

# Hemoglobin expression and regulation in glaucoma: insights into retinal ganglion cell oxygenation.

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**PURPOSE:** To determine expression, cellular distribution, and regulation of hemoglobin (Hb) in normal and glaucomatous tissues.

**METHODS:** Proteomic analysis of Hb expression was conducted on protein samples from ocular hypertensive and control rat eyes and human donor eyes with or without glaucoma. Proteomic findings were validated by quantitative (q) RT-PCR, Western blot analysis, immunohistochemistry, and the analysis of new Hb synthesis in culture. Hypoxic regulation of Hb expression was also studied in primary cultures of rat RGCs and macroglia and after transfer of the glia-conditioned medium to RGCs. The role of erythropoietin (EPO) signaling in Hb induction and cell survival was determined by applying recombinant (r) EPO treatment and performing EPO neutralization experiments by using soluble EPO receptor treatment of hypoxic cultures.

**RESULTS:** In vivo findings revealed Hb expression in the retina and optic nerve head macroglia and RGCs, suggesting an approximately two-fold upregulation in ocular hypertensive rat eyes and glaucomatous human donor eyes relative to the control eyes. In vitro findings collectively supported that hypoxia boosts glial Hb expression through hypoxia-inducible EPO signaling in an autocrine manner. Based on passive transfer experiments, hypoxia-induced production of glial EPO was also found to upregulate Hb expression in RGCs in a paracrine manner, thereby increasing the hypoxic survival of these neurons.

**CONCLUSIONS:** Findings of this study provide new insights into tissue oxygen transport in the inner retina and optic nerve head through the regulated expression of Hb in macroglia and RGCs. Upregulation of Hb expression appears to be an intrinsic protective mechanism to facilitate cellular oxygenation and may also provide free radical scavenging.

<http://www.ncbi.nlm.nih.gov/pubmed/19741249>