Abstract

Nocturnin (Noct) is a circadian deadenylase that has been implicated in hepatic lipid metabolism but its putative role in hepatic disorders such as non-alcoholic steatohepatitis and its regulation lacks clarity. This study investigates the circadian rhythmicity of hepatic Noct in high-fat-high-fructose diet (H) and/or photoperiodic manipulations induced chronodisruption (CD) amounting to NASH. Further, the role of melatonin and epigenetic factors of hepatic Noct in NASH has also been investigated. Preliminary experiments had revealed that *Noct* mRNA undergoes moderate to significant increment in liver samples of H, CD or HCD treated C57BL/6J mice or oleic acid (OA) treated HepG2 cells. Herein, the peaks of *Noct* mRNA were observed at ZT12 in control (C) and H groups whereas; CD and HCD groups recorded peaks at ZT6. OA-treated HepG2 cells recorded significant increment in Noct (mRNA and protein) with NOCT protein recording a shift in Centre of Gravity (CoG) as evidenced by CircWave analysis. These findings provided evidence that an increment in Noct has a putative role in NASH pathophysiology. Since therapeutic role of melatonin in NASH is known, exogenous melatonin was administered in H (HM), CD (CDM) or HCD (HCDM) groups wherein; melatonin treatment accounted for moderate to significant corrections in hepatic *Noct* mRNA evidenced by decrements in the relative amplitude and oscillations. HepG2 cells subjected to a combination of OA and melatonin accounted for significant decrement in *NOCT* mRNA that was evident by corrections in CoG and relative amplitude. Further, melatonin-treated HepG2 cells accounted for subtle alterations in NOCT protein that was evident by significant increments in NOCT and shifts in CoG to 32h. However, a significant decrement in the relative amplitude of NOCT was recorded in melatonin-treated HepG2 cells suggesting that, melatonin makes corrective changes in Noct rhythms but fails to lower its cellular levels. Further, preliminary *in silico* studies identified microRNA-122 (miR-122) as a putative epigenetic regulator of hepatic Noct in NASH pathology. Intrahepatic miR-122 was found to be significantly elevated at ZT 12 in liver of H, CD and HCD treated mice as well as OA treated

HepG2 cells (at 24 h). Intrahepatic *Noct* recorded a decrement at ZT12 and at 24h time points in the respective experimental groups thus implying towards its negative correlation with miR-122. These findings also corroborate the results of *in silico* studies. Treatment of HepG2 cells with miR-122-specific inhibitor accounted for a significant increment in *NOCT* expression, shift in CoG (36h to 40 h) and significant increment in the relative amplitude.

This thesis comprises of three chapters and collectively, findings of the study showcased herein highlight the relevance of altered hepatic Noct expression and its rhythmicity in experimental models of NASH and reveal the implications of an altered circadian clock in modulating hepatic Noct. Further, we report that exogenous melatonin interacts with hepatic Noct but only partially restores Noct expression and its oscillations. An altered miRNA expression profile is a hallmark of NASH pathology. Herein, we provide prima facie evidence of the epigenetic regulation of Noct in the said pathology and report that miR-122 modulates hepatic Noct rhythmicity without having any role in the regulation of Noct in NASH. Thus, our study provides novel and fascinating insights into previously unknown mechanisms operational in circadian desynchrony-induced NASH pathology and report that Noct rhythmicity in liver is shaped, in part, by melatonin and miR-122.