Award Number: W81XWH-10-2-0003

TITLE: Vision Integrating Strategies in Ophthalmology and Neurochemistry (VISION)

PRINCIPAL INVESTIGATOR: Thomas Yorio, PhD

CONTRACTING ORGANIZATION: University of North Texas Health Science
Fort Worth, TX 76107: 2553

REPORT DATE: May 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

x

Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-
4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
May 2015	Final	22 JAN 2010 - 27 Feb 2015

4. TITLE AND SUBTITLE
Vision Integrating Strategies in Ophthalmology and Neurochemistry (VISION)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0003

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Abbot Clark and Thomas Yorio

email: yoriot@hsc.unt.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of North Texas
Health Science
3500 Camp Bowie Blvd.
Fort Worth, TX 76107-2553

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel
Command Fort Detrick, Maryland 21702--5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The overall goal of the VISION project is to discover neuroprotective strategies in three separate mouse models of injury to the visual axis, in order to identify potential candidates for the treatment of combat eye injuries and preserve vision in our injured warfighters. We have established three different mouse models of ocular injury with different injury- initiating mechanisms (i.e. optic nerve crush, retinal ischemia/reperfusion, and chronic ocular hypertension). We have developed techniques to quantify damage to the retina, optic nerve, and visual axis in the brain (i.e. superior colliculus) that are damaged in these three models. We are testing neuroprotective agents and strategies, including neuroprotective estrogens, sigma-1 agonists, Brn3b, inhibitors of Jun N-terminal kinase (JNK), and inhibitors of protein stress to determine their efficacy in protecting the retina, optic nerve and superior colliculus from the damage induced by each of the 3 models.

15. SUBJECT TERMS
ocular injury; brain injury; neuroprotection; neuroprotective agents

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39

Table of Contents

Introduction

In combat situations, traumatic eye injuries are frequent, leading to irreversible damage to the visual axis (retina, optic nerve, and visual centers in the brain). Although many of these injuries are treated surgically, there is a definite need for new therapeutic agents that protect and restore normal functions to ocular and brain neural tissues following traumatic injury to the eye. In addition, our models of ocular trauma mimic some features of common degenerative ocular diseases, so our discovery of pathogenic pathways and new therapeutic agents may also identify new therapeutic interventions for these diseases.

The overall goal of the VISION project was to discover neuroprotective strategies in three separate rodent models of injury to the visual axis, in order to identify potential candidates for the treatment of combat eye injuries and preserve vision in our injured warfighters. We established and used three different in vivo rodent models of ocular injury with different injury-initiating mechanisms (i.e. optic nerve crush, retinal ischemia/reperfusion, and chronic ocular hypertension). We developed techniques to quantify damage to the retina, optic nerve, and visual axis in the brain (i.e. superior colliculus) damaged in these three models.

Identification of pathogenic pathways that are involved in traumatic injury to the retina, optic nerve, and visual centers in the brain will lead to the discovery and development of new neuroprotective and regenerative strategies for treating combat related ocular injuries and degenerative ocular diseases. This initiative focused on addressing “Inadequate mitigation and treatment of traumatic injuries, war-related injuries, and diseases to ocular structures and the visual system”. We proposed two aims that use rodent models of ocular injury (optic nerve crush and ischemia reperfusion) to: (1) test 6 neuroprotective strategies that will either delay or prevent further damage to the retina, optic nerve and visual centers of the brain, and (2) use genomic (transcriptomic) techniques to identify and characterize early and late pathogenic pathways involved in damage to the retina, optic nerve and visual centers of the brain. We used AAV2 delivery of agents that led to neuroprotection and neuroregeneration and also validated new therapeutic targets by genetic depletion of candidate pathogenic genes. Looking at initial and progressive damage to all these tissues helped us better understand the pathophysiology of neuronal damage and led to the design and testing of novel neuroprotective strategies.

We initially evaluated six specific neuroprotective strategies (neuroprotective estrogens, inhibitors of Jun N-terminal kinase (JNK), the sigma-1 receptor, Brn3b, C1q, and methylene blue) to determine their efficacy in protecting the retina, optic nerve and superior colliculus from neurodegenerative damage induced our 3 models. In addition, we evaluated time dependent, injury-induced changes in gene expression in the effected tissues and identified major pathogenic pathways involved in neurodegeneration in order to develop new therapeutic approaches for neuroprotection and neuroregeneration. This approach has allowed us to identify four new molecular pathogenic pathways, including neuritin-1, CHOP, caspase 7, and BiP. Therefore, we evaluated ten different neuroprotective/neuroregenerative strategies, all of which provided some degree of neuroprotection and several of these strategies provided profound neuroprotection to the visual axis. We readily shared our discoveries through presentations at scientific meetings, published meeting abstracts, and peer-reviewed scientific publications.

This is the final report for the VISION (Vision Integrating Strategies in Ophthalmology and Neurochemistry) project at UNTHSC. Although our original proposal was based on 5 years of funding, we only received funding for the first two years, and we have received no cost extensions for years 3-5. We made a number of important discoveries despite the reduced funding for this overall project.

Key Words

- Neuroprotection
- Retina
- Optic nerve
- Visual centers of the brain
- Axonopathy
- Ischemia/reperfusion injury
- cJun N-terminal kinase inhibition
- Sigma-1 receptor
- Brn3b
- Neuritin-1
- CHOP
- BiP
- Caspase 7
- Complement factor C1q
- Transcriptomics

Overall Project Summary

We feel that overall this project was very successful for the following reasons: (1) Ten different neuroprotective agents were tested in different models of injury to visual axis neurons (retina, optic nerve, and neurons in the visual centers of the brain). Several of these treatments provided profound neuroprotection from severe neuronal insults. (2) We have shared our discoveries with the scientific community via 39 presentations and abstracts at national/international scientific meetings as well as 20 peer-reviewed scientific publications. We sincerely hope that one or more of these discoveries will be tested and developed clinically for treating individuals, including warfighters, with traumatic neuronal injury.

Establishment and characterization of rodent models of neuronal injury to the visual axis: We established and characterized three mouse models of damage to visual axis (retina, optic nerve and visual centers in the brain). Optic nerve crush (ONC) is an acute model of axonal injury leading to subsequent degeneration of retinal ganglion cell (RGC) somas as well as target neurons in the superior colliculus of the brain (Liu et al. ARVO 2012; Liu et al. IOVS 2014). Retinal ischemia/reperfusion (I/R) injury is an acute model of ischemic damage to the inner retina, followed by degeneration of retinal and superior colliculus neurons (Kim et al. Mol Neurodegen 2013; Kim et al. 2015 submitted). Chronic elevation of intraocular pressure progressively damages optic nerve axons leading to the loss of retinal ganglion cells. We also developed quantitative methods to access neuronal damage to the visual axis in all three models, including non-invasive techniques to measure neuronal morphological changes (retinal layer thickness using spectral domain-optical coherence tomography) and functional changes to the retina (electroretinography (ERG)) (Kim et al. ARVO 2012; Kim et al. Mol Neurodegen 2013; Liu et al. ARVO 2012; Liu et al. IOVS 2014).

Neuroprotection by JNK inhibition: The cJun N-terminal kinase (JNK) is a major signaling hub that can regulate cell proliferation, differentiation, or apoptosis, depending on the circumstances and environment. We have shown early activation (phosphorylation) of JNK and its downstream substrate JUN in retinas within 1 hour of retinal I/R injury. This activation is most prominent in retinal ganglion cells (RGCs) and precedes the progressive loss of RGCs, thinning of inner retinal layers, decreased retinal function (ERG b-waves), and neuronal loss in the superior colliculus (vision center in the brain innervated by RGC axons). Daily systemic administration of the JNK inhibitor SP600125 totally protected the retina and RGCs from I/R induced retinal injury (Kim et al. ARVO 2013; Kim et al. 2015 submitted). It truly is remarkable that this therapy provided complete structural and functional protection of retinal and brain neurons from this severe insult.

Neuroprotection and neuroregeneration by gene therapy with retinal transcription factor Brn3b: Brn3b is a transcription factor responsible for the development of the majority of RGCs. This transcription factor protects and enhances neurite outgrowth in cultured neurons (Phatak et al. ARVO 2011) and neurons exposed to hypoxia (Phatak et al. Cell Mol Neurobiology 2015). A viral Brn3b expression vector (AAV2.Brn3b) was constructed to transduce RGC in vivo. Over-expression of Brn3b in the retina protected RGCs from chronically elevated IOP in a rat model of glaucoma. Brn3b also appeared to enhance RGC axonal regeneration through the optic nerve head, which is the initial site of glaucomatous damage to RGCs (Stankowska et al. ARVO 2012, 2013, 2014, 2015; Krishnamoorthy et al. ARVO 2012, 2014; Stankowska et al. IOVS 2015).

Neuroprotection by activation of the sigma-1 receptor: The sigma-1 receptor is an endoplasmic reticulum chaperone protein that modulates intracellular calcium signaling and inhibits voltage gated K+ channels. This receptor is linked to a number of additional signaling transduction pathways. The sigma-1 receptor is expressed on RGCs and attenuates the calcium response in cultured RGCs via interaction with voltage gated calcium channels (Mueller et al. ARVO 2011; Mueller et al. Exp Eye

Res. 2013) and attenuates the NMDA induced calcium influx in RGCs (Mueller et al. ARVO 2012). Sigma-1 receptors also enhance RGC synapse formation by mediating mitochondrial fusion (Yorio et al. ARVO 2011; Mueller et al, ARVO 2012). Stimulation of the sigma-1 receptor protects cultured RGCs from ischemic damage by activating ERK1/2 (Mueller et al. ARVO 2013; Mueller et al. Exp Eye Res. 2014). Sigma-1 activation protects cultured RGCs from oxygen glucose deprivation by stabilizing the mitochondrial membrane potential (Ellis et al. ISER 2014, AOPT 2015, ARVO 2015). Additional in vivo studies are being conducted in the ONC and retinal I/R models using a sigma-1 receptor agonist (pentazocine) as well as in Sigma-1 receptor deficient mice.

Neuroprotection by targeting complement factor C1q: The complement cascade plays a major role in both innate and acquired immune responses. Relatively recently, complement components C1q and C3 have been shown to play a role in developmental dendritic pruning of RGCs and their target neurons in the brain. We have shown increased expression of C1q in the retinas exposed to I/R injury that corresponds to glial activation in the retina and superior colliculus (Silverman et al. ARVO 2013, 2015). I/R retinal injury is significantly reduced morphologically (protection of retinal thickness and RGCs) and functionally (ERG) in mice deficient in C1q (*C1q*$^{+/-}$ and *C1q*$^{-/-}$ mice) (Silverman et al. ARVO 2015; Silverman et al., manuscript in preparation). This strongly suggests that C1q plays an important pathogenic role in neurodegeneration of the visual axis induced by ischemia.

Neuroprotection with non-feminizing estrogens: Estrogens are well known as female sex hormones, but recent evidence also supports their neuroprotective activities, which often can be separated from their feminizing activities. Non-feminizing estrogens can protect cultured retinal neurons from excitotoxic injury (Nixon & Simpkins, Society of Neuroscience Meeting 2011; Nixon & Simpkins, IOVS 2012). However, a single systemic dose of estradiol prior to ONC injury failed to protect RGCs from progressive degeneration. Therefore, no further in vivo studies were conducted.

Neuroprotection with methylene blue: Methylene blue has been reported to protect a variety of cells from ischemic damage, including brain neurons, via targeting mitochondrial respiration. Treatment of primary rat RGC cultures with methylene blue protects these cells from cellular senescence (Daudt et al. IOVS 2012). Further evaluation of this compound for activity in our in vivo models was not possible given the decreased funding support for the VISION project.

Approach to discover new therapeutic targets: In addition to testing the 6 neurprotective therapeutic targets listed above, we also used a variety of methods to discover new potential therapeutic targets in our models of neurodegeneration of the visual axis. We used transcriptomics to identify temporal changes in gene expression in the retina, optic nerve, and superior colliculus in our two acute models of injury to the visual axis (i.e. ONC and retinal I/R induced injury). We reported temporal changes in retinal gene expression after ONC (Putliwala et al. ARVO 2012; Sharma et al. Mol Neurodegen 2014) that led to the discovery of neuritin-1 as a new therapeutic target (Putliwala et al. ARVO 2013; Sharma et al. ARVO 2014; Sharma et al. Cell Death Disease 2015) (see below). We also used transcriptomics to discover changes in retinal gene expression after retinal I/R injury (Kim et al. Mol Neurodegen 2013). In addition, we found that the unfolded protein response (UPR) and endoplasmic reticulum associated degradation (ERAD) pathways were activated after acute retinal damage induced by I/R or ONC. This discovery led us to evaluate key targets associated with these pathways (i.e. CHOP, Caspase 7, and BiP) for potential neuroprotective activities (see below).

Neuroprotection and neuroregeneration by Neuritin-1 (NRN1) gene therapy: Examination of changes in retinal gene expression after ONC lead to the discovery that *Nrn1* expression was significantly down-regulated shortly after ONC (Sharma et al. Mol Neurodegen 2014). Neuritin-1 promotes neuritogenesis of neurons during development, appears to play a role in neuronal plasticity in adults, and is expressed in RGCs. Given the functions of neuritin-1, we hypothesized that increasing NRN1

expression in RGCs would protect these neurons from axonopathy induced neurodegeneration. We constructed a viral vector (AAV2.NRN1) to transduce mouse eyes and over-express NRN1 in RGCs. NRN1 partially protected RGCs from ONC-induced neurodegeneration and more importantly functionally protected the RGC from ERG b-wave deficits (Sharma et al. Cell Death Dis. 2015).

Neuroprotection by targeting CHOP: Endoplasmic reticulum (ER) stress occurs during a wide variety of neuronal insults. One downstream effector of ER stress is CHOP (DDIT3), which is a C/EBP transcription factor that can activate neuronal death via apoptosis. The expression of retinal CHOP is significantly increased within 3 days of retinal I/R injury, and expression is mostly localized to RGCs. Deletion of CHOP expression in *Chop*$^{-/-}$ mice partially protected RGCs morphologically and functionally from I/R induced damage (Nashine et al. ARVO 2014; Nashine et al. IOVS 2014). This clearly demonstrates that ER stress plays a major role in retinal I/R induced neurodegeneration and suggests other potential ER stress associated therapeutic targets.

Neuroprotection by targeting Caspase 7: Caspase 7 is an effector caspase induced by ER stress that is involved in apoptotic cell death. Little was known about its potential involvement in neuronal cell death, especially in the retina. ONC injury in mice increases expression and activation of caspase 7 in retinal ganglion cells. In addition, deletion of caspase 7 expression in *Casp7*$^{-/-}$ mice partially protected the retina from ONC induced loss of RGCs and functional deficits (ERG pSTR) (Choudhury et al. ARVO 2014; Choudhury et al. submitted for publication). This demonstrates that RGCs can be protected by targeting ER stress induced apoptosis.

Neuroprotection by BiP (GRP78) gene therapy: BiP is an ER molecular chaperone that plays a protective role during ER stress by translocating misfolded proteins out of the ER for proteolytic degradation. Expression of BiP in RGCs is increased shortly after ONC injury in an attempt to provide endogenous protection. We generated a viral vector to transduce and over-express BiP in RGCs (AAV2.BiP). Over-expression of BiP significantly reduced RGC loss post-ONC and partially protected ERG pSTR amplitudes, providing both morphological and functional protection (Liu et al. ARVO 2014). This demonstrates that ONC associated ER stress causes pathogenic neurodegeneration, which can be prevented by gene therapy with the molecular chaperone BiP.

Key Research Accomplishments

(1) The VISION research project was a multi-PI effort to discover new neuroprotective strategies to protect the visual axis (retina, optic nerve, and visual centers in the brain) from traumatic injury. We proposed to evaluate specific neuroprotective strategies using small molecules, gene therapy, and genetic deletion of target genes. In addition, we evaluated molecular changes in the visual axis over the course of traumatic injury in order to discover and test new therapeutic strategies. The VISION project was an ambitious 5-year project initially involving 6 faculty members, 5 postdoctoral fellows, 7 graduate students, and 4 research associates. However, we only receiving funding for the first 2 years of this 5-year project and had to make major adjustments to our budget, personnel, and research plan. Over the 5 years of this project, we evaluated 10 different therapeutic approaches and made a number of significant discoveries, despite this major budget reduction.

(2) We established and characterized three mouse models of damage to visual axis (retina, optic nerve and visual centers in the brain). Optic nerve crush (ONC) is an acute model of axonal injury leading to subsequent degeneration of retinal ganglion cell somas as well as target neurons in the superior colliculus of the brain. Retinal ischemia/reperfusion (I/R) injury is an acute model of ischemic damage to the inner retina, followed by degeneration of retinal and superior colliculus neurons. We also developed an inducible model of elevated intraocular pressure that causes optic neuropathy and retinopathy that progressively damages retinal ganglion cells. In addition, we developed quantitative methods to access structural and functional neuronal damage to the visual axis in all three models.

(3) We initially evaluated six different therapeutic neuroprotective strategies, including JNK inhibition, Brn3b, sigma-1 receptor, C1q, neuroprotective estrogens, and methylene blue. All six strategies gave varying degrees of neuroprotection. JNK inhibition totally protected the visual axis (retina and superior colliculus) structurally and morphologically from retinal ischemia/reperfusion injury. Genetic depletion of complement component C1q also protected the retina from ischemia/reperfusion injury. Gene therapy with Brn3b significantly protected retinal ganglion cells from ocular hypertension induced damage and appeared to stimulate axonal regeneration. Sigma-1 receptor agonists and methylene blue are neuroprotective in cultured neurons. We plan on testing the potential neuroprotective role of the sigma-1 receptor in our in vivo models.

(4) We used transcriptomics to identify additional neuroprotective targets, including NRN1, CHOP, caspase 7, and BiP. Gene therapy with NRN1 or with BiP partially protected the retina from ONC-induced retinal ganglion cell loss and partially protected RGC function. NRN1 also induced optic nerve axonal regeneration, so this gene is both neuroprotective and neuroregenerative. Genetic knockout of CHOP structurally and functionally protected the retina from ischemia/reperfusion injury, while genetic depletion of caspase 7 protected the retina from ONC injury.

(5) We have presented our research findings at national and international visual sciences meetings (39 presentations and published abstracts). We also have published 18 and submitted an additional 2 peer-reviewed scientific manuscripts related to this work.

(6) Over the course of this 5 year project, we have trained and at least partially supported 5 postdoctoral fellows and 7 graduate students.

Conclusion

Through the generous support of TATRC and the Department of Defense, the VISION project discovered and evaluated 10 different therapeutic neuroprotective approaches to treat traumatic injury to neurons in the visual axis (brain, optic nerve, and visual centers in the brain). Two of our experimental in vivo models caused acute and severe traumatic damage to the visual axis. We showed total structural and functional protection with one therapy (JNK inhibition), and to our knowledge no previous therapeutic approach has provided this level of protection. Several of our gene therapy approaches not only provided neuroprotection, but also showed neuroregeneration of optic nerve axons showing that we can regrow injured nerve axons. Since the retina and optic nerve are extensions of central nervous system (CNS) and we also evaluated neurons in the visual centers of the brain, our discoveries would likely also apply to other CNS neurons in the brain and spinal cord. We have readily shared all our discoveries with the scientific community through 39 presentations and published abstracts as well as 20 peer-reviewed publications.

Personnel Supported by VISION Project

Faculty (Principal Investigators)

- Abbot Clark, PhD (Program Director) (2010-2015)
- Marina Gorbatyuk, PhD (2010-2012)
- Raghu Krishnamoorthy, PhD (2010-2013)
- James Simpkins, PhD (2010-2011)
- Robert Wordinger, PhD (2010-2013)
- Thomas Yorio, PhD (2010-2015)

Postdoctoral Fellows

- Bjung-Jin Kim, PhD (2010-2014)
- Yang Liu, PhD (2010-2014)
- Everett Nixon, PhD (2010-2011)
- Dorota Stankowska, PhD (2010-2013)
- Zhang Zhang, PhD (2010-2012)

Graduate Students

- Shreyasi Choudhury (2011-2014)
- Brett Mueller (2010-2013)
- Sonali Nashine (2011-2014)
- Yong Park (2011-2015)
- Nitasha Phatak (2010-2013)
- Tasneem Sharma (2010-2014)
- Sean Silverman (2011-2015)

Technical Support

- Terri Beckwith (2011-2013)
- Sherri Harris (2014-2015)
- Sandra Neubauer (2010-2015)
- Holly Tebow (2010-2014)

Publications, Abstracts, and Presentations

Abstracts and Presentations (39)

Phatak NR, Minton AZ, Mireles CE, Krishnamoorthy R. Overexpression of POU domain transcription factor, Brn3b, cuased neurite outgrowth and axon elongation in cultured transformed 661W cells. 2011 ARVO Annual Meeting, Abstract 2659

Mueller B, Krishnamoorthy R, Daudt D, Ma H-Y, Yorio T. Interaction of sigma-1 receptors with voltage gated calcium channels attenuates calcium response in primary retinal ganglion cells. 2011 ARVO Annual Meeting, Abstract 2657

Yorio T, Daudt D, Mueller B. Effects of sigma-1 receptors on mitochondrial fusion in retinal ganglion cells. 2011 ARVO Annual Meeting, Abstract 4619

Nixon ES, Simpkins JW. Neuroprotective effects of non-feminizing estrogen analogues in retinal neurons. 2011 Society for Neuroscience Abstract 895.01.

Mueller B, Ma H-Y, Yorio T. Inhibition of NMDA induced calcium ion influx in retinal ganglion cells through sigma-1 receptor stimulation. 2012 ARVO Annual Meeting, Abstract 5320

Putliwala T, McDowell C, Liu Y, Casavant TL, Faga B, Thole D, Wordinger RJ, Braun TA, Clark AF. Temporal changes in retinal gene expression after optic nerve crush in mice. 2012 ARVO Annual Meeting, Abstract 3847

Kim B-J, Wordinger RJ, Clark AF. Pathologic progression of retinal ischemia and reperfusion injury in mice associated with defective retinal function. 2012 ARVO Annual Meeting. Abstract 2477

Liu Y, McDowell C, Tebow H, Beckwith T, Wordinger RJ, Clark AF. Pattern ERG deficits after optic nerve crush in mice. 2012 ARVO Annual Meeting. Abstract 169

Stankowska DL, Minton AZ, He S, Krishnamoorthy R. Neuroregenerative properties of transcription factor Brn3b in an elevated IOP rat model of glaucoma. 2012 ARVO Annual Meeting, Abstract 5370

Mueller B, Daudt D, Yorio T. Neuroprotective effects and enhanced retinal ganglion cell synapse formation through sigma-1 receptor mediated mitochondrial fusion. 2012 ARVO Annual Meeting Abstract 5320

Shinde V et al. Role of ER stress in retinal degeneration in S334ter rhodopsin rats. 2012 ARVO Annual Meeting Abstract 4283

Choudhury S et al. Role of ER stress-induced caspase7 in retinal degeneration of T17M rhodopsin transgenic mice. 2012 ARVO Annual Meeting Abstract 6446

Kunte M et al. ER stress is involved in retinal degeneration induced by human T17M mutant rhodopsin. 2012 ARVO Annual Meeting Abstract 6453

Nashine S et al. Deficiency in the pro-apoptotic CHOP protein, an UPR downstream marker, does not prevent vision loss in T17M Rho retina. 2012 ARVO Annual Meeting Abstract 6457

Krishnamoorthy R et al. Transcription factor Brn3b promotes neuroregeneration in an ocular hypertension rodent model of glaucoma. 2012 ARVO Annual Meeting Abstract 6958

Silverman S, McDowell CM, Kim B-J, Wordinger RJ, Clark AF. Complement and glial activity in the retinocollicular pathway of mice in a novel model of glaucoma. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 421.

Liu Y, Zhang Z, Wordinger RJ, Libby RT, Pang I-H, Clark AF. Differential activation of p38 and c-jun N-terminal kinase in the visual pathway following optic nerve crush. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 422.

Putliwala T, Liu Y, Wordinger RJ, Clark AF. Role of neuritin 1 in response to optic nerve crush. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 431.

Kim BJ, Liu Y, Silverman S, Wordinger RJ, Libby RT, Pang I-H, Clark AF. Protective effects of JNK inhibition in retinal ganglion cells and in retinal ischemia/reperfusion injury. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2624.

Mueller BH, Park YH, Ma H-Y, Yorio T. Sigma-1 receptor stimulation protects purified RGCs from ischemic insult through the phosphorylation of extracellular signal kinase 1/2. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 3699.

Stankowska DL, Minton AZ, Krishnamoorthy RR. Brn3b mediated regeneration of the optic nerve in a rodent model of glaucoma. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 6357.

Phatak N, Stankowska DL, Krishnamoorthy RR. Overexpression of the POU domain transcription factor, Brn3b, causes neurite outgrowth in cultured PC12 cells. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 6364.

Park Y, Mueller B, Ma H-Y, Yorio T. Stimulation of the AMPA Receptor in Retinal Ganglion Cells Increases Phosphorylation of CREB. 2013 Annual Meeting of Association for Research in Vision and Ophthalmology, Seattle, WA Abstract 6353

Nashine SR, Kim BJ, Clark AF, Pang I-H. Role of C/EBP homologous protein (CHOP) in the survival of retinal ganglion cells after retinal ischemia/reperfusion injury. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1729

Liu Y, Sharma TP, Wordinger RJ Gorbatyuk MS, Clark AF. Gene delivery of GRP78/BiP promotes retinal ganglion cell survival following optic nerve crush. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2426

Choudhury S, Liu Y, Clark AF, Pang I-H. Knockout Of Caspase-7 Protects Against Optic Nerve Crush-Induced Retinal Ganglion Cell Death. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2667

Sharma TP, Liu Y, Pang I-H, Wordinger RJ, Clark AF. Neuritin1 Promotes Retinal Ganglion Cell Survival And Axonal Regeneration Following Optic Nerve Crush. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2669.

Mueller BH, Park YH, Ma H-Y, Yorio T. Prolonged NMDA stimulation induces neuroprotective pathways and enhances survivability of primary retinal ganglion cells. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1903

Park YH, Mueller BH, McGrady N, Dibas A, Yorio T. Retinal ganglion cells are resistant to AMPA receptor mediated excitotoxicity. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 1904

Krishnamoorthy RR, Stankowska DL. Brn3b mediated axonal regeneration of the optic nerve in a rodent model of glaucoma. 2014 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2183

Park, Yong H.; McGrady, Nolan R.; Yorio, Thomas, “AMPAR Receptor Dual Role in RGC Survival” (2014), Association for Ocular Pharmacology and Therapeutics, Charleston, SC

Ellis, Dorette Z; Li, Linya; Park Yong H.; Mueller, Brett; Ma, Hai-Ying; Yorio, Thomas, "Sigma-1 Receptor-Induced Neuroprotection: Correlation between Mitochondrial Membrane Potential and Caspase Activity in Glucose Oxygen Deprived Retinal Ganglion Cells" (2014), Association for Ocular Pharmacology and Therapeutics, Charleston, SC

Ellis, Dorette Z; Li, Linya; Park, Yong H.; Mueller, Brett; Ma, Hai-Ying; Yorio, Thomas, “Sigma-1 Receptor Increases Mitochondrial Membrane Potential in Glucose and Oxygen Deprived Retinal Ganglion Cells” (2014), International Society for Eye Research, San Francisco, CA

Park, Yong H.; Mueller, Brett H.; Yorio, Thomas, “NMDA and AMPA Receptor Stimulation in Retinal Ganglion Cells Induces Prolonged Phosphorylation of CREB and Increases Resistance to Apoptosis” (2014), International Society for Eye Research, San Francisco, CA

Silverman S, Howell GR, John SW, Wordinger RJ, Clark AF. Neuroinflammatory response to retinal ischemia/reperfusion injury in the visual axis and complement mediated protection. 2015 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 2417

Stankowska DL, Krishnamoorthy RR. Transcription factor Brn3b mediated neuroprotection in an ocular hypertension rat model of glaucoma. 2015 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 4426

Phatak N, Stankowska DL, Krishnamoorthy RR. Overexpression of the POU domain transcription factor Brn-3b leads to upregulation of the pro-survival factor bcl-2 in rat retinal ganglion cells. 2015 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 4410

Ellis D, Li L, Park YH, Mueller B, Ma H-Y, Yorio T. Sigma-1 receptor increases mitochondrial membrane potential in glucose and oxygen deprived retinal ganglion cells. 2015 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 4258

Park YH, McGrady N, Yorio T. Oxygen glucose deprivation produced increased susceptibility of retinal ganglion cells to AMPA receptor-mediated death. 2015 Annual Meeting of Association for Research in Vision and Ophthalmology, Abstract 4960

Publications (20)

Daudt DR 3rd, Mueller B, Park YH, Wen Y, Yorio T. Methylene blue protects primary rat retinal ganglion cells from cellular senescence. Invest Ophthalmol Vis Sci. 2012;53(8):4657-67

Kunte MM, Choudhury S, Manheim JF et al. ER stress is involved in T17M rhodopsin-induced retinal degeneration. Invest Ophthalmol Vis Sci. 2012;53:3792-3800

Nixon E, Simpkins JW. Neuroprotective effects of nonfeminizing estrogens in retinal photoreceptor neurons. Invest Ophthalmol Vis Sci. 2012;53:4739-4747

Poteet E, Winters A, Yan LJ, Shufelt K, Green KN, Simpkins JW, Wen Y, Yang SH. Neuroprotective actions of methylene blue and its derivatives. PLoS One. 2012;7(10):e48279.

Mueller BH 2nd, Park Y, Daudt DR 3rd, Ma HY, Akopova I, Stankowska DL, Clark AF, Yorio T. Sigma-1 receptor stimulation attenuates calcium influx through activated L-type Voltage Gated Calcium Channels in purified retinal ganglion cells. Exp Eye Res. 2013;107:21-31.

Kim, BJ, Braun TA, Wordinger RJ, Clark AF. Progressive morphological changes and impaired retinal function associated with temporal regulation of gene expression after retinal ischemia/reperfusion injury in mice. Molecular Neurodegeneration 8:21, 2013.

Nashine S, Bhootada Y, Lewin AS, Gorbatyuk M. Ablation of C/EBP homologous protein does not protect T17M RHO mice from retinal degeneration. PLoS One. 2013 Apr 30;8(4):e63205.

Choudhury S, Bhootada Y, Gorbatyuk O, Gorbatyuk M. Caspase-7 ablation modulates UPR, reprograms TRAF2-JNK apoptosis and protects T17M rhodopsin mice from severe retinal degeneration. Cell Death Dis. 2013 Mar 7;4:e528.

Mueller BH, Park Y, Ma H-Y, Dibas A, Clark AF, Yorio T. Sigma-1 receptor stimulation protects retinal ganglion cells from ischemic-like insult through the activation of extracellular-signal-related kinases 1/2. Exp Eye Res. 2014 Nov;128:156-69.

Nashine S, Liu Y, Kim BJ, Clark AF, Pang IH. Role of C/EBP homologous protein in retinal ganglion cell death after ischemia/reperfusion injury. Invest Ophthalmol Vis Sci. 2014 Nov 20;56(1):221-31.

Liu Y, McDowell CM, Zhang Z, Tebow HE, Wordinger RJ, Clark AF. Monitoring retinal morphologic and functional changes in mice following optic nerve crush. Invest Ophthalmol Vis Sci. 2014 May 22;55(6):3766-74.

Sharma TP, McDowell CM, Liu Y, Wagner AH, Thole D, Faga BP, Wordinger RJ, Braun TA, Clark AF. Optic nerve crush induces spatial and temporal gene expression patterns in retina and optic nerve of BALB/cJ mice. Mol Neurodegener. 2014 Apr 27;9:14. doi: 10.1186/1750-1326-9-14.

Park YH, Mueller BH 2nd, McGrady NR, Ma HY, Yorio T. AMPA receptor desensitization is the determinant of AMPA receptor mediated excitotoxicity in purified retinal ganglion cells. Exp Eye Res. 2015 Jan 30;132C:136-150.

Stankowska DL, Minton AZ, Rutledge MA, Mueller BH 2nd, Phatak NR, He S, Ma HY, Forster MJ, Yorio T, Krishnamoorthy RR. Neuroprotective effects of transcription factor brn3b in an ocular hypertension rat model of glaucoma. Invest Ophthalmol Vis Sci. 2015 Jan 13;56(2):893-907.

Xie L, Choudhury GR, Wang J, Park Y, Liu R, Yuan F, Zhang CL, Yorio T, Jin K, Yang SH. Methylene blue promotes quiescence of rat neural progenitor cells. Front Cell Neurosci. 2014 Oct 7;8:315.

Phatak, NR, Stankowska DL and Krishnamoorthy RR (2015) Transcription factor Brn-3b overexpression enhances neurite outgrowth in PC12 cells under condition of hypoxia. Cellular and Molecular Neurobiology 2015, In Press.

Sharma TP, Liu Y, Wordinger RJ, Pang I-H, Clark AF. Neuritin 1 promotes retinal ganglion cell survival and regeneration following optic nerve crush. Cell Death & Disease 6:1661, 2015.

Kim BJ, Silverman SM, Liu Y, Wordinger RJ, Libby RT, Pang I-H, Clark AF. *In vitro* and *in vivo* protective effects of JNK inhibitors on retinal ganglion cells. Neurobiology of Disease (submitted)

Choudhury S, Liu Y, Clark AF, Pang I-H. Knockout Of Caspase-7 Protects Against Optic Nerve Crush-Induced Retinal Ganglion Cell Death. Molecular Neurodegeneration (submitted).

Silverman S, Howell GR, John SW, Clark AF. C1q deficiency protects from neuroinflammation and neurodegeneration of the visual axis induced by retinal ischemia/reperfusion injury. Manuscript in preparation.