Autocrine effects of IGF-I-induced VEGF and IGFBP-3 secretion in retinal pigment epithelial cell line ARPE-19

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Slomiany, Mark G., and Steven A. Rosenzweig. Autocrine effects of IGF-I-induced VEGF and IGFBP-3 secretion in retinal pigment epithelial cell line ARPE-19. Am J Physiol Cell Physiol 287: C746–C753, 2004.—Hypoxia can induce HIF-1 activity and the secretion of VEGF in RPE cells. To study this mechanism in more detail, we measured VEGF and IGFBP-3 secretion in response to IGF-I stimulation. We confirmed in this study that IGF-I stimulated VEGF and IGFBP-3 secretion, and that the autocrine and paracrine actions of VEGF and IGFBP-3 on these processes in the spontaneously transformed RPE cell line ARPE-19. Cells were treated with CoCl2, IGF-I, recombinant human (rh)IGFBP-3, and rhVEGF. Immunoblot analysis revealed IGF-I-induced upregulation of total HIF-1α protein, whereas luciferase reporter assays of HIF-1 transcriptional activity demonstrated accumulation of HIF-1α correlated with the formation of functional HIF-1 heterodimers. Western and ligand blot analyses of RPE cell-conditioned medium confirmed that IGF-I stimulated VEGF and IGFBP-3 secretion. rhVEGF stimulated IGFBP-3 secretion in an IGF-I- and HIF-1α-independent manner, whereas rhIGFBP-3 attenuated IGF-I-induced VEGF secretion. These findings demonstrate the multifaceted autocrine regulation of IGF-I-induced VEGF secretion by IGFBP-3 secreted in response to both IGF-I and, to a lesser extent, VEGF. These results provide evidence for HIF-1-dependent and -independent mechanisms by which IGF-I regulates VEGF and IGFBP-3 secretion. A number of animal models support a role for increased RPE VEGF secretion in the progression of CNV (9, 27, 50). In addition to elevated VEGF levels in the vitreous (62), the RPE and surrounding subretinal membranes express increased levels of VEGF and its receptor kinase insert domain receptor (KDR)/fetal liver kinase receptor-1 (Flk-1) in CNV (3, 48, 58); these increased levels have been attributed to the cellular hypoxic response (59). A number of factors regulate VEGF production; among them, insulin-like growth factor (IGF)-I has been shown to stimulate VEGF production. Puniglia and coworkers (40) showed that increased serum and vitreous IGF-I levels correlate with a wide variety of ischemic retinal disorders linked to neovascularization of the retina and iris. Examination of dissected postmortem RPE-choroid as well as cultured RPE cell lines has found transcription and cell membrane localization of the IGF-I and IGF-II receptors (34, 38, 53, 54, 59, 63) as well as transcription and secretion of IGF-I and IGF-II (34, 36, 38, 53, 63), along with IGF binding proteins (IGFBPs) 1–6 and the IGFBP-related protein IGFBP-rP1 (36, 38, 53, 63, 64). Because IGFs bind with higher affinity to IGFBPs than to the IGF-I receptor, IGFBPs are capable of acting as antagonists by reducing IGF bioavailability through sequestration (25, 42). Thus the RPE provides the necessary components for a subretinal autocrine-paracrine IGF system capable of modulating retinal function as well as contributing to the pathogenesis of CNV (60, 67). On the basis of a growing body of evidence demonstrating that IGF-I can induce HIF-1 activity and the secretion of VEGF and IGFBP-3 in RPE cells in vivo and in vitro (15–17, 22, 35, 39, 40).
41, 43, 45), we used the spontaneously transformed RPE cell line ARPE-19 (12) to examine the effect of IGF-I on HIF-1α protein expression, VEGF and IGFBP-3 secretion, and the autocrine effects of VEGF and IGFBP-3. Immunoblot analysis revealed IGF-I-induced upregulation of total HIF-1α protein, whereas luciferase reporter assays of HIF-1 transcriptional activity demonstrated accumulation of HIF-1α correlated with the formation of functional HIF-1 heterodimers. In contrast, addition of exogenous VEGF had no significant effect on HIF-1α protein levels in control or IGF-I-stimulated cells. Western and ligand blot analyses of conditioned medium confirmed that IGF-I induced VEGF and IGFBP-3 secretion, recombinant human (rh)VEGF induced IGFBP-3 secretion, and rhIGFBP-3 attenuated IGF-I-stimulated VEGF release. These findings demonstrate that, as seen for VEGF, IGF-I-induced stimulation of IGFBP-3 secretion in RPE cells correlates with increased HIF-1α expression and nuclear localization. We have also identified a unique autocrine function of VEGF in inducing the secretion of IGFBP-3 in control and IGF-I-stimulated ARPE-19 cells without affecting HIF-1 protein expression. Finally, our study demonstrates the negative-feedback role of IGFBP-3 in sequestering and thereby attenuating IGF-I-induced VEGF secretion.

EXPERIMENTAL PROCEDURES

Materials and reagents. ARPE-19 cells were obtained from the American Type Culture Collection (Manassas, VA). Fetal bovine serum (FBS) was purchased from Atlas Biologicals (Fort Collins, CO). DMEM was purchased from Sigma (St. Louis, MO). IGF-I and rhVEGF (bacterial origin) were generously provided by Genentech (San Francisco, CA). rhIGFBP-3 (N109D, bacterial origin) was obtained from Upstate (Lake Placid, NY). CoCl2 was from Fisher Scientific (Fair Lawn, NJ). HIF-1α monoclonal antibody was obtained from Transduction Laboratories (Lexington, KY). HIF-1α monoclonal antibody from Novus (Littleton, CO), and β-actin polyclonal antibody from Sigma. VEGF polyclonal antibody and horseradish peroxidase (HRP) were purchased from Chemicon (Temecula, CA). Neutrinovin-GRF and bicornichonic acid (BCA) reagent were obtained from Pierce (Rockford, IL). Tunicamycin was purchased from Calbiochem (San Diego, CA). ECL reagent was obtained from Amersham Biosciences (Clearbrook, IL) and Biomax film from Kodak (Rochester, NY). Fugene 6 was obtained from Roche. The Dual-Luciferase Reporter Assay System was purchased from Promega. p2.1 was a generous gift from the laboratory of Dr. Gregg L. Semenza of the Johns Hopkins University School of Medicine (Baltimore, MD), and SV40 Renilla, obtained from Promega, was provided by Dr. D. T. Kurtz, Medical University of South Carolina. All other chemicals were of reagent grade or higher.

Tissue culture. ARPE-19 cells were incubated in a 1-to-1 ratio of Dulbecco’s modified Eagle’s medium Base D-5030 and Nutrient Mixture F-12 (Ham) N-6760 with 10% FBS and 10 μl/ml penicillin-streptomycin solution. Unless otherwise stated, cells were maintained at 37°C in a humidified 5% CO2-95% air incubator.

IGF-I, IGFBP-3, CoCl2, tunicamycin, and VEGF treatments. ARPE-19 cells were seeded at a density of 8.6 x 10^4/well in six-well (9.6-cm² area) plates. Confluent cells were serum starved (FBS was eliminated in all experiments) for 24 h, to remove known stimulatory growth factors (including IGF-I), before the indicated treatment in fresh, serum-free, medium.

Immunoblot and ligand blot analysis. Confluent serum-starved cells were treated with IGF-I or CoCl2 as indicated, and whole cell lysates were prepared with a modified RIPA buffer containing (in mM) 50 Tris-HCl pH 7.4, 150 NaCl, 10 EDTA, 1 PMSE, 2 sodium orthovanadate, and 10 NaF with 1% Triton X-100 and 10 μg/ml aprotinin and leupeptin. Protein content was determined by BCA assay (Pierce), and 100-μg aliquots were solubilized in SDS sample buffer. VEGF and IGFBP-3 in conditioned medium were quantified after precipitation in 10% trichloroacetic acid (TCA), washing of the pellet with acetone, and solubilization in SDS sample buffer. Proteins so collected were resolved on 12.5% nonreducing polyacrylamide gels, transferred to nitrocellulose (Osmonics, Westborough MA) with a TE-70 Semidisc apparatus (Hoefer Scientific Instruments, San Francisco, CA), and subjected to ligand or immunoblot analysis. For ligand blot analysis, protein-containing nitrocellulose membranes were washed for 10 min at 23°C in Tris-buffered saline (TBS) containing 3% Triton X-100 and blocked for 1 h with TBS containing 0.2% gelatin. Blots were probed overnight at 4°C with 10 ng/ml tetrahydroxylated IGF-I (Robinson SA and Rosenzweig SA, unpublished data), followed by a 2-h incubation at 23°C with 200 ng/ml Neutrinovin-HRP in TBS containing 0.1% Tween 20 and 0.1% BSA. Blots were visualized with the ECL reagent (Amersham Biosciences) on Biomax film (Kodak). Films were subsequently digitized to.tif format, and band intensity was quantified with NIH Image, version II.

For immunoblots, nitrocellulose membranes were blocked for 1 h in bovine lacto fetin technique optimier (BLOTTO), a TBS solution containing 0.1% Tween 20 and 5% milk protein (reviewed in Ref. 51), before being probed with 1 μg/ml VEGF polyclonal antibody or 1 μg/ml HIF-1α monoclonal antibody, 1 μg/ml HIF-1β monoclonal antibody, or 1:10,000 β-actin monoclonal antibody in BLOTTO. HRP-linked secondary antibodies diluted 1:5,000 in BLOTTO were subsequently added for 2 h. To reprobe HIF-1α immunoblots for HIF-1β or β-actin levels, antibodies were removed from the nitrocellulose via the application of Chemicon light stripping solution according to the manufacturer’s instructions. Blots were visualized with the ECL reagent as described above.

Luciferase assays. To assay the transcriptional activity of HIF-1, we used the pGL2 basic p2.1 enolase 1 (EN01) promoter vector, which contains a 68-bp EN01 promoter fragment encompassing a HIF-1 binding site downstream from the luciferase gene (46). Each well of subconfluent ARPE-19 cells was transiently cotransfected with 100 ng of reporter plasmid and 50 ng of pRL-SV40 Renilla as a control for transfection efficiency. After 24 h, cells were treated with 100 nM IGF-I or 100 μM CoCl2 in 500 μl/well fresh serum-free medium. After an 18-h incubation, cells were lysed in 100 μl/well passive lysis buffer provided with the Dual-Luciferase Reporter Assay System. Cells were scraped and centrifuged for 10 min at 18,890 g, and 20 μl of supernatant per sample was loaded on a 96-well plate and processed for luciferase activity on the Victor2 1420 Multilabel Counter (PerkinElmer Life Sciences, Downers Grove, IL) with the firefly and Renilla luciferase buffers provided with the Dual-Luciferase kit.

RESULTS

HIF-1α expression and activity. To examine the effect of IGF-I on HIF-1α protein expression, serum-starved ARPE-19 cells were exposed to a stimulatory dose of IGF-I (100 nM) for 4 h. This treatment resulted in increased HIF-1α protein levels based on immunoblot analysis (Fig. 1A) and normalization to HIF-1β control levels (Fig. 1, B and C). As a positive control, cells were exposed to 100 μM CoCl2, a chemical hypoxia-inducing agent that abrogates HIF-1α degradation (14). The blots were stripped and reprobed for HIF-1β, the constitutively expressed HIF-1α binding partner present in the HIF-1 heterodimer (Fig. 1B). As expected, neither IGF-I nor CoCl2 induced significant changes in HIF-1β expression in ARPE-19 cells, demonstrating a specific effect of these agents on HIF-1α expression. To establish the connection between HIF-1α protein expression and functional HIF-1 heterodimer formation, transcriptional activity was analyzed (Fig. 1D). ARPE-19 cells
were cotransfected with the p2.1 HIF-1 reporter plasmid and the pRL-SV40 control plasmid and subjected to the conditions described above for 18 h. As expected, IGF-I- and CoCl₂-stimulated increases in HIF-1 transcriptional activity were consistent with increases in HIF-1α protein expression.

To determine the temporal parameters of IGF-I-induced HIF-1α protein expression, cells were stimulated with 100 nM IGF-I over a 24-h time course. HIF-1α expression peaked between 4 and 6 h of stimulation and declined thereafter (Fig. 2). HIF-1α levels increased again at 24 h, although this change was not statistically significant (P > 0.05) compared with time 0. Treatment of serum-starved ARPE-19 cells with IGF-I for 4 h resulted in a dose-dependent increase in HIF-1α protein levels, with the maximal effect occurring at 10 nM. This increase was not significantly (P > 0.05) greater than the effect of 100 nM IGF-I (Fig. 3).

VEGF and IGFBP-3 secretion. To examine VEGF secretion, serum-starved ARPE-19 cells were incubated in the presence or absence of 100 nM IGF-I for 1–24 h. As shown in Fig. 4A, immunoblot analysis of conditioned medium revealed the presence of a VEGF doublet of ~42 and 44 kDa. These represent VEGF homodimers that are singly and doubly glycosylated at each of the single consensus N-linked glycosylation sites per monomer. Time course studies indicated that VEGF accumulation exhibited a lag phase of ~4 h, with a significant level of growth factor accumulation in the medium appearing thereafter (Fig. 4B).

![Fig. 2. Time course of IGF-I-induced HIF-1α protein expression in ARPE-19 cells. Top: duplicate dishes of confluent ARPE-19 cells were serum starved for 24 h before treatment with 100 nM IGF-I in fresh serum-free medium for an additional 0–24 h. At the times indicated, cells were lysed and protein contents were determined. Lysates (100 μg) were solubilized in SDS sample buffer and analyzed for HIF-1α and β-actin as described in Fig. 1. This blot is representative of 3 experiments. Bottom: densitometric normalization of relative HIF-1α intensity to β-actin control for each treatment. Error bars represent SD between duplicate wells. Significant differences in HIF-1α protein expression were observed (*P < 0.075, **P < 0.05).](http://ajpcell.physiology.org/)

![Fig. 3. Dose-dependent effect of IGF-I on HIF-1α protein expression in ARPE-19 cells. Top: duplicate dishes of confluent ARPE-19 cells were serum starved for 24 h before treatment with a battery of IGF-I doses (10 pM-1 μM) in fresh serum-free medium. After 4 h of incubation, cell lysates were analyzed for HIF-1α and β-actin as described in Fig. 1. This blot is representative of 3 experiments. Bottom: densitometric normalization of relative HIF-1α intensity to β-actin control for each treatment. Error bars represent SD between duplicate wells. Significant differences in HIF-1α protein expression were observed (*P < 0.05, **P < 0.005).](http://ajpcell.physiology.org/)
IGF-I was shown previously to stimulate the secretion of IGFBP-3 in various retinal cells including RPE cells (36, 41, 49). Thus we next examined IGFBP-3 secretion by serum-starved ARPE-19 cells incubated in the absence or presence of 100 nM IGF-I for 1–24 h. As shown in Fig. 5A, four bands ranging in size from ~28 to ~45 kDa were identified in conditioned medium by ligand blot analysis. Immunoblot analysis confirmed that these bands were all IGFBP-3 related (not shown). IGFBP-3 contains three consensus sites for N-linked glycosylation, explaining the observed banding pattern. To confirm this, ARPE-19 cells were incubated with tunicamycin, an inhibitor of N-linked sugar transfer from dolichol precursors (55). As shown in Fig. 5A, tunicamycin treatment of unstimulated or IGF-I-stimulated cells after a 2-h tunicamycin pretreatment resulted in the expression of a single species of IGFBP-3 in conditioned medium that comigrated with recombinant nonglycosylated IGFBP-3. Bands representing mono-, di-, and triglycosylated IGFBP-3 were absent. IGFBP-1, -2, -4, -5, and -6 were not detected by ligand or immunoblot. Yang and Chaum (64) similarly observed IGFBP-1, -2, and -4 to be nearly absent, whereas expression of IGFBP-3, -5, and -6 tended to vary in an RPE cell line-dependent manner. Significantly, we have observed similar profiles for the secretion of VEGF and IGFBP-3, along with the absence of other IGFBPs, in the RPE cell line D407 (49). Bands representing non-, mono-, di-, and triglycosylated IGFBP-3 were all included when quantifying IGFBP-3 secretion. As shown in Fig. 5B, secreted IGFBP-3 was not detectable in the medium for 4 h of incubation. From that point, accumulation in the medium was approximately linear. IGF-I addition significantly increased this level of IGFBP-3 secretion over that in unstimulated cells.

As shown in Fig. 6, stimulation of ARPE-19 cells with a battery of IGF-I doses for 12 h caused a dose-dependent increase in VEGF secretion. Maximal secretion was obtained with 10 nM IGF-I, although statistically insignificant (P > 0.05) from the effect at 100 nM. Secretion at 100 nM, the IGF-I dose used in all time course studies, represents a 17-fold induction of VEGF secretion over control. Similar to VEGF, IGF-I stimulated a dose-dependent increase in IGFBP-3 secretion (Fig. 7). Again, maximal secretion was obtained with 10 nM IGF-I, although statistically insignificant (P > 0.05) from the effect at 100 nM. Secretion at 100 nM IGF-I elicited a 30-fold increase in IGFBP-3 secretion over control.

Autocrine relationship between VEGF and IGFBP-3. To examine whether VEGF secretion by ARPE-19 cells affects IGFBP-3 release, we added rVEGF at two doses in an IGF-I dose-response assay. As illustrated in Fig. 8A, coaddition of 1
or 10 ng/ml rhVEGF in the presence or absence of IGF-I to ARPE-19 cells had no significant effect on HIF-1α protein levels. In contrast, coaddition of 1 ng/ml rhVEGF to cells treated with 0.5 or 10 nM IGF-I led to increases in IGFBP-3 accumulation that were greater than each dose of IGF-I alone (Fig. 8B). A 10-fold increase in VEGF concentration had no additional effect on IGFBP-3 secretion.

Treatment of ARPE-19 cells with rhIGFBP-3 alone had no effect on VEGF secretion. However, coaddition of rhIGFBP-3 with IGF-I resulted in a dose-dependent decrease in IGF-I-stimulated VEGF secretion (Fig. 9). IGF-I-stimulated VEGF secretion was reduced to control levels with 10 nM rhIGFBP-3 in cells treated with 0.5 nM IGF-I, whereas 100 nM IGFBP-3 was required in cells treated with 10 nM IGF-I. Addition of 10 nM rhIGFBP-3 to cells treated with 10 nM IGF-I led to an ~60% reduction in VEGF secretion, approximately equivalent to the secretory response observed with the 0.5 nM IGF-I dose alone.

**DISCUSSION**

IGF-I stimulated a time- and dose-dependent increase in HIF-1α protein, the regulated member of the HIF-1 heterodimer. Luciferase reporter assays of HIF-1 transcriptional activity demonstrated accumulation of HIF-1α correlated with the formation of functional, HRE-binding, HIF-1 heterodimers. Interestingly, time courses revealed IGF-I stimulation of HIF-1α peaking at 4–6 h, with a second increase in HIF-1α sporadically occurring at 24 h. Although statistically insignificant, the potential autocrine effect of IGF-I-induced secretion and accumulation of cytokines and/or growth factors in the conditioned medium on the expression of HIF-1α protein has yet to be examined.

Although the signaling cascade leading to IGF-I-induced HIF-1α expression is still intensely debated, it is well established that the VEGF promoter contains HREs, activation of which results from the binding of HIF-1α (reviewed in Ref. 45). Similarly, a connection, although tenuous, has been established between HIF-1 activity and IGFBP-3 protein expression. Work by Feldser and colleagues (16) demonstrated that although the IGFBP-3 promoter lacks an obvious HRE, IGFBP-3 gene expression was markedly reduced in HIF-1α-deficient cells
HIF-1 responds to VEGF. Whereas rhVEGF addition did not alter (8, 21). Accordingly, we examined whether ARPE-19 cells virus-like tyrosine receptor-1 (Flt-1), colocalize to RPE cells VEGF and its receptors, including Flk-1 and feline sarcoma progression of CNV in animal models (4, 24, 27, 50, 62).

in VEGF and IGFBP-3, respectively.

and Punglia et al. (40) demonstrating IGF-I-induced increases secretion in a time- and dose-dependent manner. Together, results demonstrate that IGF-I stimulates VEGF and IGFBP-3 mechanisms by which HIF-1 levels are increased, the present

there is still controversy in the literature as to whether IGF-I level of regulation, possibly an indirect effect. In addition, additional findings extend the initial work of Randolph et al. (41) and Punglia et al. (40) demonstrating IGF-I-induced increases in VEGF and IGFBP-3, respectively.

Elevated subretinal levels of VEGF can act to trigger the progression of CNV in animal models (4, 24, 27, 50, 62). VEGF and its receptors, including Flk-1 and feline sarcoma virus-like tyrosine receptor-1 (Flt-1), colocalize to RPE cells (8, 21). Accordingly, we examined whether ARPE-19 cells respond to VEGF. Whereas rhVEGF addition did not alter HIF-1α expression, it did stimulate secretion of IGFBP-3, suggesting that VEGF may regulate RPE cell function in an autocrine manner.

Low oxygen tension (1–2%) significantly promotes angiogenesis by stimulating VEGF secretion and the upregulation of KDR (7, 61). These conditions also promote the formation of oxygen radicals through a mechanism involving the electron transport chain. It has been reported that reactive oxygen species (ROS) increase the DNA binding activity of HIF-1α (7). It is tempting to speculate that VEGF stimulation of ROS leads to greater HIF-1 binding to the HRE in the IGFBP-3 promoter, leading to increased expression of IGFBP-3. This may serve to explain the observed VEGF stimulation of IGFBP-3 secretion in the absence of detectable alterations in HIF-1α expression.

IGFBP-3 release by RPE cells may have important implications in the regulation of IGF-I/IGF-II autocrine and/or paracrine functions at the RPE and photoreceptor layers, given that IGF action may be inhibited (42) or enhanced (5, 13) by IGFBP-3. Our results demonstrate that IGF-I stimulates IGFBP-3 secretion in a time- and dose-dependent manner. Furthermore, IGF-I-induced VEGF secretion was attenuated by rhIGFBP-3 addition. In light of the fact that IGF-I upregulates the secretion of IGFBP-3 and VEGF in ARPE-19 cells and that their secretion occurs at the apical pole in polarized RPE cells (33, 49), the ability of IGFBP-3 to reduce the bioavailability of IGF-I may play a major role in modulating VEGF secretion by RPE cells in the subretinal space (25, 33, 49). As such, fluctuations in IGF-I, IGF-II, or IGFBPs may have significant implications on RPE cell proliferation and migration after choroidal capillary invasion and the subsequent leakage of circulatory IGFs from choroidal vessels (41, 48, 52, 58, 66). Consequently, dysregulation of the IGF-I system at the level of the subretina may contribute to changes in RPE morphology and increases in angiogenic factor secretion, consistent with CNV.

In summary, we have shown that IGF-I stimulates the expression of HIF-1α and the formation of functional HIF-1 dimers as well as the secretion of VEGF and IGFBP-3 in a time- and dose-dependent manner. In contrast, VEGF enhances the secretion of IGFBP-3 both in the absence and presence of IGF-I without affecting HIF-1α protein expression. Although it had no effect alone, IGFBP-3 attenuated IGF-I-induced VEGF secretion to control levels when present in 10-fold molar excess of exogenously added IGF-I. Together, these results provide further evidence for a role of an IGF-I autocrine/paracrine system in the retina, both in terms of normal ocular physiology as well as in the progression of CNV. The ability of rhVEGF to enhance IGFBP-3 expression, which in turn attenuates IGF-I-stimulated VEGF secretion, constitutes a novel negative autocrine loop regulating this potent angiogenic factor. Furthermore, the ability of rhIGFBP-3 to attenuate IGF-I stimulation of VEGF to constitutive levels presents a tempting avenue in the development of peptide mimetics that retain the IGF-I antagonistic properties of IGFBP-3. Such antagonists may be helpful in the treatment of a wide variety of ischemic retinal disorders linked to neovascularization of the retina and iris where serum and vitreous IGF-I levels are elevated (40). Although HIF-1α is primarily maintained at low levels under normoxic conditions by a degradation process involving the ubiquitin-proteasome system, several cytokines have been found to increase HIF-1 activity (17, 22, 35, 43, 45, 57). Van Obbergen and colleagues (57) reported that insulin stimulates HIF-1α translation via a phosphatidylinositol 3-kinase (PI3-kinase)-dependent signaling pathway in ARPE-19 cells. They also reported that insulin and IGF-I stimulate VEGF expression via different signaling pathways in NIH 3T3 cells (35). Whereas insulin stimulates PI 3-kinase/protein kinase B (PKB), induction by IGF-I involves ERK/mitogen-activated protein kinase (MAPK). In contrast, Semenza and colleagues (17) reported that IGF-I induces HIF-1α synthesis through both PI 3-kinase and MAPK pathways in HCT116 human colon cancer cells. We propose to carry out studies designed to elucidate the roles of reduced oxygen tension and retinal cytokines on HIF-1α expression and VEGF and IGFBP-3 secretion in the RPE and their influence on the progression of CNV. Studies at the cellular level will provide important insights into the mechanisms underlying the pathologies observed in the animal models of CNV. This will lead to a better understanding of the pathogenesis of this disease and to better treatments for this leading cause of blindness.

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