IRBP in Diabetic retinopathy – Cell Culture & Ophthalmic Pathology Studies

Background: Interphotoreceptor-Retinoid-Binding-Protein (IRBP) is restricted to the subretinal space by the external limiting membrane and RPE zonulae occludens. Its expression is reduced in diabetic retinopathy (DR) and increased expression is protective. Hypoxia decreases viable cone-like photoreceptors (661W) but increased VEGF, suggesting a role of photoreceptors in proliferative DR (PDR) (Rodriguez *et al*, Arch Clin Exp. Ophthal 3:23,2021). Here, we investigated the effects of high glucose and VEGF on IRBP expression by 661W and rod-like Y79 cells and distribution of IRBP and albumin in human globes with PDR and NPDR.

Methods:
661W and Y79 cells were cultured in DMEM or RPMI respectively with 10% FBS to 80% confluency and passaged to 65,000 cells/well for 24hrs prior to treatment and treated with 5.5mM and 30mM glucose with 10ng/mL of VEGF for 24 hrs. ELISA measured IRBP in the media. The expression of IRBP and albumin in human globes were examined by immunohistochemisry.

Results: During the 24hr treatment period, neither high glucose nor exogenous VEGF changed viable cell number. High glucose decreased of IRBP concentration in the media of 661W after the 24hrs (127$\pm$5nM IRBP in 5.5mM versus 69$\pm$5nM in 30mM glucose). VEGF also decreased in IRBP in the cell media (28$\pm$ 5nM IRBP in 5.5mM versus 0nM in 30mM glucose). Glucose and VEGF did not induce a similar decrease IRBP expression in Y79 cells (133nM in 5.5mM glucose, 133nM in 5.5mM with VEGF, 133nM in 30mM and 133nM in 30mM with VEGF). ANOVA showed significant treatment effects in 661W cells but not in Y79 ($F=0.83$ for 661W and $F=0.41$ for Y79). In control eyes IRBP was prominently expressed within the interphotoreceptor matrix (IPM); IRBP expression was markedly reduced in NPDR and PDR retina. Albumin was not detected in the IPM of control globes but was present in those with PDR.

Conclusions: High glucose and exogenous VEGF decreased IRBP expression in 661W but not Y79 cells. The decreased expression of IRBP in DR globes and entry of albumin into the subretinal space in PDR suggests a breakdown of the blood retinal barrier. Taken together, our data suggests that the mechanisms controlling the expression of IRBP are complex involving decreased IRBP transcription, and changes in the integrity of the junctional complexes normally restricting IRBP to the subretinal space.

Supported by: UTRGV/SOM, NIH/T32; VAMC/UMMC, NIH RO1, VA ORD.