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Expression of optineurin, a glaucoma-linked gene, is influenced by elevated intraocular pressure

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Abstract

Optineurin (optic neuropathy inducing protein; OPTN) was recently linked to 16.7% of families with primary open-angle glaucoma. The function of OPTN in the eye is not known, but is present in the trabecular meshwork, which is responsible for maintenance of intraocular pressure (IOP). To gain insight into the role of OPTN in the development of glaucoma we studied its expression in response to factors known to be associated with the disease: elevated IOP, tumor necrosis factor-α (TNFα), and dexamethasone (DEX). We performed the treatments in human organ cultures under conditions mimicking physiological pressure. We find OPTN significantly upregulated after 2, 4, and 7 days of sustained elevated IOP. OPTN expression is also induced 2.3-fold by TNFα and 2.6-fold by prolonged DEX treatment. These results demonstrate that OPTN is part of the transcriptome responding to glaucomatous insults and support the protective role of this protein in the trabecular meshwork.

Keywords: Optineurin; Gene expression; Human trabecular meshwork; Primary open-angle glaucoma; Elevated intraocular pressure; Perfused anterior segments; Relative quantitative RT-PCR; FIP-2; TNFα; Dexamethasone

Glaucoma is a complex, heterogeneous disease characterized by a progressive degeneration of the optic nerve. The disease has a significant genetic component and affects more than 70 million people worldwide, leading to profound visual impairment and blindness [1]. In the United States, glaucoma is the primary cause of blindness among African Americans and the second leading cause among Caucasians. The most common type of glaucoma, adult-onset primary open-angle glaucoma (POAG), is in most cases associated with elevated intraocular pressure [2].

Recently, a new gene designated optineurin (optic neuropathy inducing protein; OPTN) has been linked to POAG [3]. Mutations in the OPTN gene were found in 16.7% of 54 families with autosomal dominant adult-onset POAG, including some with normal tension glaucoma. Mutations in OPTN were also found in 13.6% of a sporadic sample of 124 glaucoma cases with predominantly normal IOP. To date, only two other genes have been linked to glaucoma. Cytochrome P4501B1 (CYP1B1) is mutated in primary congenital glaucoma [4,5] while myocilin (TIGR/MYOC) is mutated in 36% of juvenile-onset POAG [6] and in about 4% of the adult-onset form of the disease [7].

OPTN, previously identified as both FIP-2 [8] and NRP [9], is a coiled-coil protein that was first identified in a yeast two-hybrid system using an adenovirus protein (E3-14.7K) as the bait. FIP-2 blocked the anti-apoptotic activity of E3-14.7K after TNFα stimulation [8]. In addition, expression of FIP-2 was induced by TNFα treatment in a time-dependent manner [8]. Although over-expression of FIP-2 alone was not able to activate the cell death signaling pathway, the authors suggested this protein could utilize some cellular factors to activate apoptosis [8]. FIP-2 has also been shown to link Huntingtin (a Huntington's disease protein) to the Rab8 protein [10] to form a complex that regulates membrane trafficking and cellular morphogenesis [10]. Expression of OPTN has been reported in human heart, brain, placenta, liver, skeletal muscle, kidney, and pancreas

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using Northern blot analysis [8]. Upon discovery of the linkage of OPTN to glaucoma, its expression in the eye was analyzed by RT-PCR and found to be present in human trabecular meshwork, non-pigmented ciliary epithelium (NPCE), and retina [3]. Additional Northern blotting in the meshwork and NPCE revealed a major 2.0-kb transcript and a minor 3.6-kb message [3] with the same relative abundance reported to occur in other human tissues [8]. Because of the reported induction of FIP-2 by TNFα and because OPTN was linked to a number of normal tension glaucoma patients, Rezaie and collaborators [3] speculated that OPTN might play a neuroprotective role in the eye and in the optic nerve.

The role of TNFα in the eye and its potential contribution to eye diseases have been studied. For example, in the human glaucomatous optic nerve head, expression of both TNFα and its receptor TNF-R1 is significantly upregulated [11]. It has also been demonstrated that the secretion of TNFα is induced in glial cells after subjecting them to simulated ischemia and elevated hydrostatic pressure, stressors known to induce retinal ganglion cell degeneration [12]. In addition, higher levels of TNFα are present in retinal Müller glial cells obtained from Royal College of Surgeons (RCS) rats, an animal model of inherited retinal degeneration [13]. In the trabecular meshwork, TNFα was shown to be induced after laser trabeculoplasty, a procedure commonly used for the management of glaucoma [14], and to modulate secretion of extracellular matrix (ECM) metalloproteinases [15].

The role of OPTN in the eye has yet to be elucidated. Since OPTN has been found in cells of the outflow pathway, which are responsible for the maintenance of physiological IOP, in this study we investigate whether expression of this novel protein is affected by known glaucoma causing stressors. Because the outflow pathway in animals is different from that of humans [16] we used perfused whole anterior segments from post-mortem human donors. In this paper, we report that OPTN gene expression is upregulated in response to periods of elevated IOP. In addition, under conditions of normal pressure, expression of the OPTN gene was also induced by TNFα and prolonged dexamethasone (DEX) treatment. The results presented here demonstrate that the OPTN gene is part of the trabecular meshwork transcriptome responding to insults known to be involved in glaucomatous occurrences. Whether OPTN's presence in the transcriptome is part of either the harmful or the protective protein subgroups needs to be determined. The information available that OPTN mutants are associated with patients carrying the disease [3] together with these results would support the hypothesis that the role of OPTN in the trabecular meshwork might in fact be that of a protective nature.

Materials and methods

Perfusion of human anterior segment organ cultures. A diagram of the perfusion system is shown in Fig. 1. Pairs of normal, non-glaucomatous human eyes ranging in age from 47 to 90 years were obtained from national eye banks (Lions Eye Bank of Oregon, the North Carolina Eye Bank, and Carolina Eye Donor Services) following signed consent of the patients' families. All procedures were in accordance with the Tenets of the Declaration of Helsinki. The eyes were dissected within 30–40 h of death and organ cultures were prepared as described previously [17,18]. Briefly, eyes were bisected at the equator and the lens, iris, ciliary body, and vitreous were removed. The anterior segment was then secured to a modified, 2-cannula petri dish (Fig. 1) and perfused at a constant flow rate of 3–4 μl/min using a microinfusion pump (Harvard Apparatus) through one cannula. The culture medium was Dulbecco's modified Eagle's medium (DMEM) containing 100 U/ml penicillin, 0.1 mg/ml streptomycin, 170 μg/ml gentamicin, and 250 μg/ml amphotericin B. Anterior segments were maintained at 37 °C, 5% CO_2, and IOP was continuously monitored with a pressure transducer connected to the dish's second cannula and recorded via computer. Eyes were allowed to equilibrate for 24 h before subjecting them to any treatment to establish a stable baseline.

For the elevated pressure experiments, the flow in one eye of each pair was raised to obtain a ΔP of $\approx$ 35 mmHg for either 6 h, 2, 4, or 7 days. The flow of the contralateral eye, which served as a control, was maintained at 3 μl/min. For drug treatments, both eyes of each pair were maintained at the same basal flow rate of 3–4 μl/min for the duration of the experiments. One eye of each pair was perfused with complete DMEM medium containing either 0.1 μM DEX (Sigma) for

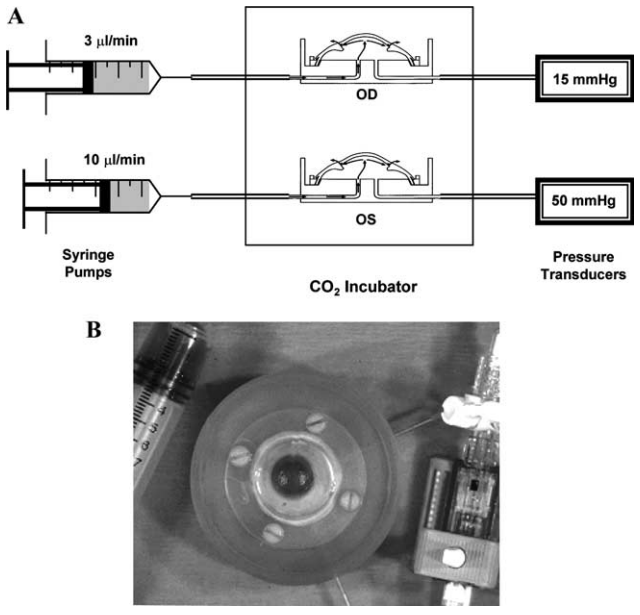

Fig. 1. (A) Schematic representation of the human anterior segment organ culture perfusion system. The culture chamber, maintained at 37 °C in an environment of 5% CO_2, has two cannulas. Through one cannula, culture medium is perfused into the anterior segment at 3 μl/min using a microinfusion syringe pump; the second cannula is connected to a pressure transducer that monitors IOP. To raise IOP in one anterior segment, the flow rate of the perfusion medium is increased to obtain a $\Delta P = 35$ mmHg. OD = right eye, OS = left eye. (B) Actual photograph of an anterior segment from a post-mortem human eye mounted to the culture chamber. A mounting ring holds the tissue in place and provides a tight seal causing perfusate to exit the eye through the normal outflow pathway (trabecular meshwork and Schlemm's canal).

7 days or 25 ng/ml TNFα (R&D Systems) for 3 days. DEX was prepared in absolute ethanol at 0.1 mM and diluted 1000× into fresh, complete DMEM every other day. The TNFα stock solution was reconstituted in sterile PBS containing 0.1% BSA to a concentration of 10 μg/ml. A 2.5-μl aliquot of the TNFα stock per ml of perfusion medium was added to make a final concentration of 25 ng/ml. The contralateral control eyes received fresh medium at the same times as the drugs and containing the same volume of drug vehicle. At the end of each experiment, anterior segments were immediately immersed in RNAlater (Ambion) and stored at 4 °C for trabecular meshwork dissection and RNA extraction.

RNA extraction. Human trabecular meshworks were excised from anterior segments under a dissecting microscope. The isolated tissue was placed into a 1.5-ml microcentrifuge tube, frozen with liquid N_2, and pulverized with a disposable pestle. Homogenization continued with the addition of 350 μl guanidine thiocyanate buffer, followed by loading onto a QIAshredder column (QIAGEN). RNA extraction continued using an RNeasy Mini kit (QIAGEN) with on-column RNase-free DNase digestion (QIAGEN) following manufacturer's directions. Total RNA averaged 2 μg per trabecular meshwork which was eluted in 30 μl RNase free water.

Relative quantitative reverse transcription-polymerase chain reaction (RQ RT-PCR) cDNA synthesis. Reverse transcription (RT) reactions were performed using random primers (RETROscript kit, Ambion). Fifteen microliters of eluted RNA (approximately 1 μg) was concentrated in a SpeedVac to 10 μl, mixed with 2 μl of 50 μM random decamers, heated at 75 °C for 2 min, and cooled on ice. The reaction continued in a 20-μl mix containing 2 μl of 10× RT buffer (500 mM Tris–HCl, pH 8.3, 750 mM KCl, 30 mM $MgCl_2$, and 50 mM DTT), 4 μl dNTP (2.5 mM each), 1 μl RNase inhibitor (10 U), and 1 μl MMLV-reverse transcriptase (100 U). The RT reactions were incubated at 42 °C for 90 min and terminated at 92 °C for 2 min.

Determination of the linear range of OPTN amplification in the samples. The OPTN cDNA was amplified using forward 5′-TCGTG TCTGAACTGCAGCTCA-3′ and reverse 5′-CCTTCTCTGCCAG TTCCAGTTT-3′ primers, yielding an amplification product of 828 bp. To eliminate the possibility of amplifying genomic DNA, OPTN primer pairs were designed to span intron–exon splice boundaries. A 2.5-μl aliquot of each RT reaction (control and treated) was amplified by polymerase chain reaction (PCR) in a 50-μl reaction mixture containing 5 μl 10× PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, and 15 mM $MgCl_2$), 4 μl dNTP (2.5 mM each), 4 μl OPTN primers (5 mM each), and 0.5 μl Ambion SuperTaq cDNA polymerase (2.5 U). The PCR mixture was split into four tubes and amplification was performed at: 94 °C for 4 min, cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 60 s, and a final extension at 72 °C for 4 min. For each treatment, the linear range of OPTN was determined by increasing the number of cycles by four in each of the four tubes. RT-PCR products were electrophoresed on Tris–borate 2% agarose gels containing 25 ng/ml ethidium bromide.

Linear range determination of 18S rRNA. 18S rRNA, an uninduced, endogenous standard, was amplified with a QuantumRNA primer:competimer set (Ambion) yielding a band of 489 bp. Since 18S rRNA is far more abundant than OPTN's mRNA, the amount of 18S rRNA amplification product was reduced by the addition of competimers, which are primers modified at their 3′ ends to block extension by DNA polymerase.

To determine the correct primer:competimer ratio to use with OPTN in each sample pair, a 2.5-μl aliquot of the control RT reaction was used in a 45-μl reaction mixture containing 5 μl 10× PCR buffer, 4 μl dNTP, 5 μl OPTN primers, and 0.5 μl SuperTaq cDNA polymerase. The mixture was split into four tubes containing 1 μl of different 18S primer:competimer ratios (1:15, 1:9, 2:8 or no primer:competimer). PCR amplifications were conducted at the pre-determined OPTN linear cycle for the given sample. Multiplex products were run on an agarose gel and the 18S rRNA band whose intensity matched that of OPTN was selected for use in the relative-quantification of OPTN.

Quantification of the OPTN PCR products. Quantification of the differential expression of the OPTN gene between treated and untreated samples was conducted by normalizing each OPTN value to its 18S rRNA internal standard. Reactions for each RT sample were performed in triplicate at conditions where the multiplexed PCR products (OPTN and 18S) were within their linear range. Typically, a PCR mixture containing the OPTN primer pair mix, predetermined 18S rRNA primer:competimer mix and SuperTaq cDNA polymerase was aliquoted into seven tubes (9.5 μl each). Tubes 1, 3, and 5 each received 0.5 μl of the control RT reaction while tubes 2, 4, and 6 each received 0.5 μl of the high-pressure RT reaction. The seventh tube received 0.5 μl water. PCR amplifications followed at the predetermined linear cycle for the given sample. Multiplex products were electrophoresed on a Tris–borate 2% agarose gel containing 25 ng/ml ethidium bromide. For quantification, the integrated optical density (IOD) for each band was captured using a UVP ChemiDoc System including a Chemi cooled CCD camera, PCI digitizing image acquisition board, EpiChemi II Darkroom with transilluminator, and LabWorks 4.0 Software. Data were exported to an excel spreadsheet for calculation of averages and standard errors.

Results

Effects of elevated IOP on OPTN gene expression

OPTN mutants have recently been genetically linked to glaucoma. To test whether expression of the OPTN gene is induced by elevated pressure we used perfused anterior segment organ cultures from post-mortem human donors. This perfusion system, in which one eye serves as the experimental subject and the contralateral eye serves as a control, is not confounded by genetic differences as both eyes are obtained from the same individual. A total of 12 pairs were used in this study with an average baseline outflow facility of 0.24 ± 0.03 ($n = 24$). Following induced elevated pressure in one eye of each pair, trabecular meshworks were dissected, total RNA was extracted, and expression of OPTN and 18S rRNA were assessed by RQ RT-PCR. All measurements were performed at the linear amplification range for both genes using a pre-determined 18S rRNA primer:competimer mix to compensate for the higher abundance of 18S rRNA (see Materials and methods). A total of four time points (6 h, 2, 4, and 7 days), with three pair of eyes per point and three measurements per eye pair were performed. The differential expression of the high-pressure eyes compared to their normotensive, contralateral controls was normalized against 18S rRNA (Fig. 2). Each value represents the average of three separate pairs of eyes at each time point ($n = 9$). Exposure to continuous elevated pressure for 6 h showed a small induction of OPTN expression in the high-pressure eye over the normotensive contralateral control of $8.2 \pm 3.9\%$ ($P = 0.07$). The induction slightly increased to $9.6 \pm 4.2\%$ ($P = 0.032$) at 2 days and was significantly higher when the eyes were subjected to elevated pressure for periods of 4 days, $40.2 \pm 4.5\%$ ($P = 0.00001$). The induction reached a peak of

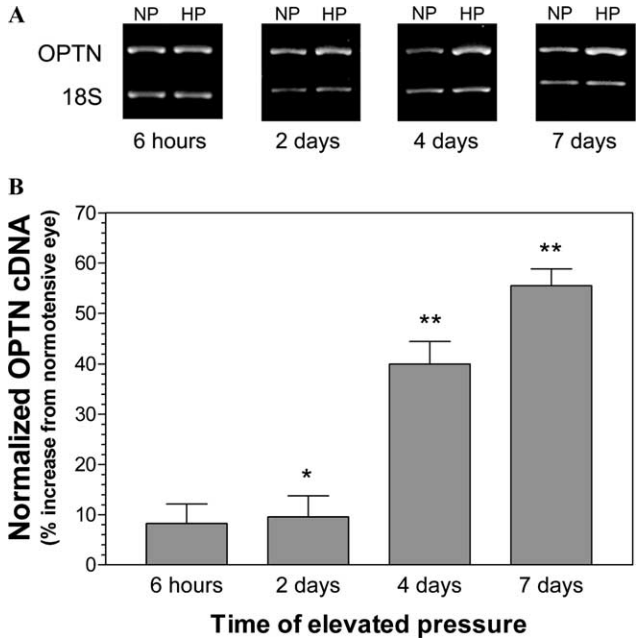

Fig. 2. Effect of elevated pressure on OPTN expression in the perfused human trabecular meshwork. One eye from each pair was subjected to a ΔP of 35 mmHg (high pressure; HP) while the contralateral eye was maintained at normal baseline pressure (NP). Pressure was sustained for 6 h, 2, 4, and 7 days ($n = 3$ pairs per time point). Total RNA from each trabecular meshwork was RT using random primers and amplified using relative quantitative RT-PCR as described in Materials and methods. Multiplex OPTN and 18S rRNA amplifications were performed at 27 cycles with an 18S rRNA primer:competimer ratio of 1:15 in all but two eye pairs (31 cycles; 1:15). PCR products were run on Tris–borate 2% agarose gels containing 25 ng/ml ethidium, bands were captured using an 8-bit CCD camera, and integrated optical density determined with LabWorks 4.0 software. (A) Representative gels of OPTN and 18S rRNA transcripts at 6 h, 2, 4, and 7 days of elevated pressure. (B) Expression of OPTN was normalized against 18S rRNA and expressed as percent increase from the normotensive, contralateral eyes. Results for each time point are expressed as means ± SEM of three independent experiments performed in triplicate ($n = 9$). $^{*}P < 0.05$, $^{**}P < 0.01$.

$55.6 \pm 3.3\%$ at 7 days ($P = 0.0002$), the longest time point measured. Thus, transcriptional regulation of OPTN in the trabecular meshwork is affected by the mechanical stress produced by elevated pressure. These observations suggest that the OPTN gene might play a role in the ability of the trabecular meshwork to respond to elevated IOP.

Response of OPTN gene expression to TNFα treatment

TNFα is a strong modulator of trabecular meshwork metalloproteinases and is induced in the anterior segment by laser trabeculoplasty [14,15]. Since FIP-2, a TNFα-inducible protein, has been suggested to be a component of the TNFα signaling pathway [8,9] we hypothesized that FIP-2/OPTN might also be induced by this cytokine in the trabecular meshwork. To validate this hypothesis we perfused one eye of each pair of

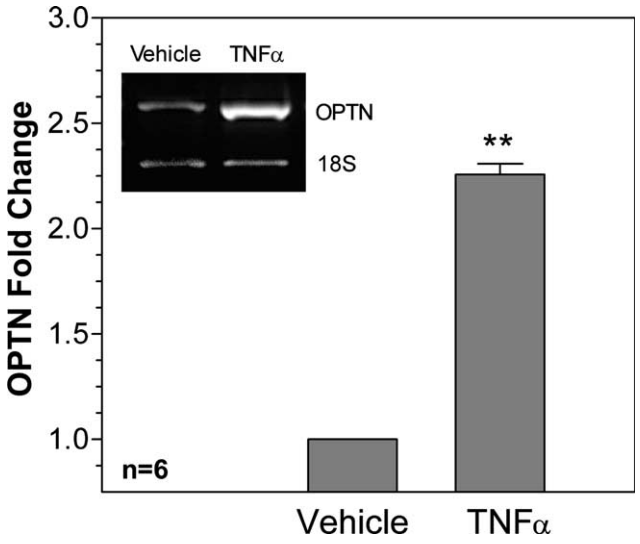

Fig. 3. Effect of TNFα on OPTN expression in the perfused human trabecular meshwork. One eye from each pair of normal human anterior segments was perfused with 25 ng/ml TNFα for 3 days while the contralateral eye received drug vehicle. Total RNA from each trabecular meshwork was RT using random primers and amplified using relative quantitative RT-PCR as described in Materials and methods. Multiplex OPTN and 18S rRNA amplifications were performed at 31 and 33 cycles with 18S rRNA primer:competimer ratios of 1:12 and 1:15, respectively. PCR products were run on Tris–borate 2% agarose gels containing 25 ng/ml ethidium, bands were captured using an 8-bit CCD camera, and integrated optical density was determined with LabWorks 4.0 software. Expression of OPTN was normalized against 18S rRNA and expressed as fold change from the untreated, contralateral eyes. Results are expressed as mean OPTN fold change ± SEM of two independent experiments performed in triplicate ($n = 6$). Inset: Representative gel of OPTN and 18S PCR products after treatment with TNFα. $^{**}P < 0.01$.

human anterior segments with TNFα ($n = 2$). After 24 h of baseline pressure stabilization, one of the cultured eyes was treated with TNFα by changing the anterior chamber medium and the perfusate to a medium containing 25 ng/ml cytokine. Baseline pressures of the control and treated eyes remained unchanged for the duration of the experiment. After 72 h of perfusion, trabecular meshworks were dissected and OPTN and 18S rRNA gene expression were analyzed by RQ-PCR as indicated above. The average of triplicate measurements for each eye ($n = 6$) is shown in Fig. 3. After normalization with 18S rRNA, OPTN expression in the TNFα treated eye was induced 2.3 ± 0.05-fold ($P = 0.008$) over the untreated, contralateral control. The induction of OPTN in trabecular cells by TNFα may indicate the presence of a common functional pathway for this protein in several tissues, including the eye.

Response of OPTN gene expression to DEX treatment

A percentage of patients undergoing steroid treatment develop glaucoma. This paradigm led to the

discovery of the linkage of the TIGR/MYOC gene to several forms of glaucoma [7,19]. To investigate whether the expression of the newly linked glaucoma gene, OPTN, could also be modulated by DEX and potentially play a role in the response of the trabecular meshwork to this steroid, we performed DEX treatments at the same physiological conditions of flow and pressure described above.

Anterior segments form post-mortem donors were affixed to perfusion chambers ($n = 3$) and allowed to equilibrate for 24 h. After which, one of eye of each pair was treated with 0.1 μM DEX for 7 days. DEX-containing medium was continuously perfused and replaced every other day for the duration of the experiment. DEX-free medium for contralateral eyes contained the same volume of drug vehicle and was also replaced at the same times. Total RNA extracted from the dissected trabecular meshworks was analyzed for the expression of OPTN and 18S rRNA. All analyses were carried out in the linear amplification range for the gene of interest together with the individually determined 18S rRNA primer:competimer ratio. For each gene, the average of triplicate measurements for each eye pair ($n = 9$) is shown in Fig. 4. After 7 days of DEX treatment and normalization against 18S rRNA, OPTN mRNA was induced 2.6 ± 0.12-fold ($P = 0.0002$) over the untreated eyes. These results suggest that OPTN could play a minor (secondary) role in the response of the trabecular meshwork to this steroid.

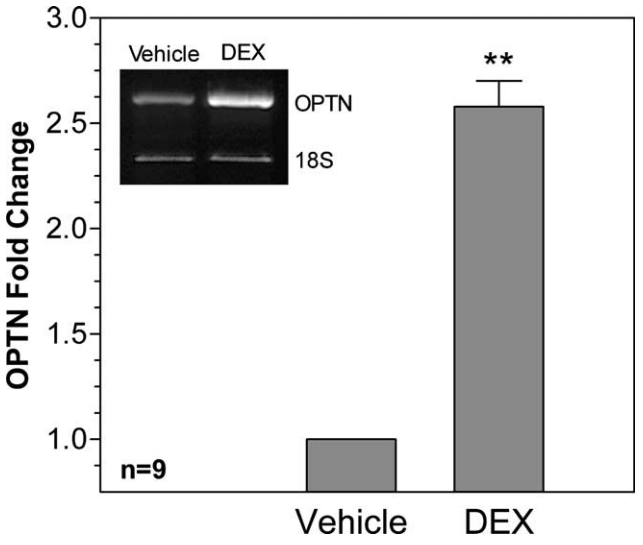

Fig. 4. Effect of DEX on OPTN expression in the perfused human trabecular meshwork. One eye from each pair of normal human anterior segments was perfused with 0.1 μM dexamethasone (DEX) for 7 days while the contralateral eye received drug vehicle. Total RNA from each trabecular meshwork was RT using random primers and amplified by relative quantitative RT-PCR as described in Materials and methods. Multiplex OPTN and 18S rRNA amplifications were performed at 27, 32, and 39 cycles with 18S rRNA primer:competimer ratios of 1:9, 1:9, and 1:7, respectively. PCR products were run on Tris–borate 2% agarose gels containing 25 ng/ml ethidium, bands were captured using an 8-bit CCD camera, and integrated optical density was determined with LabWorks 4.0 software. Expression of OPTN was normalized against 18S rRNA and expressed as fold change from the untreated, contralateral eyes. Results are expressed as mean OPTN fold change ± SEM of three independent experiments performed in triplicate ($n = 9$). Inset: Representative gel of OPTN and 18S PCR products after treatment with DEX. $^{**}P < 0.01$.

Discussion

Glaucoma is a multifaceted disease most commonly associated with elevated IOP. To date, eight map regions have been associated with glaucoma but only three genes, CYP1B1 [4,5], TIGR/MYOC [7], and OPTN [3], have been linked to this disease. Mutations in each of the genes have been associated with a limited percentage of glaucoma families, an indication that the mechanisms responsible for the development of glaucoma are varied. The molecular basis as to why deletions in the three genes cause glaucoma is not known. CYP1B1, an enzyme that metabolizes environmental pollutants, has been speculated to activate a metabolite that inhibits NaK-ATPase, an enzyme involved in aqueous humor production. TIGR/MYOC mutants have been shown to accumulate inside the cells and proposed to cause the disease by an accumulation of misfolded proteins [20,21]. OPTN, the most recently linked gene, was suggested to have a neuroprotective effect [3].

To gain insight into the functional role of OPTN in the outflow pathway, here we investigated its expression profile in the trabecular meshwork. Specifically, we questioned whether risk factors known to be associated with the development of glaucoma (IOP, TNFα, and DEX) had an effect on the expression of the OPTN gene. To carry out these studies we used a perfused organ culture system rather than human trabecular meshwork cells in culture.

The perfused anterior segment organ culture from post-mortem human donor eyes [17], offers an ideal model to study the trabecular meshwork tissue under conditions mimicking the IOP in the human eye. These cultures revitalize the donor tissue and maintain the original architecture of the trabecular meshwork. In addition, the different trabecular meshwork cell types can be exposed to mechanical insults and/or drugs in much the same way as they would be exposed to in vivo. Moreover, because a treated eye is compared with the contralateral eye from the same person, differential gene expressions are not affected by individual genetic variations.

By increasing the flow (and thus pressure) in one eye of each pair we find that OPTN expression was affected by elevated IOP. The effect was not that of an immediate response but rather of a late induction after prolonged exposure. We observed a slight 8.2% induction at 6 h, which increased to 9.6% and became significant at

2 days. The induction was considerably higher after 4 and 7 days of continuous high pressure (40% and 56%, respectively), the longest time-points studied. The significance of these findings is not yet clear since OPTN mutants are found not only in glaucoma patients with elevated pressure but also in some patients with normal tension glaucoma. Previously, we reported the presence of an adaptive mechanism in the trabecular meshwork in response to elevated pressure [22]. We showed that, between 2 and 4 days, the trabecular meshwork tissue seemed to counteract the pressure insult by increasing outflow facility. At 7 days, when TIGR/MYOC was induced, the homeostatic mechanism was no longer present.

Perhaps the induced expression of OPTN is part of the homeostatic response mechanism as well as of a mechanism that protects the trabecular meshwork against external insults. In this regard, it would be of interest to find if all genes thus far linked to glaucoma have protective properties and if their combined expression has additive or synergistic effects. Recently, Vincent et al. [23] have shown that individuals with mutations in both CYP1B1 and TIGR/MYOC genes developed glaucoma at a younger age than did individuals with only TIGR/MYOC mutations, and suggested that certain mutated glaucoma genes might be able to modify the expression of other glaucoma genes.

The function of OPTN, under the name of FIP-2, has been explored in other cell types. Interestingly, FIP-2 is induced by TNFα and binds to other proteins such as adenoviral E3-14.7K and Rab8/Huntingtin [8,10]. The binding of FIP-2 to E3-14.7K reverses the protective effect of this viral protein to favor TNFα-induced cell death [8]. In our studies, OPTN expression was also induced by TNFα. Increases of TNFα in the eye have been associated with glaucomatous conditions, elevated hydrostatic pressure, and retinal degeneration [11–13]. In the trabecular meshwork, TNFα modulates the expression of metalloproteinases and their tissue inhibitors (TIMP), key regulators of outflow facility [15]. Due to our results, it would seem reasonable to assume that such TNFα induction in the eye would also lead to an increase in OPTN expression. Since OPTN mutants rather than the wild-type form cause glaucoma, induction of OPTN expression would not likely be detrimental to the trabecular meshwork by binding, like FIP-2, to an anti-TNFα protein and allowing apoptosis. Instead, it could possibly function by influencing ECM remodeling and facilitating aqueous humor outflow.

Most interesting and probably relevant to trabecular meshwork function is the fact that FIP-2 links Rab8 and Huntingtin proteins [10]. The small GTPase Rab8 is known to cause radical changes in cell shape [24] and expression of both Rab8 and FIP-2 resulted in the formation of neurite-like extensions in fibrosarcoma HT80 cells [10]. The authors postulated that the Rab8/FIP-2/Huntingtin complex modulates cellular morphogenesis. It is conceivable that induction of OPTN in the trabecular meshwork might result in the formation of complexes that are important in modulating cell shape and, as such, have a protective effect on outflow facility. This is relevant since it has been shown that pharmacological agents that act directly to disrupt the cytoskeleton of trabecular meshwork cells lead to increased outflow facility [25].

Patients (30–40%) treated with glucocorticoids develops steroid-induced glaucoma [26]. Because steroid-induced glaucoma mimics many aspects of POAG, exposure of cells to DEX has been considered an important model for the study of this disease. TIGR/MYOC, one of the three genes linked to glaucoma, was first discovered as a protein induced with DEX in primary trabecular meshwork cell cultures. Although the mechanisms by which DEX causes glaucoma have not been clearly identified, ultrastructural studies report the presence of plaques or extracellular aggregates in the trabecular meshwork [27]. The presence of optineurin in the aqueous humor implies that this protein is also secreted [3]. We therefore questioned whether OPTN was induced by DEX as occurs with other relevant trabecular meshwork secreted proteins [28]. Previously, using early passages of non-transformed cultures of human trabecular meshwork cells and GeneChip analysis we found that the oligonucleotide corresponding to the FIP-2 gene in the chip was not induced by DEX [28]. However, here we find that the OPTN gene from intact trabecular meshwork tissue perfused under physiological pressure is induced by DEX 2.6-fold over the contralateral control. This difference between the two systems is not unexpected and reflects the importance of studying gene expression under closer physiologic conditions rather than in cultured cells.

The fact that OPTN is induced in the trabecular meshwork under three different conditions known to be involved or associated with glaucoma might be an indication of the presence of a general protective mechanism to defend the cells of the outflow pathway. Elevated IOP, TNFα, and DEX are all unrelated insults which induce OPTN gene expression. Work by Wang et al. [29] recently demonstrated that glaucoma could be explained by the activation of NF-κB, a common mediator of stress response. It would be of great interest to determine whether the NFκB pathway mediates the induction of OPTN expression observed here.

In summary, we have shown that OPTN, a gene linked to approximately 17% of glaucoma families, including some with normal tension, is induced by elevated IOP, TNFα, and DEX in the intact human trabecular meshwork. The induction by elevated IOP begins at 2 days and reaches its peak (57%) after 7 days, the last point studied. The time-course of induction coincides in part with the trabecular meshwork adaptive

mechanism previously described [22] but continues when the mechanism is no longer present. The additional induction of OPTN with TNFα and DEX is suggestive of the presence of a general defense mechanism against glaucomatous insults, whose common mediator remains to be identified. These findings demonstrate that OPTN belongs to the trabecular meshwork transcriptome that responds to insults involved in glaucomatous occurrences.

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