Circadian clocks in diabetic retinal endothelial cells

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ABSTRACT

Background: Diabetes has been reported to alter normal circadian rhythms and circadian disruption emerges as an important factor in the disease prognosis and treatment success. Our objective was to investigate whether diabetes affects circadian gene expression in endothelial cells and the mechanisms involved.

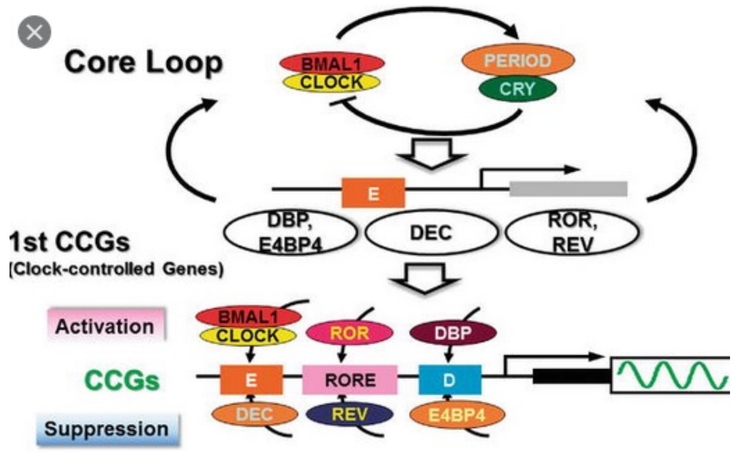
Methods: Induced Pluripotent Stem Cell-Derived Endothelial Cells (iPS-ECs) from healthy and diabetic patients were sequenced and differential analysis was performed. Genes related to circadian rhythms were identified. Primary human retinal endothelial cells (HRECs) were cultured in vivo in hyperglycaemic and hypoxic conditions to validate the results. Cells were synchronised with 50% serum shock and repeated samples collected every 2 hours over a 36 hour period. Circadian gene expression was measured using RT-PCR.

Results: ip-ECs from diabetic patients had a 5.7 fold reduction in *Dec2* mRNA expression and a 4.0 fold increase in *Bmal-2.* Four weeks of hyperglycaemic conditions resulted in a slight increase of *Bmal-1* and a reduction in *Dec2* mRNA. Hypoxia had a significant effect in reducing the expression of the majority of circadian genes and in synchronised HRECs under hypoxic conditions gained a more robust circadian oscillation but lower amplitude of *Bmal-1* indicating an effect of hypoxia on circadian rhythmicity.

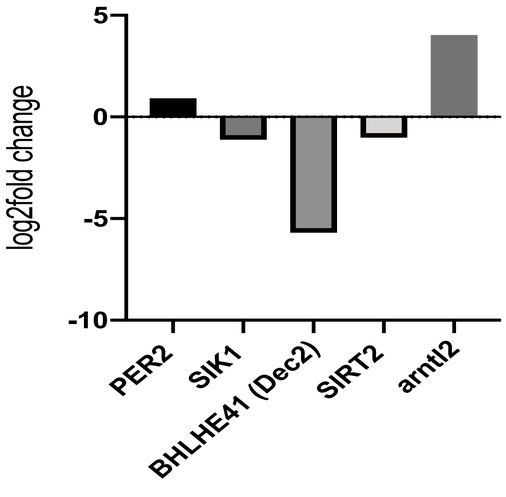
Conclusions: Diabetic conditions resulted in a specific reduction of *Dec2* expression in both patient derived iPS-ECs and HRECS in hyperglycaemic conditions. Hypoxia alone had a more pronounced effect on circadian gene expression and rhythmicity compared to hyperglycaemia alone.

Results

The molecular organization of the circadian clock



Circadian genes that are significantly different between human derived ip-ECs from healthy and diabetic patients



Per2 was slightly upregulated, showing increased transcriptional activity of Arntl-1 on E boxes.

Arntl-2, is a paralogue of Arntl-1 which also forms heterodimers with Clock and Npas2 to regulate E boxes on promoters of circadian controlled genes.

Bhlhe41 (Dec2) was downregulated. It binds to E boxes and inhibits Arntl-1 binding.

Figure 1. mRNA sequencing was performed in human derived ip-ECs from diabetic and control patients. Fold change indicates the differential expression in diabetes over control. Here we looked on circadian core genes and only shown the identified significantly different genes related to circadian rhythms. No difference was found for the major core genes with the exception of Per2.

High glucose can alter the expression of circadian genes in primary human retinal endothelial cells (HRECs)

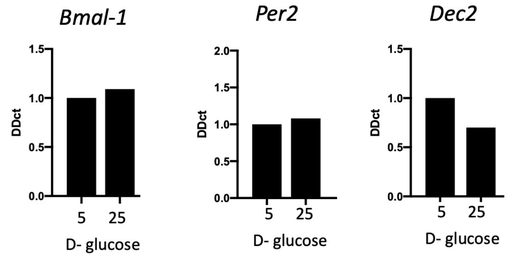


Figure 2. High glucose retinal primary endothelial cells had similar results to retinal primary endothelial cells as in the ip-ECs from diabetic patients (n=1, currently we analyze samples)

Hypoxia in combination with high glucose also alters the expression of circadian genes in HRECS

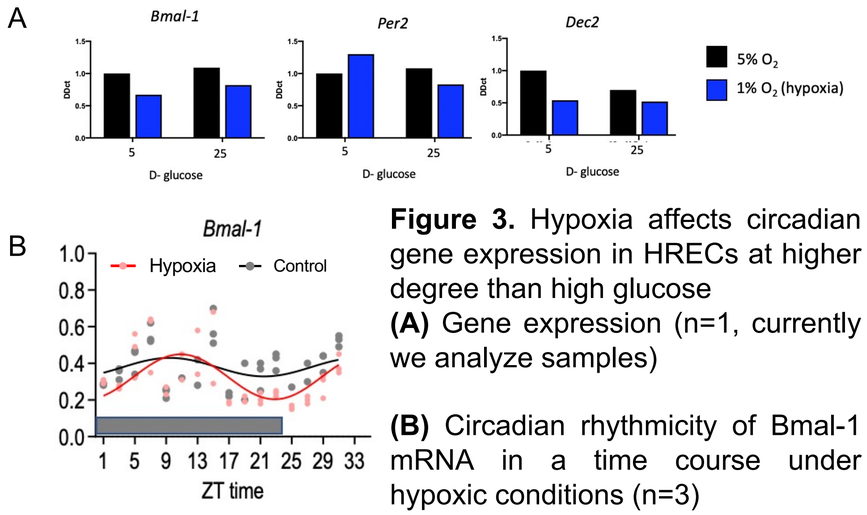


Figure 3. Hypoxia affects circadian gene expression in HRECs at higher degree than high glucose

(A) Gene expression (n=1, currently we analyze samples)

(B) Circadian rhythmicity of Bmal-1 mRNA in a time course under hypoxic conditions (n=3)

Conclusions

- Diabetic ip- ECs have reduced Dec2 and increased Bmal-2
- High glucose specifically downregulated Dec2 expression and increased Bmal-1
- Hypoxia also affected overall circadian gene expression and rhythmicity, with reduced Bmal-1, Per1, Cry1 and Dec2 but made the rhythms more robust