Visible light exposure induces VEGF gene expression through activation of retinoic acid receptor-α in retinoblastoma Y79 cells

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Akiyama, Hideo, Toru Tanaka, Hiroshi Doi, Hiroyoshi Kanai, Toshitaka Maeno, Hirotaka Itakura, Tomohiro Iida, Yasutaka Kimura, Shoji Kishi, and Masahiko Kurabayashi. Visible light exposure induces VEGF gene expression in retinoblastoma Y79 cells. Am J Physiol Cell Physiol 288: C913–C920, 2005. First published December 21, 2004; doi:10.1152/ajpcell.00116.2004.—Neovascularization of the retina and choroids is the pathological hallmark of many retinopathies, but its molecular mechanisms remain unclear. Vascular endothelial growth factor (VEGF), which is induced by hypoxia or cytokines, plays a critical role in the abnormal growth of blood vessels. In this study, we report that visible light exposure induces VEGF gene expression in retinoblastoma Y79 cells. Fluorescent light exposure (700 lux, wavelength 400–740 nm) caused a significant increase in VEGF transcripts and protein levels. Such an induction seemed to be specific to certain cells, including photoreceptor cells, because light-induced VEGF expression was not observed in either nontransformed cells, such as retinal pigment epithelium cells, and bovine aortic endothelial cells or transformed cells, such as CV-1 and HepG2 cells. Pertussis toxin and guanosine 5′-thio[γ]diphosphate, specific inhibitors for rhodopsin-associated G protein, blunted this induction. Progressive deletion and site-specific mutation analyses indicate that light stimulation increases VEGF promoter activity through G+C-rich sequence, which is proven by Sp1 binding sites by supershift assays. Electrophoretic mobility shift assays show that light stimulation increases Sp1 binding. Synthetic retinoic acid receptor-α (RARα) antagonist completely abrogated light-mediated increase in VEGF expression. Transfection of Y79 cells with dominant negative mutant of RARα significantly attenuated the light-mediated induction of VEGF promoter activity. In conclusion, our data indicate that light exposure increases VEGF expression through the mechanisms involving activation of Sp1 and RARα signaling in Y79 cells. This study provides new insight into the role of visible light in the transcription and induction of VEGF gene expression.

angiogenesis; transcription factors; retinoid

RETINAL AND CHOROIDAL NEOVASCULAR diseases, including age-related macular degeneration, proliferative diabetic retinopathy, and retinopathy of prematurity, are major causes of blindness. Among the potential mediators of angiogenesis, vascular endothelial growth factor (VEGF) plays a critical role in the abnormal growth of blood vessels across the retinas (24). Tissue hypoxia may be the primary stimulus for VEGF gene induction in most ocular angiogenic diseases (35). Hypoxia induces the binding of the hypoxia-inducible factor-1α/aryl hydrocarbon receptor translocator heterodimer to its cognate binding sequence (hypoxia response element) located upstream of the VEGF promoter (26, 23). Furthermore, evidence indicates that proinflammatory cytokines, such as IL-1β, TNF-α, TGF-β, and IL-6, increase the expression of VEGF transcripts in several cell lines (6, 11, 22, 31). These studies showed that induction of VEGF gene expression in response to IL-1β and TNF-α is mediated by Sp1 binding sites, whereas TGF-β-induced expression is predominantly mediated by AP-2 binding sites. However, the precise molecular mechanisms for the regulation of the VEGF gene expression have not been described in ocular cells.

The molecular mechanisms underlying the intraocular neovascularization have been the focus of intensive research. VEGF is an angiogenic peptide that is increased greatly in response to hypoxia in retinal cells (25). To date, it has been well established that hypoxia-mediated increase in VEGF expression plays a major role in development of many retinal diseases such as retinopathy of prematurity and diabetic retinopathy (19, 27). However, the molecular mechanisms underlying VEGF induction independent of hypoxia remain unclear. Thus the identification of the mediators that are capable of inducing VEGF expression may provide new insight into the mechanism of hypoxia-independent ocular disease. In this regard, our study, which indicates that light exposure increases VEGF expression in retinal cells, seems to provide a clue to an important clinical relevance.

Light exposure initiates a cascade leading to the transmission of a visual signal through the phototransduction pathway. During this process, 11-cis retinal is converted to the all-trans retinal, which is then reduced to all-trans retinol by reduced nicotinamide adenine dinucleotide phosphate and photoreceptor retinol dehydrogenase within the rod outer segment (12). Rhodopsin undergoes conformational change by which it catalytically activates a G protein and transmits a visual signal (3). These events could be blocked by guanosine 5′-[β-thio]diphosphate (GDPβS) (18a), and many biological effects of pertussis toxin are the result of a toxin-catalyzed transfer of an ADP ribose moiety from NAD to the α-subunits of signal-transducing G protein (43). All-trans retinal subsequently dissociates from opsin, leaving the chromophore pocket empty (3). Recently, one report (28) has shown that exposure of retinal tissue to light induces retinoic acid (RA) synthesis. Our previous studies have shown that treatment of Y79 cells, a cultured human retinoblastoma cell line, with all-trans RA increases the VEGF expression (1). These lines of evidence led us to
hypothese that light exposure increases VEGF expression in photoreceptor cells.

One of the major findings in this study is that a cis element involving light-mediated increase in VEGF expression is localized at −89 and −68 of VEGF promoter, whose sequence matches Sp1 binding sites. In fact, the results of electrophoretic mobility shift assays (EMSA) showed that Sp1 protein binds to this site and light stimulation increases the binding of nuclear factors to the Sp1 site. We also showed that light increased Sp1 protein levels but not Sp1 mRNA levels, thus suggesting that light has a potential to enhance the stability of Sp1 protein. Previous studies done by us and others support the idea that Sp1 plays a role in mediating the inducible expression of various genes, such as superoxide dismutase (37), glucose activation of the carboxylase (9), plasminogen activator inhibitor-1 (6), and VEGF genes (31). In addition, a role of Sp1 has been described in PMA-induced expression of the WAF1/CIP1 (4) gene and the platelet thromboxane receptor gene (8).

In this study, we determined the effects of light on the VEGF gene expression in human retinoblastoma cell line Y79 and showed that the exposure of Y79 cells to visible light induces VEGF transcription via RA-mediated activation of RA receptor-α (RARα). These data suggest a novel and important role of light for the induction of VEGF gene in ocular tissues.

MATERIALS AND METHODS

Materials. SB-203580, wortmannin, PD-98059, and PP1 were purchased from Calbiochem (La Jolla, CA). Actinomycin D and GDPBPS were purchased from Sigma (St. Louis, MO). Affinity-purified rabbit antibody for Sp1 was purchased from Santa Cruz (Santa Cruz, CA). α-[32P]dCTP (3,000 Ci/mmole) and γ-[32P]ATP (6,000 Ci/mmole) were obtained from Amersham (Princeton, NJ). RAR antagonist (LE-135, C29H30N2O2) (17) was kindly provided by Dr. K. Shudo (National Institute of Health Sciences, Tokyo, Japan).

Fluorescent light exposure. A fluorescent lamp (Neoball Z 40W EFAREL, Toshiba) provided visible light for culture dishes in a 37°C incubator in 5% CO2. According to the data sheet provided by the manufacturer, the wavelengths of this illumination range from 400 to 740 nm with the major peaks at 550 and 620 nm, and with the minor peaks at 410, 440, 500, 640, and 730 nm. The fluorescent lamp was adjusted to 50 cm above culture dishes. The wavelengths of this illumination range from 400 to 740 nm. A control dish was shaded with aluminum foil. The length of exposure time and illumination intensity are indicated in the figure legends. Illumination light intensity was measured with the use of a SUN ILLUMIND SLX-1330 (Sanyo, Tokyo, Japan), and medium temperature was taken with a measuring kit (model 950, Testo, Yokohama, Japan) as stated in the figure legends.

Plasmid constructions. We obtained the VEGF promoter/pGL3 from Dr. J. A. Abraham, which contained DNA fragment from −1,180 to +338 of the human VEGF gene fused to luciferase reporter plasmid (41). Plasmids −480 and −89LUC were made by subcloning the BgIII and SmaI insert from −1180LUC into the corresponding site of pGL3 (Promega, Madison, WI). The RA response element, RARα dominant-negative/pCMX, was a generous gift from Dr. A. Kakizuka (32) (Osaka Bioscience Institute).

Cell culture and transfection. Y79 retinoblastoma and retinal pigment epithelium cells were obtained from the American Type Culture Collection and cultured in RPMI 1640 supplemented with 10% FBS and antibiotics at 37°C in 5% CO2. Transfection into Y79 cells was performed with a modified calcium phosphate coprecipitation technique as previously described (14). The cells were transfected with 1 μg of reporter plasmid. Twenty-four hours after transfection, the cells were washed twice with phosphate-buffered saline and treated with or without light (700 lux) stimulation for 12 h. After 24 h of incubation, the cells were harvested for luciferase assay. Luciferase activity was measured with the use of a luminometer (Lumat model LB9501, Berthold, Bad Wildbad, Germany) and was normalized to cellular protein concentration. Each transfection was repeated, and means ± SE were presented.

EMSA and supershift assays. Nuclear extracts from Y79 cells were prepared as previously described (14). The sequences of double-stranded oligonucleotides used as probes or competitors in EMSAs were as follows, with the mutations of wild-type sequences in boldface: VEGF-89/67, 5′-CCCCGGGGGCAGGGGGGGGGG-3′; VEGF-89/67 (Sp1m), 5′-CCCCGGGAAAGCCGGGGAGGGG-3′; Sp1, 5′-ATTCGATCGGGGCGGGCGGAGC-3′; cAMP response element binding protein (CREB), 5′-AGAGATGCTGAGGCTGACTG-3′; and AP-2, 5′-GATCGAAGCTGACTGAGCCGGGCGCGG-3′.

Binding reactions, EMSAs, and supershift assays were performed as previously described (14).

Northern and Western blot analyses. A 642-bp fragment of human VEGF cdNA sequence and 2.0-kb fragment of the rat Sp1 cdNA sequence were used as a probe for Northern blot analyses. Nuclear extracts from vehicle- or light-treated Y79 cells were directly subjected to immunoblotting for Sp1. Western blot analyses were performed essentially as previously described (14). Sp1 was visualized by using an affinity-purified rabbit polyclonal antibody and a horseradish peroxidase-linked anti-rabbit IgG secondary antibody (Amersham).

ELISA for VEGF. The concentration of VEGF produced was measured using a commercially available ELISA kit (Immunobiological Laboratories, Fujioka, Japan), as stated in the Fig. 2 legend. The culture supernatants were collected after stimulation for 48 h, and the absorbency was measured at 450 nm. VEGF production was normalized to the volume of the medium and cell number.

Statistical analysis. Statistical analysis were performed by Student’s t-test with significant differences determined as P < 0.05. Correlation was performed with the use of simple regression analysis.

RESULTS

Light induces VEGF mRNA in Y79 cells. Exposure of human retinoblastoma Y79 cells to fluorescent light at 700 lux increased the levels of VEGF mRNA in a time-dependent manner (Fig. 1A and B), reaching a maximum at 72 h, followed by a gradual decline (data not shown). Such a response was specific to Y79 cells because light exposure had no effects on VEGF expression in either nontransformed cells, such as retinal pigment epithelium cells and bovine aortic endothelial cells or transformed cells, such as CV-1 and HepG2 cells (Fig. 1C). To rule out the possibility that heat elicited by a lamp may cause the VEGF induction, we shaded the dish with aluminum foil and measured the median temperature. Light exposure increased the medium temperature to the comparable level between noncovered and covered dishes with aluminum foil after 3 h, and the median temperature stayed at the same level thereafter (Fig. 1D). Thus light exposure is a reliable inducer of the VEGF gene expression in Y79 cells.

Light induces VEGF production in Y79 cells. To assess whether the observed increase in VEGF transcripts represents upregulation of VEGF production, we performed specific ELISA of the conditioned medium with which confluent cultures of Y79 cells were incubated for 48 h with or without light exposure. Light exposure significantly increased VEGF production (Fig. 2; 1.250 ± 30 vs. 1.720 ± 25 ng/ml medium: 10 cells; P < 0.01).
Specific inhibitors for G protein, pertussis toxin, and GDPβS, repress light-induced VEGF expression. To define the signaling pathways responsible for the effects of light stimulation on the transcription of VEGF gene, we tested the effects on light stimulation of a set of different protein kinase inhibitors, including SB-203580 (inhibitor of p38 mitogen-activated protein kinase) (38), wortmannin (phosphatidylinositol 3-kinase inhibitor) (40), PD-98059 (mitogen-activated protein kinase inhibitor) (38), and PP1 (Src family kinase inhibitor) (33), as well as pertussis toxin and GDPβS of G protein inhibitor. Northern blot analyses showed that PP1 partly inhibited and GDPβS and pertussis toxin completely abolished the induction of light-mediated increase in VEGF mRNA expression (Fig. 3). These results suggest that light induces the VEGF gene expression through phototransduction, given that the transduction of light into a neural signal in rod and cone receptor goes through signaling by G protein.

Light induces VEGF mRNA at the transcriptional level. To determine whether light induces VEGF mRNA at the transcriptional level, we performed a standard mRNA decay assay using actinomycin D, which prevents the transcription of the genes. Y79 cells were stimulated with light for 24 h. After incubation with or without light exposure, cells were treated with actinomycin D and were harvested after various intervals for Northern blot analyses. A rate of decrease in VEGF mRNA levels was comparable between control and light-treated cells, thus suggesting that light treatment did not affect the stability of VEGF transcripts (Fig. 4A).

Next, we tested whether light increases the promoter activity of a plasmid containing human VEGF promoter sequence between −1,180 and +338 relative to the transcription start site. The luciferase activity was increased about 2-fold by light (Fig. 4B), whereas β-actin promoter was not responsive to light (data not shown).

We then attempted to map the regions that confer both basal and light-induced activities on the VEGF promoter. Light significantly increased the promoter activity of both −480Luc

**Fig. 1.** Effect of light exposure on vascular endothelial growth factor (VEGF) mRNA. A: total cellular RNA (20 μg) prepared from Y79 cells at the indicated times after stimulation with light (700 lux) was analyzed by Northern blot analysis for VEGF mRNA. 28S ribosomal RNA indicates that comparable amount of total RNA actually blotted onto a membrane. B: densitometric analysis of the Northern blots. VEGF mRNA levels yielded by scanning the autoradiographs were normalized to the 28S signal. The results are indicated as values relative to VEGF mRNA levels in the control cells. Values of the control cells are set at 1.0. C, top: total cellular RNA (20 μg) prepared from retinal pigment epithelium (RPE) cells at the indicated times after exposure to fluorescent light at 700 lux was analyzed by Northern blot analysis for VEGF mRNA. Bottom: total cellular RNA (20 μg) prepared from Y79, bovine aortic endothelial cells (BAEC), CV-1, and HepG2 cells, which were exposed to fluorescent light (700 lux) for 24 h was studied using Northern blot analysis for VEGF mRNA. 28S ribosomal RNA indicates that comparable amount of total RNA actually blotted onto a membrane. D: light stimulated and shaded median (control) temperatures by aluminum foil were measured at 0, 3, 6, 12, and 24 h with the use of a Testo 950 measuring kit.

**Fig. 2.** VEGF production in Y79 cells in response to light stimulation. Confluent cells were serum starved for 24 h and then incubated with light stimulation (700 lux) for 48 h. Culture medium was harvested, and the levels of VEGF protein were measured by specific ELISA. Standard curves were constructed from dilutions of purified VEGF. *P < 0.05 compared with untreated control cells (n = 5).

**Fig. 3.** Effects of protein kinase inhibitors on light-induced VEGF mRNA expression. Top: Y79 cells were pretreated with various protein kinase inhibitors, SB-203580 (SB; 20 μM), wortmannin (Wor; 1 μM), PD-98059 (PD; 20 μM), PP1 (5 μM), pertussis toxin (PT; 100 ng/ml), and guanosine 5′-[β-thio]diphosphate (GDPβS; 100 μM) for 1 h, and were then exposed to light for 24 h. Total cellular RNA (20 μg) was analyzed with a Northern blot for VEGF mRNA. Bottom: bar graphs show VEGF mRNA levels normalized by intensity of 28S RNA. *P < 0.05 compared with untreated control cells (n = 5).
and −89Luc (Fig. 4B). Luciferase activity of −89Luc was significantly higher than that of promoterless construct pGL3, suggesting that the sequences downstream of −89 contain elements required for both basal and light-induced expression of the VEGF promoter. A response of the VEGF promoter activity to light appears to be promoter specific because β-actin promoter was totally unresponsive (data not shown). These results indicate that a sequence between −89 and +338 is necessary for light-induced VEGF gene transcription as well as for basal expression.

Sp1 sites play a critical role in light-mediated VEGF expression. We have previously shown that a sequence between −89 and +338 contains two functional Sp1 sites at −85 and −74 and a consensus AP-2 site at −80 located upstream of the transcription start site. To test whether Sp1 sites serve as the light-regulatory element, −89(Sp1m)Luc, a plasmid that contains mutations within the two Sp1 binding sites, was transiently transfected into Y79 cells. The disruption of both Sp1 sites markedly impaired the responsiveness to light stimulation (Fig. 4B). Such a loss of responsiveness of −89(Sp1m)Luc to light was not due to the disruption of the essential elements for the basal transcription, because the luciferase activity of −89(Sp1m)Luc was significantly higher than that of pGL3. These results indicate that the activation of VEGF promoter in response to light depends on the integrity of at least one of the two Sp1 sites.

Identification of nuclear factors binding to light-responsive elements in human VEGF promoter. To examine the ability of these sites to interact with Sp1 or related factors, EMSAs were performed using nuclear extracts prepared from either un-treated or light-treated Y79 cells and the 32P-labeled double-stranded oligonucleotide probe containing the sequence between −89 and −67. As shown in Fig. 5A, the 32P-labeled VEGF −89/−67 probe gave rise to two specific DNA:protein complexes (C1 and C2). The intensity of C1 complex was enhanced by light. By contrast, the C2 complex was not affected (Fig. 5A). Both C1 and C2 complexes were proved to be sequence specific because formation of these complexes was competed by wild type but not by a mutated version of the probe sequence (Fig. 5B). These complexes could also be competed by consensus Sp1 sequence but not by CREB and AP-2 binding sequence (Fig. 5B). To verify that C1 and C2 complexes contain Sp1- or Sp1-related proteins, we carried out supershift assays using Sp1- or Sp3-antisera (Fig. 5C). The addition of an Sp1 antibody resulted in a supershift of a complex C1, indicating that Sp1 is a principal DNA binding component of this complex. An Sp3 antibody completely supershifted complex C2. The addition of the CREB and AP-2 antibodies had no effect on complex formation. These results provide the evidence that Sp1 and Sp3 but not AP-2 or CREB bind to the VEGF −89/−67 probe, and light increased the binding of Sp1 to this sequence.

Effects of all-trans RA on Sp1 mRNA and protein levels. An increase in Sp1 binding as assessed by EMSAs led us to determine whether light treatment increases the levels of Sp1 transcripts. As shown in Fig. 6A, Northern blot analyses indicated that Sp1 mRNA levels were not measurably affected by light treatment in Y79 cells at any time points. In contrast, Western blot analyses using nuclear extracts from vehicle- or light-treated Y79 cells showed that light stimulation increased Sp1 protein levels, which correspond to the two distinct bands whose molecular mass is either 95 or 105 kDa (Fig. 6, B and C). These two protein species are generated as the result of differential posttranslational modification of the single Sp1 polypeptide. Both species of Sp1 protein in light-treated Y79 cells were significantly increased. SDS-polyacrylamide gel stained with Coomassie brilliant blue showed that nuclear extracts prepared from either control or light-treated Y79 cells
contain the same quantity of protein (Fig. 6D). Taken together, our data suggest that light exposure increases Sp1 protein levels without affecting its mRNA levels. An increase in Sp1 protein then induces VEGF promoter by binding to its proximal promoter region.

**Dominant negative mutant of RA receptor-α inhibits VEGF promoter activation in response to light stimulation.** To test the involvement of RA and its receptor in light-mediated increase in VEGF gene transcription, we performed cotransfection experiments using a RA receptor-α expression vector (RARα). As shown in Fig. 7A, cotransfection of RARα enhanced the light-mediated increase in −1180Luc activity compared with the control, in which −1180Luc is cotransfected with empty vector (Fig. 6A; 2.1 ± 0.2 vs. 4.2 ± 0.5, P < 0.01). Furthermore, cotransfection of RA response element (32), which acts as a dominant negative mutant of RARα by forming a nonfunctional RAR/retinoid X receptor heterodimer, abolished the light-mediated increase in −1180Luc activity.

**Effect of RARα antagonist LE-135 on response to light stimulation.** To further determine the role of RA in the upregulation of VEGF expression, we examined the effects of RARα antagonist (LE-135) on the transcription of the VEGF gene. Representative Northern blot analysis was performed on total RNA prepared from Y79 cells with or without light stimulation for 24 h in the presence or absence of LE-135 (1 and 5 μM) (Fig. 7B). LE-135 completely abolished the increase in VEGF mRNA by light stimulation. These findings imply that RARα can lead to the activation of the VEGF promoter and that retinoic acid plays an important role in mediating light-induced VEGF expression.

**DISCUSSION**

In this study, we investigated the effects of visible light exposure on the VEGF gene expression in retinoblastoma Y79 cells. Results suggest that light exposure may indeed contribute to VEGF production and all-trans RA (atRA) plays a central role in mediating this response in retinal tissue, including photoreceptor cells. Neovascularization is a hallmark of many retinal and choroidal diseases, shared by patients with age-related macular degeneration, proliferative diabetic retinopathy, and retinopathy of prematurity. Previous studies (24) have
established the role of tissue hypoxia and inflammatory cytokines as primary stimuli for the induction of VEGF in a variety of tissues, including the retina. The identification of the mediators that are capable of inducing VEGF expression may provide new insight into the mechanism of hypoxia-independent ocular disease (Fig. 8).

To date, several studies (5, 12, 20, 24) have documented the role of light as an initiator of photoreceptor cell death in vivo and in vitro. Those studies have shown that exposure of animals or retinal tumor-derived cells to constant light resulted in apoptosis of photoreceptor cells. In the current study, we have identified the role of light as a stimulus to induce VEGF expression in the Y79 retinoblastoma cell line. This cell line has been known to express several markers of differentiated photoreceptors, including opsin, arrestin, phosducin, and interphotoreceptor retinol binding protein (7, 21, 30, 34). However, it remains unknown whether light exerts its effects on regulating the expression of the genes whose primary function is relevant to neovascularization in Y79 cells. To the best of our knowledge, this is the first report indicating the induction of VEGF gene expression by light exposure in vitro.

In addition to the pleiotropic effects of RA, such as the regulation of cellular growth and differentiation of the retinal tissue (16, 18, 29, 42), most of the earlier studies on RA in the retinal tissues focused on its role in visual cycles. Among them, a significant observation was that light exposure induces RA synthesis in retinal tissue in vivo (28). Along a similar line, we have recently reported that atRA induces the VEGF gene expression in Y79 cells (1). Thus we tested whether Y79 cells afford a model to analyze the molecular events accounting for some forms of light-induced retinopathy. Here, we specified the role of RA in light-induced VEGF expression by testing the effects of RARα antagonist (LE-135), a RARα antagonist (17), potently inhibited the light-mediated VEGF induction. We also showed that cotransfection of dominant negative mutant of RARα attenuates the light-induced VEGF promoter activity. Thus these data suggest that atRA-activated RARα mediate the light-induced VEGF expression in Y79 cells.

In view of the mechanisms of photoreceptor cell death via apoptosis that characterize retinal dystrophies, previous studies (10, 39) have shown that light exposure creates the condition of
oxidative stress leading to oxidative damage. Although oxidative events are important for a variety of biological processes, such as signal transduction and gene expression, we should emphasize that light-induced VEGF expression is not mainly due to the oxidative stress, because an inhibitor for G protein and antagonist of RARs inhibited the response. In this regard, we propose that light serves as a regulator of gene expression via ligand-activated nuclear receptors.

We have identified RARα as a mediator of the light-induced VEGF expression. In mice disrupted with RARα, the eyes are extremely small with gross morphological defects in choroid, sclera, and retinal dysplasia (13). Furthermore, previous reports (36) indicated that RA produces rod photoreceptor-selective apoptosis in developing mammalian retina. Future studies on the light-induced VEGF expression using the conditional knockout of RARα loci will uncover the role of RARα in the mature retina.

In conclusion, we found that light induces the VEGF gene expression through Sp1-binding sites of the human VEGF promoter. In addition, light increases the levels of Sp1 protein and enhances its binding activity to Sp1 sites within the VEGF promoter. Given that light induces RA synthesis in retinal tissue in vivo, RA may be one of the critical mediators for neovascularization through induction of VEGF expression. Therefore, our findings raise the possibility that pharmacological intervention that inhibits the signals elicited by light or RA may be effective in treating VEGF-mediated retinopathies.

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GRANTS

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