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Molecular Mechanism Analysis of Intensive Light-Induced Retinal Damages

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Abstract

Introduction: The retina is a light-sensitive tissue, and intensive light exposure leads to light-induced retinal damage. It is pointed out that photoreceptor damage is responsible for the decrease in retina function. The aim of this study was to detect the main genes and biological terms which are involved in retinal response to intensive light exposure.

Methods: The effect of intensive light on the mouse retina function was searched in the Gene Expression Omnibus (GEO) database. The data of GSE22818 were assessed by the GEO2R program. The significant differentially expressed genes (DEGs) were determined and evaluated via directed protein-protein interaction (PPI) network analysis. The critical significant DEGs were enriched via gene ontology analysis to find the related biological processes, molecular function, and biochemical pathways.

Results: Data analysis indicates that the high intensity of light induces gene expression alteration in the retina. 105 significant DEGs were identified as the main responsive genes to light damage in the retina. STAT3, JUN, IL6ST, SOCS3, ATF3, JUNB, FOSL1, CCL2, ICAM1, FGF2, AGT, MYC, LIF, CISH, and EGR1 were introduced as the critical affected genes. STAT3, JUN, IL6ST, SOCS3, and ATF3 and "Positive regulation of the receptor signaling pathway via JAK-STAT" were highlighted as the key elements of molecular events.

Conclusion: It can be concluded that regulation of the key DEGs and the dependent biological terms can effectively provide tools to prevent the development of light-induced retinal damage.

Keywords: Light; Retina; Mouse; Gene expression change; Network analysis.

Introduction

Photoreceptor cells in the retina are responsible for recognizing light, and this process is the beginning of vision. The retina as light-sensitive tissue is located at the back of the eye. Rod photoreceptors are responsible for detecting "low-light vision and motion detection", while cone photoreceptors are involved in "high-acuity daytime and trichromatic color vision".[1] Investigations indicate that in response to the higher intensity of environmental light, the function of the retina in mice has decreased. This process is associated with light-dependent photoreceptor loss which leads to morphological changes and loss of synaptic connectivity. Various types of damage, including an increased number of inflammatory cells, oxidative stress markers, and impaired reactive gliosis, are highlighted as consequence of light-dependent retinal degeneration.[2]

Gene expression analysis is a suitable method for studying the molecular mechanism of biological, physiological and pathological conditions. Due to the dynamic situation in the gene expression of a biological sample, results can provide a clear perspective of the molecular events.[3,4] Genomics studies are tied to bioinformatics. Bioinformatics as a powerful tool is applied to interpret the expression change of the studied genes and to assess the relationship between genes.[5,6] Protein-protein interaction (PPI) network analysis is an attractive

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method for exploring the properties of the investigated interactomes. The queried genes interact with each other based on specific properties of the genes to construct a network. The network includes the genes as nodes and the connection that links the nodes. The nodes may be linked via undirected connections or directed edges. Detecting regulatory relationships between genes, such as activation, inhibition, and expression, can provide a new insight into molecular events in pathological conditions.[7-9]

Gene ontology is another approach that is applied to find the molecular function, biological processes, and biochemical pathways that are associated with the studied genes. Xie et al. published a document about the toxicity effect of white light-emitting diodes (LEDs) on mouse retina.[10] As reported in the mentioned study, a considerable number of significant differentially expressed genes (DEGs) responded to light exposure. Gene ontology revealed that the significant DEGs were involved in 341 biological terms for the intense exposure of light. The single pathways that were associated with ubiquitin were the key part of light-induced retinal relapse.[10] In the present study, the mouse retina DEGs in response to intensive exposure to light were analyzed and enriched via gene ontology to introduce a new perspective of molecular events.

Methods

Data Collection

To explore the effect of 1000 lux bright light (intense exposure of light) on photoreceptor damage, we compared the gene expression profiles of albino Sprague Dawley rats with the gene expression profiles of control samples. Data are presented in GSE22818 of the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse22818). Details of the experiment are described in GSE22818. The albino Sprague Dawley rats raised in dim cyclic illumination (12 h 5 lux, 12 h darkness) were challenged by 24-hour exposure to bright (1000 lux) light.

Pre-evaluation Analysis

The data were assessed via the GEO2R program to find the possible significant DEGs. The results were visualized as a volcano plot and a Uniform Manifold Approximation and Projection (UMAP) diagram. The significant DEGs were identified and inserted in Excel for more analysis. The uncharacterized genes and repeated DEGs were removed from the file, and the remaining significant DEGs were selected for PPI network analysis.

PPI Network Analysis

Activation, inhibition, expression, binding, catalysis, reaction, and post-translational modification relationships between the selected significant DEGs were investigated via PPI network analysis by CluePedia application v 1.5.7 of Cytoscape software v 3.7.2. The network was created via directed edges that connect the query DEGs. The nodes that were characterized by total degree (degree in + degree out) > 6 were identified as the critical targeted genes.

Gene Ontology Enrichment

The critical DEGs were evaluated via gene ontology enrichment by ClueGO v 2.5.7 application of Cytoscape software to explore the affected biological processes, molecular function, and biochemical pathways. The pathways were determined and grouped based on the Kappa score and network specificity criteria.

Data Validation

The introduced critical DEGs were searched in the literature and confirmed.

Statistical Analysis

The significant DEGs were identified based on adjusted P value < 0.05. The PPI network was performed by considering confidence score = 0.4. The biological terms were introduced in view of Kappa score threshold = 0.4, term p-value, term p-value corrected with Bonferroni step-down, group P value, and group P value corrected with Bonferroni step-down less than 0.05. About medium, "Network specificity" was applied. Merge redundant groups with > 50% overlap were considered in this study.

Results

GEO2R analysis led to the introduction of 136 significant DEGs among 29,078 dysregulated genes. Further analysis showed that the treated samples were discriminated from control individuals by 105 significant DEGs. Box plot evaluation (Figure 1) and UMAP assessment (Figure 2) indicate that analyses explore the suitable differences between the studied samples. As depicted in the UMAP plot, the light damage group is completely separated from the controls. This finding refers to the differences between the gene expression profiles of the two compared groups of samples. Among 105 significant DEGs, 100 individuals were recognized by CluePedia and included in the PPI network. The network including 56 isolated genes, six paired DEGs, one triple subnetwork, and a main connected component of 35 nodes was constructed via directed edges. The main connected component is shown in Figure 3.

The list of 15 elements (STAT3, JUN, IL6ST, SOCS3, ATF3, JUNB, FOSL1, CCL2, ICAM1, FGF2, AGT, MYC, LIF, CISH, and EGR1) of the main component of the PPI network with total degree > 6 is presented in Table 1. Seven classes including 35 biological terms (biological processes, molecular function, and biochemical pathways) were determined as the associated terms with the critical targeted significant DEGs (see Figure 4).

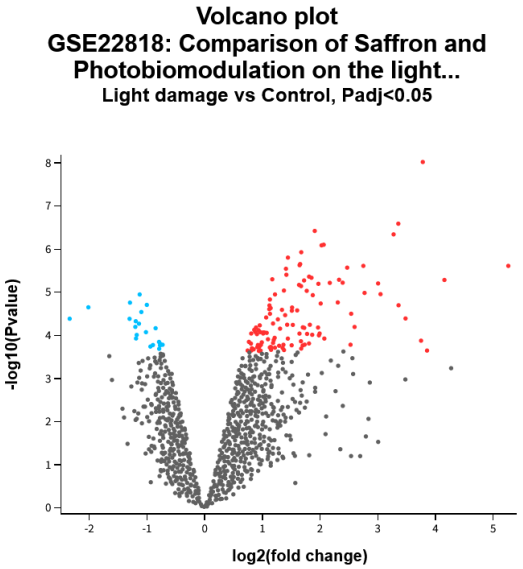

Figure 1. Volcano Plot for the Analysis of the Light-Damaged Group Versus the Control. Blue and red dots refer to significant downregulated and upregulated DEGs

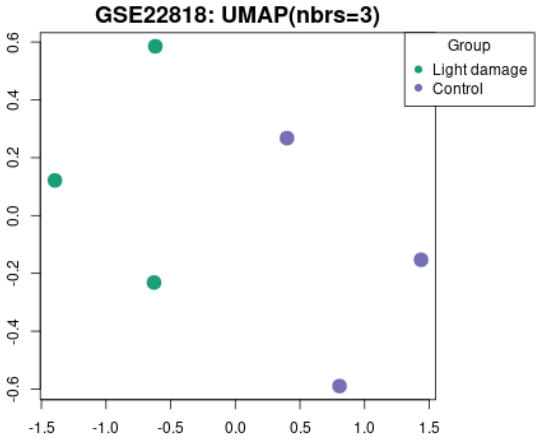

Figure 2. UMAP Plot for the Analysis of the Light-Damaged Group Versus the Control. Blue and red dots refer to significant downregulated and upregulated DEGs

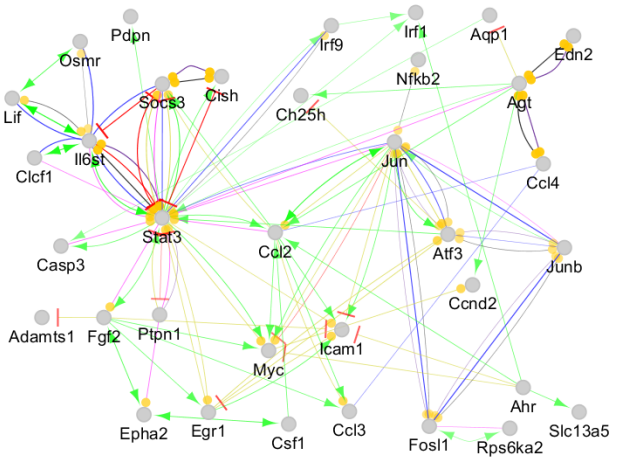

Figure 3. Activation (Green), Inhibition (Red), Expression (Yellow), Binding (Blue), Catalysis (Purple), Reaction (Black), and Post-translational Modification (Pink) Relationships Between the Selected Significant DEGs of the Main Connected Component of the PPI Network

Table 1. List of the nodes of the main connected component characterized by degree total > 6

No.	Gene	Degree in	Degree out	Degree total	LogFC
1	STAT3	20	24	44	2.03
2	JUN	12	18	30	2.07
3	IL6ST	14	14	28	1.13
4	SOCS3	11	9	20	3.01
5	ATF3	11	9	20	3.78
6	JUNB	7	9	16	2.01
7	FOSL1	7	8	15	2.47
8	CCL2	6	9	15	5.26
9	ICAM1	8	3	11	2.39
10	FGF2	3	8	11	1.98
11	AGT	2	8	10	-1.14
12	MYC	8	1	9	1.38
13	LIF	4	4	8	2.54
14	CISH	4	3	7	3.36
15	EGR1	3	4	7	1.82

Discussion

Based on Figure 1, there are differences between the gene expression profiles of the mouse retina in the presence of intensive light exposure and the controls. It seems that many genes are affected by intensive light. Apoptosis and mitotic catastrophe are pointed out as the two major cell deaths induced by radiation.[11] It is reported that apoptosis occurs in light-induced lesions in the retina.[12] The significant up- and downregulated genes appear in the volcano plot. It can be sure that the number of upregulated populations of significant DEGs is considerably larger than the number of downregulated individuals. UMAP analysis (see Figure 2) indicates that the samples are compared and separated correctly. Escobedo and colleagues' investigation showed the aging effects of light stress on transcriptome of photoreceptors.[13]

Action map analysis (see Figure 3) revealed that a large number of the significant DEGs are connected via regulatory relationships. The top 15 interacted DEGs are shown in Table 1. The members of this list are characterized by the amounts of gene expression changes and total degree values. Amounts of gene expression changes are considered in many studies to interpret molecular events in the studied situations.[14,15] The genes with higher values of degree can be considered hub genes. The hub genes are regarded as the critical genes that are involved in important physiological or pathological activities in studied samples.[16] Considering total degree value, top 10% of nodes including STAT3, JUN, IL6ST, and SOCS3 were identified as hub genes. As it is shown in Table 1, the total degree of ATF3 is equal to the total degree value of the last hub gene (SOCS3). Then, SOcS3 was measured as a hub. At first glance, the introduced five

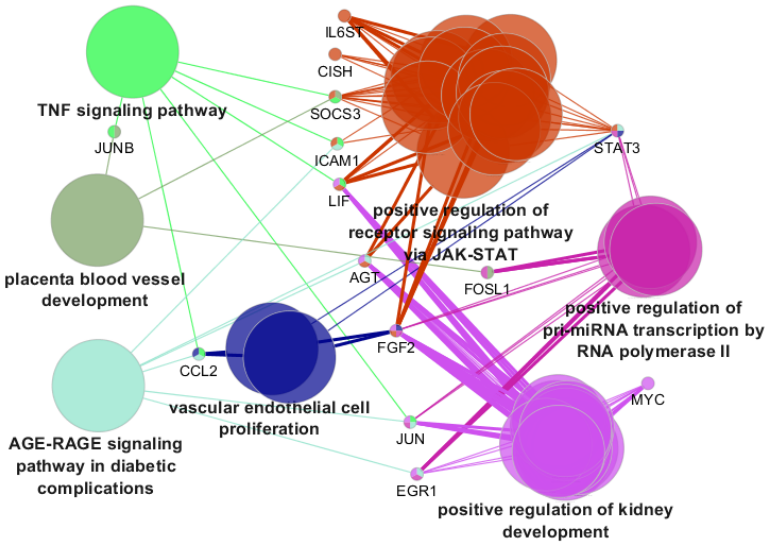

Figure 4. The Grouped Biological Processes, Molecular Function, and Biochemical Pathways Associated With Critical DEGs

hub genes are upregulated.

Signal transducer and activator of transcription 3 (STAT3) is the first-ranked hub gene. It is known as a pro-inflammatory transcription factor with a critical role in response to vaccine, infectious diseases, metabolism, autoimmunity, and malignancy. The substantial role of STAT3 in immunity against some pathogens, dental and musculoskeletal systems, and connective tissue is confirmed. It is pointed out that the function of STAT3 is required to maintain immune homeostasis.[17] Yun and colleagues' investigation indicates that STAT3 activation of STAT3 in microglia leads to a rise in pericyte apoptosis in diabetic retinas, a process that occurs via TNF-α/AKT/p70S6 kinase signaling.[18] This finding confirms the role of STAT3 in light-related retina damage in the present study.

Jun proto-oncogene, AP-1 transcription factor subunit (JUN) is the second introduced hub gene. The induction of oncogenic transformation is attributed to JUN oncogene.[19] Grimm and colleagues' examination revealed that the activation of transcription factor AP-1 plays a key role in the apoptotic cell death of photoreceptors in response to high levels of visible light. The experiment showed that the c-JUN mRNA level is upregulated fourfold in the light-related damaged retina.[20] The fourfold upregulation of the JUN gene is presented in Table 1.

Interleukin 6 signal transducer (IL6ST) is the third hub DEG which has upregulated about twofold. The reception of interleukin 6 (IL-6) signal by the IL-6 receptor requires gp130 function.[21] Izumi-Nagai et al published the special role of IL-6 as a potent proinflammatory cytokine which is involved in the progress of choroidal neovascularization.[22] It is discussed that IL-6 and its downstream STAT3 pathway play a critical role in choroidal neovascularization which is induced by a laser.[22] Valle and colleagues' investigation showed that the inhibition of interleukin-6 trans-signaling is accompanied by the anticipation of inflammation and endothelial barrier interruption in retinal endothelial cells.[23]

The suppressor of cytokine signaling 3 (SOCS3) as the 4th hub is a main controller of inflammation and infection.[24] SOCS3 is suggested as a potential therapeutic target molecule to keep the function of photoreceptor cells during the inflammation. Upregulation of SOCS3 in the present study refers to light-induced inflammation and retina damage. The last hub is activating transcription factor 3 (ATF3). Like SOCS3, the activation of AFT3 is associated with the protection of retinal ganglion cells after retina damage due to optic nerve crush.[25,26]

The results of gene ontology assessment are depicted in Figure 4. "Positive regulation of kidney development" and "Positive regulation of receptor signaling pathway via JAK-STAT" appear as the largest classes of affected biological processes, molecular function, and biochemical pathways. "Positive regulation of receptor signaling pathway via JAK-STAT" is connected to STAT3, IL6ST, SOCS3, ICAM1, FGF2, AGT, LIF, and CISH. As it was discussed, STAT3, IL6ST, and SOCS3 are the potent hubs. This class of biological terms is related to other classes of biological terms except "Vascular endothelial cell proliferation".

Conclusion

In conclusion, the intensive exposure of light is accompanied by light-induced retinal damage. Dysregulation of STAT3, JUN, IL6ST, SOCS3, and ATF3 is the main molecular event following light exposure. "Positive regulation of receptor signaling pathway via JAK-STAT" is highlighted as the critical class of biological terms involved in response to light-induced retinal damage. Regulation of the introduced critical genes and the dependent biological terms can prevent the

development of damages.

Authors' Contribution

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Resources: Aliasghar Keramatinia.
Software: Zahra Razzaghi.
Supervision: Aliasghar Keramatinia, Reza M Robati, Babak Arjmand.
Validation: Babak Arjmand.
Visualization: Zahra Razaghi.
Writing–original draft: Mohammad Rostami Nejad, Mostafa Rezaei-Tavirani, Zahra Razzaghi.
Writing–review & editing: Mostafa Rezaei-Tavirani, Babak Arjmand, Reza M Robati.

Competing Interests

The authors have no conflicts of interest to declare.

Ethical Approval

This project is approved by IR.SBMU.RETECH.REC.1402.721 ethical code.

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