

# Vitamin C Reduces IGF-1 and VEGF Signaling in Retinal Endothelial Cells

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#### Purpose

Glucose acts as a competitive inhibitor for vitamin C, an important cofactor for DNA methylation, when crossing the blood retinal barrier. The purpose is to investigate potential role of vitamin C in gene expression pathways that may lead to the development of diabetic retinopathy.

#### Methods

- Primary, human retinal endothelial cells were treated either with or without 50  $\mu$ M vitamin C.
- RNA was extracted and RNA-seq was used to determine transcription changes.
- Pathway analysis was performed using EnrichR, Gorilla, and Gene Set Enrichment Analysis (GSEA).

#### Results

437 genes upregulated and 308 genes downregulated transcription after treatment with vitamin C. Several pathways that may contribute to the pathogenesis of diabetic retinopathy were down regulated including IGF-1 and VEGF signaling in retinal endothelial cells.

### Discussion

Although the contribution of IGF-1 to diabetic retinopathy has been largely attributed to stimulation of production of vascular endothelial growth factor A (VEGFA) in retinal pigment epithelial cells (RPE), knockout of IGF-1 receptors in retinal vascular endothelial cells was shown to reduce

neovascularization in an oxygen-induced retinopathy mouse model. New blood vessels from proliferative diabetic retinopathy have been found to regress after anti-VEGF treatments, and anti-VEGF is similar in efficiency to panretinal photocoagulation of the blood retinal barrier in diabetic retinopathy.

### Conclusion

Local vitamin C deficiencies in the eyes of diabetics affect signaling in the retinal endothelial cells which may contribute to the breakdown of the blood-retinal barrier in diabetic retinopathy.



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#### Vitamin C regulates pathways related to diabetic retinopathy Database Term Cellular response to vascular endothelial growth GO Bio Process factor stimulus GO Bio Process Leukocyte adhesion to vascular endothelial cell GO Bio Process Leukocyte tethering or rolling GO Bio Process Negative regulation of endothelial cell migration Transport across blood-brain barrier GO Bio Process **GO Molecular Function** Insulin-like growth factor binding GO Molecular Function Insulin-like growth factor I binding **VEGF-A** complex Jensen Compartments 50 **Diabetic retinopathy** Jensen Diseases Z Hypoxia MSigDB Hallmark 2020 MSigDB Hallmark 2020 Inflammatory Response Neovascularisation processes WP4331 WIKI Figure 1. Heatmap showing the relative expression of differential genes. Red VEGFA-VEGFR2 Signaling Pathway WIKI represents upregulated expression and blue represents downregulated expression. Regulation of endothelial cell migration GO Bio Process Table 1. Table of pathways significantly downregulated in expression after treatment with vitamin c. All p-values are adjusted with ological proces the Banjamini-Hocherberg method. biological regulation response to stimulus cellular process prichment plot: INSULIN-LIKE GROWT FACTOR BINDING (GO:000552) biologica Process process Function cellular response regulation of response to Componen to stimulus chemical iological proces biological cellular localization adhesion process 0:0070887 ):0048519 0 2,000 4.000 6,000 8,000 10,000 12,000 Rank in Ordered Dataset 0 2.000 (negatively correlated) 0 2.000 4.000 6.000 8.000 10.000 12.000 Rank in Ordered Dataset e 2,000 4,000 6,000 8,000 10,000 12,000 Rank in Ordered Dataset negative regulation of regulation of ellular response regulation of response to Part of - Enrichment profile -- Hits -- Ranking metric scores to chemical response to cellular process organic substance :0051674 GO:004001 piological process stimulus stimulus Enrichment plot: REGULATION OF ENDOTHELIAL nent plot: VEGFA-VEGFR2 SIGNALING Regulates movement o ocalization of cell adhesion cell or locomotion subcellula Positively regulates cellular respons Negatively regulates response to 0048870 to organic growth factor substance immune cell-cell Occurs in cell motility system adhesion process 0048523 Capable of negative regulation of GO:0016477 negative regulation of cellular response 0 'na\_neg/(negatively correlated) 0 2,000 4,000 6,000 8,000 10,000 12,000 Rank in Ordered Dataset 2,000 4,000 6,000 8,000 10,000 12,000 Rank in Ordered Dataset o growth factor 0 2,000 4,000 6,000 8,000 10,000 12,000 Rank in Ordered Dataset A Capable of part of response to cellular process leukocyte stimulus cell-cell cell migration Figure 4. QuickGO nrichment plot: DIABETIC RETINOPAT adhesion Figure 2. QuickGO regulation of || cellular response cellular response to growth factor stimulus factor stimulus leukocyte flow-chart representing migration 60:004512 Gene Ontology pathway 0:0061756 with regulation of regulation of leukocyte cellular

rollina QuickGO - https://www.ebi.ac.uk/QuickGO

adhesion to

vascular

leukocyte

tethering or

extravasation

interactions involving terms related to leukocyte tethering or rolling.

Database ID	EnrichR	GSEA	GOrilla
GO:0035924	0.036	0.052	N/A
GO:0061756	0.046	0.053	0.019
GO:0050901	0.042	0.020	0.016
GO:0010596	0.045	0.000	N/A
GO:0150104	0.040	0.036	N/A
GO:0005520	0.008	0.005	2.16E-04
GO:0031994	0.032	800.0	0.003
	2.69E-06	0.000	N/A
	0.015	0.005	N/A
M5891	2.15E-04	0.006	N/A
M5932	0.035	0.121	N/A
WP4331	3.54E-04	0.016	N/A
WP3888	3.95E-04	0.000	N/A
GO:0010594	0.015	0.000	0.004





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