Vitamin C contributes to epigenetic regulation of genes related to diabetic retinopathy in retinal endothelial cells

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Background

Glucose acts as a competitive inhibitor for vitamin C transport across the blood retinal barrier, and individuals with diabetes are known to have significantly lower vitamin C levels in the eyes than the general population.

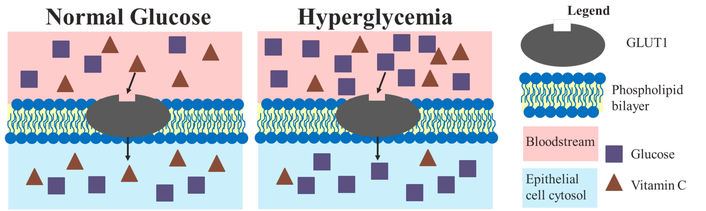

Figure 1. Diagram representing the competitive inhibition of glucose for vitamin C's entry into the epithelial cells, therefore inhibiting entry into the retina.

Vitamin C is an important cofactor for DNA methylation. A lack of vitamin C in the eyes of diabetics may contribute to dysregulation of the transcriptome and contribute to diabetic retinopathy.

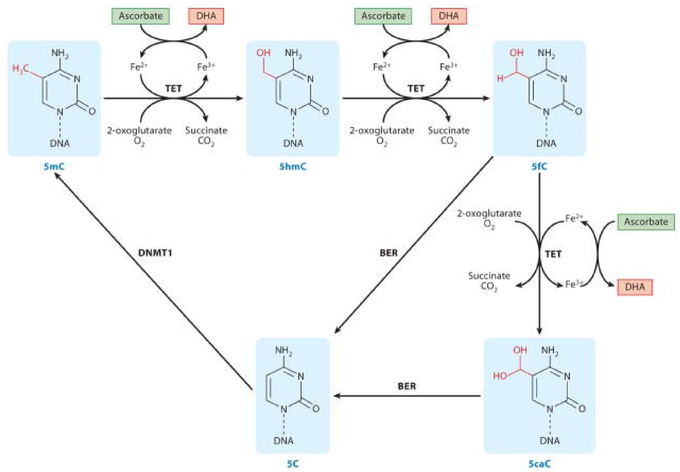

Figure 2. copied from Young *et al.* 2015 Annu Rev Nutr. As a cofactor, ascorbate participates in the cascade oxidation of 5mC, to 5hmC catalyzed by TET dioxygenases.

Primary, human retinal endothelial cells grown in 15.7 mM glucose were treated either with or without 50 µM vitamin C. RNA was extracted and RNA-seq was performed to determine transcriptional changes. Pathway analysis was performed using EnrichR, GOrilla, and Gene Set Enrichment Analysis (GSEA).

GSEA Gene Set Enrichment Analysis

Enrichr

GORILLA

Gene Ontology enRIchment anaLysis and visuaLIzAtion tool

Results

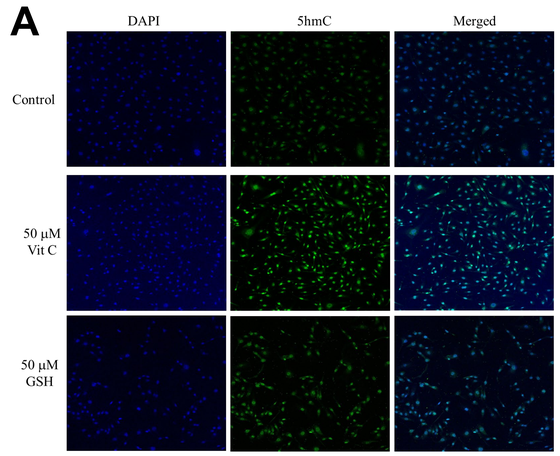

Figure 3. (A) Confocal imaging of 5hmC in primary retinal endothelial cells with or without 50 µM vitamin C or glutathione (GSH) for 5 days shows that global levels of 5hmC increase with vitamin C treatment, but not GSH. **(B)** Mean Fluorescent Intensity measurements of 5hmC staining of retinal endothelial cells treated with or without vitamin C or glutathione show that 5hmC levels increase dramatically with vitamin C. * $P < 10^{-15}$, *** $P < 10^{-200}$

Term	Database	Database ID	EnrichR	GSEA	GOrilla
Leukocyte tethering or rolling	GO Bio Process	GO:0050901	0.042	0.020	0.016
Negative regulation of endothelial cell migration	GO Bio Process	GO:0010596	0.045	0.000	N/A
Transport across blood-brain barrier	GO Bio Process	GO:0150104	0.040	0.036	N/A
Insulin-like growth factor binding	GO Molecular Function	GO:0005520	0.008	0.005	2.16E-04
Insulin-like growth factor I binding	GO Molecular Function	GO:0031994	0.032	0.008	0.003
VEGF-A complex	Jensen Compartments		2.69E-06	0.000	N/A
Diabetic retinopathy	Jensen Diseases		0.015	0.005	N/A
Hypoxia	MSigDB Hallmark 2020	M5891	2.15E-04	0.006	N/A
Neovascularisation processes WP4331	WIKI	WP4331	3.54E-04	0.016	N/A
VEGFA-VEGFR2 Signaling Pathway	WIKI	WP3888	3.95E-04	0.000	N/A
Regulation of endothelial cell migration	GO Bio Process	GO:0010594	0.015	0.000	0.004

Table 1. Table of pathways significantly downregulated in expression after treatment with vitamin c. All p-values are adjusted with the Banjamini-Hocherberg method.

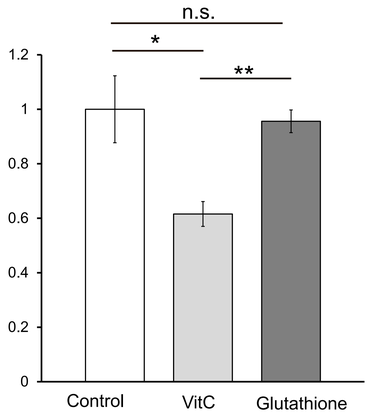

Figure 6. qPCR confirmation of reduction in *SELP* gene. ANOVA p=0.0269, *p<0.05, **p<0.01

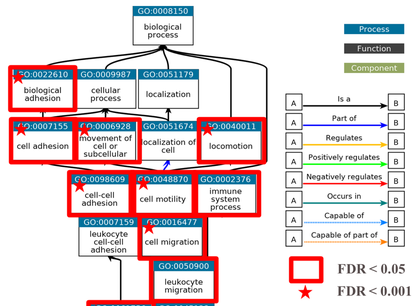

Figure 7. QuickGO flow-chart representing Gene Ontology pathway interactions involving terms related to leukocyte tethering or rolling. Red markings indicate pathways called as differential by GOrilla.

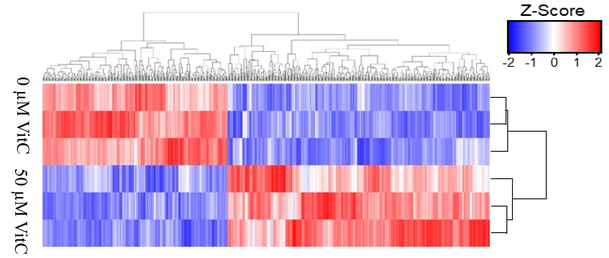

Figure 4. Heatmap showing the relative expression of differential genes. Red represents upregulated expression and blue represents downregulated expression. 437 genes were upregulated, and 308 genes were downregulated after treatment with vitamin C.

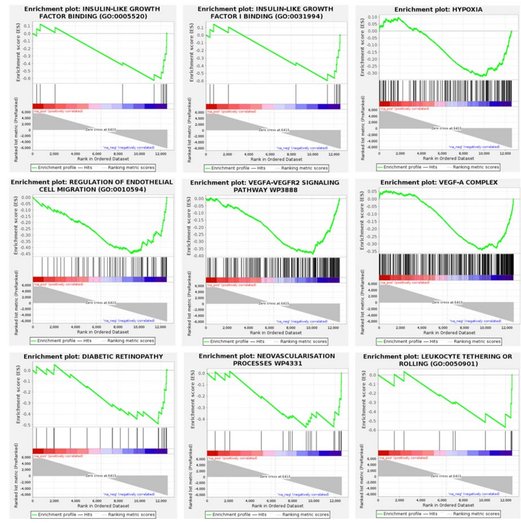

Figure 5. GSEA Traces from selected differential pathways.

Conclusion

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