Objective: To examine tissue hypoxia in the retina and optic nerve head of glaucomatous eyes by the assessment of a transcription factor, hypoxia-inducible factor 1α (HIF-1α), which is tightly regulated by the cellular oxygen concentration.

Methods: Using immunohistochemical analysis, the cellular localization of HIF-1α was studied in the retina and optic nerve head of 28 human donor eyes with glaucoma compared with 20 control eyes from healthy donors matched for several characteristics. The relationship between the retinal regions that exhibited immunostaining for HIF-1α and functional damage was examined using visual field data.

Results: There was an increase in the immunostaining for HIF-1α in the retina and optic nerve head of glaucomatous donor eyes compared with the control eyes. In addition, the retinal location of the increased immunostaining for HIF-1α in some of the glaucomatous eyes was closely concordant with the location of visual field defects recorded in these eyes.

Conclusions: Because the regions of HIF-1α induction represent the areas of decreased oxygen delivery and hypoxic stress, information obtained from this study provides direct evidence that tissue hypoxia is present in the retina and optic nerve head of glaucomatous eyes, and hypoxic signaling is a likely component of the pathogenic mechanisms of glaucomatous neurodegeneration.

Clinical Relevance: These findings support the presence of tissue hypoxia in the retina and optic nerve head of glaucomatous patients.

Arch Ophthalmol. 2004;122:1348-1356

ISSUE HYPOXIA IN THE OPTIC NERVE HEAD AND/OR RETINA IS thought to develop secondary to or independent from the elevated intraocular pressure in glaucomatous eyes and has been proposed to be associated with pathogenic mechanisms underlying optic nerve degeneration in glaucoma. Considerable evidence suggests that tissue hypoxia in the retina may adversely affect the survival of retinal ganglion cells by inducing apoptosis. An alteration in the microcirculation of the optic nerve head blood supply, which may lead to an oligemic-hypoxic insult, has also been suggested to contribute to retinal ganglion cell death in glaucoma. Furthermore, chronic ischemia of the primate anterior optic nerve is accompanied by the diffuse loss of axons similar to that detected in glaucomatous human eyes. The pathophysiology of neuronal hypoxic damage involves glutamate excitotoxicity, calcium overload, and oxidative stress, including that caused by nitric oxide. In addition, tumor necrosis factor α has been implicated in hypoxia-induced neuronal apoptosis. Current knowledge recognizes that most of these hypoxia-associated mediator mechanisms identified in the brain are likely involved in glaucomatous optic nerve degeneration.

Hypoxia has been postulated to occur in glaucomatous eyes on the basis of blood flow studies. Clinical evidence of vascular abnormalities in glaucoma patients, such as vasospasm, systemic hypotension, angiographic vascular perfusion defects, and alterations in blood flow variables, has been suggested to result in reduced vascular perfusion in the optic nerve head and/or retina. However, there is no direct evidence demonstrating that tissue hypoxia is present in glaucomatous eyes.

The identification of the molecular mechanisms responsible for the expression of hypoxia-induced genes provides an opportunity to better understand the presence of a hypoxic component of the neurodegeneration process in glaucoma. Hypoxia-inducible factor 1 (HIF-1) is an oxygen-regulated transcriptional activator that functions as a master regulator of oxygen homeostasis. Under hypoxic condi-
tions, HIF-1 activates the transcription of a broad variety of genes, including those encoding erythropoietin, glucose transporters, glycolytic enzymes, vascular endothelial growth factor, inducible nitric oxide synthase, heme oxygenase 1, and other genes whose protein products increase oxygen delivery or facilitate metabolic adaptation to hypoxia.24-30

HIF-1 is a heterodimer composed of α (120 kDa) and β (approximately 92 kDa) subunits that belongs to the basic helix-loop-helix Per/Arnt/Sim (PAS) protein family.27,31 Although HIF-1α is constitutively expressed under normoxic conditions, it is rapidly degraded by the ubiquitin-proteosome system.32 However, under hypoxic conditions, HIF-1α is stabilized and accumulated, allowing the transcriptional activation of several genes.30,33 The expression and activity of HIF-1α have been shown to tightly regulate by the cellular oxygen concentration. For example, the expression of HIF-1α is exponentially increased as cells are exposed to oxygen concentrations less than 6%.34

HIF-1α has been identified to be expressed in the mammalian central nervous system,35 including neurons.36,37 Although increased levels of HIF-1 have been detected in the ischemic retina,36 the expression of HIF-1α has not been studied in glaucoma. To determine tissue hypoxia in glaucoma, we studied the cellular localization of the HIF-1α protein in the retina and optic nerve head of human donor eyes with glaucoma compared with control eyes from healthy donors, using immunohistochemical analysis. Our findings demonstrated an increased immunostaining for HIF-1α in the retina and optic nerve head of glaucomatous eyes, which indicates that hypoxic tissue stress is present in these eyes. In addition, the retinal immunostaining for HIF-1α in some of the glaucomatous eyes was found to exhibit a spatial relationship with functional damage recorded in these eyes. These findings support the pathophysiological role of hypoxic signaling in glaucomatous neurodegeneration.

METHODS

HUMAN DONOR EYES

Twenty-eight donor eyes with a diagnosis of glaucoma (donor age, 56-94 years) and 20 eyes from age-matched, normal donors (donor age, 55-96 years) were obtained from the Glaucoma Research Foundation (San Francisco, Calif), the Mid-America Eye Bank (St Louis, Mo), one of us (M.B.W.) (Alcon Laboratories, Ft Worth, Tex), and Douglas H. Johnson, MD (Mayo Clinic, Rochester, Minn). All of the human donor eyes were handled according to the tenets of the Declaration of Helsinki. Clinical findings of glaucomatous donor eyes were well documented, which included intraocular pressure readings, optic disc assessments, and visual field tests (Table 1). Normal donors had no history of eye disease. There was no diabetes, collagen vascular disease, infection, or sepsis in any of the donors. The cause of death for all of the donors used in this study was acute myocardial infarction or cardiopulmonary failure.

All of the donor eyes were enucleated within 2 to 4 hours after death and fixed within 6 to 12 hours. To know retinal orientation of histologic sections and to be able to examine the relationship between the pattern of immunostaining and functional damage, 10 freshly obtained globes from glaucomatous donors were marked for nasal, temporal, superior, and inferior sites before their processing. The posterior poles were dissected from the surrounding tissues, washed extensively in 0.2% glycine in phosphate-buffered saline at pH 7.4, embedded in paraffin, and oriented sagittally to obtain 5-mm sections. Microscopic examination of histologic slides revealed that the retina and optic nerve head tissues were relatively well preserved, with rare perimortem and/or processing-related alterations of photoreceptors.

Table 1. Clinical Data of Glaucomatous Donor Eyes

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<tr>
<th>Donor No./</th>
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Abbreviations: C/D, cup-to-disc ratio; IOP, intraocular pressure; NA, not applicable; NPG, normal-pressure glaucoma; POAG, primary open-angle glaucoma; VF, visual field.

PROCEDURES

Immunoperoxidase staining and double immunofluorescence labeling were used to study the cellular localization of HIF-1α. All of the histologic slides subjected to immunohistochemical analysis were masked for the identity and diagnosis of donors and numbered by a technician unfamiliar with the retina and optic nerve head condition before their immunostaining. To control variations in the immunostaining, slides obtained from glaucomatous and control eyes, as well as the negative control slides, were simultaneously subjected to immunohistochemical analysis. The intensity of immunostaining was first qualitatively graded as negative (--), faint (+), moderate (++), and strong (++++) using at least 5 histologic sections from each donor eye. To obtain complementary information, we then performed quantitative image analysis as recently described.39 For this purpose, chromogen quantity per pixel was measured using the TIFFalyzer program,40 and the value obtained from the negative control slide was subtracted from the experimental slide to determine the intensity of immunostaining.

To determine whether there is a relationship between the retinal regions that exhibit increased immunostaining for HIF-1α and the location of visual field defects, we correlated the gradings of HIF-1α immunostaining and visual field defects in glau-
comatous eyes. Although immunoreactivity may vary among different individuals, we considered that a masked evaluation could be informative in determining the correlation of glaucomatous damage with HIF-1α immunostaining in corresponding retinal quadrants of individual eyes.

Visual field defects in 4 quadrants were classified in these 10 eyes in a masked fashion. These classifications were based on the last available visual field test results (at most 2 years before death) in the patients’ clinical records. The visual field test results evaluated had been obtained using the Humphrey Field Analyzer (30-2 program) and had met the reliability criteria of a fixation loss less than 20% and false-positive and false-negative rates less than 30%. We calculated the mean of visual field indices within quadrants of the total deviation plot, and the quadrants were defined as having no, mild, moderate, or advanced visual field deficit if the mean defect was more than −2 dB, −2 to −6 dB, −7 to −15 dB, or less than −15 dB, respectively.41,42

RESULTS

Immunoperoxidase staining using a monoclonal antibody against HIF-1α was virtually negative in the retina of healthy donor eyes, except for very faint immunostaining in limited regions (Figure 1A). However, immunostaining for HIF-1α was detectable in retina sections obtained from the glaucomatous donor eyes (Figure 1B). HIF-1α immunostaining in the glaucomatous retina was detectable in all of the slides examined, although the intensity of immunostaining and the number of cells immunostained for HIF-1α exhibited individual and regional differences. Immunostaining for HIF-1α in the glaucomatous retina was qualitatively graded as moderate or strong. Using digital image analysis, the intensity (mean ± SD) of HIF-1α immunostaining was 76 ± 12 energy units per pixel in the glaucomatous retina but fewer than 16 energy units per pixel in the control retina.

Immunostaining for HIF-1α in the glaucomatous retina was predominant in the inner retinal layers, mostly in the retinal ganglion cell layer. In this layer, immunostaining was detectable in cells with large cell bodies, which likely correspond to retinal ganglion cells. In addition, based on the morphologic characteristics and known localization of retinal cell types, some glial cells, including astrocytes and Müller cells, exhibited immunostaining for HIF-1α in the glaucomatous retina. For example, retinal astrocytes are localized in the inner retina and can be differentiated from the retinal ganglion cells by their characteristic darker, smaller, and irregular nucleus relative to that of ganglion cells and by their proximity to the blood vessels of the inner retina.44,45 Another macroglial cell type in the retina, Müller cells, are characterized by their radial orientation and processes that extend all through the retina, although their cell bodies are located in the inner nuclear layer.44,45 Gliarial immunostaining for HIF-1α was mostly associated with the nuclei, although glial cell processes that extend all through the retina exhibited some faint immunostaining. However, immunostaining of retinal ganglion cells for HIF-1α was mostly not exclusively, localized to the cytoplasm (Figure 1C).

Some retinal immunostaining for HIF-1α was also detectable in scattered cells located in the inner nuclear or inner plexiform layers, which were likely amacrine cells, based on their morphologic characteristics. In addition, faint immunostaining was detectable at the inner wall of the blood vessels in the glaucomatous retina, as well as in the perivascular glial cells (Figure 1D). Control slides in which the primary antibody was omitted or replaced with nonimmune sera were all negative for specific immunostaining for HIF-1α.

Although increased immunostaining for HIF-1α in the glaucomatous retina was mostly detectable in the inner retinal layers, HIF-1α immunostaining was also detectable in the photoreceptor cells in the eyes of a patient with normal-pressure glaucoma (eyes 20 and 21), whose clinical and histopathologic findings were previously documented47 (Figure 2). This observation suggests that individual factors play a role in determining tissue hypoxia.
and/or cellular responses to tissue hypoxia and/or immunoreactivity in different retinal layers.

Similar to the control retina, immunostaining of the control optic nerve head for HIF-1α was virtually negative (Figure 1E). However, optic nerve head sections from glaucomatous eyes exhibited immunostaining of glial cells for HIF-1α (Figure 1F) in all of the slides examined, despite interindividual differences. Immunostaining for

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**Figure 1.** Immunoperoxidase staining for hypoxia-inducible factor 1α (HIF-1α) in the human retina. A, Retina section from a healthy donor eye; virtually no immunostaining for HIF-1α is detectable. B-D, Retina sections obtained from donor eyes with primary open-angle glaucoma with moderate damage (gc indicates ganglion cell; in, inner nuclear; on, outer nuclear layers); prominent immunostaining for HIF-1α is apparent in these eyes, which is mostly associated with the inner retinal layers. C and D, Higher-magnification images of the inner retina in glaucomatous eyes (original magnification ×500). Both retinal ganglion cells (arrows) and glial cells (arrowheads) exhibit detectable immunostaining for HIF-1α in the glaucomatous retina. Some immunostaining is detectable in the retinal blood vessels (V). E and F, Optic nerve head sections from a healthy donor eye and a donor eye with primary open-angle glaucoma with moderate damage, respectively. Although no immunostaining is detectable in the control optic nerve head (E), there is prominent immunostaining of the glaucomatous optic nerve head for HIF-1α (F), in which both glial cells (arrowheads) and nerve bundles (nb) exhibit immunostaining (Chromagen: 3,3-diaminobenzidine tetrahydrochloride [Sigma-Aldrich, St Louis, Mo]; nuclear counterstain: Mayer hematoxylin) (original magnification ×120 [A, B, E, and F]).
HIF-1α in the glaucomatous optic nerve head was qualitatively graded as moderate or strong. Using digital image analysis, the intensity of the HIF-1α immunostaining was 84±14 energy units per pixel in the glaucomatous optic nerve head but 16±5 energy units per pixel in the control optic nerve head. Glial immunostaining for HIF-1α was mostly detectable at the prelaminar and laminar regions of the glaucomatous optic nerve head. Both the processes and the nuclei of some glial cells around the nerve bundles and blood vessels were immunostained for HIF-1α. HIF-1α immunostaining was also detectable in nerve bundles that passed through the prelaminar region of the optic nerve head.

To identify retinal cell types that exhibit HIF-1α immunostaining, double immunofluorescence labeling was performed. We observed that the increased immunostaining for HIF-1α in glaucomatous eyes was localized to the retinal ganglion cells and glial cells. As shown in Figure 3, HIF-1α immunostaining was colocalized with the immunostaining for Brn-3a, which is a marker for retinal ganglion cells (Figure 3A-C), or with the immunostaining for GFAP (Figure 3D-F), which is a marker for astrocytes and Müller cells in glaucomatous eyes. The cellular pattern of HIF-1α immunostaining using double immunofluorescence labeling was consistent with the observations using immunoperoxidase staining, in which HIF-1α immunostaining was detectable mostly in the cytoplasm of Brn-3a-positive ganglion cells, although the immunostaining of GFAP-positive glial cells was mostly localized to the nuclei.

An examination of the relationship between the retinal regions that exhibited increased immunostaining for HIF-1α and the location of visual field defects in 10 glaucomatous donor eyes, in which visual field data could be referenced to histologic sections, revealed a close correspondence between the increased immunostaining for HIF-1α and the functional damage. As shown in Figure 4, retinal immunostaining for HIF-1α was more prominent in retinal regions corresponding to visual field defects. Although the intensity of immunostaining var-

![Figure 2. Immunoperoxidase staining for hypoxia-inducible factor 1α (HIF-1α) in the human retina. Although HIF-1α immunostaining in the glaucomatous retina is most prominent in the inner layers, in a patient with normal-pressure glaucoma, retinal photoreceptor cells also exhibited prominent immunostaining for this protein (gc indicates ganglion cell; in, inner nuclear; on, outer nuclear layers; V, blood vessel) (Chromagen: 3,3-diaminobenzidine tetrahydrochloride [Sigma-Aldrich, St Louis, Mo]; nuclear counterstain: Mayer hematoxylin) (original magnification ×120).](https://archopht.jamanetwork.com/content/122/9/1352.f2)

![Figure 3. Double immunofluorescence labeling in the glaucomatous retina. Images were obtained from a donor eye with primary open-angle glaucoma and moderate damage. In many instances, immunostaining for hypoxia-inducible factor 1α (HIF-1α) (red, A and D) was colocalized with the immunostaining for Brn-3a (Chemicon International Inc, Temecula, Calif) (green, B), a cell marker of retinal ganglion cells, or is colocalized with the immunostaining for glial fibrillary acidic protein (GFAP) (green, E), a cell marker of glial cells (V indicates blood vessel). C, Merged images of A and B (arrows illustrate cells immunostained for both Brn-3a and HIF-1α) (gc indicates ganglion cell; in, inner nuclear; on, outer nuclear layers). F, Merged images of D and E (arrows indicate HIF-1α–positive but GFAP-negative cells, which likely correspond to retinal ganglion cells). Another HIF-1α–positive but GFAP-negative cell (arrowhead) is likely an amacrine cell; other cells exhibiting HIF-1α immunostaining are GFAP-positive glial cells, either astrocytes or Müller cells (original magnification ×120 for all).](https://archopht.jamanetwork.com/content/122/9/1352.f3)
ied among different individuals, the grading of immunostaining was generally faint or moderate in retinal regions, corresponding to relatively normal visual field sensitivity in individual eyes. **Table 2** documents the corresponding gradings of visual field defects and HIF-1α immunostaining in 10 glaucomatous eyes. Because the expression of HIF-1α is an indicator of hypoxic tissue stress, the retinal areas corresponding to visual field defects in these glaucomatous eyes are the retinal areas in which tissue hypoxia is predominant.

**COMMENT**

Our findings demonstrate that immunostaining for HIF-1α in the retina and optic nerve head of glaucomatous eyes is greater than in the control eyes matched for several characteristics. Additionally, we examined the relationship between the retinal regions exhibiting increased immunostaining for HIF-1α and the location of visual field defects recorded in some of these glaucomatous eyes. This demonstrated that the increased immunostaining for HIF-1α in glaucomatous eyes was most prominent in retinal regions that correspond to areas of decreased oxygen delivery and hypoxic stress, these observations suggest that tissue hypoxia is present in glaucomatous eyes, and hypoxic signaling is a likely component underlying the pathogenic mechanisms of glaucomatous neurodegeneration.

Immunostaining for HIF-1α was virtually negative in the control tissues. This is consistent with the fact that HIF-1α is constitutively expressed yet hardly detectable in normoxic cells due to its rapid degradation. Tissue oxygen tension is not homogeneously distributed in the retina as a direct consequence of oxygen delivery to the tissue by diffusion from the capillary network, which varies throughout the retina. Thus, there are regions of

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**Figure 4.** Spatial relationship between the retinal immunostaining for hypoxia-inducible factor 1α (HIF-1α) and functional damage in a donor eye with normal-pressure glaucoma. A, Last visual field test obtained 1 year before death (SN indicates superior nasal; ST, superior temporal; IN, inferior nasal; IT, inferior temporal). B-D, HIF-1α immunostaining in the histologic sections of the retina obtained from this eye. Compared with the retina section from the superior temporal quadrant that corresponds to normal visual field sensitivity at the inferior nasal quadrant, retina sections from the inferior nasal or the inferior temporal quadrants exhibit more prominent immunostaining for HIF-1α, which corresponds to the advanced and moderate defects detected in the superior nasal and superior temporal quadrants of the visual field, respectively. Both the intensity of HIF-1α immunostaining and the number of immunostained cells are greatest in the inferior temporal retinal quadrant, corresponding to the most advanced defect in the superior nasal visual field (gc indicates ganglion cell; in, inner nuclear; on, outer nuclear layers; V, blood vessel) (Chromagen: 3,3-diaminobenzidine tetrahydrochloride [Sigma-Aldrich, St Louis, Mo]; nuclear counterstain: Mayer hematoxylin; original magnification ×120).
though choroidal and retinal vasculature may be differing of the glaucomatous optic nerve head for HIF-1
senses. However, the predominant increase of HIF-1
retinal circulation with cilioretinal capillary anastomo-
optic nerve head are known to be supplied mostly by the
suggests that tissue hypoxia is present in the retina as well.

Signal to their cell bodies to up-regulate HIF-1
axon at the optic nerve head can initiate a retrograde
inside the retina should be ground for its transcriptional activ-
and/or proteosomal degradation of
imunostaining in the inner layers. This observation signifies the role of indi-
immunoreactivity and/or cellular responses to tissue hyp-
Alternatively, differences in the intensity of immuno-
staining between different glaucomatous donor eyes might have been associated, in part, with the fixation time of

Immunostaining for HIF-1α in glaucoma-
tous eyes was mostly associated with cells located in the
inner retinal layers. This observation is consistent with pre-
vious observations in the ischemic retina. Immuno-
unistaining of the glaucomatous optic nerve head for HIF-1α was
similarly most prominent in the inner layers. Ischemia of the axons at the optic nerve head can initiate a retrograde signal to their cell bodies to up-regulate HIF-1α. How-
ever, in addition to the immunolabeling of retinal gан-
glion cells, immunolabeling of retinal glial cells for HIF-1α
suggests that tissue hypoxia is present in the retina as well.

Similar to the inner retina, superficial layers of the
optic nerve head are known to be supplied mostly by the
retinal circulation with cilioretinal capillary anastomo-
ses. However, the predominant increase of HIF-1α im-
munostaining in the inner layers of the retina and optic
nerve head in glaucomatous eyes does not necessarily mean
that tissue hypoxia is manifest mainly in the regions sup-
plied by the retinal circulation. First, blood flow studies

demonstrate vascular abnormalities in both the retinal and
the choroidal systems in glaucomatous eyes, although
choroidal and retinal vasculature may be differ-
ently affected in primary open-angle glaucoma and normal-
pressure glaucoma. Second, it is feasible that all layers
of the retina and/or optic nerve head sustain an equal
amount of hypoxic stress in glaucomatous eyes, whereas
hypoxia-induced regulation of HIF-1α expression may vary
among their different layers, resulting in the prominent
up-regulation in the inner layers. For example, recent ob-
servations in glaucomatous eyes provide evidence that the
signaling molecules, also involved in the regulation of HIF-
α, are differentially activated through the retina. In ad-
in, one of the patients with normal-pressure glau-
coma, whose clinical and histopathologic findings were
previously documented, HIF-1α immunostaining was also
detectable in the outer retinal layer, including the photo-
ceptor cells. This observation signifies the role of indi-
vidual factors in determining tissue hypoxia and/or cel-
lar responses to tissue hypoxia in different retinal layers.
Individual factors that determine immunoreactivity may
also be associated with the differential pattern of immu-
nostaining among different eyes.

We also noticed cellular differences in the immuno-
 staining for HIF-1α in glaucomatous eyes. For example,
HIF-1α immunostaining was more prominent within the
cytosol of retinal ganglion cells, whereas glial immuno-
 staining for HIF-1α occurred predominantly in the
nucleus. Nuclear immunostaining for HIF-1α is consist-
ent with the fact that HIF-1α is a DNA binding protein
and is localized to the nucleus for its transcriptional activ-
ity. However, cytoplasmic immunostaining may be asso-
ciated with the insufficient transcriptional activity and
thereby stabilized cytoplasmic expression of HIF-1α and/or
its insufficient degradation in the ubiquitin-proteosome sys-
tem. It has been demonstrated that the stabilization, transcrip-
tional activation, and proteosomal degradation of
HIF-1α are independently regulated. The signaling com-
ponents controlling the regulation of these intracellular
events, which require phosphorylation, may vary among
different cell types, depending on kinase activity. There-
fore, the variability we observed in the intracellular local-
ization of HIF-1α immunostaining in retinal cell types may
be associated, in part, with the differential activity of the
regulatory signaling cascades between retinal ganglion cells

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</tr>
<tr>
<td>18</td>
<td>POAG</td>
<td>Advanced/strong (72) Advanced/strong (67) Advanced/strong (63) Advanced/strong (71)</td>
</tr>
<tr>
<td>19</td>
<td>POAG</td>
<td>Moderate/faint (33) Moderate/faint (25) Moderate/faint (22) Moderate/faint (39)</td>
</tr>
<tr>
<td>27</td>
<td>NPG</td>
<td>Moderate/strong (133) Mild/moderate (66) Advanced/strong (141) Mild/moderate (64)</td>
</tr>
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<td>28</td>
<td>NPG</td>
<td>Mild/faint (40) Mild/faint (31) Mild/faint (27) Mild/faint (36)</td>
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</table>

Abbreviations: HIF-1α, hypoxia-inducible factor 1α; IN, inferior nasal; IT, inferior temporal; NPG, normal-pressure glaucoma; POAG, primary open-angle glaucoma; SN, superior nasal; ST, superior temporal.

*Visual field defects were classified as mild, moderate, and advanced. The intensity of immunostaining was qualitatively classified as faint, moderate, and strong, and the quantitative data were obtained using digital image analysis (mean energy units per pixel).
and glial cells, which is evident in glaucomatous eyes. Additional studies are warranted to identify the regulation of HIF-1α and the associated signaling cascades in glaucoma. Such information may also explain why the sensitivity of these cell types to glaucomatous damage, as well as to hypoxic stress, is not uniform, despite the fact that both neuronal and glial cells are exposed to similar hypoxic stress and exhibit increased immunostaining for HIF-1α in the glaucomatous eyes.

In addition to retinal ganglion cells and glial cells, some immunostaining for HIF-1α was also detectable in the blood vessels. This may support the proposed role of HIF-1α in metabolic adaptation to hypoxia, such as an increase in oxygen delivery and glucose transport through the up-regulation of vascular endothelial growth factor and glucose transporters.

During prolonged exposure to hypoxia, HIF-1α is expressed as long as the balance between oxygen supply and tissue utilization has not been reached. For example, it has been shown that the expression of HIF-1α rapidly increases during the onset of hypoxia in rat brain and remains increased for at least 14 days. Despite the continuously low arterial oxygen tension, however, it returns to normal within 21 days following compensatory adaptations. Therefore, our results demonstrating immunostaining for HIF-1α in a diverse sample of glaucomatous donor eyes may signify a sustained hypoxic insult or possibly recurrent episodes of tissue hypoxia in these eyes.

Glaucomatous donors whose eyes were used in immunohistochemical analysis were under antiglaucomatous treatment, and their last available intraocular pressure readings were within normal limits. Because of this and the retrospective nature of our data collection, we considered that the determination of a relationship between the intraocular pressure and HIF-1α immunostaining in these eyes would not be precisely informative. No evidence is currently present that any medication could induce HIF-1α expression and explain HIF-1α immunostaining in glaucomatous eyes, which exhibits a specific pattern closely concordant with the functional damage. Whether it is secondary to or independent from the elevated intraocular pressure, our findings provide evidence for the presence of hypoxic stress in the glaucomatous retina and optic nerve head and suggest that hypoxic signaling is associated with the initiation and/or progression of neuronal damage. HIF-1α has been shown to coordinate the expression of not only adaptive but also pathogenic genes, such as p53, and has therefore been implicated to promote delayed neuronal cell death. HIF-1α may similarly be associated with the activation of a cell death program in glaucomatous eyes through p53, which has been suggested to be a transcriptional activator of neuronal apoptosis in glaucoma.

Molecular mechanisms responsible for oxygen sensing are still poorly understood. Recent experimental studies have provided evidence in support of the hypothesis that mitochondrial generation of reactive oxygen species (which are implicated in glaucomatous neurodegeneration) is required for the induction of HIF-1α expression and activity. Although the signal transduction pathways remain enigmatic, recent advances in this field imply that HIF-1 activity is also regulated by different signals, including nitric oxide and cytokines, such as tumor necrosis factor α, which are also implicated in glaucoma. Thus, it is clear that both the expression and the transcriptional activity of HIF-1α are regulated by the cellular oxygen concentration and the redox modifications of the protein, although many details remain elusive.

Despite the unique informative value of immunohistochemical studies of postmortem human tissues, their findings can be difficult to interpret mainly due to perimortem tissue alterations. Obviously, such tissue alterations cannot fully be ruled out for the tissues used in our study, because the photoreceptor layer shown in the figures may be suspected of having such abnormalities. However, all of the tissues used in this study were fixed within 12 hours after death. Within this period, retinal neurons, including human photoreceptor cells, have been reported to maintain high viability. In addition to the death-to-tissue fixation period, glaucomatous and control eyes were also carefully matched for donor age and cause of death, and any eyes with ocular vascular diseases were excluded. These features should minimize the vulnerabilities intrinsic to such a study and validate the interpretation of immunostaining differences between glaucomatous eyes and control eyes from healthy donors.

In conclusion, immunostaining for HIF-1α is increased in the retina and optic nerve head of glaucomatous eyes and exhibits a spatial relationship with functional damage recorded in these eyes. Because the expression of HIF-1α is a direct indicator of hypoxic stress, our findings demonstrate that hypoxic stress is present in the glaucomatous retina and optic nerve head and HIF-1α signaling may have a pathophysiologic role in the development and/or progression of neurodegeneration in these eyes. HIF-1α is known to play a central role in the cellular regulation of a broad variety of hypoxia-inducible proteins and is critical for the determination of ultimate cell fate in response to hypoxic stress. Therefore, better understanding of the regulation of HIF-1α and the associated signaling cascades can provide insights into the pathogenic mechanisms of glaucomatous neurodegeneration, thereby providing new strategies for neuroprotection in glaucoma.

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