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Liver circadian genes are modulated by high fat feeding in mice: Investigation of microRNA-mediated mechanisms

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major public health issue, with a recent estimated global prevalence of 30%[(1)]. The pathogenesis of MASLD is complex, multifactorial, and incompletely understood[(2)]. MicroRNAs (miRNAs) are small non-coding RNAs that typically inhibit gene expression as post-transcriptional regulators[(3)]. MiRNAs are increasingly recognised for their potential as diagnostic biomarkers and therapeutic targets in liver diseases[(3)]. The aim of this research was to consider the role of miRNAs in MASLD pathogenesis and their regulation by diet.

Liver RNA samples were extracted from male C57BL/6J mice (n = 6/group) fed for 8 weeks either a chow diet (CD) or 60% fat (high fat diet, HFD) and sent for RNA sequencing. The raw data obtained from next-generation sequencing (Novogene, Cambridge) were passed through a quality control pipeline. Differential expression analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and target predictions were performed in the R environment. Enriched GO terms were summarised and clustered by online tool Revigo. Rich factor (RF) was used to describe the intensity of dysregulated pathways, calculated as the ratio of differentially expressed genes (DEGs) to the total genes included in each pathway. MicroRNAs that targeted the DEGs were identified using two validated miRNA-mRNA interaction databases, Tarbase and miRTarbase.

A total of 1087 DEGs were found in HFD fed mice relative to CD (adjusted $P<0.05$) and their biological functions were examined using GO and KEGG enrichment analyses. In aggregate, significantly enriched components and functions included: small molecule metabolic processes, collagen-containing extracellular matrix and lipoprotein particles, and oxidoreductase activity. Circadian rhythm was the most enriched pathway (RF = 0.29) among 12 enriched KEGG pathways, which also included fatty acid degradation (RF = 0.21), PPAR signalling (RF = 0.16), retinol (RF = 0.17) and cholesterol metabolism (RF = 0.22) pathways. Indeed, out of the 34 genes that comprise the KEGG circadian rhythm pathway, 10 (29%) were found dysregulated by HFD, including the critical. Period genes (*Per2*, *Per3*). Further exploration in human found hsa-miR-133a-3p targets *PER2* and *PER3* via examination of validated miRNA-mRNA interaction databases. In addition to circadian rhythm, hsa-miR-133a-3p was found to target genes that function in macroautophagy, cold-induced thermogenesis, and insulin response pathways.

Peripheral clocks of circadian rhythm (liver, pancreas, adipose tissue and muscle) are regulated by miRNAs that contribute to the pathogenesis of MASLD through disruption of lipid metabolism and the insulin response. Future work includes the confirmation of miR-133a-3p expression and the related pathways in mouse liver and human primary hepatocytes.

References

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