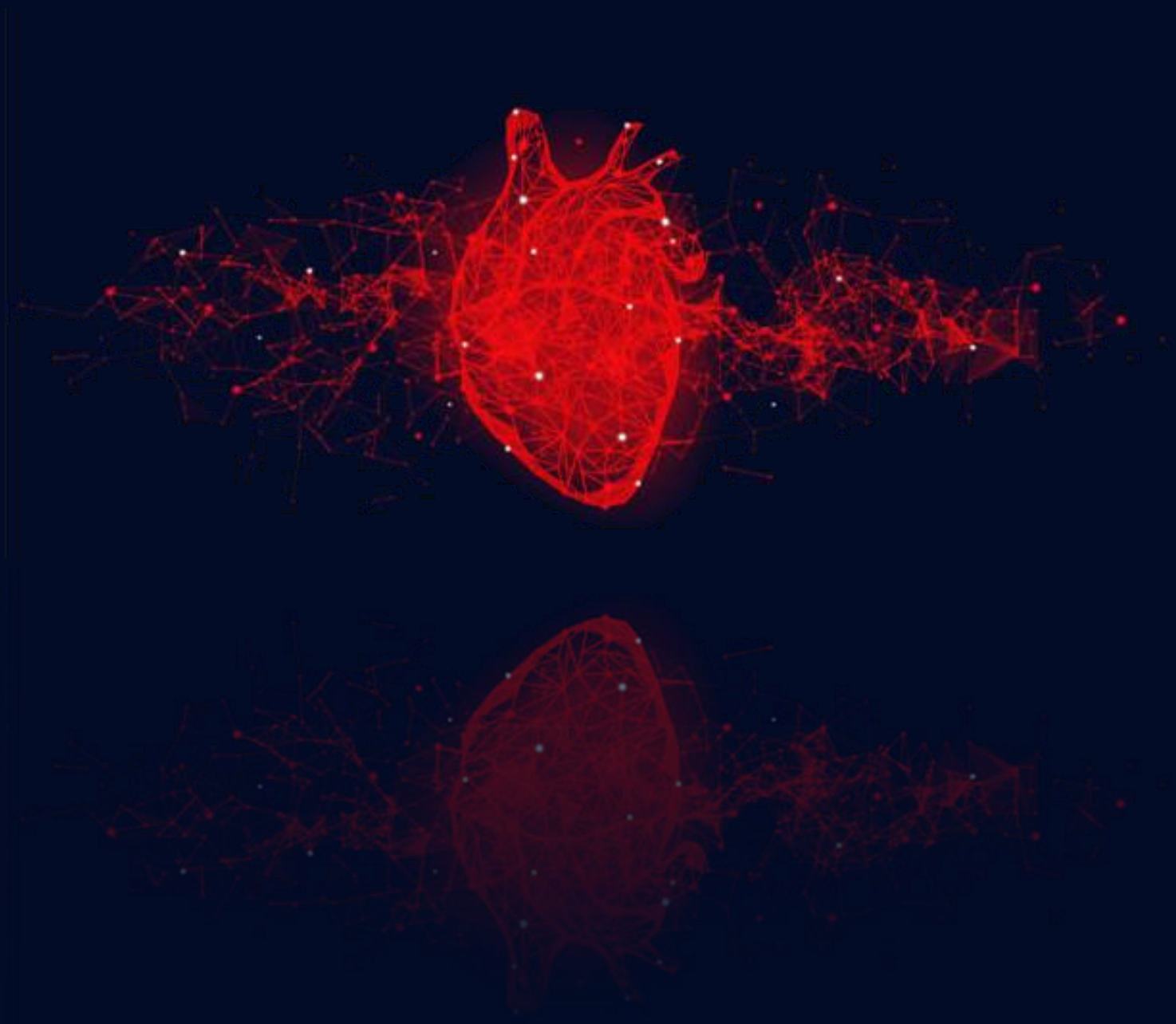
Hypothesis and Objectives



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Hypothesis

Alterations in biological clocks coupled with high calorie intake forms the basis of majority of the lifestyle disorders including atherosclerosis. Circadian basis of atherogenic changes have been variedly reported by several research groups. However, a clear cause-consequence link between the two still lacks clarity. Herein, it is hypothesized that chronodisruptive miRNAs plausibly play a bigger role in initiation and progression of atherosclerosis. The miRNAs regulated by set of Clock genes and their altered function conceivably regulate the synergy of metabolic genes that consequentially drive manifestation and/or progression of atherogenic changes. Further an attempt is made to understand the role of circadian clock associated endogenous gasotransmitter, Carbon Monoxide (CO), in altering miRNA expressions and subsequent pathological implications.

Aim of the study

Aim of the study is to assess a mechanistic link between chronodisruption induced atherosclerosis pertaining to systemic regulation of Clock associated miRNAs.

Objectives:

1. **Deciphering Clock gene associated miRNAs**

In-silico algorithms were employed to deduce Clock associated miRNAs. These shortlisted miRNAs were further subjected to analyze their role in CVDs. miR34a-5p was construed from the data analysis and the same was validated in cellular (serum synchronized HUVEC) and chronodisruptive rodent model system (male C57BL/6J mice). Confirming on to miR34a-5p – CLOCK association, the miRNA was further looked upon for it atherogenic target genes that are studied in detail in further chapters.

2. **Elucidating mechanism of miR34a-5p expression in experimental atherogenic models and its modulation via CORM-A1.**

miR34a-5p expression was assessed in experimental atherogenic cellular (HUVEC & MDMs) and rodent models (male Sprague Dawley rats). The elevated expression was further investigated for mechanistic details in the pathological milieu. Furthermore, CORM-A1 meditated alterations in miR34a-5p expression were also assessed that formed the bases for detailed investigation of miR34a-5p functions in atherogenic systems.

3. **To assess implication of miR34a-5p – KLF4 axis pertaining to inflammation in atherogenic milieu.**

In-silico target prediction software showed presence of Watson-Crick base pair complementary seed sequence for miR34a-5p, in 3'UTR of KLF4 gene. The same was validated in cellular system (HUVEC & MDMs). Further an attempt to assess role of miR34a-5p in KLF4 – NF-κB mediated inflammatory response in atherogenic conditions was studied in cell specific manner. CORM-A1 mediated downregulation of miR34a-5p and overall impact on atherogenicity was also studied herein.

4. **To assess implication of miR34a-5p – SIRT1 axis pertaining to mitochondrial biogenesis and function in atherogenic milieu.**

Improving mitochondrial health is a current hotspot as therapeutic target in atherosclerosis. Mitochondrial damage dictates cellular health in a potent manner. miR34a-5p is well documented to regulate SIRT1 expression by inhibiting its 3'UTR activity. Herein, the same was validated in our model systems. Further this section studies CORM-A1 mediated improvement in mitochondrial function and biogenesis in miR34a-5p dependent and independent manner.