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Differential expression and epigenetic regulation of β1, β2 and β3-Adrenergic receptors in retinal endothelial cells

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Objective: Epigenetic mechanisms are increasingly accepted factors in the pathophysiology of diabetes and its associated complications. This study aims to examine the correlation between methylation and expression of β1, β2 and β3 ARs and to analyze the differential variability of promoter methylation in hyperglycemic retinal endothelial cells

Methods: Human retinal endothelial cells were cultured in CSC complete medium under high and low glucose conditions. DNA, RNA and protein were extracted, using respective commercial kits. RT-PCR and Western Blotting were performed to examine the expression of beta adrenergic receptors. CpG island and promoter sequences were identified and retrieved from NCBI, UCSC and EPD databases. DNA was modified through bisulfite conversion, cloned, transformed into DH5α and sequenced.

Results: β1, β2 and β3ARs are expressed in retinal endothelial cells more like in a differential manner. According to reverse transcription and Western analysis results, β1 and β3 ARs have higher exprssion when RECs are grown in the presence of 25mM glucose, while mRNA of β2 AR is hardly detectable in both high and low glucose conditions. Promoter of β1and β3 ARs are hypomethylated while methylation in β2 AR is relatively high and significantly associated with the level of expression. Upstream and downstream promoter methylation levels of β1 and β2 ARs are not significantly different

Conclusion: Our results provide new insights into the promoter methylation of β-adrenergic receptors, its associations with expression levels and effect of hyperglycemia in retinal endothelial cells. It supports the established hypothesis that methylation could mediate the expression levels of genes

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