. Confirm Ybx1:mRNA interactions by ChIP-qPCR
3. Test Ybx1's role in mRNA stability with RT-qPCR

References and Acknowledgements

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)