# UNDERSTANDING THE METABOLIC CONTROL OF GENE TRANSCRIPTION IN METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE

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**KUSUMA SCHOOL OF BIOLOGICAL SCIENCES**
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# UNDERSTANDING THE METABOLIC CONTROL OF GENE TRANSCRIPTION IN METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE

**by**

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# CERTIFICATE

This is to certify that the thesis titled "**Understanding the metabolic control of gene transcription in Metabolic Dysfunction-Associated Fatty Liver Disease**" submitted by **Ms. Debashruti Bhattacharya** to the Indian Institute of Technology Delhi for the award of the degree of **"Doctor of Philosophy"** is a record of the Bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of the Indian Institute of Technology Delhi, India. The results prescribed in it have not been submitted in part or full to any other University for the award of any degree or diploma.

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Debashruti Bhattacharya

# 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-Cre 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.

# सार

मेटाबोलिक डिसफंक्शन-संबंधित फैटी लिवर डिजीज़ (MAFLD) लंबे समय तक हेटैटोसेलुलर लिपिड ओवरलोड के कारण होती है, जो यकृत के मेटाबोलिक नेटवर्क को गड़बड़ कर देती है और यकृत के ट्रांसक्रिप्शनल प्रोग्राम में महत्वपूर्ण बदलाव भी लाती है। हेटैटिक मेटाबॉलिक और ट्रांसक्रिप्शनल रिमॉडलिंग के साथ होने वाली आणविक घटनाओं को समझने के लिए, स्टेटियोसिस की स्थितियों में, हमने पामिटिक एसिड से समृद्ध, डाइट-इंड्यूस्ड ज़ेब्राफिश मॉडल तैयार किया और इसे अन्य इन वाइवो और इन विट्रो तरीकों के साथ मिलाकर कैरेक्टराइज किया। यह सिस्टम विश्वसनीय रूप से हेटैटोसेलुलर लिपिड के जमा होने, माइटोकॉन्ड्रियल और मेटाबॉलिक स्ट्रेस, और ट्रांसक्रिप्शनल रीप्रोग्रामिंग को उत्तेजित करता है, जिससे स्टेटियोसिस-संबंधित रेगुलेटरी पाथवेज़ के मैकेनिस्टिक इंटरोगेशन के लिए एक नियंत्रित प्लेटफ़ॉर्म मिलता है।

स्टीएटोटिक और कंट्रोल लिवर्स की ट्रांसक्रिप्टोमिक प्रोफाइलिंग ने लिपिड मेटाबोलिक जीन, ऑक्सीडेटिव और ER स्ट्रेस पाथवे, और क्रोमैटिन-लिंक्ड रेगुलेटरी माड्यूल्स की समन्वित एक्टिवेशन को उजागर किया। ये खोजें दिखाती हैं कि पोषण-अधिकता की स्थितियों में ट्रांसक्रिप्शनल नियंत्रण का व्यापक रीवायरिंग होता है, और मेटाबोलाइट-संवेदनशील रेगुलेटरी फैक्टर्स इसके संभावित मध्यस्थ के रूप में शामिल हो सकते हैं जो हेपेटिक मेटाबॉलिक होमियोस्टेसिस को नियंत्रित करते हैं।

इन निष्कर्षों के आधार पर, हमने होस्ट सेल फैक्टर-1 (HCF-1) और O-लिंक्ड N-एसिटाइलग्लुकोसामाइन ट्रांसफरेज़ (OGT) अक्ष पर ध्यान केंद्रित किया, जो एक पोषण-संवेदनशील नियामक मॉड्यूल के रूप में जाना जाता है और मेटाबोलिक संकेतों को ट्रांस्क्रिप्शनल रेगुलेशन के साथ जोड़ता है। इंटीग्रेटिव ट्रांसक्रिप्टोमिक और प्रोटिओमिक विश्लेषणों ने दिखाया कि HCF-1:OGT अक्ष में गड़बड़ी से जिगर में लिपोटॉक्सिसिटी होती है, जबकि विशेष रूप से हेपेटोसाइट्स में HCF-1 की अभिव्यक्ति खो जाने से क्रोमैटिन-संस्थागत प्रोटीन इंटरैक्शन में बदलाव होते हैं, जिससे मेटाबोलिक होमियोस्टेसिस को नियंत्रित करने वाले ट्रांस्क्रिप्शनल प्रोग्राम बाधित हो जाते हैं। ये आणविक बदलाव व्यापक ट्रांस्क्रिप्शनल डिसरेगुलेशन, सेलुलर स्ट्रेस पाथवे की सक्रियता, भिन्न जीन अभिव्यक्ति पैटर्न और प्रो-इन्फ्लेमेटरी विशेषताओं के साथ मेल खाते हैं, जो सभी जानते हैं कि सरल स्टीटोसिस से लेकर अधिक उन्नत MAFLD-संबंधित पैथोलॉजी तक की प्रगति के अनुरूप हैं।

इस अक्ष की कार्यात्मक भूमिका को सीधे परीक्षण करने के लिए, हमने ज़ेब्राफिश में hcfc1a और hcfc1b का लक्षित नॉकडाउन किया और OGT की O-GlcNAcylation फ़ंक्शन को अवरोधक के माध्यम से बाधित किया। hcfc1a/b पैरालॉग्स का नुकसान और OGT गतिविधि का अवरोधन, वसायुक्तता से संबंधित ट्रांसक्रिप्शनल प्रोग्राम को बाधित कर दिया, साथ ही लगातार मेटाबॉलिक जीन एक्सप्रेशन प्रतिक्रियाओं को कम कर दिया, जो HCF-1:OGT अक्ष की पोषण स्थिति को ट्रांसक्रिप्शनल आउटपुट से जोड़ने में यांत्रिक भूमिका का समर्थन करता है। विकासात्मक संरक्षण का मूल्यांकन करने के लिए, ज़ेब्राफिश डेटा सेट को माउस के लिवर-स्पेसिफिक Alb-Cre मध्यस्थ HCF-1 लोस-ऑफ-फ़ंक्शन ट्रांसक्रिप्टोम्स के साथ तुलना की गई। अंतर-प्रजातीय एकीकरण ने संरक्षित ट्रांसक्रिप्शनल संकेतक दिखाए, जिनमें मेटाबॉलिक और सिग्नलिंग पाथवेज़ में साझा गड़बड़ियां, स्ट्रेस-रिस्पांस मॉड्यूल और अन्य रोग-संबंधित जीन एक्सप्रेशन पैटर्न शामिल हैं।

साथ मिलकर, ये निष्कर्ष एक मजबूत डाइट-इंड्यूस्ड ज़ेब्राफिश मॉडल स्थापित करते हैं जो हेपेटिक स्टीटोसिस के मैकेनिस्टिक अध्ययन के लिए है और HCF-1:OGT एक्सिस को एक न्यूट्रिएंट-सेंसिटिव रेगुलेटरी हब के रूप में पहचानते हैं जो मेटाबॉलिक स्ट्रेस को क्रोमैटिन-असोसिएटेड ट्रांसक्रिप्शनल कंट्रोल से जोड़ता है। यह काम यह बताता है कि कैसे मेटाबॉलिक ओवरलोड ट्रांसक्रिप्शनल होमियोस्टेसिस को अस्थिर कर देता है और HCF-1:OGT के माध्यम से रेगुलेशन को डायज़ीज़ प्रोग्रेशन का संभावित ड्राइवर के रूप में हाइलाइट करता है।

# TABLE OF CONTENTS

1. **Chapter 1: Introduction** ..... **21**

1.1. Pathophysiology of MAFLD: A progressive disruption of hepatic homeostasis ..... 21

1.2. Transcriptional and chromatin regulation of hepatic metabolism in MAFLD ..... 22

1.2.1. Nutrient-responsive transcription factors in hepatic lipid metabolism ..... 23

1.2.2. SREBP signaling and selective insulin resistance in MAFLD ..... 23

1.2.3. ChREBP-mediated coupling of carbohydrate flux to lipogenesis ..... 24

1.2.4. Integration of transcriptional dysregulation with mitochondrial and ER stress… 24

1.2.5. Modulatory roles of PPAR signaling in hepatic metabolic homeostasis ..... 25

1.2.6. Chromatin remodeling and epigenetic control of lipogenic transcription ..... 25

1.3. Nutrient responsive regulation of hepatic metabolism and transcription in MAFLD ..... 26

1.3.1. Integration of PI3K/AKT, AMPK, and mTOR pathways in hepatic nutrient regulation ..... 27

1.3.2. O-GlcNAcylation as a nutrient-sensing regulator in hepatic metabolism and MAFLD ..... 28

1.4. HCF 1 and OGT as nutrient responsive regulators of hepatic transcription in MAFLD ..... 31

1.5. *In vivo* models are essential tools in investigating hepatic metabolic regulation in MAFLD ..... 34

1.6. Cross-species conservation of regulatory mechanisms in MAFLD ..... 36

1.7. Rationale and objectives of the study ..... 38

2. **Chapter 2: Review of literature**

2.1. Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD): an overview ..... 41

2.2. Pathogenesis of MAFLD ..... 44

2.2.1. Hepatic steatosis ..... 46

2.2.2. Mitochondrial dysfunction and oxidative stress in MAFLD ..... 52
2.2.3. Endoplasmic reticulum (ER) stress in MAFLD ..... 56
2.2.4. Inflammation in MAFLD ..... 58

2.3. Genetic and epigenetic factors contributing to the MAFLD development ..... 59
2.3.1. Genetic Factors ..... 60
2.3.2. Epigenetic Factors ..... 62
2.3.2.1. DNA methylation in MAFLD pathogenesis ..... 63
2.3.2.2. Histone modifications and hepatic lipid metabolism ..... 63
2.3.2.3. MicroRNAs in MAFLD development and progression ..... 64

2.4. Existing *in vitro* and *in vivo* models for MAFLD research ..... 66
2.4.1. Rodent models of MAFLD ..... 67
2.4.2. Zebrafish as a complementary model system ..... 68
2.4.3. *In vitro* models of MAFLD ..... 71

2.5. Impairment of liver regeneration in the background of MAFLD ..... 73
2.5.1. Acute lipotoxicity, altered hepatic lipid metabolism and prolonged activation of ER stress pathways in MAFLD impacts liver regeneration ..... 76
2.5.2. Hepatic steatosis impairs hepatocyte proliferation and increases cell death ..... 78
2.5.3. ATP depletion and altered glucose metabolism results in impaired regeneration ..... 78

2.6. Sex, gender, and therapeutic implications in MAFLD/MASH ..... 79

2.7. Role of HCF-1-OGT axis in transcriptional regulation of MAFLD ..... 80
2.7.1. HCF-1 undergoes proteolytic cleavage and maturation by unusual protease OGT ..... 82
2.7.2. Role of HCF-1 in cell cycle regulation, development and regeneration ..... 85
2.7.3. Role of HCF-1 as a central integrator of metabolic and

mitochondrial gene regulation ..... 89
2.7.4. Role of HCF-1/OGT in hepatic metabolism ..... 90
2.7.5. Loss of HCF-1 leads to hepatic steatosis and mitochondrial abnormalities in murine models ..... 92
2.7.6. Role of HCF-1 in liver regeneration ..... 94
2.7.7. Role of OGT as a nutrient sensor in regulating transcription of metabolic genes and its implications in liver diseases ..... 97
2.7.8. Role of OGT in modulation of gene transcription and epigenomic landscape ..... 100
2.7.9. Therapeutic modulation of O-GlcNAcylation in the Treatment of MAFLD ..... 102
2.7.10. Targeting HCF-1 in MAFLD/MASH ..... 104

**3. Chapter 3: Objectives of the study ..... 106**
3.1. Objective 1: To establish and characterize experimental models of fatty liver disease ..... 109
3.2. Objective 2: To define the regulatory contribution of the HCF-1:OGT axis to metabolic liver pathology ..... 110
3.3. Objective 3: To elucidate transcriptional and chromatin-associated changes accompanying disease progression ..... 111

**4. Chapter 4: Establishing and characterizing experimental models of fatty liver disease ..... 113**
4.1. Introduction ..... 113
4.1.1. Existing model systems for MAFLD ..... 113
4.1.2. Zebrafish as a model organism ..... 114
4.1.3. Clinical relevance of diet-induced models ..... 115
4.1.4. Palmitic acid supplementation strategy ..... 116
4.1.5. Integration of zebrafish and *in vitro* models ..... 117

4.2. Methodology ..... 118
4.2.1. Zebrafish husbandry and feeding regime ..... 118
4.2.2. *In vitro* steatosis model ..... 119
4.2.3. Annexin V staining ..... 120
4.2.4. ROS assay ..... 121

4.2.5. Immunoblotting ..... 121
4.2.6. Histological assessments ..... 122
4.2.7. RNA isolation ..... 124
4.2.8. Quantitative real-time PCR (qPCR) ..... 124
4.2.9. Calorie restriction paradigm ..... 124
4.2.10. Whole genome transcriptomic profiling ..... 125
4.2.11. Statistical analysis ..... 125

4.3. Results ..... 126
4.3.1. Overconsumption of PA-supplemented diet in zebrafish leads to increased body weight and hepatic lipid overload ..... 126
4.3.2. PA-supplementation leads to metabolic perturbations, dysregulation of cellular stress pathways and mitochondrial functioning ..... 129
4.3.3. Calorie restriction is effective in ameliorating steatosis induced by PA-supplementation ..... 134
4.3.4. PA-supplementation significantly perturbs zebrafish liver transcriptome ..... 135
4.3.5. Free fatty acid supplementation of HepG2 cells leads to *in vitro* model of steatosis ..... 143

4.4. Discussion ..... 144
4.4.1. Molecular and cellular pathways underlying MAFLD in the PA-supplemented zebrafish model ..... 145
4.4.2. Reversibility of steatosis in the PA-supplemented zebrafish model ..... 146
4.4.3. Transcriptomic insights into MAFLD pathogenesis in the PA-supplemented zebrafish model ..... 147

**5. Chapter 5: Defining the regulatory contribution of the HCF-1:OGT axis to metabolic liver pathology ..... 149**
5.1. Introduction ..... 149
5.1.1. Transcriptional regulators in MAFLD pathogenesis ..... 149
5.1.2. Molecular features of HCF-1 and its emerging role in MAFLD ..... 150

5.1.3. HCF-1:OGT axis in hepatic metabolism and MAFLD ..... 151

5.2. Methodology ..... 153
5.2.1. Zebrafish husbandry and morpholino injections ..... 153
5.2.2. Histological assessments ..... 154
5.2.3. Immunoblotting ..... 155
5.2.4. RNA isolation ..... 156
5.2.5. Quantitative real-time PCR (qPCR) ..... 156
5.2.6. OGT inhibition by OSMI-1 treatment ..... 156
5.2.7. Whole genome transcriptomic profiling ..... 157
5.2.8. Whole mount *in-situ* hybridization ..... 157
5.2.9. Statistical analysis ..... 159

5.3. Results ..... 160
5.3.1. Hcfc1 in zebrafish is expressed during development and shows uniform expression in the liver ..... 160
5.3.2. PA-supplementation leads to an increase in HCFC1 and OGT expression in zebrafish ..... 161
5.3.3. Loss of Hcfc1 leads to dyslipidemia, mitochondrial dysfunction and transcriptional dysregulation in zebrafish larvae ..... 163
5.3.4. Inhibition of OGT activity impacts hepatic lipid metabolism in a context dependent manner ..... 167
5.3.5. Loss of HCF-1 in HepG2 cells leads to spontaneous development of steatotic features ..... 171

5.4. Discussion ..... 171
5.4.1. *Hcfc1* gene shows constitutive expression in zebrafish development ..... 172
5.4.2. HCF-1 and OGT are elevated upon PA-supplementation in zebrafish ..... 172
5.4.3. *Hcfc1* depletion leads to dyslipidemia, mitochondrial dysfunction, and transcriptomic reprogramming ..... 172
5.4.4. OGT inhibition reveals its context-dependent role in hepatic metabolism and

MAFLD.....174

**6. Chapter 6: Elucidating transcriptional and chromatin-associated changes accompanying disease progression.....175**

6.1. Introduction.....175

6.1.1. Transcriptomic and epigenetic reprogramming in MAFLD.....176

6.1.2. HCF-1 depletion reshapes chromatin and metabolic programs in MAFLD.....178

6.2. Methodology.....180

6.2.1. Mice.....180

6.2.2. DNA isolation and genotyping.....181

6.2.3. Tamoxifen administration.....181

6.2.4. Immunohistochemistry.....182

6.2.5. TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling) assay.....183

6.2.6. Electron microscopy.....183

6.2.7. Histological assessments.....184

6.2.8. Immunoblotting.....184

6.2.9. ChIP-sequencing.....185

6.2.10. Statistical analysis.....185

6.3. Results.....187

6.3.1. Tamoxifen administration leads to hepatocyte specific loss of HCF-1 in murine liver.....187

6.3.2. HCF-1 deletion leads to extensive liver injury.....189

6.3.3. Loss of HCF-1 leads to impaired liver regeneration.....193

6.3.4. HCF-1 occupancy at transcriptional start sites is associated with active transcription.....198

6.3.5. Genomic and functional annotation of ChIP-Seq peaks signals associated with HCF-1, POL2, and H3K4me3.....200

6.3.6. Hepatocyte specific disruption of HCF-1 in mice leads to significant perturbations

in cellular metabolism and signal transduction ..... 209

6.4. Discussion ..... 215
6.4.1. HCF-1 depletion drives metabolic dysfunction and epigenetic reprogramming in MAFLD ..... 215
6.4.2. HCF-1 loss impairs hepatic regeneration ..... 217

**7. Chapter 7: Conclusions ..... 218**

7.1. MAFLD pathogenesis involves metabolic, mitochondrial and inflammatory dysregulation ..... 220
7.2. The HCF-1:OGT axis as a central coordinator of hepatic epigenetic and transcriptional programs ..... 221
7.3. Epigenetic dysregulation and regenerative failure suggest towards a crucial role of HCF-1 MAFLD progression and pathogenesis ..... 222
7.4. Mechanistic insights, therapeutic implications, and limitations of the study ..... 223

**8. Chapter 8: Future Perspectives ..... 227**

**9. Data Availability ..... 230**

**10. Ethics Statement ..... 230**

**11. References ..... 231**

**12. Appendix ..... 279**

**13. Conferences attended ..... 301**

**14. Publications ..... 301**

**15. Resume ..... 302**

## LIST OF FIGURES

Figure 1: MAFLD progression from simple fatty liver to more advanced stages… ..... 41
Figure 2: Overview of the mechanisms driving MAFLD ..... 45
Figure 3: Lipid handling by the liver ..... 46
Figure 4: Transcriptional regulation of hepatic *de novo* lipogenesis pathway by ChREBP and SREBP-1c ..... 48
Figure 5: Metabolic perturbations leading to development of fatty liver…. ..... 49
Figure 6: Triglyceride metabolism in hepatocytes ..... 52
Figure 7: Mitochondrial unfolded protein response ..... 53
Figure 8: Endoplasmic reticulum (ER) stress during MAFLD ..... 57
Figure 9: Decline of hepatic regeneration during MAFLD progression… ..... 74
Figure 10: Lipid metabolism is crucial for liver regeneration…. ..... 76
Figure 11: HCF-1 shows strong evolutionary conservation across species. ..... 83
Figure 12: OGT cleaves HCF-1… ..... 84
Figure 13: Domain structure and isoforms of O-GlcNAc transferase ..... 84
Figure 14: Overall structure of human HCF-1 with structural elements and interacting complexes…. ..... 86
Figure 15: HCF-1: OGT plays context-dependent role in hepatic metabolism…. ..... 91
Figure 16: Hepatocyte-specific loss of hcfc1 in mice causes increased steatosis ..... 92
Figure 17: RNA-Seq and ChIP-Seq reveal alterations in HCF-1-bound mitochondrial genes…. ..... 93
Figure 18: Hepatocytes deficient in HCF-1 are unable to enter the cell division cycle following partial hepatectomy ..... 95
Figure 19: Interaction between OGT and transcription factors is enhanced by HCF-1…. ..... 98
Figure 20: O-GlcNAc cycling and RNAP II-mediated transcription…. ..... 100
Figure 21: TET/OGT complex as an epigenetic regulator in DNA demethylation…. ..... 101
Figure 22: Schematic Representation of the Study ..... 109
Figure 23: Applications of zebrafish MAFLD models… ..... 114
Figure 24: Schematic of methodology used for generating *in vivo* and *in vitro* diet-induced MAFLD models… ..... 120
Figure 25: Schematic of the PA-supplementation paradigm ..... 126

Figure 26: Assessment of cell proliferation after PA-supplemented diet… ..... 127
Figure 27: Measurement of cell death in zebrafish liver (Annexin V & PI uptake)… ..... 127
Figure 28: PA-enriched diet alters liver morphology and increases steatosis..... 128
Figure 29: BODIPY staining of adult control and PA-fed liver cryosections…..... 129
Figure 30: PA-supplemented diet disrupts hepatic metabolism… .....130
Figure 31: PA-supplemented diet alters ER stress and UPR gene expression… .....131
Figure 32: PA-supplementation impairs oxidative phosphorylation…..... 132
Figure 33: PA-supplemented diet alters expression of metabolic genes…..... 133
Figure 34: Calorie restriction improves weight, lipid accumulation and metabolic gene expression…..... 135
Figure 35: Identification and annotation of DEGs..... 136
Figure 36: Heatmap showing dysregulated signaling pathways… ..... 139
Figure 37: STRING analysis of protein–protein interactions among dysregulated genes…. 142
Figure 38: OA:PA (2:1) treatment induces steatosis in HepG2 cells…..... 144
Figure 39: Hcfc1 expression during development and in adult liver… ..... 161
Figure 40: PA-supplemented diet increases Hcfc1 and Ogt expression…..... 162
Figure 41: Phenotypic assessment of hcfc1a/b loss-of-function in zebrafish… ..... 163
Figure 42: Loss of hcfc1 causes hepatic steatosis in zebrafish larvae..... 163
Figure 43: Dysregulated genes after MO-mediated knockdown of hcfc1… ..... 165
Figure 44: Loss of hcfc1 alters expression of lipid metabolism, apoptosis, proliferation and mitochondrial genes… ..... 166
Figure 45: OGT inhibition in zebrafish larvae..... 169
Figure 46: OGT inhibition in PA-fed larvae ameliorates hepatic steatosis…..... 170
Figure 47: Loss of HCF-1 in HepG2 cells induces spontaneous steatosis… .....171
Figure 48: Tamoxifen induction causes rapid loss of hepatocyte-specific HCF-1… ..... 188
Figure 49: Loss of Hcfc1 increases liver injury marker AST… ..... 189
Figure 50: Hcfc1 knockout leads to severe liver injury… ..... 190
Figure 51: Loss of Hcfc1 accelerates progression to MASH-like pathology…..... 192
Figure 52: Heterozygous Hcfc1 expression eventually resolves liver pathology… ..... 193
Figure 53: HCF-1-depleted hepatocytes fail to proliferate after partial hepatectomy…..... 195
Figure 54: Hcfc1 depletion impairs regenerative response after partial hepatectomy… ..... 197

Figure 55: HCF-1-deficient hepatocytes show reduced H3K4me3 signal…. 199
Figure 56: Circos plot of HCF-1, POL2 and H3K4me3 peak distributions 200
Figure 57: Top transcription factor motifs enriched in HCF-1 peak regions(HOMER)…. 201
Figure 58: Heatmap showing decreasing HCF-1 peak signals. 203
Figure 59: Heatmap showing increasing H3K4me3 peak signals…. 205
Figure 60: Heatmap showing increasing POL2 peak signals…. 207
Figure 61: Pathway enrichment of genes common to POL2 and H3K4me3 peaks…. 209
Figure 62: GO enrichment analysis of upregulated and downregulated genes…. 211
Figure 63: Loss of Hcfc1 affects mitochondrial structure 212
Figure 64: Loss of Hcfc1 reduces OXPHOS complex expression…. 213
Figure 65: Loss of Hcfc1 impairs electron transport chain complex levels…. 214
Figure 66: Schematic summarizing key findings of this study… 226

## LIST OF TABLES

Table 1: Comparative staging of liver fibrosis across Ishak, METAVIR, and Batts Ludwig systems…..43
Table 2: Rodent Models for MAFLD research.....68
Table 3: Genetic models of zebrafish for MAFLD research. ..... 70
Table 4: Transgenic models of zebrafish for MAFLD research ..... 70
Table 5: Dietary- or chemical-treated zebrafish models of liver disease.....71
Table 6: HCF-1 interacting proteins. ..... 87
Table 7: Comparative Features of Experimental Models for MAFLD Research .....113
Table 8: Comparative advantages and limitations of experimental models for liver disease research ..... 115
Table 9: Comparative features of palmitic acid (PA) and oleic acid (OA) in metabolic dysfunction.....116
Table 10: Major Transcriptional Regulators Implicated in MAFLD and Their Functional Domains ..... 149
Table 11: Structural Domains of HCF-1 and Their Regulatory Functions relevant for MAFLD .....150
Table 12: Primers for in-situ hybridization.....158
Table 13: Known HCF-1 interacting partners relevant to MAFLD… .....178
Table 14: Primers for genotyping ..... 181

# ABBREVIATIONS

**Asxl1:** Additional sex comb like transcriptional regulator1

**CC:** Cellular Component

**ChIP-Seq:** Chromatin Immunoprecipitation sequencing

**CpG:** Cytosine-phosphate-Guanine

**CR:** Calorie restriction

**DAPI:** 4',6-diamidino-2-phenylindole

**DAVID:** The Database for Annotation, Visualization and Integrated Discovery

**DE:** Dysregulated gene

**ECM:** Extracellular matrix

**ER:** Endoplasmic reticulum

**Dpf:** Days post fertilization

**ERK:** Extracellular signal regulated kinases

**GO:** Gene ontology

**GSEA:** Gene set enrichment analysis

**H3K4me3:** Histone H3 Lysine 4 trimethylation

**HE:** Hematoxylin Eosin

**HBM:** HCF-1 binding motif

**HCF-1/HCFC1:** Host Cell Factor 1

**HET:** Heterozygous

**HOMER:** Hypergeometric Optimization of Motif EnRichment

**Hpf:** Hours post fertilization

**HSV:** Herpes Simplex Virus

**JNK:** c-Jun N terminal Kinase

**KD:** Knock down

**kDa:** Kilodalton

**KO:** Knockout

**MAFLD:** Metabolic dysfunction associated- fatty liver disease

**MAPK:** Mitogen activated protein kinase

**MS222:** Tricaine methanesulfonate

**MASH:** Metabolic dysfunction associated- steatohepatitis

**MF:** Molecular function

**OGT:** O-linked β-N-acetylglucosamine transferase

**PA:** Palmitic acid

**PCNA:** Proliferating cell nuclear antigen

**PH:** 2/3$^{rd}$ Partial hepatectomy

**POL2:** RNA polymerase II

**SMA:** Smooth muscle α-actin

**TSS:** Transcriptional start sites

**PTM:** Post-Translation modification

**THAP11:** Thanatos-associated protein domain containing 11

**TPR:** Tetratricopeptide repeat

**UPR:** Unfolded protein response

**VLDLs:** Very low-density lipoproteins

**Wnt:** Wingless related integration site

**YAP:** Yes associated protein