EGR1 Is a Critical Gene in Response of Human Keratinocyte to Blue Light Radiation

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Abstract

Introduction: Investigating the molecular mechanism of cellular response to light radiation has attracted many researchers' attention. In the present study, the critically affected gene by 7.5 min blue light radiation in human keratinocytes was investigated via network analysis.

Methods: Gene expression profiles of human keratinocytes exposed to 7.5 min blue light radiation plus controls were extracted from Gene Expression Omnibus (GEO). The significant dysregulated genes plus 100 first neighbors were investigated by Cytoscape software and its applications. The central nodes of the network based on four centrality parameters were determined and discussed.

Results: Among 6 significant dysregulated genes, 4 individuals were recognized by the STRING database. The network was constructed by using the 4 queried genes and 100 first neighbors. EGR1, STAT1, and ISG15 were identified as central nodes; however, the prominent role of EGR1 was highlighted.

Conclusion: EGR1 appeared as a critically affected gene after blue light irradiation. It seems that this upregulated gene is responsible for protecting human keratinocytes against stress and cancer. Therefore, the application of blue light may be accompanied by antistress effects in the human body.

Keywords: Blue light; Human keratinocyte; Gene expression; Network analysis; Radiation.

Introduction

Several types of lasers are applied in different fields of medicine and also for cosmetic purposes.^{1,2} Since the benefits of laser application are significant, it can be expected that the use of lasers will achieve considerable progress in the near future.³ On the other hand, the molecular mechanism of laser effects on the human body is not clear yet.⁴ There are many documents about the molecular mechanism regarding the gene expression change of many genes after the treatment of the human body by diverse lasers.⁵ In vitro and also in vivo investigations represent useful results, along with contradictory data about the effect of low-level lasers in medicine.⁵

Gene expression studies have attempted to elucidate the molecular mechanism of many diseases and administrate

interventions.⁶ In such studies, gene expression changes in the treated samples were compared with the controls to find pure effects of the applied condition or probes.⁷ Since a large number of genes usually dysregulate, it is necessary to find the critical dysregulated gene or genes. Network analysis is a useful method to screen the dysregulated genes based on the role of the studied genes in interaction with the other elements of the network.⁸

A network contains nodes which are connected together via edges.⁹ In the scale-free network, there are a few nodes that play an important role in constructing the network. These nodes are known as central nodes.¹⁰ One type of important central node is recognized as hubs. The hub nodes are characterized by a large number of connections (adages) which the hub node makes with the other nodes directly (this centrality parameter is identified as

degree).¹¹ Betweenness is the other centrality parameter that refers to the related shortest paths of a node. A node that is characterized by a high value of betweenness is known as bottleneck. A node that is hub and bottleneck is determined as hub-bottleneck which is a strong central node.^{12,13} There are other centrality parameters such as closeness centrality and stress that refer to the more effective role of the studied node in the structure of the constructed network.¹⁴ A node characterized by a greater number of high-value centrality parameters plays a critical role in the analyzed network.¹⁵

In the present study, the gene expression profiles of human keratinocytes which were treated by one-hour blue light radiation were compared with the controls and the significant differentially expressed genes (DEGs) were determined to construct a network. The critical elements of the network were determined and discussed to find a prominent effect of blue light on the human body.

Methods

Data of GSE89086 (which is entitled “Photobiomodulation by blue light induces a dose dependent biphasic proliferation curve in human keratinocytes”) were downloaded from Gene Expression Omnibus (GEO). (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89086>). As it is reported in this document, three treated samples of human keratinocytes were exposed to 7.5 minutes blue light irradiation and then were harvested 1h after irradiation. These samples were characterized as GSM2358577, GSM2358579, and GSM235881. As the treated individuals without light irradiation, the three control samples (including GSM2358578, GSM2358580, and GSM235882) were similar. The gene expression profiles of the treated samples versus the control cells were analyzed via GEO2R.

A volcano plot, a Meandiff plot, and an expression density curve were provided to compare the treated and control gene expression profiles. Considering P value <0.05 and fold change >1.5 , the significant DEGs were extracted. The name and description of the significant DEGs were searched via GEO “Accession viewer-NCBI” (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi>). The names of the selected DEGs were searched in the STRING database¹⁶ and the recognized ones plus 100 first neighbors from the STRING database were interacted by Cytoscape software.¹⁷ The network was analyzed by “networkAnalyzer” application of Cytoscape. To find the central nodes, 10% of the top nodes based on degree value, betweenness centrality, closeness centrality, and stress were identified. The common nodes among the central nodes were determined as critical nodes. Based on the amounts of the centrality parameters, the crucial nodes were selected among the critical nodes. The relationship between the crucial nodes and their first neighbors was investigated via CluePedia application of Cytoscape software.

Results

Gene expression profile analysis by GEO2R revealed that the profiles of treated and control cells are comparable. As it is shown in Figure 1, only a few numbers of genes are dysregulated statistically and most dysregulated genes do not have significant expression changes. In this figure, asymmetric distribution between up- and down-regulated genes is presented. The distribution of the dysregulated genes based on the expression value versus the logarithm of fold change is shown in Figure 2. As it is presented in this figure, an increase of fold change or gene expression change is accompanied by a decrease in the numbers of genes without considering the up- or down-regulation modes of expression change. The density of expression for the dysregulated genes is shown in Figure 3. Based on the presented curve in Figure 3, the density of expression is maximized in the low value of the intensity of expression. It can be considered that the contribution of highly dysregulated genes to the density of expression versus the low or mild individuals is characterized by lower or

Figure 1. The Volcano Plot of the Gene Expression Profile of Human Keratinocytes in the Presence of 7.5 min Blue Light Radiation Versus the Controls.

Figure 2. The Meandiff Plot of the Gene Expression Profile of Human Keratinocytes in the Presence of 7.5 min Blue Light Radiation Versus the Controls. The logarithm of expression change values versus the logarithm of fold changes is presented for the related dysregulated genes.

minimum quantities.

The analysis led to the introduction of 6 characterized significant DEGs. The names and descriptions of these

Figure 3. Presentation of the Density of Expression Versus the Intensity of Expression for Gene Expression Profile of Human Keratinocytes in the Presence of 7.5 min Blue Light Radiation Versus the Controls.

genes are tabulated in Table 1. Four characterized significant DEGs including EID3, ARRDC3, PLEKHM1, and EGR1 were recognized by the STRING database. The network was constructed for the 4 recognized DEGs plus 100 first neighbors (see Figure 4). The 104 elements of the network were connected by 1530 undirected connections. The network was analyzed by “NetworkAnalyzer” and visualized based on degree value. Among the 40 central nodes 3 critical individuals including EGR1, STAT1, and IGS15, which are presented in Table 2, were identified. Since EGR1 was eligible to be selected as a crucial node, 10 first neighbors of this gene were extracted from the STRING database. The action map of EGR1 and its 10 first neighbors are shown in Figure 5.

Discussion

There are many documents about gene expression changes and photobiomodulation (PBM). In such references, researchers have shown that after light radiation several genes are dysregulated.^{18,19} Since the understanding of the molecular mechanism of PBM effects on the body and human cells is an important feature of the therapeutic

Figure 4. A Network Including 4 Queried DEGs and the 100 First Neighbors Which Differentiate Human Keratinocytes in the Presence of 7.5 min Blue Light Radiation From the Control Individuals. The nodes are layout based on degree value; the bigger size and green color refer to a higher value of degree.

Table 1. The Characterized Significant DEGs Which Differentiate Human Keratinocytes in the Presence of 7.5 min Blue Light Radiation From the Controls.

No.	Gene ID	Gene description	Display name	P Value	LogFC
1	344887_at	NmrA-like family domain containing 1 pseudogene	NMRAL2P	0.0002	0.922
2	493861_at	EP300 interacting inhibitor of differentiation 3	EID3	0.0004	0.907
3	28809_at	Immunoglobulin lambda variable 3-1	IGLV3-1	0.0014	0.761
4	9842_at	Pleckstrin homology domain containing family M (with RUN domain) member 1	PLEKHM1	0.0000	0.727
5	1958_at	Early growth response 1	EGR1	0.0020	0.687
6	57561_at	Arrestin domain containing 3	ARRDC3	0.0048	-0.642

Table 2. Critical Nodes of the Analyzed Network

No.	Gene Display Name	Degree	BC	CC	Stress
1	EGR1	80 ¹	0.196 ¹	0.792 ¹	8738 ¹
2	STAT1	61 ²	0.044 ⁵	0.696 ²	5318 ⁵
3	ISG15	50 ⁷	0.027 ⁸	0.656 ³	3482 ⁸

Note: BC and CC refer to betweenness centrality and closeness centrality respectively. Superscript number corresponds to the rank of the node among the central individuals based on the titled centrality parameters.

Figure 5. The Action Map of EGR1 and its 10 First Neighbors. Green, red, and yellow colors refer to activation, inhibition, and expression actions respectively.

properties of PBM, great efforts have been made to find the key targets of the exposure. On the other hand, network analysis is a well-established method to study dysregulated genes.²⁰

As it is shown in [Figure 1](#), most genes of the human keratinocytes are not affected by blue light significantly. Therefore, it can be concluded that a limited number of dysregulated genes can be identified. Dispersion of genes on the right side of [Figure 1](#) indicates that up-regulation is a dominant process versus downregulation in the cells which were exposed to blue light. This finding corresponds with other researches that refer to more upregulated DEGs relative to less downregulated ones.²¹ The Meandiff plot (see [Figure 2](#)) shows gross changes of upregulated DEGs versus the downregulate genes obviously. As it is depicted in [Figure 3](#), the expression of most genes is not affected noticeably by exposure. On the other hand, 7.5 min blue light radiation is accompanied by mild alteration in the gene expression process of human keratinocytes. Mild effects of PBM are reported by several researchers that applied PBM as a therapeutic tool.²²

As it is depicted in [Table 1](#), there are 6 characterized DEGs that differentiate the treated samples from the control cells. However, only four DEGs were recognized by the database. Network analysis revealed that EGR1 is the crucial dysregulated gene which is targeted by blue light radiation. Based on the results of this analysis, EGR1 occupies the first rank among the central nodes (including the queried DEGs and the added first neighbor genes) regarding degree value, betweenness centrality, closeness centrality, and stress as the four important centrality

parameters (see Table 2). Degree and betweenness of EGR1 are 33% and 168%, which are higher than STAT1 and UBE2I as the second hub and second bottleneck genes respectively. It can be concluded that EGR1 is the key gene that is targeted by blue light radiation. As it is represented in Table 1, EGR1 is upregulated after 1-hour radiation.

It is reported that EGR1 belongs to the family of immediate early response transcription factors which plays a role in several important cellular processes such as stress responses, cell development, cell growth, and regulating of homeostasis of stem cells in many tissues.²³ Several key roles such as the gatekeeper of inflammatory enhancers of human macrophages, the multifaceted regulator of matrix production in tendons and other connective tissues, the regulator of cell cycle progression and tumorigenesis in gastric cancer are attributed to EGR1.²⁴⁻²⁶

As it is depicted in [Figure 5](#), CREB1, EP300, FOS, FOSB, JUN, NAB1, NAB2, NFATC1, and TP53 are the first neighbors of EGR1. CREB1, EP300, JUN, NAB2, and TP53 are directly connected to EGR1. Since TP53, EP300, and JUN are central nodes, the relationship between EGR1 and these 3 first neighbors could be important. As it is shown in [Figure 5](#), EGR1 activates EP300 but upregulates TP53. The single connection between JUN and EGR1 refers to the upregulation of EGR1 by JUN. Many years ago, JUN was characterized as a proto-oncogene.²⁷ The upregulation of EGR1 by JUN refers to the anticancer property of EGR1 which increases against tumor development. There are many documents about cancer onset and autism due to mutation and partial deletion of EP300.^{28,29} It seems that the activation of EP300 by EGR1 is a protective role of EGR1 against the mentioned diseases. TP53 is a well-known tumor suppressor gene.³⁰ The upregulation of TP53 by upregulated EGR1 due to blue light radiation indicates the cancer prevention effect of PBM.

Conclusion

It can be concluded that 1-hour blue light radiation induced the gene expression change of many genes of human keratinocytes; however, only a few genes were affected significantly. The results indicate that upregulation is a prominent process. ERG1 as a key upregulated gene was highlighted in the network analysis. The finding indicates that EGR1 plays a protecting role against cancer and stress in the irradiated cells. More investigations by using different cell types are suggested.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare they have no conflicts of interest.

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