PERSISTENT CHANGES IN THE GENE EXPRESSION PROFILE OF RETINAL ENDOTHELIAL CELLS

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Abstract: Diabetic retinopathy is a microvascular disease and a leading cause of blindness in adults. Leading causes of diabetic retinopathy are not well understood. Recent advances in diabetic retinopathy research demonstrate that metabolic memory leading to persistent changes in the retinal gene expression profile is involved in the pathogenesis of the disease. In this study we examined the effect of diabetes and age on gene expression profile of the central tissue affected by diabetic retinopathy, human Retinal Endothelial cells (HREC). This study is to identify changes in gene expression and potential ways to reverse metabolic memory as a way to control diabetic cells from undergoing retinopathy. Cells from human diabetic and control donors were cultured and harvested in endothelial cell growth media in BD falcon flasks then cultured in 10cm plates coated with gelatin. Cells were then harvest for mRNA extraction using trizol as the main reagent. With the final concentration of mRNA obtained from the optic density measurement, copy Dioxyriboneucliacid (cDNA) was extracted. Special reagents used in cDNA extraction are primer (1μl of 50μM Oligo (dT)20), annealing buffer, 2x First-strand Rxn mix (10μl) and Superscript™III/RNaseout™ Enzyme mix (2μl). Acid sphingomelinase (ASM), intercellular adhesion molecule (ICAM1) and Receptor tyrosine kinases (Tie2) mRNA levels were analyzed by real-time PCR using SYBR Green. Comparison of HRECs isolated from control and diabetic donors from the same age group below 30 demonstrated a persistent increase in ASM, ICAM-1 and Tie2 mRNA expression level even after 4 passages in normal endothelial cell growth media. Moreover, the effect of donor’s age on the ASM, ICAM-1 and Tie2 expression was similar to the effect of diabetes. Persistent changes in gene expression profile of HREC indicate that diabetes and aging can induce metabolic memory changes that may lead to the pathogenesis of the disease.