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Novel Therapeutic Targets in Ocular Neovascular Diseases: From Extracellular Matrix Remodeling to Autophagy Modulation

Md Shahariar Islam[1], Mohammadi Matin[2], Romee Paul[3], Siddhant Pandey[4]
[1]Researcher, Far Eastern Federal University, Vladivostok, Russia
[2]Physician, Far Eastern Federal University, Vladivostok, Russia
[3]General Medicine student, Far Eastern Federal University, Vladivostok, Russia
[4]General Medicine student, Far Eastern Federal University, Vladivostok, Russia
Email address: dr_s_islam@yahoo.com

Abstract*—Purpose**: *Ocular neovascular diseases, including proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP), and neovascular glaucoma (NVG), remain leading causes of vision loss despite anti-VEGF therapies. This review synthesizes emerging evidence on non-canonical pathogenic mechanisms and novel therapeutic targets beyond VEGF signaling.* ***Methods: *We review recent preclinical studies examining the urokinase plasminogen activator receptor (uPAR) system in iris neovascularization and autophagic mechanisms in retinal neurodegeneration, using ex vivo human models, in vivo mouse models of rubeosis iridis, and rat models of oxygen-induced retinopathy.* ***Results***: *The uPAR antagonist UPARANT demonstrated superior efficacy compared to anti-VEGF therapy in mitigating iris neovascularization through multifactorial mechanisms involving extracellular matrix remodeling, inflammation modulation, and novel uPAR/LRP-1 interaction inhibition. In retinal development and disease, autophagy plays a dual role: supporting physiological vascularization during hypoxic developmental phases, but contributing to neurodegeneration when dysregulated in pathological conditions. Autophagy inhibition with 3-methyladenine reduced necroptosis in OIR retinas, revealing autophagy-necroptosis crosstalk as a pathogenic mechanism.* ***Conclusions***: *Targeting the uPAR system offers advantages over VEGF-exclusive approaches by addressing multiple angiogenic pathways simultaneously and demonstrating efficacy via systemic administration. Modulating autophagy represents a promising neuroprotective strategy for retinal diseases. These findings support a paradigm shift toward multifactorial therapeutic approaches addressing both vascular and neurodegenerative components of ocular diseases, with potential for improved clinical outcomes in patients with PDR, ROP, and NVG.*

***Keywords*—** *Ocular neovascularization; uPAR; UPARANT; autophagy; retinopathy of prematurity; diabetic retinopathy; rubeosis iridis; neuroprotection.*

I. Introduction

Ocular neovascular diseases affect approximately 3.9 million people worldwide and represent a leading cause of vision impairment and blindness. Proliferative diabetic retinopathy (PDR), retinopathy of prematurity (ROP), and neovascular glaucoma (NVG) share common pathogenic mechanisms characterized by aberrant blood vessel formation in response to hypoxia and ischemia. While anti-vascular endothelial growth factor (VEGF) therapies have revolutionized treatment paradigms, significant limitations persist, including variable treatment response, need for frequent intravitreal injections, and potential systemic complications.

Beyond the visible vascular pathology, these diseases involve complex neurodegenerative processes affecting retinal neurons, contributing to irreversible visual dysfunction even after successful vascular treatment. The relationship between microvascular abnormalities and neuronal cell death remains incompletely understood, highlighting critical gaps in our therapeutic approach.

Recent evidence suggests that ocular neovascularization involves multiple non-VEGF pathways, including extracellular matrix (ECM) remodeling mediated by the urokinase plasminogen activator (uPA)/uPA receptor (uPAR) system, and cellular stress responses such as autophagy. The uPAR system, through its interactome with formyl peptide receptors (FPRs), integrins, and other transmembrane receptors, regulates endothelial cell migration, proliferation, and inflammatory responses. Meanwhile, autophagy serves as a critical homeostatic mechanism that, when dysregulated, can trigger cell death pathways contributing to retinal neurodegeneration.

This review synthesizes emerging preclinical evidence examining two novel therapeutic targets: (1) the uPAR system in iris and retinal neovascularization, and (2) autophagic mechanisms in retinal development and pathology. We present data from complementary model systems including ex vivo human iris organotypic cultures, in vivo mouse models of rubeosis iridis associated with proliferative retinopathy (RI-PR), and rat models of oxygen-induced retinopathy (OIR). Our findings support a paradigm shift toward multifactorial therapeutic strategies addressing both angiogenic and neurodegenerative components of ocular disease.

II. The uPAR System as a Multifactorial Angiogenic Target

2.1 uPAR Biology and Ocular Neovascularization

The urokinase plasminogen activator receptor (uPAR) is a glycosylphosphatidylinositol (GPI)-anchored protein that plays a pivotal role in ECM remodeling, cell migration, and angiogenesis. uPAR lacks intrinsic signaling capacity but functions through lateral interactions with transmembrane co-

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receptors including integrins (particularly α5β1), formyl peptide receptors (FPR1, FPR2), epidermal growth factor receptor (EGFR), and low-density lipoprotein receptor-related protein-1 (LRP-1). This multiprotein signaling complex coordinates diverse cellular processes essential for angiogenesis.

In ocular tissues, uPAR expression increases under hypoxic and inflammatory conditions. While VEGF-driven angiogenesis has been extensively studied, the uPAR system represents a complementary pathway that mediates ECM degradation, endothelial cell invasion, and inflammatory cell recruitment—processes inadequately addressed by anti-VEGF monotherapy.

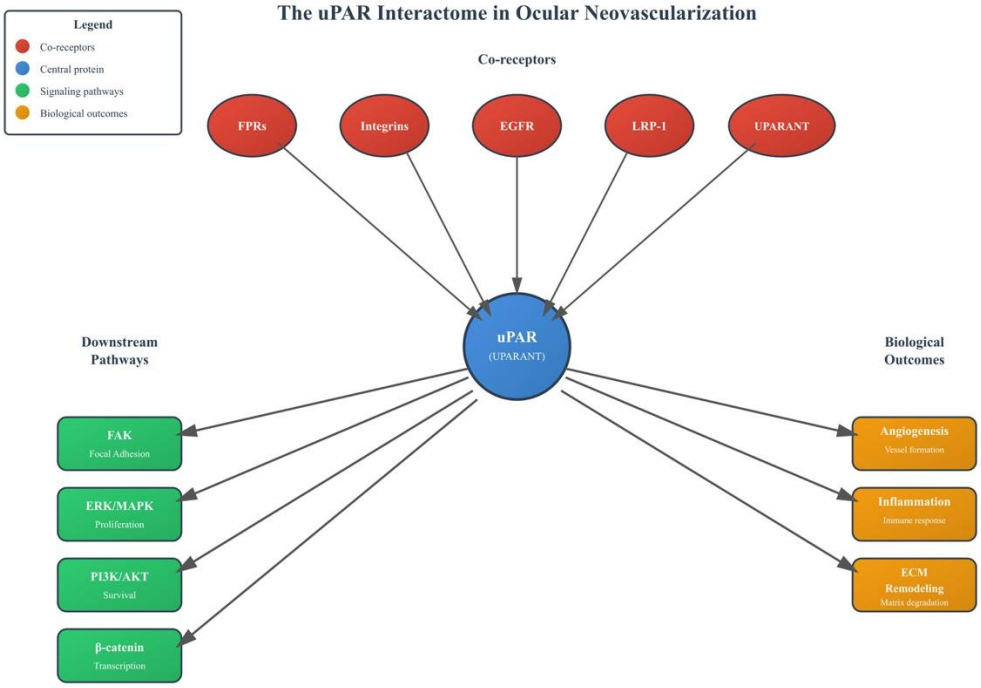

Figure 1. The uPAR Interactome in Ocular Neovascularization

TABLE 1. Comparison of Anti-VEGF vs. UPARANT Mechanisms and Effects

Feature	Anti-VEGF Therapy	UPARANT
Primary Target	VEGF/VEGFR signaling exclusively	uPAR system (multifactorial)
Angiogenic pathways	VEGF-dependent only	VEGF-independent: ECM remodeling, inflammation, cell migration
Transcriptional effects	Limited to VEGF signaling	HIF-1α, NFκB, STAT3, β-catenin
Anti-inflammatory Activity	Indirect	Direct (via FPR antagonism)
ECM Modulation	None	Reduces MMP2/9, uPA
Administration Route (preclinical)	Intravitreal only	Intravitreal or systemic
Neuroprotection Potential	May be neurotoxic (VEGF depletion)	Potential via anti-inflammatory effects
Efficacy in Iris Neovascularization	Moderate, delayed	Superior, rapid
Novel Mechanism	N/A	uPAR/LRP-1 complex disruption

Schematic representation showing uPAR interactions with multiple co-receptors (FPRs, integrins, EGFR, LRP-1) and downstream signaling pathways (FAK, ERK/MAPK, PI3K/AKT, β-catenin) involved in angiogenesis, inflammation, and ECM remodeling. UPARANT antagonism blocks these interactions, providing multifactorial inhibition of neovascularization.

2.2 UPARANT: A Novel uPAR Antagonist

UPARANT is a synthetic peptide derived from the uPAR sequence (amino acids 88-92) that acts as a competitive antagonist at the uPAR binding site for both uPA and FPRs. Unlike anti-VEGF agents that exclusively target VEGF-driven signaling, UPARANT modulates multiple angiogenic pathways through uPAR antagonism.

2.3 UPARANT Efficacy in Human Ex Vivo Iris Angiogenesis

To investigate UPARANT's effects in a physiologically relevant human model, we developed a novel ex vivo human iris organotypic culture system. Under hypoxia-stimulated conditions (1% O_2), human iris explants embedded in extracellular matrix formed capillary sprouts expressing VEGFR2 and PECAM-1, confirming active sprouting angiogenesis.

Key Findings:

Cellular Specificity: In vitro assays revealed that UPARANT potently inhibited human retinal endothelial cell (hREC) sprouting but did not affect human iris epithelial cell (hIEC) migration. This differential effect correlated with expression patterns: while uPAR and its ligands (uPA, PAI-1)

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were expressed in both cell types, the canonical uPAR co-receptors FPR2 and FPR3 were detectable only in hRECs. Immunofluorescence confirmed predominant uPAR and FPR expression in human iris vasculature.

Superior Angiogenic Inhibition: UPARANT treatment reduced ex vivo iris angiogenic sprout area more effectively than the clinically-used anti-VEGF agent aflibercept, despite equivalent hypoxic stimulus.

Transcriptional Regulation: Western blot analysis demonstrated that UPARANT decreased HIF-1α protein levels and phosphorylation of pro-inflammatory transcription factors (NFκB, STAT3) compared to both vehicle and aflibercept-treated irises, indicating modulation of both hypoxia and inflammatory pathways.

Phospho-Proteome Profiling: Comprehensive phospho-proteome array analysis revealed that UPARANT decreased phosphorylation of multiple angiogenesis-associated proteins including EGFR, FAK, Src, ERK1/2, and notably β-catenin—suggesting interference with canonical and non-canonical angiogenic pathways.

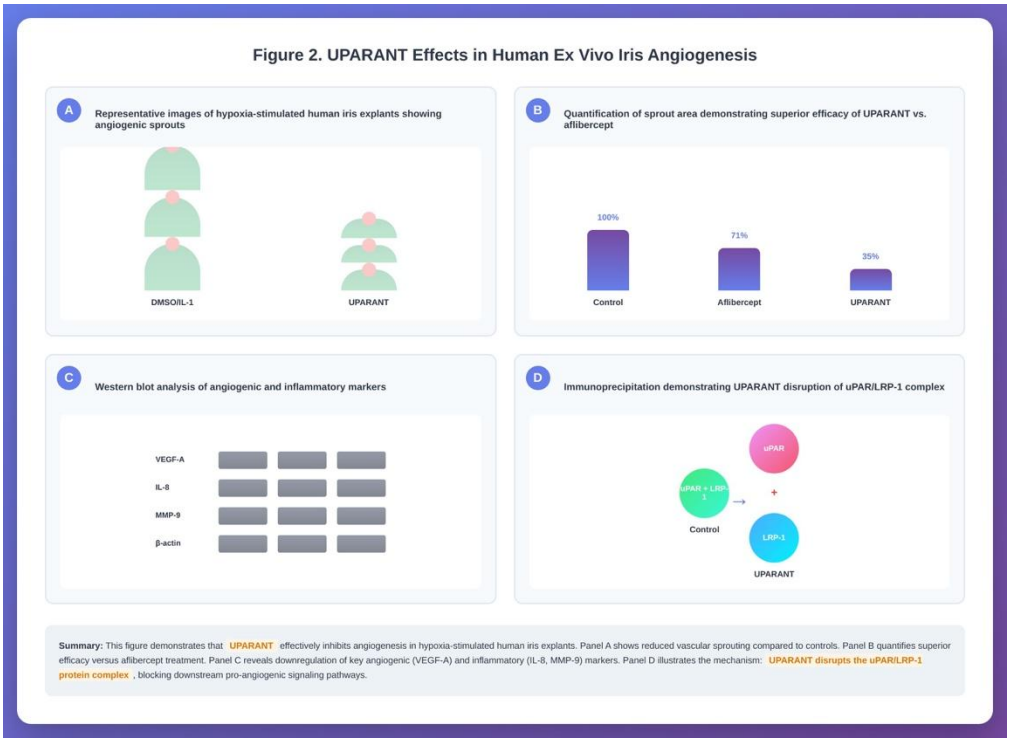

Figure 2

2.4 Novel Mechanism: UPARANT Inhibition of uPAR/LRP-1 Interaction

A previously unrecognized mechanism emerged from our ex vivo studies: UPARANT interfered with the interaction between uPAR and LRP-1. Immunoprecipitation experiments confirmed a physical association between uPAR and LRP-1 in hypoxia-stimulated human iris tissue. UPARANT treatment disrupted this complex without affecting total protein levels of either uPAR or LRP-1. The uPAR/LRP-1 interaction has been implicated in β-catenin-mediated cell motility and angiogenesis. PAI-1, when cleaved by proteases, can simultaneously bind uPA and LRP-1, inducing cellular migration through β-catenin activation and modulating LRP-1 lipid raft partitioning. Our data indicate that UPARANT antagonism of the uPAR/LRP-1 complex inhibits β-catenin signaling, providing a VEGF-independent mechanism for suppressing angiogenesis. Confocal microscopy confirmed colocalization of uPAR and LRP-1 in human iris vascular structures, with uPA and PAI-1 expression in iris epithelial cells suggesting a paracrine mechanism whereby epithelial-derived ligands activate endothelial uPAR/LRP-1 signaling to promote neovascularization.

2.5 In Vivo Efficacy in Rubeosis Iridis Associated with Proliferative Retinopathy

To translate ex vivo findings to an in vivo context, we developed a murine model of puncture-induced rubeosis iridis associated with proliferative retinopathy (RI-PR). This model combines mechanical iris puncture with intravitreal injection of hypoxia-exposed retinal pigment epithelium (RPE) culture media containing multiple pro-angiogenic factors, mimicking the complex molecular milieu present in advanced PDR.

Intravitreal Administration:

RI-PR induction increased iris vasculature by approximately 35% compared to controls. Intravitreal UPARANT rapidly reduced macrovascular density as assessed by non-invasive iris imaging, with effects observable earlier than anti-VEGF treatment (VEGFR1 chimera). Microvasculature analysis via PECAM-1 immunofluorescence demonstrated that UPARANT-treated eyes were indistinguishable from controls regarding vessel number,

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sprouting, and branching—metrics that remained partially elevated in anti-VEGF-treated eyes.

Molecular Mechanisms:

Gene expression and protein analyses revealed that UPARANT effectively downregulated:

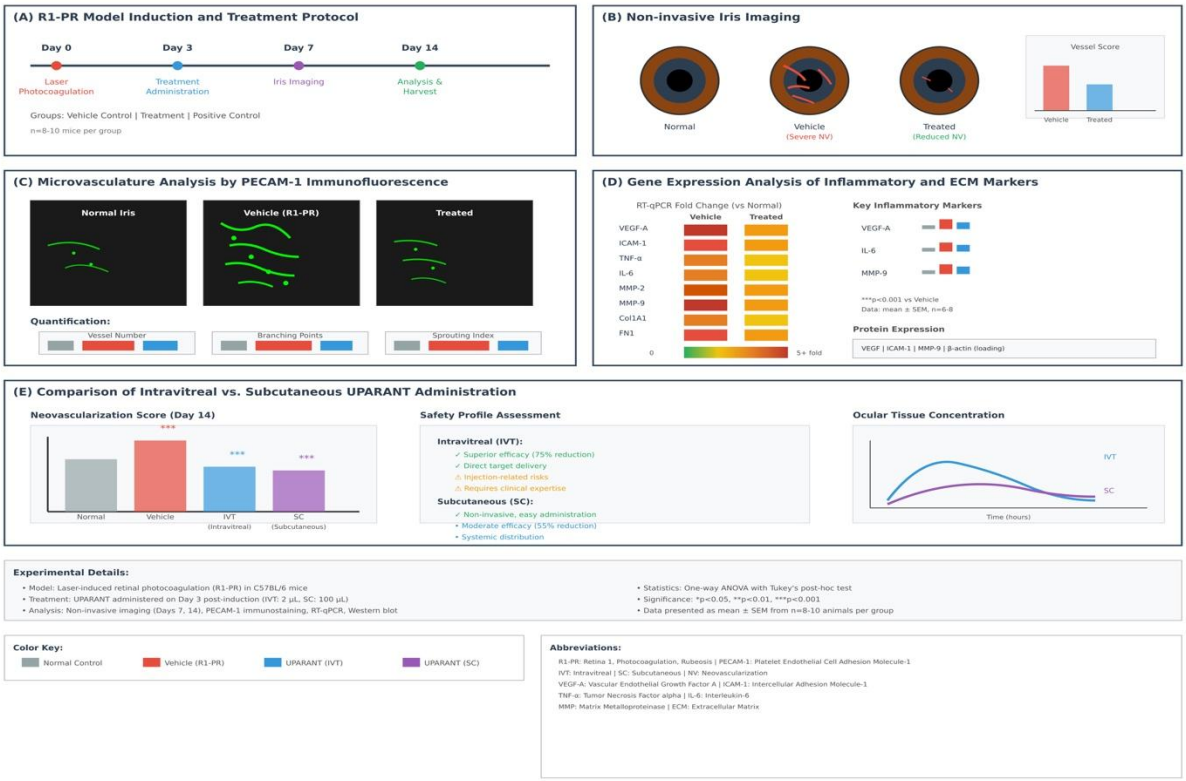
- Inflammatory transcripts (Il6, Tnfα, Ccl2)
- ECM degradation markers (Mmp2, Mmp9, uPA)
- Phosphorylated NFκB

Notably, canonical hypoxia markers (HIF-1α, VEGF-A) were not significantly elevated in the RI-PR model, and UPARANT did not modulate these pathways—supporting the concept that in mouse iris neovascularization, inflammation and ECM remodeling predominate over hypoxia-driven mechanisms, even in the presence of exogenous pro-angiogenic factors.

Systemic Administration:

Given that systemic UPARANT achieves pharmacological levels in ocular tissues, we evaluated subcutaneous administration in the RI-PR model. Systemic UPARANT mitigated neovascularization comparably to local administration, reducing blood vessel density, ECM remodeling markers, and inflammatory transcripts to control levels while downregulating FPR1 expression. This finding has significant clinical implications: systemic administration could benefit patients with bilateral disease or provide prophylactic treatment for the fellow eye in asymmetric presentations of PDR or NVG, eliminating risks associated with repeated intravitreal injections.

Figure 3. In Vivo Efficacy in Mouse Model of Rubeosis Iridis



2.6 Clinical Implications of uPAR Targeting

The superiority of UPARANT over anti-VEGF therapy in preclinical models stems from its multifactorial mechanism:

1. Simultaneous pathway inhibition: UPARANT modulates inflammation (via FPR antagonism), ECM degradation (via uPA inhibition), and cell migration (via integrin and LRP-1 interactions)—processes that continue despite VEGF blockade.
2. Upstream regulation: By interfering with uPAR signaling, UPARANT affects transcriptional programs that regulate multiple growth factors and cytokines, not exclusively VEGF.
3. Systemic delivery potential: Effective subcutaneous administration could improve patient compliance, reduce injection-related complications (endophthalmitis, retinal detachment, increased intraocular pressure), and enable treatment of bilateral disease.
4. Neuroprotection potential: Unlike anti-VEGF agents, which may have neurotoxic effects by depleting VEGF's neuroprotective actions, UPARANT's anti-inflammatory effects could preserve neuronal function.

III. Autophagy In Retinal Development And Disease

3.1 Autophagy: A Double-Edged Sword in the Retina

Macroautophagy (hereafter autophagy) is an evolutionarily conserved catabolic process involving sequestration of cytoplasmic contents within double-membrane autophagosomes, fusion with lysosomes, and degradation of cargo to recycle macromolecules. In the metabolically

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demanding retina, autophagy serves critical homeostatic functions including organelle turnover, protein quality control, and bioenergetic adaptation to stress. However, autophagy exhibits context-dependent effects: physiological autophagy supports cellular adaptation and survival, while dysregulated autophagy can contribute to cell death. This duality is particularly relevant in retinal diseases where autophagy upregulation may represent either an adaptive response to ischemia/hypoxia or a pathogenic mechanism exacerbating neurodegeneration.

3.2 Autophagy in Postnatal Retinal Development

To establish baseline autophagy dynamics during normal retinal maturation, we characterized autophagic markers in rat retinas from birth through postnatal day 18 (P18), encompassing the period of retinal vascularization.

Vascular Development Timeline:

In rats, retinal vascularization occurs postnatally. At birth, the retina is partially avascular with persistent hyaloid vessels. By P7, hyaloid regression is nearly complete and retinal vasculature covers most of the retinal surface, though the blood-retinal barrier (BRB) remains immature (evidenced by low expression of tight junction proteins Claudin-5 and Occludin). By P14-P18, retinal vasculature is complete and the BRB is functional with robust tight junction expression.

Hypoxia During Development:

The transition from hyaloid to retinal circulation creates a physiological hypoxic period at P7. We observed peak expression of HIF-1α and VEGF-A at P7, correlating with maximal hypoxic stimulus driving angiogenesis. These markers decreased by P14-P18 when vascularization was complete.

Autophagy Dynamics:

Autophagy markers (LC3-II, Beclin-1, ATG5) showed significant upregulation at P7, coinciding with the hypoxic peak, and decreased at P14 and P18. Immunohistochemistry revealed LC3 expression predominantly in the inner plexiform layer (IPL) and outer plexiform layer (OPL) at P7, with notable colocalization with vascular markers (PECAM-1) in developing retinal vessels.

Mechanistic Insights:

The temporal correlation between hypoxia and autophagy induction suggests that HIF-mediated signaling, in conjunction with energy stress, activates autophagy via AMPK to support the bioenergetic and biosynthetic demands of rapidly proliferating endothelial and retinal cells. Autophagy likely facilitates:

- Endothelial cell proliferation and migration during angiogenesis
- Neuronal remodeling and synapse formation
- Quality control of newly synthesized proteins and organelles
- Energy homeostasis during the metabolically demanding developmental phase

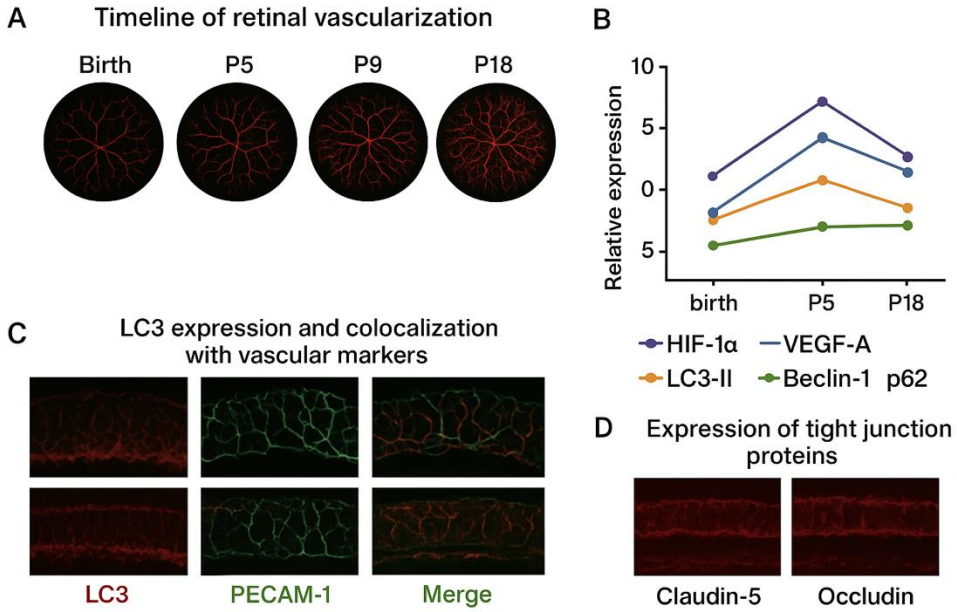

Figure 4. Autophagy Dynamics During Rat Retinal Development
(A) Timeline of retinal vascularization from birth to P18 with whole-mount retinal vasculature images. (B) Temporal expression profiles of HIF-1α, VEGF-A, and autophagy markers (LC3-II, Beclin-1, p62). (C) Immunohistochemistry showing LC3 expression and colocalization with vascular markers (PECAM-1) at critical developmental stages. (D) Expression of tight junction proteins (Claudin-5, Occludin) indicating blood-retinal barrier maturation.

3.3 Autophagy Dysregulation in Oxygen-Induced Retinopathy

To investigate autophagy in pathological neovascularization, we employed the 50/10 oxygen-induced retinopathy (OIR) model in rats—an established model of ROP. Rat pups were exposed to alternating cycles of 50% and 10% oxygen every 24 hours from birth to P14, then returned to room air until P18.

Vascular Phenotype:

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OIR rats exhibited characteristic ROP features: peripheral retinal avascularity at P14 and pathological neovascular tufts at P18, confirmed by whole-mount retinal vasculature staining. These vascular abnormalities were accompanied by retinal dysfunction measured by electroretinography (ERG).

VEGF-A Upregulation:

OIR retinas showed significantly elevated VEGF-A at both P14 and P18, consistent with hypoxia-driven neovascularization. Surprisingly, HIF-1α protein levels were not significantly different from controls, suggesting HIF-independent VEGF-A upregulation potentially mediated by reactive oxygen species (ROS) and AMPK activation—mechanisms previously described in RPE cells.

Autophagy Induction:

OIR retinas displayed marked increases in:

- Phosphorylated AMPKα (p-AMPKα)
- LC3-II (autophagosome marker)
- Beclin-1 and ATG5 (autophagy initiators)

Concomitantly, mTOR pathway activity was suppressed (decreased p-mTOR, p-4EBP1, p-S6), consistent with AMPK-mediated mTOR inhibition and autophagy activation.

Impaired Autophagic Flux:

Despite increased autophagy induction markers, p62/SQSTM1 protein—which is normally degraded by autophagy—accumulated in OIR retinas. This paradoxical finding indicates impaired autophagic flux: autophagosomes form but fail to fuse efficiently with lysosomes, leading to incomplete cargo degradation. The accumulation of autophagosomes without corresponding degradation suggests lysosomal dysfunction, previously reported in chronic stress conditions. This "autophagy gridlock" prevents effective recycling of macromolecules and may trigger pathogenic signaling cascades.

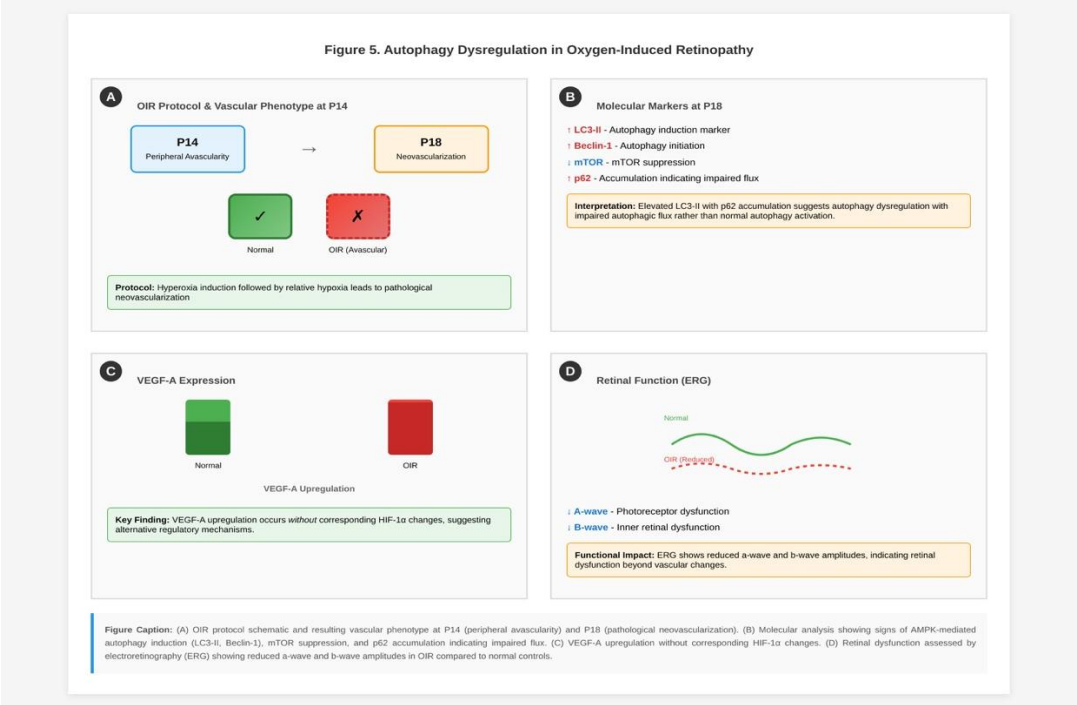

TABLE 2. Autophagy in Retinal Development vs. Disease

Parameter	Normal Development (P7)	OIR Pathology (P14-P18)
Vascular Status	Physiological hypoxia, active vascularization	Pathological: peripheral avascularity + neovascular tufts
HIF-1α	Peak expression	Not significantly elevated
VEGF-A	Peak expression	Significantly elevated
AMPK Activation	Moderate	Markedly increased
mTOR Activity	Regulated	Suppressed
LC3-II (autophagosome)	Increased, transient	Persistently elevated
p62 (flux marker)	Normal degradation	Accumulated (impaired flux)
Autophagic Flux	Functional	Impaired
Cell Death	Minimal	Necroptosis (RIPK-1+) in neurons; Apoptosis in vessels
Functional Outcome	Normal ERG development	Impaired ERG responses
Biological Role	Adaptive (supports vascularization)	Maladaptive (contributes to neurodegeneration)

3.4 Autophagy-Necroptosis Crosstalk in OIR

To determine whether autophagy dysregulation contributes to retinal cell death in OIR, we examined the spatial and molecular relationship between autophagy and cell death markers.

Cell Death Mechanisms:

Immunohistochemistry revealed:

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- LC3: Increased expression in ganglion cell layer (GCL), IPL, OPL, and photoreceptor outer segments (OS) in OIR retinas
- RIPK-1 (necroptosis marker): Markedly increased in the same layers (GCL, IPL, OPL, OS) in OIR, with substantial colocalization with LC3
- Cleaved Caspase-8 (apoptosis marker): Predominantly expressed in retinal endothelial cells in both control and OIR retinas, consistent with its role in physiological vascular remodeling

Mechanistic Link:

The spatial colocalization of LC3 and RIPK-1 specifically in neuronal layers, combined with their temporal upregulation, suggests autophagy-dependent necroptosis in retinal neurons. This mechanism has been described in other neurodegenerative contexts: when autophagic flux is blocked, accumulated autophagosomes can trigger necroptosis through p62-mediated RIPK-1 activation and subsequent NFκB signaling. The selective presence of caspase-8 in endothelial cells without RIPK-1 colocalization indicates that vascular cells undergo apoptosis (necessary for vascular remodeling), while neuronal cells preferentially undergo necroptosis associated with autophagy dysfunction.

Therapeutic Intervention:

To test whether autophagy drives necroptosis in OIR, we administered 3-methyladenine (3-MA), a phosphatidylinositol 3-kinase inhibitor that blocks autophagosome formation. Intravitreal 3-MA treatment in OIR rats:

- Significantly reduced LC3 and RIPK-1 expression in GCL, IPL, OPL, and OS
- Did not affect cleaved caspase-8 in retinal vessels
- Did not improve ERG responses (a-wave and b-wave amplitudes)

The reduction in necroptosis markers without functional improvement suggests that while autophagy-necroptosis contributes to neuronal loss, additional cell death mechanisms (potentially apoptosis-independent pathways, excitotoxicity, or oxidative damage) also impair retinal function. Alternatively, by P18 when ERG was measured, irreversible neuronal damage may have already occurred despite reducing ongoing necroptosis.

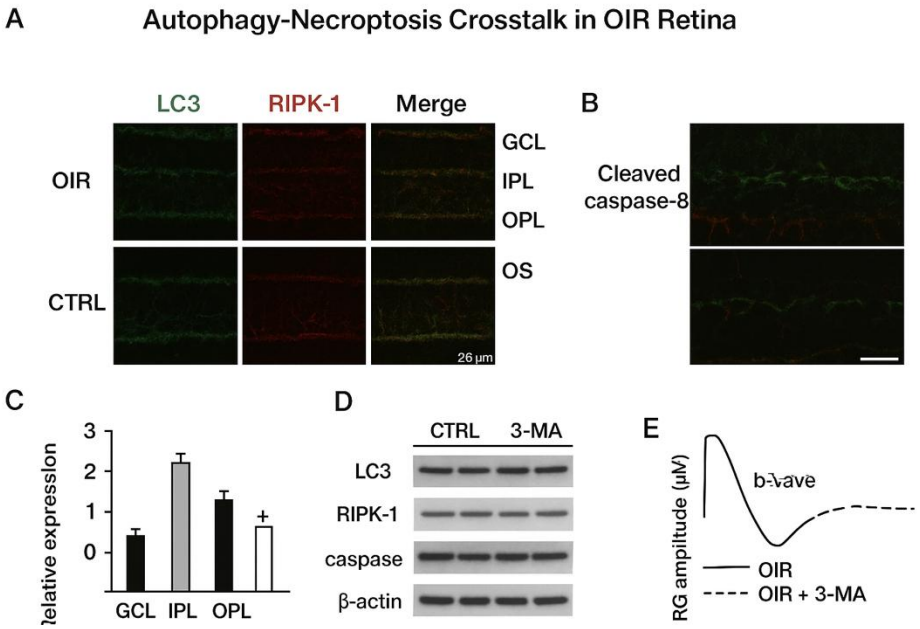

Figure 6. Autophagy-Necroptosis Crosstalk in OIR Retina

(A) Representative immunofluorescence images showing colocalization of LC3 (autophagy marker) and RIPK-1 (necroptosis marker) in retinal layers (GCL, IPL, OPL, OS). (B) Cleaved caspase-8 expression predominantly in retinal vessels (PECAM-1+) in both control and OIR retinas. (C) Quantification of LC3, RIPK-1, and caspase-8 expression across retinal layers. (D) Effects of 3-methyladenine (3-MA) treatment on autophagy and necroptosis markers. (E) ERG responses in OIR rats with and without 3-MA treatment showing lack of functional recovery despite reduced necroptosis.

3.5 Clinical Implications of Autophagy Modulation

Our findings reveal autophagy as a context-dependent factor in retinal health:

Physiological Role: During normal development, hypoxia-induced autophagy supports retinal vascularization and neuronal maturation—arguing against chronic autophagy inhibition as a therapeutic strategy.

Pathological Role: In OIR/ROP, chronic oxygen fluctuations induce excessive autophagy with impaired flux, leading to autophagosome accumulation and necroptosis in retinal neurons. This represents a potentially targetable pathogenic mechanism.

Therapeutic Considerations:

1. Timing is critical: Autophagy inhibition should be considered only after establishing pathological vascular changes, not during early developmental vascularization.
2. Combination therapy: Given that autophagy inhibition alone did not restore function, combining autophagy modulation with anti-angiogenic therapy and/or agents targeting other cell death pathways may be necessary.

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3. Flux restoration vs. inhibition: Rather than blocking autophagy initiation, therapies aimed at restoring lysosomal function and autophagic flux completion might be more physiologically sound.
4. Biomarker development: Identifying patients with autophagy dysregulation (potentially via OCT imaging of retinal layer thinning or serum biomarkers) could enable personalized intervention.

IV. Integrated Perspective: Multifactorial Therapeutic Strategies

4.1 Complementary Pathogenic Mechanisms

Our studies reveal that ocular neovascular diseases involve parallel pathogenic processes:
Vascular compartment:
- VEGF-driven angiogenesis (canonical pathway, target of current therapy)
- uPAR-mediated ECM remodeling and inflammation (non-canonical pathway, inadequately addressed)
- Endothelial cell apoptosis (physiological remodeling vs. pathological loss)
Neuronal compartment:
- Hypoxia/ischemia-induced stress
- Autophagy dysregulation with impaired flux
- Necroptosis and other cell death mechanisms
- Synaptic dysfunction and electrophysiological impairment
Current anti-VEGF monotherapy addresses only one of these mechanisms, explaining variable treatment responses and incomplete visual recovery in many patients.

4.2 Advantages of Multi-Target Approaches

uPAR Antagonism:
- Inhibits multiple pro-angiogenic pathways simultaneously
- Reduces inflammation, potentially protecting neurons
- Demonstrates efficacy via systemic administration
- May avoid neurotoxicity associated with VEGF depletion
Autophagy Modulation:
- Addresses neurodegeneration directly
- Targets a mechanism common to multiple retinal diseases
- Potential for combination with anti-angiogenic therapy
- May require individualized timing based on disease stage

4.3 Proposed Therapeutic Paradigm

Based on our findings, we propose a stage-dependent, multifactorial treatment approach:
Early/Proliferative Stage:
- Primary: uPAR antagonist (systemic or intravitreal) to address angiogenesis, inflammation, and ECM remodeling
- Adjunct: Anti-VEGF (reduced frequency) for VEGF-specific signaling
- Goal: Prevent neovascularization while preserving VEGF's neuroprotective effects
Established/Chronic Stage:
- Continue uPAR antagonist
- Add autophagy modulator (if biomarkers indicate dysregulated flux) to reduce necroptosis
- Consider antioxidants to reduce ROS-driven damage
- Goal: Stabilize vasculature and prevent progressive neurodegeneration
Post-Acute Stage:
- Maintenance therapy with reduced frequency
- Monitor for autophagy-related biomarkers
- Goal: Prevent recurrence while supporting retinal metabolic health

V. Limitations and Future Directions

5.1 Study Limitations

Model Systems:
- Ex vivo human iris cultures lack systemic factors and hemodynamic influences
- Mouse and rat models incompletely recapitulate human disease progression
- OIR model emphasizes oxygen fluctuation, which may differ from gradual ischemia in clinical disease
- ERG measurements at single time points may miss dynamic functional changes
Mechanistic Understanding:
- Precise molecular mechanisms linking uPAR to LRP-1 require further elucidation
- Autophagy-necroptosis crosstalk details remain incomplete
- Cell-type-specific contributions need clarification using conditional knockout models
Translational Gap:
- Human trials required to confirm preclinical efficacy
- Optimal dosing, timing, and delivery routes need clinical determination
- Long-term safety profiles must be established

5.2 Future Research Priorities

uPAR System:
1. Clinical trials of UPARANT in PDR, ROP, and NVG patients
2. Comparative effectiveness studies vs. current anti-VEGF agents
3. Pharmacokinetic/pharmacodynamic studies in humans
4. Biomarker development to identify patients likely to benefit
5. Investigation of UPARANT effects on retinal function (ERG, OCT angiography)
6. Structural studies of uPAR/LRP-1 complex for rational drug design
Autophagy:
1. Longitudinal assessment of autophagy flux during disease progression
2. Cell-type-specific autophagy manipulation using Cre-lox systems
3. Screening for autophagy flux restoring agents (rather than inhibitors)
4. Non-invasive biomarkers of retinal autophagy dysfunction
5. Clinical correlation studies: retinal layer thickness, autophagy markers, and visual outcomes
6. Combination therapy studies: autophagy modulators + anti-angiogenic agents
Integrated Approaches:
1. Systems biology analyses integrating transcriptomics, proteomics, and metabolomics

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2. Computational modeling of multi-target therapy effects
3. Patient stratification strategies based on predominant pathogenic mechanism
4. Development of combination drug formulations
5. Real-world evidence studies to assess clinical effectiveness

VI. Conclusions

Ocular neovascular diseases represent complex pathologies involving coordinated vascular and neurodegenerative processes. Current anti-VEGF monotherapy, while transformative, incompletely addresses disease pathogenesis and fails to prevent vision loss in many patients. Our preclinical studies demonstrate that:

1. The uPAR system is a superior therapeutic target for iris and retinal neovascularization, offering multifactorial pathway inhibition, anti-inflammatory effects, potential neuroprotection, and systemic delivery feasibility. UPARANT's novel mechanism involving uPAR/LRP-1 complex disruption provides a VEGF-independent means of suppressing angiogenesis.
2. Autophagy plays dual roles in retinal health supporting physiological development during hypoxic phases, but contributing to neurodegeneration when dysregulated in chronic disease. Autophagy-necroptosis crosstalk represents a targetable mechanism in ROP and potentially other retinopathies.
3. Multifactorial therapeutic approaches are necessary to address both vascular and neuronal compartments, ideally in a stage-dependent manner guided by disease biomarkers.

These findings support a paradigm shift from single-target to multi-target therapies in ocular neovascular diseases. Translation of these preclinical discoveries to clinical practice has the potential to improve outcomes for millions of patients affected by PDR, ROP, and NVG, reducing the global burden of vision loss. The integration of anti-angiogenic strategies (targeting both VEGF and uPAR pathways) with neuroprotective approaches (modulating autophagy and cell death mechanisms) represents the next frontier in treating these complex diseases. As we move toward precision medicine in ophthalmology, identifying which pathogenic mechanisms predominate in individual patients will enable tailored therapeutic strategies that address the specific molecular drivers of their disease.

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Declaration of Interests

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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