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Thesis Title: **Understanding the metabolic control of gene transcription in Metabolic Dysfunction-Associated Fatty Liver Disease**

Abstract

Metabolic dysfunction-associated fatty liver disease (MAFLD) arises from sustained hepatocellular lipid overload that perturbs hepatic metabolic circuitry along with significant alteration of the hepatic transcriptional program. To dissect the molecular events that accompany hepatic metabolic and transcriptional remodelling, under conditions of steatosis, we established a palmitic acid enriched, diet-induced zebrafish model of hepatic steatosis and characterized it by integrating other *in vivo* and *in vitro* approaches. This system reliably induces hepatocellular lipid accumulation, mitochondrial and metabolic stress, and transcriptional reprogramming, providing a controlled platform for mechanistic interrogation of steatosis-associated regulatory pathways.

Transcriptomic profiling of steatotic versus control livers revealed coordinated activation of lipid metabolic genes, oxidative and ER stress pathways, and chromatin-linked regulatory modules. These findings point to a broader rewiring of transcriptional control under nutrient-excess conditions, implicating the involvement of metabolite-responsive regulatory factors as potential mediators of hepatic metabolic homeostasis.

Building on these observations, we focused on the Host Cell Factor-1 (HCF-1) and O-linked N-acetylglucosamine transferase (OGT) axis, a nutrient-sensing regulatory module known to integrate metabolic cues with transcriptional regulation. Integrative transcriptomic and proteomic analyses demonstrated that perturbation of the HCF-1:OGT axis caused hepatic lipotoxicity, while loss of HCF-1 expression specifically in hepatocytes, led to alterations in chromatin-associated protein interactions thereby disrupting transcriptional programs that govern metabolic homeostasis. These molecular changes coincide with extensive transcriptional dysregulation, activation of cellular stress pathways, differential gene expression patterns and proinflammatory features, all of which are known to be consistent with progression from simple steatosis toward more advanced MAFLD-related pathology.

To directly test the functional contribution of this axis, we performed targeted knockdown of *hcfc1a* and *hcfc1b* and inhibitor-mediated disruption of OGT's O-GlcNAcylation function in zebrafish. Loss of *hcfc1a/b* paralogs and inhibition of OGT activity, disrupted the steatosis-associated transcriptional programs, in addition to consistently attenuating metabolic gene expression responses, supporting a mechanistic role for the HCF-1:OGT axis in coupling nutrient status to transcriptional output. To evaluate evolutionary conservation, zebrafish datasets were compared with liver-specific Alb-CreERT2 mediated HCF-1 loss-of-function transcriptomes from mice. Cross-species integration revealed conserved transcriptional signatures, including shared perturbations in metabolic and signaling pathways, stress-response modules along with other disease-associated gene expression patterns.

Together, these findings establish a robust diet-induced zebrafish model for mechanistic studies of hepatic steatosis and identifies HCF-1:OGT axis as a nutrient-sensitive regulatory hub that links metabolic stress to chromatin-associated transcriptional control. This work provides molecular insight into how metabolic overload destabilizes transcriptional homeostasis in MAFLD and highlights HCF-1:OGT mediated regulation as a potential driver of disease progression.