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| 13. SUPPLEMENTARY NOTES | | | | | |
| 14. ABSTRACT Purpose: The present research project is designed to define the overall change in gene expression in the eye following a blast injury to the eye. In this process the genetic networks activated by injury will be defined along with biological markers of retinal injury. Scope: The proposal will examine the changes in gene expression that occur in a mouse genetic reference panel, the BXD recombinant inbred (RI) strain set. This analysis will define genomic loci modulating the response of the eye to a blast injury and the genetic networks activated by the injury. Major Finding: Collected retinas from 40 normal strains with 148 microarrays run. We have collected phenotypic data on corneal thickness, IOP and lens opacity on 27 strains of normal mice and 27 strains following a 50psi blast injury. There was no difference in corneal thickness 5 days following the blast injury. We have added 8 strains (32 microarrays) to the TATRC Normal Retinal Database that contains 148 microarrays from 38 normal strains. We have opened the DoD TATRC Normal Retina Database to the public at GeneNetwork.org. We have identified SOX11 as a good marker for neuronal injury in the retina and the manuscripts describing these results are being completed for publication. In January, the lab was moved to Emory University. We have constructed a new blast gun for use at Emory. With Dr. Mike Iuvone we have characterized the injury created by this blast (publication in preparation). We have all animal protocols in place and have established a colony of BXD mice to use in the remaining portions of the projects. | | | | | |
| 15. SUBJECT TERMS Genomics, Blast Injury, Eye, Retina | | | | | |
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1. INTRODUCTION

Our laboratory developed a mouse model of blast injury to the eye, which accurately mimics the traumatic blast injury increasingly suffered by warriors under current battlefield conditions (Hines-Beard et al., 2012). We are taking full advantage of this mouse model in combination with a powerful combination of systems biology, microarray analysis, expression genetics, and bioinformatics. At the heart of our approach is a genetic reference panel of mice, the unique resource of BXD recombinant inbred (RI) strain set. The set of RI strains was produced from a genetic cross between the C57BL/6J mouse and the DBA/2J mouse. Using 60 BXD strains provides a new and powerful method to defining elements in the genome regulating the response of the eye to blast injury. This allows us to generate specific, testable hypotheses to define the pathways that regulate the response of the eye to blast injury and reactive responses in the retina. As more diverse gene expression data sets become available, comparison of gene expression and regulation in different biological contexts will help identify the regulatory elements controlling the injury response of the eye and the retina. We will identify genetic networks activated by blast injury and the genomic loci modulating these genes. In addition, we hope to identify new markers for retinal injury as well as potential targets for intervention.

2. KEYWORDS

Mouse Genomics, Blast Injury, Eye, Retina, Gene Expression, Microarray

3. ACCOMPLISHMENTS

Major Goals:

Task 1) Quantify the strain-to-strain differences in the severity of blast-induced ocular pathologies, using a set of 60 BXD RI mouse strains and map the genomic loci that regulate the response of the eye to blast injury. In this Task we were measuring intraocular pressure (IOP), central corneal thickness (CCT) and visual acuity.

Task 2) Define the genetic networks activated by blast injury in the eye and in the retina, using transcriptome-wide profiling across the BXD RI strain set. We are using the Affymetrix GeneChip Gene 2.0 ST Mouse Array to characterize the changes occurring following a blast injury to the eye in 60 BXD strains. There were several major benefits to using the new Affymetrix array. Specifically, there are probes for 7,000 non-coding RNAs (RNA that is not converted to protein but does affect the functioning of the cell). We are now finding out that many of these non-coding RNAs play extremely important roles in the body. Within these 7,000 probes, 588 encode microRNAs (small RNAs that regulate protein expression). We are creating an entire normal retina dataset using the Affymetrix GeneChip Gene 2.0 Mouse Array and comparing this data set to a dataset from retinas 5 days after a 50psi blast injury to the eye.

Task 3) Define biomarkers that can predict the severity of injury and eventual outcomes.

This portion of our study was to begin in the latter years of the grant (Months 40 to 48). We are using this to characterize the 50-psi blast injury in advance of resuming the blast microarray study on the BXD RI strain set. Immunostaining sections of retina revealed that SOX11 was upregulated in the neurons of the inner retina following blast. SOX11 labeled cells in the ganglion cell layer and the inner nuclear layer. In the ganglion cell layer SOX11 labeled a majority of the cells, indicating that it was labeling most ganglion cells and displaced amacrine cells. Once the datasets are fully implemented, we will be able to accurately define the changes occurring within the injured retina.

Accomplishments Under These Goals:

Task1:

At the present time we have measured IOP and central corneal thickness on 27 strains of mice. We have also begun to measure the changes occurring following 50psi blast injury to the eye of 27 BXD Strains. The total number of animals, pre and post blast now sits at 88 and the data is presented in Table 1. These data were all collected at the University of Tennessee. We have our animal protocols in

place at Emory University and are waiting for ACURO to approve our animal protocol before beginning data collection. Personnel are trained on taking these phenotypic measurements. We have our CCT measurements and IOP measurements on our first strain here at Emory.

Table 1 Pre and Sd Post-Blast IOP Data To Date (October 1 2013)

| BXD Strain | Pre-Blast OO Avg IOP (mmHg) | Sd Post-Blast OD Avg IOP {mmHg} | Pre-Blast OS Avg IOP (mmHg) | Sd Post-Blast OS Avg IOP {mmHg} | No. of Mice |
|------------|--------------------------------|---------------------------------|-----------------------------|---------------------------------|-------------|
| C57BL/6l | 12.0 | 17.1 | 11.2 | 14.7 | 6 |
| 2 | 13.8 | 10.7 | 13.6 | 13.4 | 4 |
| 5 | 12.3 | 15.0 | 13.2 | 13.2 | 4 |
| 6 | 10.8 | 16.2 | 12.2 | 16.2 | 2 |
| 8 | 11.5 | 15.2 | 10.2 | 14.4 | 3 |
| 9 | 13.9 | 16.5 | 15.7 | 9.7 | 2 |
| 12 | 14.2 | 13.8 | 15.0 | 12.8 | 4 |
| 14 | 17.1 | 15.1 | 16.9 | 18.6 | 2 |
| 15 | 11.6 | 12.9 | 15.0 | 15.5 | 3 |
| 18 | 14.6 | 17.8 | 15.7 | 16.1 | 4 |
| 19 | 18.1 | 10.5 | 16.7 | 13.1 | 4 |
| 21 | 19.8 | 17.7 | 16.8 | 17.1 | 4 |
| 22 | 11.4 | 12.6 | 12.8 | 11.5 | 4 |
| 40 | 11.4 | 7.4 | 14.0 | 7.1 | 2 |
| 48 | 16.4 | 12.4 | 12.0 | 10.4 | 4 |
| 51 | 14.6 | 12.9 | 17.8 | 12.8 | 4 |
| 60 | 16.2 | 14.0 | 14.5 | 15.8 | 2 |
| 63 | 17.0 | 12.9 | 18.3 | 13.9 | 4 |
| 65 | 13.5 | 13.6 | 14.8 | 12.6 | 3 |
| 69 | 15.3 | 10.0 | 16.0 | 14.4 | 2 |
| 73 | 15.9 | 12.0 | 15.4 | 20.8 | 1 |
| 86 | 11.2 | 18.8 | 10.8 | 13.6 | 2 |
| 87 | 14.1 | 13.0 | 14.1 | 11.9 | 3 |
| 90 | 10.6 | 9.0 | 11.6 | 10.3 | 1 |
| 92A | 11.4 | 9.9 | 12.9 | 11.9 | 4 |
| 96 | 8.5 | 14.3 | 11.5 | 7.5 | 1 |
| 100 | 9.3 | 14.1 | 9.4 | 18.3 | 2 |
| 101 | 11.8 | 15.6 | 15.0 | 13.1 | 4 |
| 102 | 15.9 | 15.0 | 16.1 | 12.5 | 3 |
| 27 | Total Number of Strains | | TotalJ Number of Mice_____ | | BB |

Characterizing the effect of blast directed at the eye on visual function and the retina.

With Dr. Mike Iuvone at the University of Emory, we are setting up a new blast gun and characterizing the effect of blast to the eye on the retina. We delivered a single 50psi blast to the left eye of 18 mice (14 Thy1-CFP and 4 wild typeC57BL/6 mice). All mice survived the first week following blast. Seven of the mice were euthanized at 1 week (on April 9, 2014) for an initial assessment of retinal anatomy. Fundus images of the remaining mice were obtained at 1, 2, 3 and 6 weeks. Flat mounts of the retinas and retinal sections were examined for Thy1-CFP (retinal ganglion cells), Iba1 (microglia), and glial fibrillary acid protein (GFAP). Figure 1 shows the effects of blast injury at 7 days on Thy-CFP fluorescence, as

observed in fundus images. The blast injury induced a statistically significant increase in fluorescence. In addition, we observed swelling of the RGC cell bodies, indicative of cellular damage. These changes were accompanied by an increase in Ibal-immunopositive microglia (Fig. 2), suggestive of an inflammatory response to blast, and an increase in GFAP, indicative of reactive gliosis.

Behavioral testing (Optokinetic tracking) was used to assess visual acuity and contrast sensitivity at 7 weeks after blast. There was a highly significant reduction in contrast sensitivity in blasted mice compared to controls ($p < 0.001$; Figure 4). There was also a trend for a small reduction in visual acuity in the blasted mice, but it was not statistically significant ($p = 0.08$; Figure 4). Two months after exposure to blast, the remaining mice were sacrificed and retinas prepared as flat mount preparations to count Thy1-CFP ganglion cells. A small, but statistically significant reduction in retinal ganglion cells was observed in both the central and peripheral. To confirm the effect of blast on visual function and to determine how soon after injury we could detect a change in contrast sensitivity, we recently exposed another cohort of mice to 50 psi blast. Taken together these data indicate that the loss of visual function occurs after blast injury, along with a loss of retinal ganglion cells.

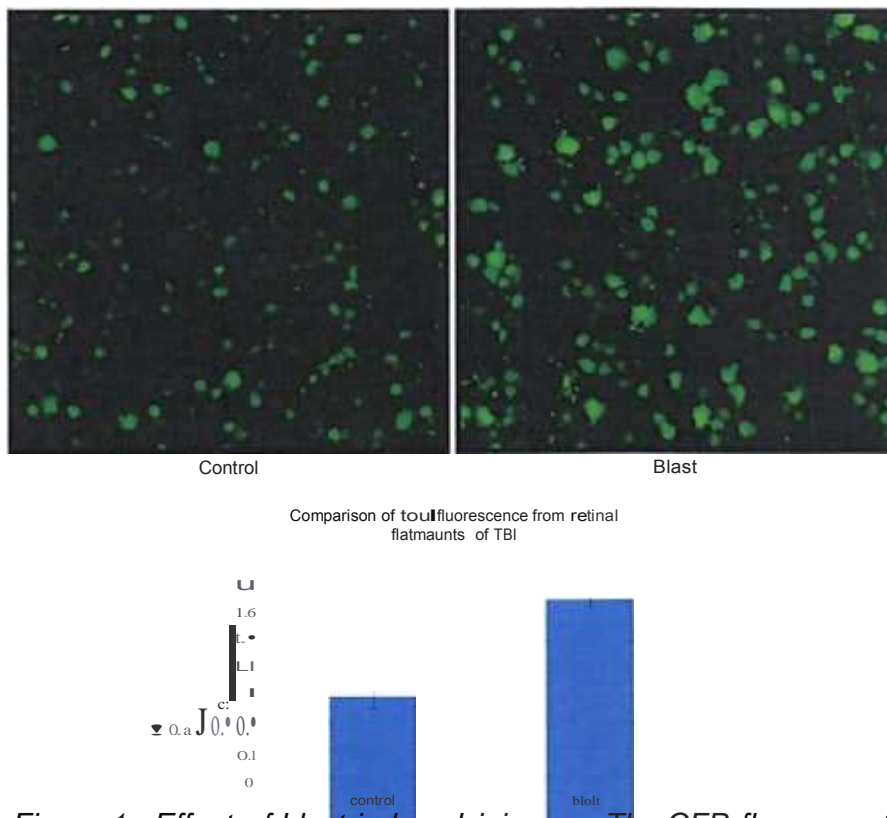


Figure 1. Effect of blast-induced injury on Thy-CFP fluorescent RGCs.

GFAP in red / Iba1 in green

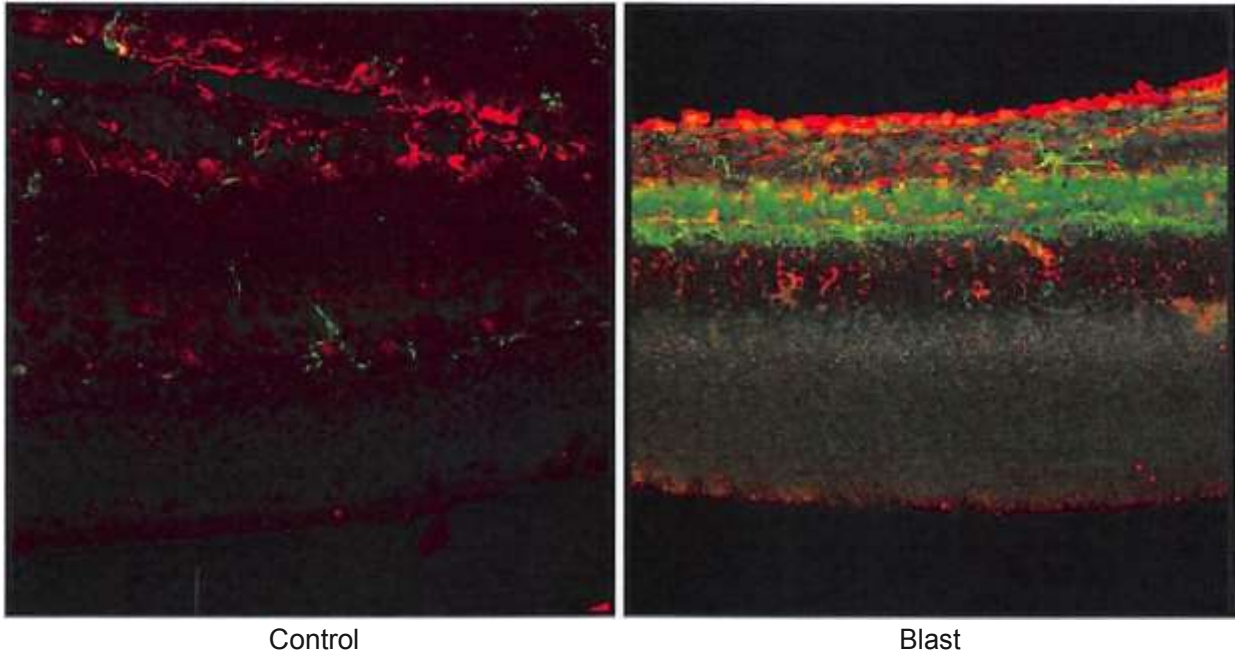


Figure 2. Representative vibratome sections 7 days following blast stained for GFAP (red) and Iba1 (green).

Task 2.

A) Before leaving the University of Tennessee we ran microarrays on 38 strains of mice and collected retinas from additional strains that are stored at -80 degrees C awaiting the transfer of the grant to run the microarrays these retinas are in the freezer until sufficient numbers are available to isolate the RNA.

B) The research group has just purchased a new Qiagen that will be used to isolate RNA for this project.

C) We are using a new array from Affymetrix. It is the GeneChip Gene 2.0 Mouse Array. This array was just released in 2012 and has many features that are not found in any other array. Specifically, there are probes for non-coding RNAs (RNA that is not converted to protein but does effect the functioning of the cell) and probes for 590 microRNAs (small RNAs that regulate protein expression). The new Affymetrix array will allow us to more fully characterize RNA expression in the retina and the changes that occur following Blast injury. We currently have

arrays for 38 normal strains in the DoD TATRC Retina Database on GeneNetwork (Genenetwork.org) with a total number of microarrays at 148. We have released the dataset to the public in April 2014. For the blast injury eyes we have collected data from 50psi blast retinas for 32 strains.

Task 3) We have identified a list of potential biomarkers for injury to the retinal ganglion cells. The best marker is SOX11 (manuscript being revised). We are using this to characterize the 50psi blast injury in advance of resuming the blast microarray study on the BXD RI strain set. Immunostaining sections of retina revealed that SOX11 was upregulated in the neurons of the inner retina following blast. SOX11 labeled cells in the ganglion cell layer and the inner nuclear layer. In the ganglion cell layer SOX11 labeled a majority of the cells, indicating that it was labeling most ganglion cells and displaced amacrine cells. Amacrine cells in the inner nuclear layer were also lightly labeled by SOX11. On immunoblots there was approximately a 2-fold increase in the intensity of the SOX11 band. We are in the final phases of writing the manuscript describing these results and should submit this year.

Training and Professional Development Opportunities:

Nothing to Report

Dissemination of Results:

- 1) SOX11 labeled cells in the ganglion cell layer and the inner nuclear layer. In the ganglion cell layer SOX11 labeled a majority of the cells, indicating that it was labeling most ganglion cells and displaced amacrine cells. This work on SOX11 was presented at the ARVO meeting in Orlando 2014. We are in the final phases of writing the manuscript describing these results and should submit the paper in this year.
- 2) The characterization of the effects of a 50psi blast on the retina is being prepared for publication.
- 3) The DoD TATRC Retina Database was released on GeneNetwork (Genenetwork.org) with a total 148 microarrays from 32 strains. The database was released to the public on April 1, 2014.

Plans for Next Reporting Period to Accomplish the Goals:

- 1) Our first goal is to work with the DoD to complete the transfer of the grant to Emory University.

2) Using seed money I have hired a technician and trained her in many of the techniques necessary to conduct this research. Once the grant is activated we will transfer her to the DoD grant to conduct the research on the BXD strains.

3) The blast gun is constructed and working. Once the grant is activated we will immediately begin blasting the eyes of BXD mice and collecting the retinas for Microarrays.

4.IMPACT

Impact on the Development of the Principal Discipline of the Project:

Once the proposed studies are completed they will provide a comprehensive analysis of the molecular pathways activated in the retina by blast injury to the eye.

Impact on Other Disciplines:

When developing Biomarkers for retinal injury, our microarray dataset will provide a means to determine if any specific biomarker could have originated from the retinal injury itself.

Impact on Society Beyond Science and Technology:

Nothing to Report

5) Changes/Problems

Changes in Approach and Reasons for Change:

None

Actual or Anticipated Problems or Delays and Actions or Plans to Resolve Them:

In January 2014, the lab moved to Emory University. We are currently in the process of transferring the grant to Emory University. I have hired a technician using start up money and she is now fully trained to begin working on the DoD grant once it is fully funded at Emory University.

Changes that had Significant Impact on Expenditures:

None

Significant Changes in the Use or Care of Human Subjects Vertebrate Animals Biohazards, or Select Agents:

At Emory we have an approved IACUC protocol for the DoD grant and are currently awaiting ACURO approval. The protocol is basically the same as was being used at the University of Tennessee.

6.PRODUCTS

Publications, conference papers, and presentations:

Conference Presentations:

Hart, Steven G; Wang, XiangDi; Rex, Tonia S.; Geisert, Eldon E. Biomarkers for Neuronal Injury Following Blast Trauma to the Eye. Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, May 5-9,2013, Seattle, Washington.

Bricker-Anthony, Courtney, Jessica Hines-Beard, Lauren D'Sumey, Eldon E Geisert, Tonia S Rex, Ocular Blast Trauma in the DBA/2J Mouse. Association for Research in Vision and Ophthalmology (ARVO) Annual Meeting, May 5-9,2013, Seattle, Washington.

Geisert E.E., Joe Caron, XiangDi Wang, SOX11 Marks injured retinal ganglion cells. Association for Research in Vision and Ophthalmology (ARVO) Orlando Florida 2014.

Website(s) or other Internet site(s):

The DoD TATRC Retina Affy MoGene 2.0 ST Database and the DoD TATRC Retina Affy MoGene 2.0 ST Exon Level Database are hosted on GeneNetwork.org. This database was open to the public in April 2014. These datasets describe gene expression in the normal retina of the BXD Strains. Both databases can be found under Mice, BXD, retina and then either DoD TATRC Retina Affy MoGene 2.0 ST Database or DoD TATRC Retina Affy MoGene 2.0 ST Exon Level Database.

Technologies or techniques:

None

Inventions, patent applications, and/or licenses:

None

Other products:

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

At the University of Tennessee (7/15/13 to 12/31/13):

| | |
|-----------------------------|------------------------------|
| Name: | Eldon E. Geisert PhD |
| Project Role | PI, Oversight of the project |
| Nearest Person Month Worked | 4 |

| | |
|-----------------------------|--|
| Name: | XiangDi Wang MD, PhD |
| Project Role | Research Associate, Dr. Wang collected the retinas and ran the visual phenotyping of the mice. |
| Nearest Person Month Worked | 6 |

| | |
|-----------------------------|---------------------|
| Name: | Justin Templeton MD |
| Project Role | Postdoctoral Fellow |
| Nearest Person Month Worked | 3 |

At Emory University (1/1/14 to present):

We have been collaborating with Dr. Mike Iuvone to construct and test a new blast gun. We are currently in the process of writing a manuscript describing the effects of a 50psi blast to the mouse eye. We are awaiting final approval of our DoD Grant to put specific people on the grant.

Has there been a change in the other active support of the PD/PI(s) or senior/key personnel since the last reporting period?

I have moved my laboratory to Emory. I have transferred my NEI R01 grant to Emory and am in the process of completing the transfer of this DoD grant.

What other organizations have been involved as partners?
None

8. SPECIAL REPORTING REQUIREMENTS

None

9. APPENDICES

None