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Deciphering Pathogenic Cytokine Interplay in Retinal Inflammation, Angiogenesis and Fibrosis: A Transcriptomic Approach

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Introduction: Dysregulation of cytokine signaling drives endothelial cell (EC) dysfunction, a key feature of retinal neovascular diseases, including neovascular age-related macular degeneration (nAMD). This is characterized by progressive inflammation, angiogenesis, and eventual fibrosis – a transdifferentiation of ECs to a mesenchymal phenotype, impeding tissue function. We explored the transcriptional changes in ECs exposed to six nAMD-associated cytokines (individual and combined effect) in an *in vitro* pathogenic retinal model. **Methods:** Primary human retinal ECs (HRECs) were treated with either no treatment (control), transforming growth factor-beta 1 (TGF-β1), TGF-β2, tumor necrosis factor-alpha (TNF-α), thrombin, interleukin-6 (IL-6), or vascular endothelial growth factor (VEGF), individually (10 ng/mL) or combined at the same concentration (n = 6). After 24h, cells were harvested, and high-throughput, bulk transcriptomic sequencing was performed (Azenta Life Sciences). Differential expression analysis ($|Log_2FC| > 1$, $FDR < 0.05$) was performed for all genes (DEGs) with reference to control. Pathway enrichment analysis (PEA) was employed to characterize gene pathways relative to disease phenotypes. DEGs were compared to nAMD patient eye RNA data (GSE135922) to assess clinical relevance. **Results:** Pro-inflammatory cytokines TNF-α (1823 DEGs) and thrombin (1019) induced a broader DEG profile than IL-6 (6). TGF-β2 (323) enriched mesenchymal pathways more potently than TGF-β1 (17). Angiogenic VEGF (32) induced a narrow, targeted DEG profile. PEA showed pro-fibrotic overlap in TNF- α, thrombin and TGF-β2 and significant enrichment of extracellular matrix and cell migration pathways. The combination group (2559) resulted in novel enhancement to differential expression revealing 884 unique DEGs, including VEGF-encoding *VEGFA*, enriching angiogenic cell-junction pathways. nAMD patient hallmark genes suggested the cytokine combination reflected disease better than individuals. **Conclusions:** Transcriptomic findings in HRECs suggest fibrosis-associated cytokines (TNF-α, thrombin and TGF-β2) to induce a strong transcriptional response in the pathogenic retina. TGF-β2 was confirmed as the dominant TGF-β isoform in HRECs. Combining cytokines presents a more disease-reflective *in vitro* model of nAMD, which may be utilized for pre-clinical drug testing, and extended to model other neovascular retinal diseases such as diabetic retinopathy and retinopathy of prematurity.