The interplay of the ticking clock and miRNA in differentiation of endothelial precursors

Our day-today activities like sleep-wake cycle, hormone release, body temperature and other important bodily functions follow a characteristic rhythm over 24 hours called a circadian rhythm. A ‘master clock’ in brain termed as suprachiasmatic nucleus (SCN) co-ordinates the circadian rhythms centrally. What is known now is that every cell has its own clock maintained by a complex set of self-regulatory genes, termed clock genes. These clock genes relay signals from SCN and control almost 10 % of genes of our body. Abnormal circadian rhythms are associated with insomnia, obesity, diabetes, depression, cancer, alcohol dependence and seasonal affective disorders. Previously, we showed in diabetes that stem/progenitor cells which help in building blood vessels (i.e. endothelial cell) possess defects in their clock gene expression. We also observed that these defective stem cells are unable to repair damaged vessels resulting in complications of eye called diabetic retinopathy (DR).

Our recent study published in *Diabetes* highlights that stem cells (i.e. $CD34^+$cells) when they differentiate toward an endothelial cell gain oscillations of their clock, thus bringing together the field of stem cell research and the molecular clock. In this study, we identified that one particular clock gene, *Per2* is critical for directing the fate of stem cells towards an endothelial cell. Next, we extended our studies to diabetic individuals which possess defects in their stem/progenitor cells. We show that their $CD34^+$ cells cannot differentiate into endothelial cells. We selected two additional populations of stem cells called as early endothelial precursors and endothelial colony forming cells and performed a novel technique to screen thousands of tiny RNAs (i.e. miRNA) to identify key miRNAs that are changing in diabetic individuals and are also known to regulate clock function. We identified miRNAs that were only seen in stem cells of individuals with diabetes; moreover, there was a significant difference in levels of miRNA between non-diabetic and diabetic individuals. We identified one unique miRNA called as miR-92a that was expressed at very low levels in diabetic stem cells.

It is well-known that duration is the strongest predictor of DR, however there are some diabetic individuals which in spite of long-term diabetes do not develop DR. Strikingly, an estimation of miR-92a levels in diabetic individuals protected from DR showed that these diabetic individuals maintain miR-92a levels similar to healthy volunteers. In contrast, miR-92a levels were lower in individuals that developed DR. We observed that lower levels of miR-92a were coupled to increase of toll like receptors (TLR) in $CD34^+$ cells of diabetics. TLR receptors are known to be involved in inflammation. We reasoned that restoration of normal miR-92a levels in $CD34^+$ cells of diabetic individuals would correct defects in $CD34^+$ cells. We did observe that restoration of miR-92a levels to normal corrected inflammation in diabetic $CD34^+$ cells.

Overall, our studies suggest that clock regulatory miRNAs play an important role in directing fate of stem cells towards differentiation. miRNA mediated repair of stem cells may represent a novel

therapeutic strategy that may be utilized for correction of defects in diabetic stem cells and miR-92a in particular may serve this important role.

Publication

miR-92a Corrects CD34+ Cell Dysfunction in Diabetes by Modulating Core Circadian Genes Involved in Progenitor Differentiation.
Bhatwadekar AD, Yan Y, Stepps V, Hazra S, Korah M, Bartelmez S, Chaqour B, Grant MB
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