Renin-Angiotensin system (RAS) in Hematopoietic Stem/Progenitor Cells (HS/PC) Predicts Vaso-reparative Dysfunction and Progression of Diabetic Retinopathy (DR)

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Purpose: We tested the hypothesis that loss of angiotensin converting enzyme 2 (ACE2) within diabetic HS/PCs would be detrimental to HS/PC reparative function, and alter their ability to contribute to vascular remodeling in human subjects and rodent models of DR.

Methods: Subjects (n=52) were recruited as controls (n=13) or diabetics (n=39) with either no DR, mild non-proliferative DR (NPDR), moderate NPDR, severe NPDR or proliferative DR (PDR). Fluorescein angiograms were analyzed using Vessel Generation Analysis (VESGEN) software in a cohort of subjects. $CD34^+$ HS/PCs were isolated from peripheral blood. RAS gene expression and migration was measured. Diabetic ACE2 knockout (KO)/C57BL/6-*Ins2* (Akita) mice at 3, 6 and 9 months of diabetes were compared to age-matched controls. Bone marrow HS/PC populations were analyzed by flow cytometry and migration and proliferation studies performed.

Results: ACE2 gene expression in human $CD34^+$ cells from diabetics without DR was increased compared to controls (p=0.0437). Mas receptor mRNA was also increased in diabetics without DR, but reduced with the onset of NPDR (p=0.0002), suggesting a loss of compensation. DR was associated with $CD34^+$ cell migratory dysfunction. By VESGEN analysis, vessel density measured by several confirming parameters in early NPDR (n=3) was greater than in normal retina (n=6) in both arteries and veins, which suggests active retinal remodeling. ACE2KO-Akita and Akita cohorts showed reduced retinal thickness by OCT at 9 months of diabetes. Absence of ACE2 in 9-month Akita mice led to an accelerated

increase in acellular capillaries compared to diabetic alone. Electroretinogram (ERG) in ACE2KO-Akita mice resulted in persistent deterioration of the neural retina. Reparative function studies showed that ACE2KO exacerbated diabetes-induced impairment of LK cell migration and proliferative functions as early as 3-month of diabetes (p=0.0019).
Conclusions: Retinopathy and adverse vascular remodeling in subjects with diabetes was associated with a loss of the protective arm of RAS in HS/PCs. Loss of ACE2 exacerbated vascular dysfunction in diabetic mice.