

Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University
2. **Reporting Period (start and end date of grant award period):** 1/1/2009- 12/31/2012
3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA
4. **Grant Contact Person's Telephone Number:** 814-935-1081
5. **Grant SAP Number:** # 4100047645
6. **Project Number and Title of Research Project:** 19 - Changes in Oxygen-induced Proliferative Retinopathy in 4E-BP1/2 Knockout Mice
7. **Start and End Date of Research Project:** 05/01/2009 – 06/30/2011
8. **Name of Principal Investigator for the Research Project:** Jeffrey S. Shenberger, MD
9. **Research Project Expenses.**

9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

\$ 61,973 _____

9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of all persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Schrufner	Graduate Student	50% - (Y1)	\$12,438
Zhang	Research Associate	12.5% - (Y1)	\$8,468

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Fang	Research Associate	20 (Y2-3)
Jefferson	Co-PI	5% - (Y1-3)
Shenberger	Principal Investigator	10% - (Y1-3)

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes _____ No X

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes X No _____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert “not funded” in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Role of Translational Regulation in the Mouse Model of Retinopathy of Prematurity	<input checked="" type="checkbox"/> NIH <input type="checkbox"/> Other federal (specify: _____) <input type="checkbox"/> Nonfederal source (specify: _____)	02/2010; 10/2010	\$275,000	\$0

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes _____ No X

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

We plan to finish the BiP studies within the next 6 months once additional funds are available. That should complete the project in terms of a publishable study.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes X No _____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female			1	
Unknown				
Total			1	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic			1	
Unknown				
Total			1	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian				
Other				
Unknown				
Total			1	

14. Recruitment of Out-of-State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes _____ No X

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes X No _____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This project contributed to the development of a stronger science base for research in pediatrics which is a high priority for Penn State's new Children's Hospital.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes _____ No X

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes _____ No X

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes _____ No X

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (β) should not print as boxes (\square) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Planned Project Overview

Premature infants exposed to high concentrations of oxygen to treat pulmonary disease often develop retinopathy of prematurity (ROP), a condition associated with long-term visual impairment and blindness. The objective of this project was to determine the contribution of protein synthetic regulatory pathways in the development of oxygen-induced retinopathy in newborn mice. Specifically, we planned to investigate the role of cap-dependent mRNA translation on the expression of vascular endothelial growth factor (VEGF) protein expression and the subsequent development of neovascularization. We hypothesized that loss of cap-dependent mRNA regulatory proteins, 4E-BP1/2, would reduce retinal neovascularization and retinal VEGF protein expression during the proliferative stage of retinopathy. The project utilized 7-day-old BALB/c and 4E-BP1/2 knockout mice exposed to 75% O₂ for 5 days followed by 5 days of room air recovery; a well-established model of proliferative retinopathy. In *Aim 1*, we assessed the effect of 4E-BP1/2 on the magnitude of oxygen-induced retinal neovascularization using immunohistochemistry. *Aim 2* was designed to delineate changes in VEGF protein expression secondary to the altered regulation of cap-dependent mRNA translation induced by the loss of 4E-BP1/2. Specifically, changes in VEGF and hypoxia-inducible factor-1 (HIF-1) α expression would be determined by immunoblotting and real-time PCR, respectively. Overall, this project was designed to provide evidence for a role of translational regulation of protein synthesis in the pathogenesis of retinopathy of prematurity.

We anticipated that retinal development under room air conditions would be unchanged in DKO animals. Under hyperoxia, however, we believed that the total avascular area and neovascular tuft formation would be decreased in animals without 4E-BP1/2. This opinion was based upon our work in the diabetic model of retinopathy and on previous work showing an inhibition of O₂-induced retinopathy in REDD1-deficient mice and mice treated with rapamycin (1, 2). Based upon work performed in DKO mice and the diabetic retinopathy model, we also anticipated that retinal VEGF protein expression would be decreased following room air recovery (P17) and that HIF-1 protein and mRNA and VEGF mRNA levels would be unchanged.

Original Specific Aims:

Specific Aim #1. Loss of 4E-BP1/2 will reduce hypoxia-induced neovascularization in the mouse model of oxygen-induced retinopathy (OIR). Exposure of 7-day-old mice to 75% O₂ for 5 days followed by 5 days of room air recovery results in profound retinal neovascularization. We proposed that loss of 4E-BP1/2 will reduce the magnitude of neovascularization (NV) observed by immunohistochemistry.

Specific Aim #2. Reductions in neovascularization will coincide with decreased VEGF protein expression. We proposed that the enhanced cap-dependent mRNA translation induced by the loss of 4E-BP1/2 would lead to less efficient translation of VEGF. We planned to determine changes in VEGF and HIF-1 α expression by immunoblotting and real-time PCR, respectively.

Modification of Objectives and Specific Aim:

In order to provide an accurate assessment of retinal NV in the knockout animals, it was necessary to establish the degree of NV in wild-type animals. As there have been no reported studies of NV in BALB/c mice (the background strain of the 4E-BP1/2^{-/-} mice), we needed to establish that retinal vascular regression (VR) and NV occurred in the Smith model of OIR. Accordingly, Aim #1 was modified to include the study of BALB/c mice during OIR. As will

be shown, we could identify no difference in NV due to the loss of 4E-BP1/2. Accordingly, Aim #2 was modified to define the pattern of mTOR activation during OIR. In this manner, we planned to identify the potential for the direct modulation of mTOR activity to induce regression of NV. With these modifications, the project was completed within the context of the funding provided, but investigation in this area continues in the laboratory.

Methods:

Experimental Design. All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University College of Medicine in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Studies were conducted on postnatal day 7 (P7), 4E-BP1/2 knockout mice (DKO) and BALB/c mice (wild-type, WT). Knockout mice were generated as previously described by intercrossing BALB/c 4E-BP1^{-/-} and 4E-BP2^{-/-} heterozygous mice (3). Dam/pup pairings were kept one per cage in the general animal facilities through P6. Animals were placed into Plexiglas exposure chambers overnight, in room air, for habituation. Dams were supplied with standard mouse chow and water *ad libitum* in bulk at the onset of the exposure period. Food and water were replenished at P12 and daily as needed thereafter. Routine day/light cycles of 12 hours were used and temperature and humidity maintained at 26°C and 75–80%, respectively. The hyperoxic mouse model of retinopathy was developed by Smith et al in 1994 and has been established as a reproducible model for the study of human ROP (4-7). In the hyperoxia protocol, mice were exposed to 75% O₂ for 5 days (P12) after which they were returned to room air for an additional 5 days (P17). Delivery of O₂ at these time points and concentrations recapitulates human ROP more accurately than earlier delivery or higher concentrations (4). Administration of O₂ was continually adjusted and monitored using a computerized system (BioSpherix Oxylyer, Reming Bioinstruments, Redfield, NY). In the control protocol, animals were kept in identical chambers through P17. In both protocols, CO₂ concentrations were adjusted by the degree of chamber "leak" and kept <0.5%. Animals were studied on P12 -P17.

Measurement of retinal avascular area (R_a) and NV. On P12 - P17, WT and DKO exposed to either the control or hyperoxia protocol were sacrificed and eyes enucleated. Retinas were fixed in 4% paraformaldehyde, flat-mounted on glass slides, and incubated with isolectin B4-Alexa 594 (Invitrogen) (8). Digital images of each quadrant were acquired on an immunofluorescent microscopy at 5X and merged in Photoshop CS4 (Adobe). Total retinal (R_t) and vascular (R_v) areas were measured using Photoshop and R_a area determined using $R_a = R_t - R_v$ (8). Times of maximal regression identified by isolectin were confirmed using fluorescein angiography as described by our lab (9, 10). The extent of NV was determined by counting the number of retinal vascular cell nuclei anterior to the internal limiting membrane in H&E-stained retinal cross-sections using the protocol described by Davies et al (11). The number of nuclei per 5 μm section obtained 40-μm apart was calculated on 15 sections obtained from the mid-portion of each eye.

Immunoblotting. Retinas were flash-frozen in liquid N₂ and homogenized in 30 μl of RIPA buffer. Homogenates were cleared by centrifugation and protein concentration determined using the BCA assay. Twenty μg of sample were separated by SDS-PAGE, transferred to PVDF, and blocked with 5% milk in TBST. Membranes were incubated overnight with total and phosphorylation-specific antibodies directed against the mTOR substrates S6K1 and 4E-BP1,

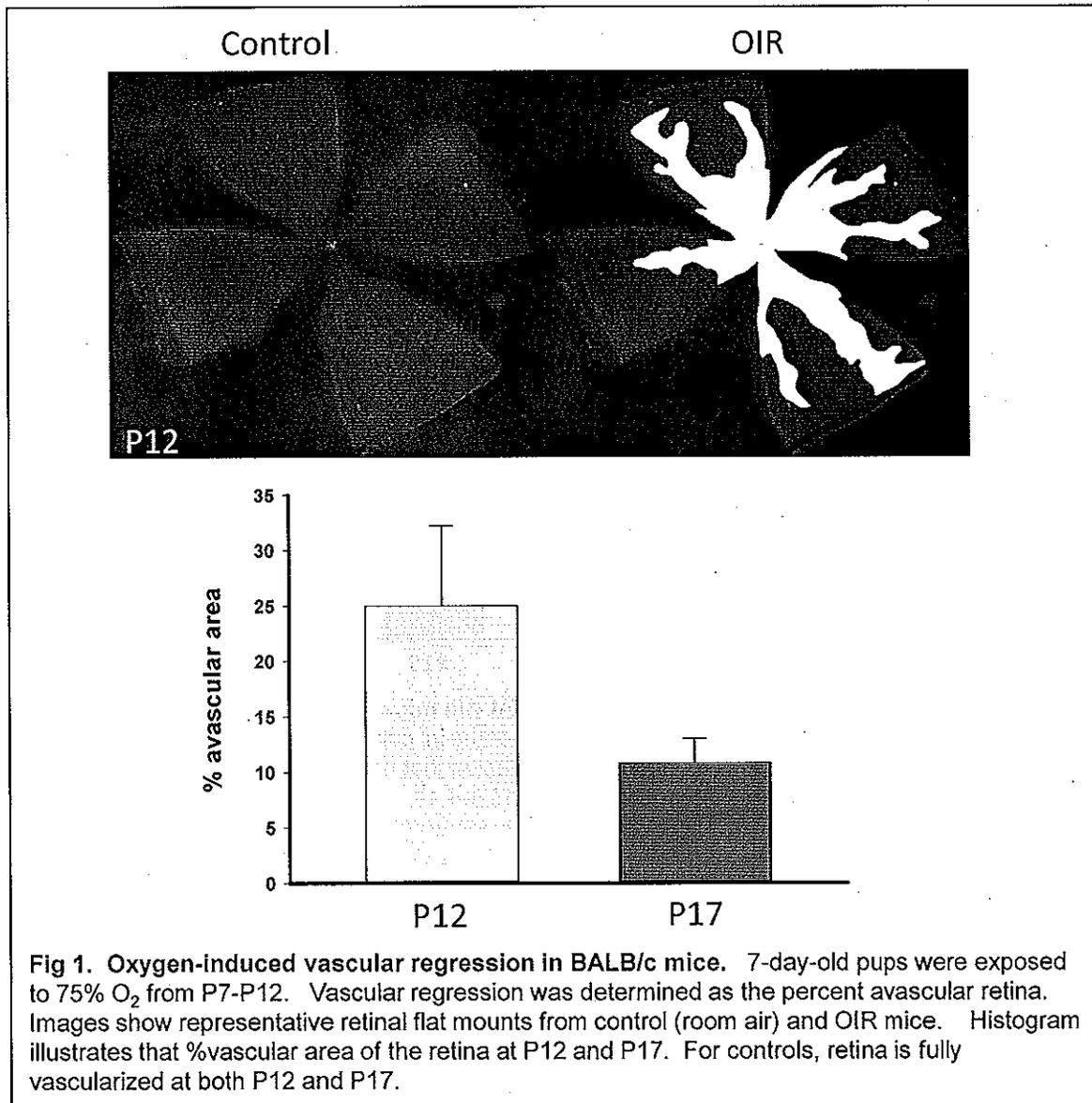
and the downstream effector of mTOR, S6RP. Blots were then incubated with the corresponding HRP-conjugated secondary antibody. Blots were developed using chemiluminescence and quantified using the GeneGnome imaging system (SynGene, Frederick, MD) and ratio of phosphorylated protein to total protein calculated as previously described (12).

Intravitreal injections. At P14 (onset of mTOR activation), pups were anesthetized with intraperitoneal injection of tribromoethanol (Sigma, Avertin). Injections were performed 1 mm posterior to the limbus with a 33 gauge stainless needle attached to a Hamilton syringe. A total volume of 0.2-0.5 μ l, providing 200-500 ng of rapamycin, respectively, was delivered. The opposite eye received an equal volume of DMSO (vehicle). Following injection, each eye will be doused with a drop of sterile H₂O to dilute residual drug in order to limit conjunctival absorbance. A dose of 500 ng given intravitreally has been shown to induce regression of hyaloid vessels in the newborn mouse eye without significant side effects (13). Control studies with saline were also performed to preclude DMSO effects and ascertain the potential of volume-related inactivation. After injection, pups were returned to the litter.

Results:

Aim #1. Loss of 4E-BP1/2 will reduce hypoxia-induced neovascularization in the mouse model of oxygen-induced retinopathy (OIR).

Since its development, the “Smith Model” has become the most-widely accepted approach to study retinal angiogenesis (14). In this model, newborn mice are exposed to 75% O₂ from postnatal (P) day P7-12, a period of rapid retinal vascularization. Angiography demonstrates that hyperoxia induces vessel regression and cessation of radial growth (Phase I) (4, 15, 16). Returning the pups to room air until P17 induces NV and increases VEGF expression (Phase II) which generally regresses by P25 (4, 17, 18).



The first step in defining the role of 4E-BP1/2 in OIR was to determine the ability of the OIR protocol to induce vascular regression and neovascularization in WT BALB/c mice – the background strain for the DKO mice. As shown in Figure 1, WT mice developed significant vascular regression at P12, though somewhat less than reported in the literature in C57BL/6 mice. Following the return to room air from P12 through P17, regression improved, but was not fully resolved at P17. Returning the oxygen-exposed mice to room air induces NV. This first becomes apparent at P14-15 and is maximal at P17 in C57BL/6 mice. Previous investigations

have not found evidence of NV in BALB/cByJ mice (19). We, however, observed clear and reproducible NV in BALB/cJ mice (Fig. 2). Nuclei anterior to the internal limiting membrane represent those of the “new” aberrant vessels. Our analysis found pre-retinal nuclei at P15 and more at P17, analogous to that reported in C57BL/6 mice (4-7).

Our next step was to determine the ability of the OIR model to induce vascular regression and NV in the DKO mice. At P12, DKO mice underwent a similar degree of vascular regression to the WT mice (not shown). At P17, DKO mice also grossly developed a similar degree of NV compared to WT mice (Fig. 3). This was replicated on two separate litters using retinal flat mounts. These findings indicated that loss of 4E-BP1/2 did not influence retinal vascularization or NV in OIR. As such Aim #2, which was to identify alterations in VEGF expression that were consistent with a role of 4E-BP1/2 in growth factor expression, was not performed. Instead, the Aims of the grant were modified to define mTOR activity during OIR, given that mTOR is the exclusive kinase for 4E-BP1/2 and one with known selective chemical inhibitors.

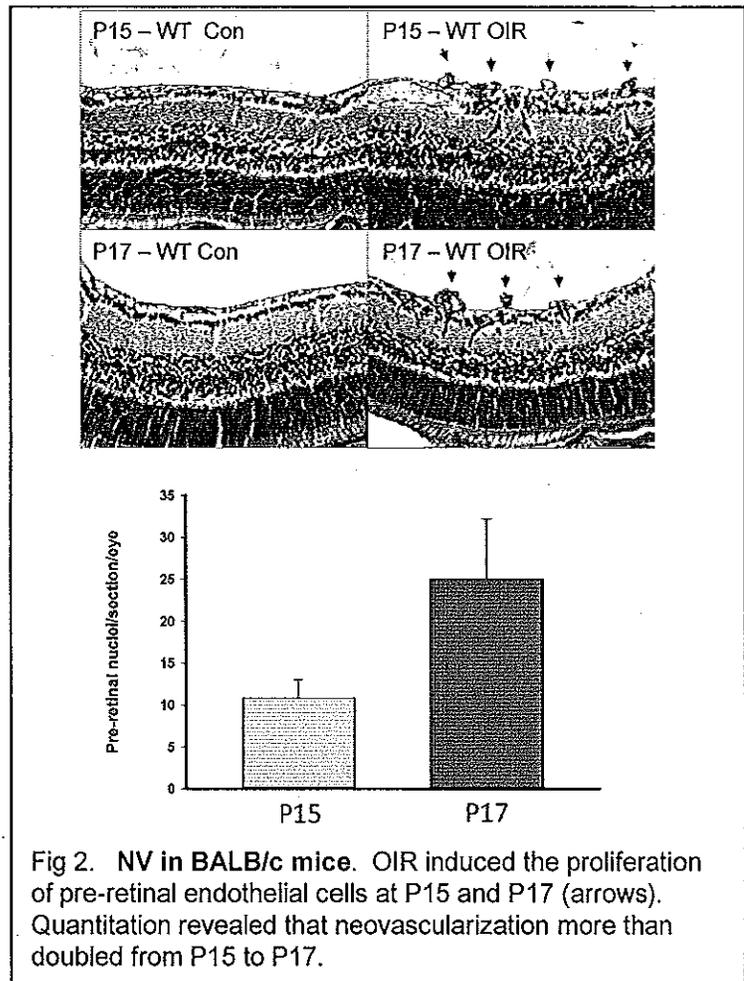


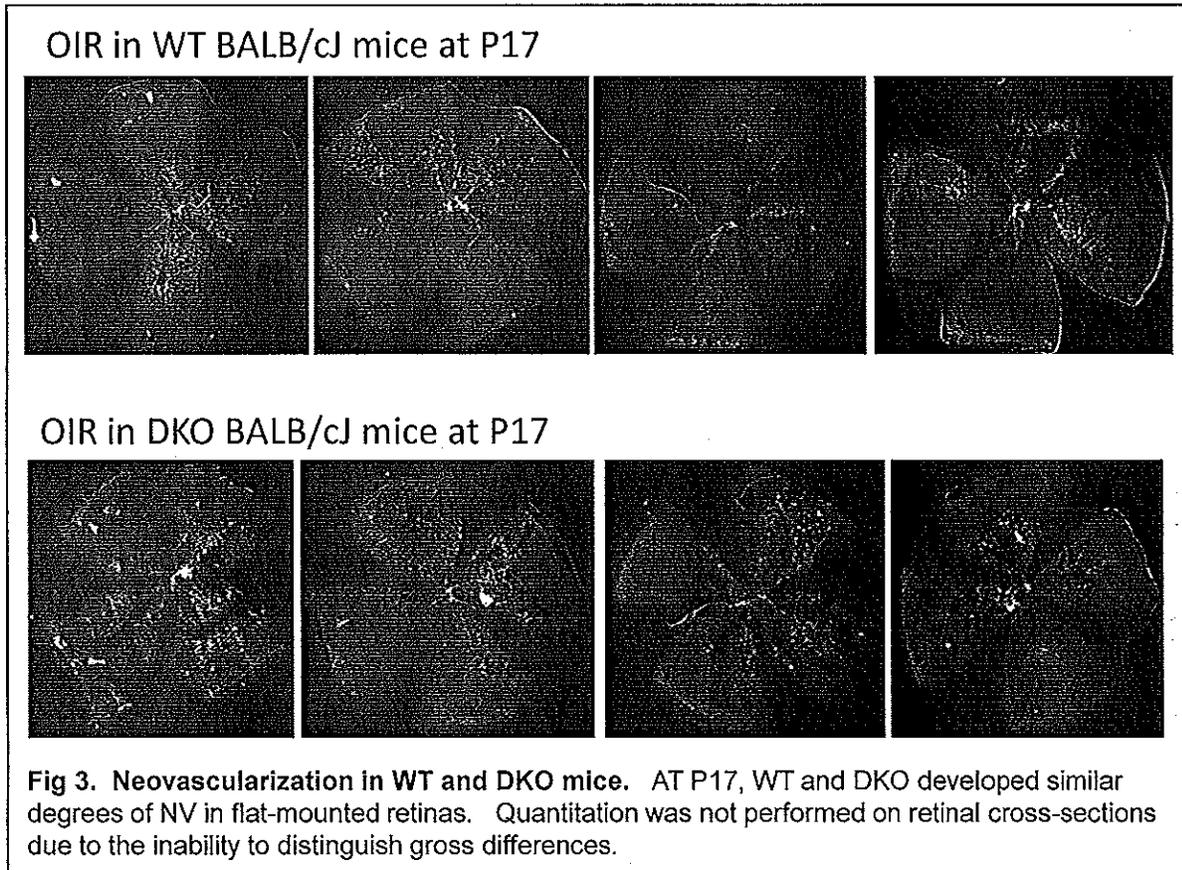
Fig 2. NV in BALB/c mice. OIR induced the proliferation of pre-retinal endothelial cells at P15 and P17 (arrows). Quantitation revealed that neovascularization more than doubled from P15 to P17.

Revised Aim #2. Delineate alterations in mTOR activity during OIR.

Due to the lack of differences in vascular regression and NV in the DKO mice, we refocused our investigation on determining the changes in mTOR activity during OIR as a means to identify the timing for potential pharmacologic interventions which may alter mTOR-driven vascular growth factor production. To this end, we used immunoblotting to identify changes in mTOR substrate phosphorylation - well-defined markers for mTOR activity. Phosphorylation of ribosomal S6 kinase 1 (S6K1) and 4E-BP1 are exclusively via mTOR. We studied the level of phosphorylation of S6K1 on Thr³⁸⁹ and 4E-BP1 on Ser⁶⁵ and Thr^{37/46} at P8, P12, P13, P15, and P17. We also measured the phosphorylation of ribosome protein S6 (S6RP) on Thr²³⁵⁻²³⁶, a general marker of translational activation, and Akt Thr³⁰⁸, an upstream activator of mTOR (Fig.

4).

We found no alterations in S6K1, 4E-BP1, S6RP, or Akt phosphorylation at P8, the first 24 hours of hyperoxic exposure. At P12, however, we were able to document suppression of 4E-BP1 phosphorylation on Ser⁶⁵ (Con: 1.05±0.11 vs. OIR: 0.70±0.08 arbitrary units, p<0.05), but not Thr^{37/46}, as well as a decrease in S6RP phosphorylation (Con: 1.88±0.24 vs. OIR: 1.17±0.10 arbitrary units, p<0.05). At P13, alterations in Ser⁶⁵ phosphorylation were no longer apparent while the phosphorylation on Thr^{37/46} was decreased (Fig. 5). In addition, retinas from OIR mice at P13 displayed increased S6RP phosphorylation relative to controls despite a suppression



of Akt phosphorylation. By P15, we observed clear indications that mTOR activation in the OIR mice with increased 4E-BP1 on both Ser⁶⁵ (Con: 0.72±0.07 vs. OIR: 1.08±0.13 arbitrary units, p<0.05) and Thr^{37/46} and S6RP (Con: 1.06±0.05 vs. OIR: 1.39±0.07 arbitrary units, p<0.05). By P17, phosphorylation of S6K1, 4E-BP1, S6RP, and Akt were no longer different between controls and OIR mice despite documented increases in NV.

These findings indicate that in the latter stages of Phase I, that mTOR activity is suppressed. Thereafter, mTOR activation increases and coincides with the development of NV. Temporally, intervention would seem appropriate at P13 or P14, the onset of mTOR activity and aberrant vascular development. Accordingly, we have begun work on the intravitreal administration of the specific mTOR inhibitor rapamycin on P14. As shown in Figure 6, administration of 500 ng of rapamycin inhibits the phosphorylation of S6K1, 4E-BP1, and S6RP compared to the contralateral eye injection with DMSO vehicle. Studies are ongoing as part of a CMN project to ascertain if rapamycin alters NV in this animals.

Summary of Project Findings

These studies define mTOR activity during OIR and illustrate that alterations in NV are not influenced by the presence of 4E-BP1/2. The precise delineation of mTOR activation in Phase II of OIR has identified a potential therapeutic target which may prove clinically useful in limiting NV. As such, we have completed and satisfied the objectives of Aim #1 and the revised Aim #2. Ongoing work in the laboratory is focused on determining if rapamycin injections limit NV.

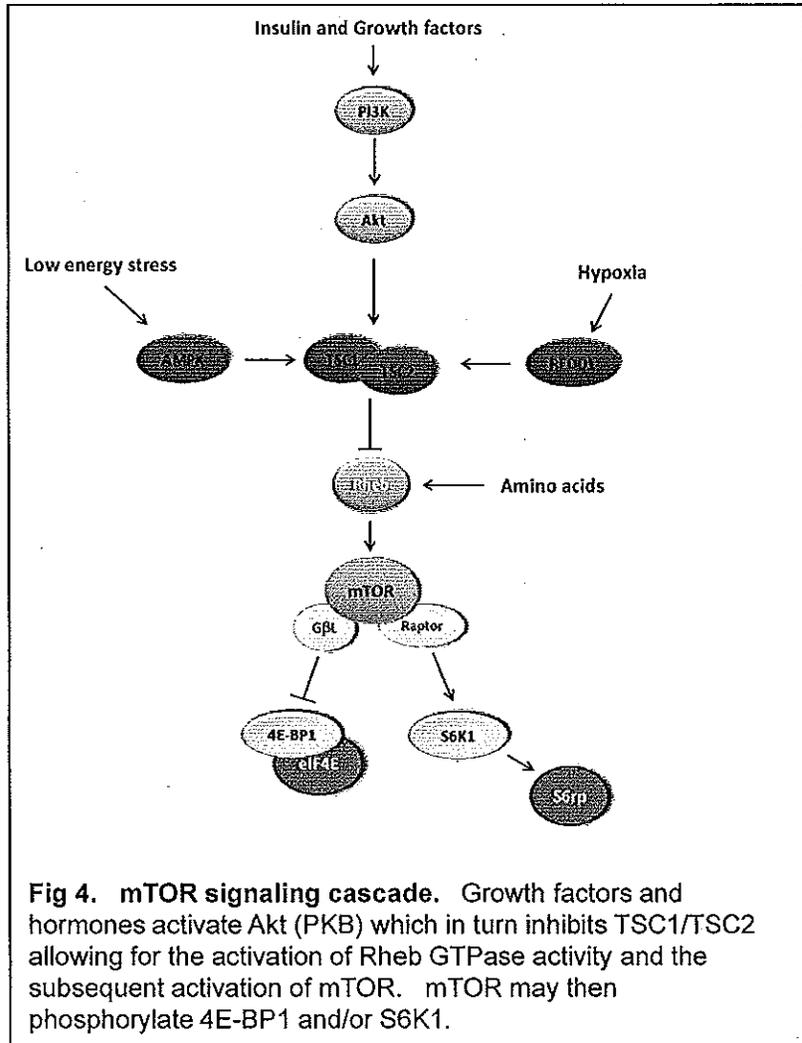
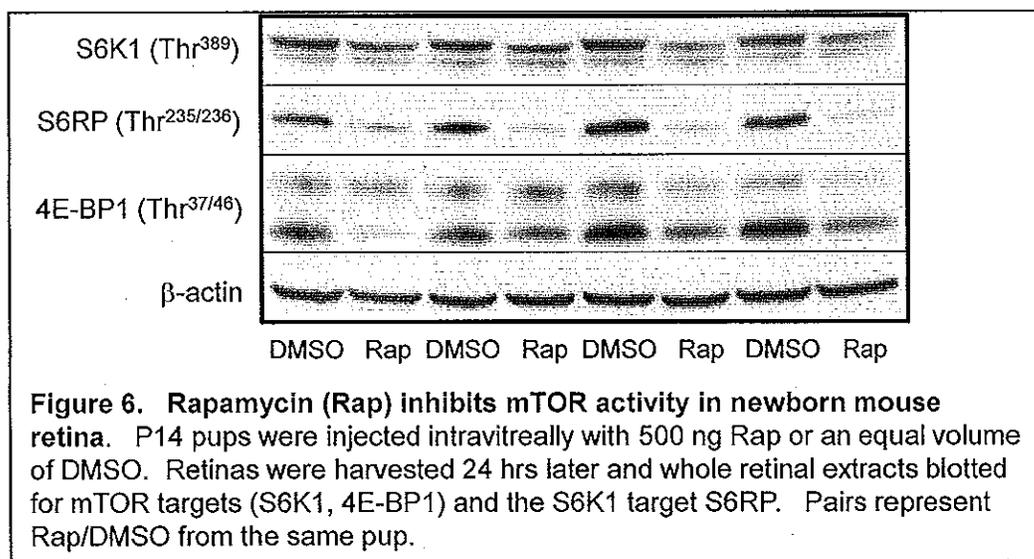
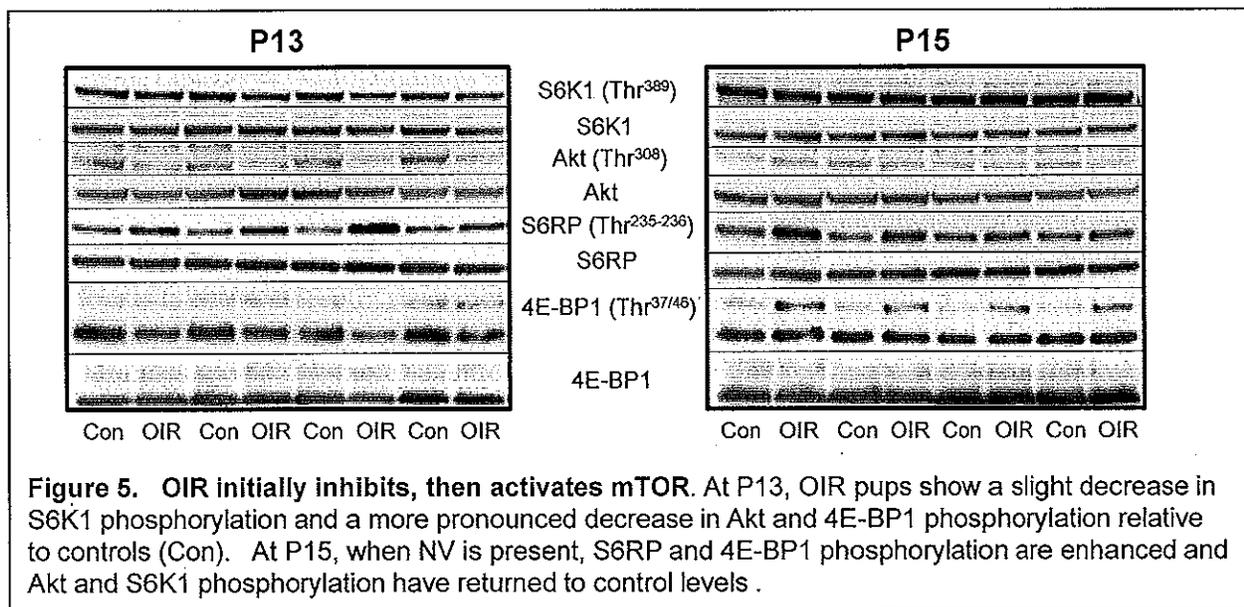


Fig 4. mTOR signaling cascade. Growth factors and hormones activate Akt (PKB) which in turn inhibits TSC1/TSC2 allowing for the activation of Rheb GTPase activity and the subsequent activation of mTOR. mTOR may then phosphorylate 4E-BP1 and/or S6K1.

To this point, the work from this grant has generated one published abstract presented at national meeting (Zhang L, Kimball S, Jefferson LS, and JS Shenberger. "Mammalian Target of Rapamycin Activation in the Mouse Model of Retinopathy of Prematurity" *Pediatr Acad Soc*, 2011). We are presently completing analysis of additional data not funded by this grant for

inclusion in a manuscript to be submitted later this summer (planned Investigative Ophthalmology and Vision Science).



References

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18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____ Yes
 X No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____ Yes
 X No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____ Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____ Number of subjects originally targeted to be included in the study
_____ Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____ Males
_____ Females
_____ Unknown

Ethnicity:

- Latinos or Hispanics
 Not Latinos or Hispanics
 Unknown

Race:

- American Indian or Alaska Native
 Asian
 Blacks or African American
 Native Hawaiian or Other Pacific Islander
 White
 Other, specify: _____
 Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?

- Yes
 No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?

- Yes
 No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic

copy of each publication, listed in the table, in a PDF version 5.0.5 format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the “Cognition and MRI in Older Adults” research project (Project 1), and two publications for PI Zhang for the “Lung Cancer” research project (Project 3), the filenames should be:

- Project 1 – Smith – Publication 1 – Cognition and MRI
- Project 1 – Smith – Publication 2 – Cognition and MRI
- Project 3 – Zhang – Publication 1 – Lung Cancer
- Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				<input type="checkbox"/> Submitted <input type="checkbox"/> Accepted <input type="checkbox"/> Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes X No _____

We are finishing the additional data collection in WT animals that will allow us to submit a manuscript illustrating the effects of OIR on mTOR signaling in the retina. Potential journals include *Pediatric Research* and *Investigative Ophthalmology and Vision Science*.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert “None”; do not use “Not applicable.” Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. **DO NOT DELETE THESE INSTRUCTIONS.** There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes _____ No X

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

- a. Title of Invention:
- b. Name of Inventor(s):
- c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):
- d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes _____ No _____

If yes, indicate date patent was filed:

- e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes _____ No _____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

- f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes _____ No _____

If yes, how many licenses were granted? _____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No___

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes _____ No X _____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. DO NOT EXCEED FOUR PAGES.

NAME Jeffrey S. Shenberger, M.D.		POSITION TITLE Associated Professor of Pediatrics and Cellular & Molecular Physiology	
eRA COMMONS USER NAME JSHENBERGER			
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)			
INSTITUTION AND LOCATION	DEGREE (if applicable)	YEAR(s)	FIELD OF STUDY
Franklin and Marshall College, Lancaster, PA	B.A.	1984	Chemistry
Pennsylvania State University, Hershey, PA	M.D.	1989	Medicine
Univ. of Minnesota Hospitals, Minneapolis, MN	Residency	1992	Pediatrics
Univ. of Minnesota Hospitals, Minneapolis, MN	Fellowship	1995	Newborn Medicine

A. Positions and Honors

Positions and Employment:

- 1995-1999 Staff Neonatologist, Wilford Hall USAF Medical Center, Lackland AFB, TX
Staff Neonatologist, Darnall Army Community Hospital, Fort Hood, TX
- 1996-1999 Director, Neonatal Research, Wilford Hall USAF Medical Center, Lackland AFB, TX
- 1998-1999 Clinical Assistant Professor, Dept. of Pediatrics, Div. of Neonatology, University of Texas San Antonio School of Medicine, San Antonio, TX
- 1999-2000 Assistant Director of Extracorporeal Membrane Oxygenation Program, Dept. of Pediatrics, Div. of Neonatology, University of Kentucky Medical School, Lexington, KY
- 1999-2001 Assistant Professor, Dept. of Pediatrics, Div. of Neonatology, University of Kentucky Medical School, Lexington, KY
- 2000-2001 Staff Neonatologist, St. Joseph's East Hospital, Lexington, KY
- 2000-2001 Director of Extracorporeal Membrane Oxygenation Program, Dept. of Pediatrics, Div. of Neonatology, University of Kentucky Medical School, Lexington, KY
- 2001-2002 Assistant Professor, Dept. of Pediatrics, Div. of Neonatology, Dartmouth Medical School, Hanover, NH
- 2002-2006 Associate Professor, Dept. of Pediatrics, Div. of Neonatology, Dartmouth Medical School, Hanover, NH
- 2006-2009 Physician-Scientist Program, Pennsylvania State University College of Medicine, Hershey, PA
- 2006- Associate Professor, Dept. of Pediatrics, Div. of Newborn Medicine, Dept. of Cellular and Molecular Physiology, Pennsylvania State University College of Medicine, Hershey, PA
- 2006- MD/PhD Training Program Faculty member, Pennsylvania State University College of Medicine, Hershey, PA
- 2009- Director of Neonatal Extracorporeal Membrane Oxygenation, Div. of Neonatology, Penn State Children's Hospital, Hershey, PA

Other Experience and Professional Memberships

- 1995- Member, American Academy of Pediatrics
- 1999- Member, American Thoracic Society
- 1998- Member, Society for Pediatric Research
- 2001- Member, Society for Free Radical Biology and Medicine
- 2012- Council member, Eastern Society for Pediatric Research

Honors and Awards:

- 1995-1999 Military Indoctrination for Medical Officers, Top Performer (1995)
- Ogden Bruton Award for Basic Science Research, Winner (1997)
- Meritorious Achievement Medal (1997)
- Ogden Bruton Award for Basic Science Research, Semi-finalist (1998),
- Meritorious Achievement Medal, First Oak Leaf Cluster (1998)
- Ogden Bruton Award for Basic Science Research, Finalist, (1999)
- Meritorious Achievement Medal, Second Oak Leaf Cluster, (1999) - United States Air Force.

B. Selected Peer-reviewed publications

Most relevant to current application

1. Shenberger JS, Shew RL, and DE Johnson. Hyperoxia-induced airway remodeling and neuroendocrine cell hyperplasia in the weanling rat. *Pediatr Res* 42(4):539-544, 1997.
2. Shenberger JS and PS Dixon. Oxygen induces S phase growth arrest and increases p53 and p21^{WAF1/CIP1} expression in human bronchial smooth muscle cells. *Am J Respir Cell Mol Biol* 21:395-403, 1999.
3. Shenberger JS, Adams MH, and SG Zimmer. Oxidant-induced hypertrophy of A549 cells is accompanied by alterations in eukaryotic translation initiation factor 4E and 4E-binding protein. *Am J Respir Cell Mol Biol* 27:250-256, 2002.
4. Shenberger JS, Myers JL, Zimmer SG, Powell RJ, and A Barchowsky. Hyperoxia alters the phosphorylation and binding of multiple factors regulating translation initiation. *Am J Physiol Lung Cell Mol Physiol* 288:L442-L449 2005 (PMC2675186).
5. McAdams RM, Mustafa SB, Shenberger JS, Dixon PS, and RJ DiGeronimo. Cyclic stretch attenuates the effects of hyperoxia on cell proliferation and viability in human alveolar epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 291:L166-174, 2006 (PMC2683386).
6. Shenberger JS, Zhang L, Hughlock M, Ueda T, Watanabe-Fukunaga R, and R Fukunaga. Roles of mitogen-activated protein kinase signal-integrating kinases 1 and 2 in oxidant-mediated eIF4E phosphorylation and translational repression. *Int J Biochem Cell Biol* 39(10):1828-42, 2007 (PMC2001257).
7. Shenberger JS, Zhang L, Powell R, and A Barchowsky. Hyperoxia enhances VEGF release from A549 cells via post-transcriptional processes. *Free Radic Biol Med* 43(5):844-52, 2007 (PMC1959513).
8. Zhang L, Kimball SR, Jefferson LS, and JS Shenberger. Hydrogen peroxide impairs insulin-stimulated assembly of mTORC1. *Free Radic Biol Med* 46(11):1500-9, 2009 (PMC2677139).
9. Konsavage W, Zhang L, Vary T, and JS Shenberger. Hyperoxia inhibits protein synthesis and increases eIF2 α phosphorylation in the newborn rat lung. *Am J Physiol Lung Cell Mol Physiol* 298(5):L678-86, 2010.

10. Floros J, Londono D, Gordon D, Silveyra P, DiAngelo S, Viscardi R, Worthen G, Shenberger J, Wang G, Lin Z, and N. Thomas. IL-18R1 and IL-18RAP SNPs may associate with bronchopulmonary dysplasia in African American infants. *Pediatr Res* 71(1):107-114, 2012.
11. Konsavage W, Zhang L, Wu Y, and Shenberger J. Hyperoxia-induced activation of the integrated stress response in the newborn rat lung. *Am J Physiol - Lung Cell Mol Physiol*, 302(1):L27-35, 2012.

Select additional publications

1. Shenberger JS, Shew RL, Johnson DE, and Kannan MS. Relaxation of porcine tracheal smooth muscle by parathyroid hormone-related protein. *Respiration Physiol* 107:59-66, 1997.
2. Shenberger JS, Dixon PS, Choate J, Helal K, Shew RL, and W Barth. Pregnancy and labor increase the capacity of human myometrial cells to secrete parathyroid hormone-related protein. *Life Sciences* 68:1557-66, 2001.
3. Reed-Thurston D, Shenberger J, Qiu F, and A Ündar. "Neonatal Extracorporeal Life Support (ECLS): Will the newest technology reduce morbidity?" *Artificial Organs* 35(11):989-996, 2011.
4. Reed-Thurston D, Ündar A, Haidat KK, and JS Shenberger. "Pediatric and neonatal extracorporeal life support (ECLS) technology component utilization: Are U.S. clinicians implementing new technology?" *Artificial Organs*, 1525-1594, 2012 (In Press).

C. Research Support

Ongoing Research Support

Children's Miracle Network, Penn State College of Medicine Jul 1, 2010 - Aug 30, 2012

"Role of mTOR in Oxygen-induced Retinopathy"

The goal of this project is to investigate alterations in mTOR activation during the hypoxic and hyperoxic phases on OIR in mice.

Role: PI

Pending Support

National Institutes of Health, NICHD, R21, "GM-CSF in lung modulation of alveolar development and antiviral immunity", Co-investigator, 5%.

Budgetary Overlap: The current project does not involve budgetary or scientific overlap with any of the currently funded or pending grant applications.