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Glycine is Dysregulated in Human Retinal Endothelial Cells and Proliferative Diabetic Retinopathy

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Introduction: Diabetic retinopathy (DR) is a leading cause of blindness when it progresses to the proliferative diabetic retinopathy (PDR) stage. However, the alterations in amino acid (AA) profiles in PDR are largely unknown. In the present study, we aimed to characterize the AA profiles and identify the enriched pathways that are dysregulated commonly in both patients with PDR and human retinal endothelial cells (HRECs) subjected to the dual effect of high glucose (HG) and hypoxia, which are common risk factors associated with PDR.

Methods: HRECs were treated with osmotic control (Mannitol, 25 mM) or high glucose (HG, 25 mM) for 5 days, followed by normoxia or hypoxia (Hyp, 2% O_2) for 24 hours. Thereafter, the Liquid Chromatography-Tandem Mass Spectrometry (LC-MS)/MS-based targeted AA platform was used to quantitatively profile the intracellular AAs in HRECs, followed by a pathway enrichment analysis using MetaboAnalyst. In parallel, vitreous humor samples from patients with PDR who had undergone pars plana vitrectomy (PPV) were assessed for their AA profile and compared to the control groups, including patients with diabetes but without clinical evidence of PDR and patients without diabetes. Principal component analysis (PCA) was performed to assess the differences in the AA profiles between these 3 groups of patients, with a false discovery rate (FDR) < 0.2 set as the threshold for significance.

Results: An increasing trend in the levels of AA with non-polar, polar, or basic side chains was observed between the Hyp, HG, and HG+Hyp versus (vs.) control groups. Specifically, a significant difference between the HG+Hyp and control groups was observed in the levels of non-essential AAs with aliphatic non-polar side chains. Dissecting this further, there were significantly higher concentrations of glycine in the HG+Hyp treatment relative to the control group. Pathway enrichment analysis revealed significant associations between the HG+Hyp vs. control comparison, all of which were related to glycine metabolism. Importantly, vitreous humor samples demonstrated higher levels of glycine in the PDR group compared to the non-diabetic and diabetics without PDR groups. Furthermore, PCA analysis revealed a clear separation in the principal components between the controls and the PDR group.

Conclusion: Our findings show that non-essential AAs with aliphatic non-polar side chains, and more precisely, glycine was significantly elevated in the HRECs treated with

HG+Hyp as well as in vitreous humor samples from patients with PDR. These results indicate AAs may be used as potential biomarkers in assessing the development of PDR, which may set the stage for the design of targeted therapies for patients with PDR.