Published in final edited form as:

Adv Exp Med Biol. 2012 ; 723: 649–656. doi:10.1007/978-1-4614-0631-0_82.

Networks Modulating the Retinal Response to Injury: Insights from Microarrays, Expression Genetics, and Bioinformatics

F.R. Vázquez-Chona and

Department of Ophthalmology, University of Utah, 65 Mario Capecchi Drive, Salt Lake City, UT 84132, USA

E.E. Geisert

Department of Ophthalmology, The Hamilton Eye Institute University of Tennessee Health Science Center, 930 Madison Avenue, Suite 731, Memphis, TN 38163, USA

F.R. Vázquez-Chona: felix.vazquez@utah.edu; E.E. Geisert: egeisert@uthsc.edu

Keywords

Retinal injury; Neuronal degeneration; Genetic networks; Crystallin; Glaucoma; Microarray; Lipin 1; QTL analysis; Ganglion cell survival

82.1 Introduction

While our knowledge of the morphological and cellular changes of retinal wound healing is extensive, a select few of the phenotypic changes has been related to molecular makers and regulatory mechanisms. We used microarray technology to catalog gene expression changes after retinal injury and to relate the expression profiles to the biochemical and cellular context of retinal healing (Vazquez-Chona et al. 2004; Templeton et al. 2009).

82.2 Gene Expression After Retinal Injury

Our initial studies of retinal injury in adult rats revealed widespread changes at the site of injury and throughout the retina (Fig. 82.1a). From a technical aspect, we have shown that microarrays reliably predict the direction of mRNA and protein changes, but provide qualitatively incomplete assessments of posttranscriptional and posttranslational modifications (Rogojina et al. 2003; Vazquez-Chona et al. 2004). Surprisingly, the large number of expression changes was highly regulated into three temporal patterns of expression – early acute (within hours), delayed subacute (within days), and late chronic phases (within weeks) (Fig. 82.1b). Genes within each phase are functionally related and reflect the sequelae of retinal wound healing (Fig. 82.1c). Of interest was the coordinated upregulation of crystallins α , β , and γ . Traditionally, crystallins were thought to function as structural lens components, but gene-sequence homology studies show that crystallins share homology with stress response genes such as heat-shock genes (Piatigorsky 1998; Horwitz 2003). Supporting their stress response function is the correlation of enhanced survival during degeneration with crystallin upregulation. For example, C57BL/6 mice express higher levels of crystallins and are more resistant to increased intraocular pressure than DBA/2J mice (Steele et al. 2006). The highly coordinated expression of crystallins and other functionally related groups suggested the presence of networks controlling the retinal response to injury.

82.3 Expression Genetics of Retinal Injury Genes

To discover the networks modulating gene expression, we used quantitative trait locus (QTL) analysis in mouse strains with known neurological differences (Vazquez-Chona et al. 2005, 2007). The C57BL/6 and DBA/2J strains differ in their response to increased intraocular pressure, optic nerve crush, neurotoxicity, audiogenic stress, and adult neurogenesis (Schauwecker and Steward 1997; Inman et al. 2002; John 2005; Templeton et al. 2009). To reveal the genetic networks modulating neurological phenotypes, members of our group combined expression profiling with linkage analysis in BXD recombinant inbred (RI) lines derived from the C57BL/6 (B) and DBA/2J (D) mouse strains (Chesler et al. 2005). Expression QTL (eQTL) analyses in BXD RI strains revealed that loci modulating expression phenotypes are also related to neurological phenotypes, and that distinct regulatory loci modulate functionally related genes (Chesler et al. 2005). Basal expression data from forebrain, striatum, and cerebellum as well as phenotypes and genetic linkage analyses for BXD RI strains are publicly available at www.GeneNetwork.org. We mined the expression genetics data at GeneNetwork to identify the eQTLs that modulate the expression of CNS wound-healing genes (Vazquez-Chona et al. 2005, 2007).

In BXD RI mouse forebrains, we found that the basal expression of acute phase genes is modulated by eQTLs on chromosomes 6, 12, and 14 (Fig. 82.2a) (Vazquez-Chona et al. 2005). Specificity and reliability were determined by comparing eQTLs across functional groups and tissues. For example, synaptic-related genes were also controlled by eQTLs on Chr. 6 and 14 in mouse forebrains, whereas wound-healing genes shared no eQTLs in analyses from hematopoietic stem cells (Vazquez-Chona et al. 2007). Thus the eQTL within the 10–30 Mb interval of Chr. 12 modulates acute phase genes known to be involved in CNS healing, neurogenesis, and cell death (Vazquez-Chona et al. 2007) (Fig. 82.3). BXD neurological phenotypes further support the role Chr. 12 network in the CNS response to degeneration (Fig. 82.2b). In BXD RI mouse strains, the DBA/2J allele for the Chr. 12 locus is one of the loci associated with susceptibility to noise-induced hearing loss and audiogenic seizures when exposed to intense auditory stimulation (Neumann and Collins 1991; Willott and Erway 1998). By contrast, the C57BL/6 allele is associated with enhanced neurogenesis and survival of new neurons and astrocytes in adult hippocampus (Kempermann and Gage 2002). Together BXD phenotypic and expression data suggest that a polymorphic gene within the Chr. 12 locus modulates the CNS response to degeneration (Vazquez-Chona et al. 2007).

82.4 Bioinformatics Can Predict Candidate Modulators

We used a suite of bioinformatic analyses and online databases (Table 82.1) to identify polymorphic genes with (a) expression variability correlating to their loci (*cis*-eQTLs), (b) single-nucleotide polymorphisms (SNPs) in motifs that can alter expression, and (c) biological significance to CNS wound healing (Vazquez-Chona et al. 2005, 2007). These analyses suggested that *Id2* and *Lpin1* were good candidate genes. Their expression variability in forebrain, cerebellum, and striatum of BXD RI mouse strains displayed highly significant *cis*-eQTLs (Fig. 82.2c). This means that a genetic variant (or variants) within or near the loci for *Id2* and *Lpin1* alters their expression. For example, our bioinformatic analyses of functional motifs showed that SNPs in *Id2* and *Lpin1* might affect transcription factor binding sites and splice variants, respectively (Vazquez-Chona et al. 2007). Publicly available microarray data also revealed that *Id2* and *Lpin1* are differentially displayed during retinal development and healing. Moreover, *Id2* and *Lpin1* were expressed in reactive glia of optic nerve heads and diabetic retinas (Vazquez-Chona et al. 2007). Together these bioinformatic analyses provided the rationale for focusing on transcription regulator *Id2* and the nuclear protein *Lpin1* as the best, current candidate genes.

82.5 Predicted Networks Require Validation

Evidence supporting the regulatory mechanism predicted by expression genetics and bioinformatics can include relevant cellular localization as well as relevant phenotypes from expression manipulation in cell and animal studies. For example, *Id2* is upregulated by reactive glia in brain, spinal cord, and optic nerve head (Vázquez-Chona et al. 2005). Moreover, our knockdown of *Id2* mRNA in cultured cerebellar astrocytes resulted in decreased migration and proliferation (Vázquez-Chona et al. 2007). Genetic studies show that *ID2* deficiency enhances ocular metastasis and apoptotic rates in epithelial cells (Yokota and Mori 2002; Agapova et al. 2010). Currently, we are also measuring the wound-healing response in a mouse line expressing a nonfunctional Lipin 1 protein, the *fl d* (fat-liver dystrophy) mouse (Peterfy et al. 2001). After optic nerve crush injury, *fl d* mice displayed a 29% increase in surviving NeuN⁺ cells relative to wild-type mice ($p < 0.05$, student *t*-test). The enhanced survival of ganglion cells in *fl d* mice correlated with the upregulation of the crystallin family; whereas in wild-type mice the increased cell death correlated with crystallin downregulation (Fig. 82.2d). Similarly, DBA/2J mice upregulated crystallins and is more resistant to optic nerve crushes than C57BL/6 mice (Templeton et al. 2009). These data suggest that the Crystallin Network is associated with increased survival of ganglion cells after optic nerve crush, and that Lipin 1 is an upstream modulator of the Crystallin Network and Chr. 12 network.

82.6 Conclusion

The present series of studies defined global changes that occur after ocular injury. They also defined a genetic network that modulates the retinal wound-healing response. Characterizing the interaction of Lipin 1 and the Crystallin Network may point to therapeutic strategies for enhancing ganglion cell survival. It may also lead to a genetic fingerprint or biomarker for predicting patients at higher risk of ganglion cell loss in blinding diseases such as glaucoma and ocular neuropathy. Our work is also moving microarray analyses from cataloging expression changes toward the discovery of expression networks and their modulators. During this process, we have developed approaches to define genetic networks by integrating gene expression profiling and higher-level bioinformatic analyses.

Acknowledgments

EEG received support from PHS grant RO1EY017841, NIH/NEI Core Grant 5P30 EY13080-04S1, and unrestricted grant from Research to Prevent Blindness. FVC received support from Daniel L. Gerwin Fellowship, Fight For Sight fellowships SF04031 and PD07010, International Retinal Research Foundation (Charles D. Kelman, MD Postdoctoral Scholar award), NIH Training Grant 5T32 HD07491, and Knights Templar Eye Foundation.

References

- Agapova OA, Person E, Harbour JW. *Id2* deficiency promotes metastasis in a mouse model of ocular cancer. *Clin Exp Metastasis*. 2010; 27:91–96. [PubMed: 20127274]
- Chesler EJ, Lu L, Shou S, et al. Complex trait analysis of gene expression uncovers poly-genic and pleiotropic networks that modulate nervous system function. *Nat Genet*. 2005; 37:233–242. [PubMed: 15711545]
- Horwitz J. Alpha-crystallin. *Exp Eye Res*. 2003; 76:145–153. [PubMed: 12565801]
- Inman D, Guth L, Steward O. Genetic influences on secondary degeneration and wound healing following spinal cord injury in various strains of mice. *J Comp Neurol*. 2002; 451:225–235. [PubMed: 12210135]
- John SW. Mechanistic insights into glaucoma provided by experimental genetics the cogan lecture. *Invest Ophthalmol Vis Sci*. 2005; 46:2649–2661. [PubMed: 16043833]

- Kempermann G, Gage FH. Genetic influence on phenotypic differentiation in adult hippocampal neurogenesis. *Brain Res.* 2002; 134:1–12.
- Neumann PE, Collins RL. Genetic dissection of susceptibility to audiogenic seizures in inbred mice. *Proc Natl Acad Sci USA.* 1991; 88:5408–5412. [PubMed: 2052619]
- Peterfy M, Phan J, Xu P, et al. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet.* 2001; 27:121–124. [PubMed: 11138012]
- Piatigorsky J. Multifunctional lens crystallins and corneal enzymes. More than meets the eye. *Annals N Y Acad Sci.* 1998; 842:7–15.
- Rogojina AT, Orr WE, Song BK, et al. Comparing the use of Affymetrix to spotted oligo-nucleotide microarrays using two retinal pigment epithelium cell lines. *Mol Vis.* 2003; 9:482–496. [PubMed: 14551534]
- Schauwecker PE, Steward O. Genetic determinants of susceptibility to excitotoxic cell death: implications for gene targeting approaches. *Proc Natl Acad Sci USA.* 1997; 94:4103–4108. [PubMed: 9108112]
- Steele MR, Inman DM, Calkins DJ, et al. Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. *Invest Ophthalmol Vis Sci.* 2006; 47:977–985. [PubMed: 16505032]
- Templeton JP, Nassr M, Vazquez-Chona F, et al. Differential response of C57BL/6J mouse and DBA/2J mouse to optic nerve crush. *BMC Neurosci.* 2009; 10:90. [PubMed: 19643015]
- Vazquez-Chona F, Song BK, Geisert EE Jr. Temporal changes in gene expression after injury in the rat retina. *Invest Ophthalmol Vis Sci.* 2004; 45:2737–2746. [PubMed: 15277499]
- Vazquez-Chona FR, Lu L, Williams RW, et al. Genomic loci modulating the retinal transcriptome in wound healing. *Gene regulation and systems biology.* 2007; 1:327–348. [PubMed: 19936100]
- Vazquez-Chona FR, Khan AN, Chan CK, et al. Genetic networks controlling retinal injury. *Mol Vis [electronic resource].* 2005; 11:958–970.
- Willott JF, Erway LC. Genetics of age-related hearing loss in mice. IV. Cochlear pathology and hearing loss in 25 BXD recombinant inbred mouse strains. *Hearing Res.* 1998; 119:27–36.
- Yokota Y, Mori S. Role of Id family proteins in growth control. *J Cell Physiol.* 2002; 190:21–28. [PubMed: 11807807]

Fig. 82.1.

Gene expression after retinal injury is highly regulated into distinct temporal groups and functionally related groups. (a) Heat map represents expression upregulation at 0 and 4 h, as well as 3, 7, and 30 days postinjury. *Dark hues* represent basal levels, whereas *bright hues* represent upregulation. Genes are listed in Fig. 82.3a. (b) Expression patterns after injury. (c) Genes within profiles are functionally related. Data adapted from Vázquez-Chona et al. (2004) (Copyright 2004 Association for Research in Vision and Ophthalmology, adapted and reproduced with permission)

Fig. 82.2.

Chromosome 12 locus modulates expression of wound-healing genes. **(a)** Quantitative trait locus (QTL) analysis maps the regulation of basal gene expression in BXD recombinant inbred (RI) strains. This regulation is based on the genetic correlation of expression (individual rows on the *y*-axis) to genomic markers across the mouse genome (*x*-axis). *Blue hues* represent correlations for elevated expression in mice with the C57BL/6 allele at a given locus, and *orange hues* represent correlations for elevated expression in mice with the DBA/2J allele. eQTLs on Chr. 6, 12, and 14 control basal expression of wound-healing genes in mouse forebrains. **(b)** Published data from phenotypes in BXD RI mouse strains further support that Chr. 12 locus associates with neurological phenotypes. **(c)** Linkage analyses for *Id2* and *Lpin1* expression across CNS tissues. **(d)** Gene expression changes in the crystallin family after optic nerve crush in *Lpin1^{fl d/fl d}* and *Lpin1^{+/+}* retina. *fl d* (fat-liver dystrophy) mice express a nonfunctional Lipin 1 protein. Expression changes were measured using a full mouse Illumina microarray and represent averages from biological replicates. **(a–c)** Adapted from Vázquez-Chona et al. (2007), under the Creative Commons Attribution By License (<http://creativecommons.org/licenses/by/3.0/>)

Fig. 82.3.

Chromosome 12 locus modulates transcription, differentiation, proliferation, and apoptotic mechanisms. **(a)** Genetic networks were derived from transcripts sharing eQTLs as shown in Fig. 82.1. *Blue lines* connecting specific genes to the locus represent correlations for elevated gene expression in mice with the C57BL/6 allele, and *orange lines* represent correlation for elevated gene expression in mice with the DBA/2J allele. Genes located within the eQTLs (*cis*-eQTLs) are indicated with a *two-arrow line*. **(b)** The major functional themes described by the network's gene functions are the regulation of transcription, differentiation, proliferation, and cell death. A nonbiased, statistical approach to defining the function of the network ($n = 44$ genes) is to compare the observed number of regulated genes as compared to the expected number in a population belonging to a particular functional category. For the chromosome 12 network, we observed 32% (14 out of 44 genes) of genes to be related to the regulation of neural development and differentiation. This percentage is higher than the percentage (7%) observed among the total population of retinal reactive transcripts and much higher than the percentage of expected genes in the entire genome. **(c)** We queried the biological literature using text-mining tools to illustrate networks within the transcripts grouped into the neurogenesis category (*Pax6*, *Neurod1*, *Neurod2*, *Id2*, *Nfib*, *Egr1*, *Hes3*, *Bcl2*, *Robo1*, *Ets1*, *Sox11*, *Casp3*, *Itgb1*, and *Sdc1*). The literature search documents the number of known molecular interactions of these genes, including activation and inhibition, that occur during neurogenesis. *Asterisk* probe set for *Lpin1* is not available in Affy U34 chip; however, post meta-analysis predicted and experimental models of gene expression confirmed the role of *Lpin1* as a wound-healing gene. Reproduced from Vázquez-Chona et al. (2007), under the Creative Commons Attribution By License. For further information go to <http://creativecommons.org/licenses/by/3.0/>

Table 82.1

Bioinformatic analyses and online databases

Analysis	Database	Website
Gene expression		
Retinal development	Retina developmental gene expression	www.scripps.edu/cb/friedlander/gene_expression
	Mouse retina SAGE library	http://bricweb.partners.org/cepko/default.asp
All tissues	Gene Expression Omnibus (GEO)	www.ncbi.nlm.nih.gov/geo
Expression QTL	GeneNetwork	www.genenetwork.org
Genes within QTLs	Genome browser	http://genome.ucsc.edu
	Ensembl	www.ensembl.org/Mus_musculus
	NCBI MapViewer	www.ncbi.nlm.nih.gov/mapview
Genes and loci causing retinal diseases	RetNet	www.sph.uth.tmc.edu/Retnet/
Loci associated with neurological phenotypes	BXD published phenotypes database	www.genenetwork.org
Single nucleotide polymorphisms (SNPs)	SNP browser	www.genenetwork.org/beta/snpBrowser.py?
	Ensembl Mouse SNPView	
	Entrez SNP databases	www.ensembl.org/Mus_musculus
		www.ncbi.nlm.nih.gov/SNP
Functional motifs		
Transcription factor binding sites	MOTIF	http://motif.genome.jp
Protein domains	Scansite	http://scansite.mit.edu
Cellular distribution of transcript		
Retina	Mouse Retina SAGE Library	http://bricweb.partners.org/cepko/default.asp
Brain	Gene Expression Nervous System Atlas (GENSAT)	www.ncbi.nlm.nih.gov/gensat
Gene ontology	WEB-based GENE SeT Analysis Toolkit (WebGestalt)	http://bioinfo.vanderbilt.edu/webgestalt/
Mining NCBI literature for interactions	Chilibot	www.chilibot.net

Table reproduced from Vázquez-Chona et al. (2007), under the Creative Commons Attribution By License

For further information go to <http://creativecommons.org/licenses/by/3.0/>