Association between Increased Vascular Density and Loss of Protective RAS in Early-Stage NPDR

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PURPOSE

Our hypothesis predicts that retinal blood vessels increase in density during early-stage progression to moderate nonproliferative diabetic retinopathy (NPDR). The prevailing paradigm of NPDR progression is that vessels drop out prior to abnormal, vision-imparing regrowth at late-stage proliferative diabetic retinopathy (DR). However, surprising results for our previous preliminary study1 with NASA’s VESSEL GENeration Analysis (VESGEN) software showed that vessels proliferated considerably during moderate NPDR compared to dropout at both mild and severe NPDR. Validation of our hypothesis will support development of successful early-stage regenerative therapies such as vascular repair by circulating angiogenic cells (CACs). The renin-angiotensin system (RAS) is implicated in the pathogenesis of DR and in the function of CACs, a critical bone marrow-derived population that is instrumental in vascular repair.

METHODS

Arterial and venous patterns were extracted from images of 6 normal control subjects and 3 early NPDR subjects (mild and moderate) obtained by Heidelberg Spectralis® 30 degree imaging following fluorescein angiography (FA). The binary vascular patterns were mapped by VESGEN to yield branching generaions (Gn) and quantified that includes densities of vessel length (Lv), area (Av), and number (Nv). Peripheral blood of diabetics and controls was collected for CD34+ CAC isolation. RAS gene expressions in CACs were measured by qPCR for Mas receptor (Mas) and its ligands Ang II (angiotensin II) and Ang-(1-7) using qCM 5.6-well chemotaxis cell migration assay.

RESULTS

By VESGEN analysis, vessel density measured by Lv, Av, and Nv in early NPDR was greater than in normal retina (Figure 1). For example, Lv was 2.00 ± 0.06E-2 px/px2 in NPDR veins for all branching generations compared to 9.65 ± 0.68E-3 px/px2 in normal retinas, and 1.64 ± 0.13E-2 px/px2 compared to 9.18 ± 0.98E-3 px/px2 in arteries. Results, which are slightly updated from our abstract submission, were confirmed by other parameters such as Av and Nv. The expression of Mas in CACs was reduced in NPDR relative to control, indicating possible loss of compensation of the protective RAS at this stage of DR. NPDR was associated with CD34+ CAC migratory dysfunction toward CXCL12, which was corrected with Ang-(1-7) pretreatment prior to CXCL12 exposure.

CONCLUSIONS

For our ongoing longitudinal study, preliminary evidence by VESGEN indicates that vascular density increased in early NPDR compared to normal retinas. The results are the first independent confirmation of our previous study1 If validated by more complete analysis, the VESGEN discovery is potentially valuable for determining optimal therapies at early stages of NPDR, when regenerative vascular treatments are more likely to be successful. These data further support the protective RAS axis within diabetic CACs is lost early in DR and is associated with increased vascular remodeling evidenced by VESGEN analysis.

REFERENCES

5. Verma A, Shen Z, Lei B, Yuan L, Li X, Nakagawa T, Grant MB, Lopeschuk GD, Oudit GY: ACE2 Deficiency Worsens Epicardial Adipose Tissue Inflammation and Cardiac Dysfunction in Response to Diet-Induced Obesity, Diabetes 2016, 65:85-95
17. Verma A, Shen Z, Lei B, Yuan L, Li X, Nakagawa T, Grant MB, Lopeschuk GD, Oudit GY: ACE2 Deficiency Worsens Epicardial Adipose Tissue Inflammation and Cardiac Dysfunction in Response to Diet-Induced Obesity, Diabetes 2016, 65:85-95