IGF-1 Role in VEGF-dependent Angiogenesis of Human Retinal Microvascular Endothelial Cells

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Retinopathy of prematurity (ROP) has abnormal temporal and spatial regulations of vascular endothelial growth factor (VEGF) and insulin-like growth factor-1 (IGF-1). These are manifested as retinal neovascularization that requires the activation of receptor tyrosine kinases (RTKs) by VEGF and IGF-1 ligands resulted in downstream pathways promoting cell survival, cell proliferation, vascular permeability and cell migration. In addition, hypoxia-inducible factor (HIF) has been shown to be the transcription factor important in inducing VEGF secretion and expression of the VEGF receptor: VEGFR2 in a variety type of cells. The goals of this study are to demonstrate *in vitro* tube formation assay as a model of ROP using human retinal microvascular endothelial cells; and to determine the role of HIF-1α in angiogenesis affected by VEGF and IGF1 synergy.

Immortalized human retinal pigment epithelial cells (ARPE-19) were grown in Lab Tek II chamber slides, once confluent, the cells were treated with VEGF, IGF-1 or both to observe the upregulation of VEGFR2 and HIF-1α using confocal microscopy. In separate experiments, human retinal microvascular endothelial cell (HRMVEC) lines were seeded in growth factor-reduced matrigel medium on 96-well plate for 3 days with activated growth medium in the incubator, then serum free media for 24 hours. The cells were then treated with serum free media supplemented with VEGF, IGF-1 or both under normoxic incubator environment and photographs were taken for 48 - 72 hours. The VEGFR2 and HIF-1α in ARPE-19 cells were upregulated with the IGF-1 treatment. Tube formation assay showed more prominent with the presence of both growth factors, but not on control or in the presence of a single growth factor. This study demonstrates the crucial roles of IGF-1, and the signaling proteins involved in VEGF-dependent angiogenesis in human retinal microvascular endothelial cells.

Fig. 1: VEGFR2 expression without IGF-1 treatment (left) and with IGF-1 treatment (middle) observed by confocal microscopy with statistical graph (right). IGF-1 significantly increases VEGFR2 expression. Scale markers = 33μm for controls and 35 μm for treated ARPE-19 cells.
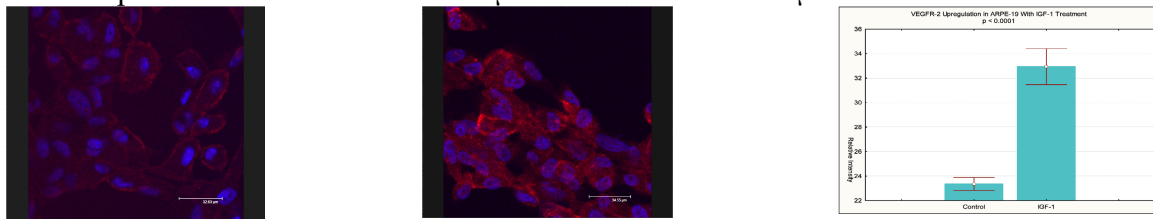

Fig. 2: IGF-1 significantly increases expression of HIF-1α in ARPE-19 cells (middle) compared to control (left) observed with 63x magnification confocal microscopy and the statistical graph (right).
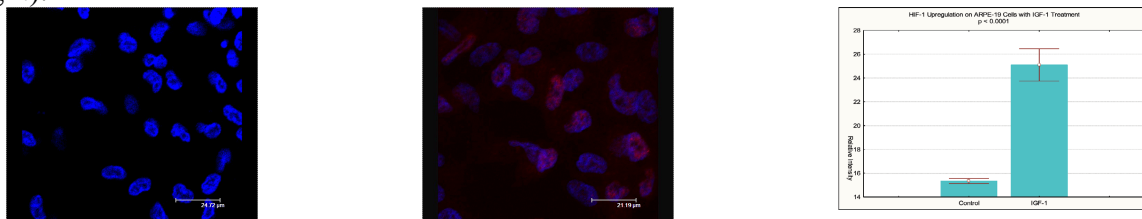

Fig. 3: HRMVECs in vitro tube formation assay with serum free media. Observed with light microscope 100x magnification after 24 hours of treatment. A. Control (SFM only). B. IGF-1 only. C. VEGF only. D. IGF-1 + VEGF

A.

B.

C.

D.