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Evaluation of Laser Intensity Effect on Photodynamic Therapy Efficacy

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Abstract

Introduction: Intensity is one of the important parameters of laser radiation in photodynamic therapy. Effective treatment requires the selection of a suitable power of laser. This study aimed to evaluate laser effectiveness in photodynamic therapy via high and low intensity by the analysis of the gene expression profiles of the treated cells.

Methods: The gene expression profiles of human SK-ChA-1 cells which are treated by 500mW and 50mW laser radiation were retrieved from the Gene Expression Omnibus (GEO) database. Data were assessed by the GEO2R program, and the significant differentially expressed genes (DEGs) were investigated via expression examination and protein-protein interaction (PPI) network analysis.

Results: Analyses revealed that the higher intensity of radiation is associated with wide gene expression changes relative to the lower mode. 196 significant DEGs were identified and assessed. The extremely dysregulated DEGs except MMP1 were down-regulated. STAT1, IRF7, IL1B, DDX58, ISG15, RSAD2, DHX58, OASL, OAS1, STAT2, DDX60, OAS2, USP18, and IFI44L were introduced as hubs of the main component of the PPI network. Final analysis showed that STAT1, IRF7, IL1B, DDX58, and STAT2 are the critical DEGs.

Conclusion: Compared to the 50 mW mode of radiation, 500 mW laser intensity effectively changed apoptosis, differentiation, cell proliferation and angiogenesis, regulation of other inflammation-related molecules, innate immunity, and maintaining immune homeostasis.

Keywords: Laser; Gene photodynamic therapy; Human SK-ChA-1 cell; Network analysis.

Introduction

Laser therapy is applied widely in medicine. There are many documents about the treatment of diseases such as knee osteoarthritis, skin diseases, diabetic retinoscopy and diabetic macular edema, eye disorders, types of heart diseases, kidney diseases, and other diseases that are improved and treated with different types of lasers.[1-5] Several aspects of lasers in medicine such as the physics of the applied laser and its interaction with the biological tissue, the mode of laser and its intensity and power, and the mode of laser radiation are challenges of laser application in therapeutic methods in clinics.[6]

Investigations have indicated that laser radiation affects the gene expression profiles of the irradiated tissue. Rocha EA et al. reported that the gene expression of the postnatal human dental pulp stem cells changed after photobiomodulation. The applied laser led to alterations in cell proliferation, growth, and differentiation. The gene

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expression change of 85 individuals which was related to inflammation and osteogenesis was highlighted.[7] Since protein-protein interaction (PPI) network analysis is a well-known method for assessing the gene expression profiles of the studied samples, the effects of laser radiation on the gene expression profiles of biological samples are examined via PPI network analysis. Razzaghi et al have studied the effects of long-term and short-term laser therapy on human skin gene expression changes via PPI network analysis. Based on this investigation, 63 significant differentially expressed genes (DEGs) and 6 classes of biological terms were pointed out as differences between the characterized samples with long-term and short-term laser therapy.[8] Our previous study via PPI network analysis showed that laser radiation affects the "Positive Regulation of Telomere Maintenance".[9] There are many genomic and bioinformatic studies about the biological and medical effects of lasers on treated patients.[10,11] In the present study, the gene expression profiles of the human SK-ChA-1 cell line which were exposed to 500 mW of laser power versus the cells treated with 50mW were extracted from the GEO database and were evaluated via PPI network analysis to find the critical targets which discriminated the two samples. Findings can be considered a criterion for assessing the efficacy of the two modes of radiation.

Methods

Data Collection

GSE68292 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse68292) was selected from GEO to be analyzed. As reported in the recorded data, the gene expression profiles of the cells treated with PDT in the presence of high and low laser power were evaluated. To assess the effect of high laser power (500 mW) on the human SK-ChA-1 cell line relative to the effect of low laser power (50 mW) on the survival of the mentioned cell line, the cells were subjected to PDT with zinc phthalocyanine (ZnPC)-encapsulating liposomes. The cultured cells were harvested 90 minutes after PDT to analyze whole genome expression.

Pre-evaluation Analysis

The gene expression profiles of the samples were evaluated by GEO2R to find the possible significant DEGs that discriminate the two treated cells. The significant DEGs were selected based on adjusted P value < 0.05. The top 250 significant DEGs were chosen for more analysis. The significant DEGs were limited by considering "fold change" > 2.

Expression Analysis

The significant DEGs were limited by considering "fold change" > 2. The selected significant DEGs were grouped to find the extra dysregulated genes. The top dysregulated genes were determined and introduced.

Network Analysis

The significant DEGs were assessed by PPI network analysis. The recognized selected DEGs were linked via undirected edges by Cytoscape software v 3.7.2.[12] The main connected component of the PPI network was analyzed by the "Network Analyzer" application of Cytoscape to find the central nodes. The top 10% of nodes based on degree value were pointed out as hubs. Activation, inhibition, and expression relationships between the nodes of the main connected component were investigated by action map analysis. Out-degree and in-degree for the elements of the action map were identified and recorded.

Results

The results of GEO2R analyses are presented in Figures 1 and 2. As depicted in Figure 1, among 44 825 dysregulated genes, there are 2498 significant DEGs that differentiate the cells which were treated with 500 mW from the individuals that were exposed to 50mW of laser radiation. The distribution of DEGs based on adjusted p-value is presented in Figure 2. As shown in Figure 2, most of the DEGs are characterized by adjusted P value > 0.5. Considering adjusted P value < 0.05 and (fold change) > 2, 196 top significant DEGs were determined.

As shown in Figure 3, the 196 significant DEGs were categorized in five groups. The last three groups of DEGs were identified as the extremely dysregulated DEGs (see Figure 4). As depicted in Figure 4, 21 down-regulated genes versus one up-regulated individual appeared.

192 genes were recognized by the STITCH–Protein/Compound query data source. Among the 192 queried DEGs, 136 individuals were included in the main connected component of the constructed network (see Figure 5). Forty-eight isolated nodes and eight paired genes were identified. Fourteen top nodes of the main connected component based on degree value, including STAT1, IRF7, IL1B, DDX58, ISG15, RSAD2, DHX58, OASL, OAS1, STAT2, DDX60, OAS2, USP18, and IFI44L, were identified as hubs.

As presented in Figure 6, among the 136 elements of the main connected component, 78 DEGs remained as isolated individuals and two nodes were paired. The other 56 genes were connected by activation, inhibition and expression actions via directed edges. The in-degree (deg_{in}) and out-degree (deg_{out}) values of the nodes of the action map (except total degree = 1) are tabulated in Table 1.

Discussion

The intensity of radiation is an important parameter in the treatment of diseases. Thus, applying optimal intensity is accompanied by effective treatment and minimal side effects.[13,14] As shown in Figure 1, the higher intensity of

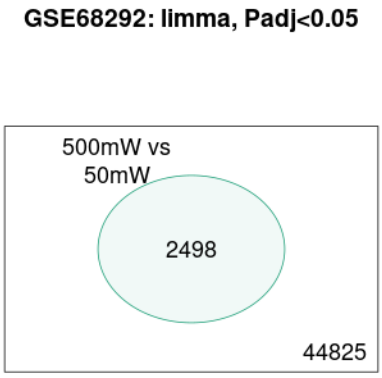

Figure 1. Venn Diagram Presentation of the 44825 Dysregulated Genes and the 4498 Significant DEGs Which Discriminate the Treated Cells With a 500 Mw Laser From the Individuals Radiated by 50mW of Laser Radiation

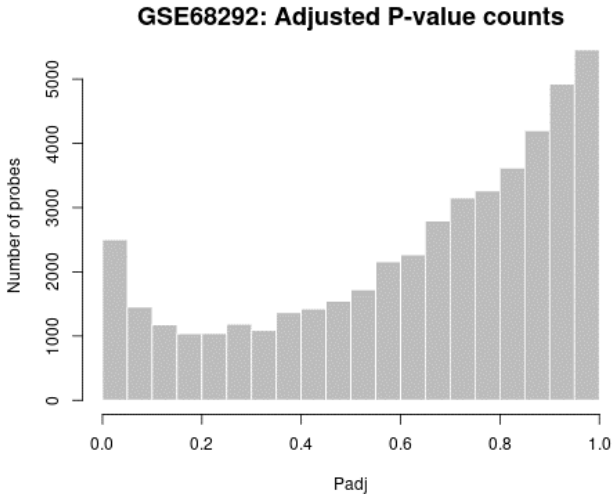

Figure 2. Adjusted *P* Value (P_{adj}) Counts for 44825 Dysregulated Genes From the Compared Gene Expression Profiles of Treated Cells With a 500 mw Laser Versus the Radiated Individuals With 50 mW of Laser Radiation

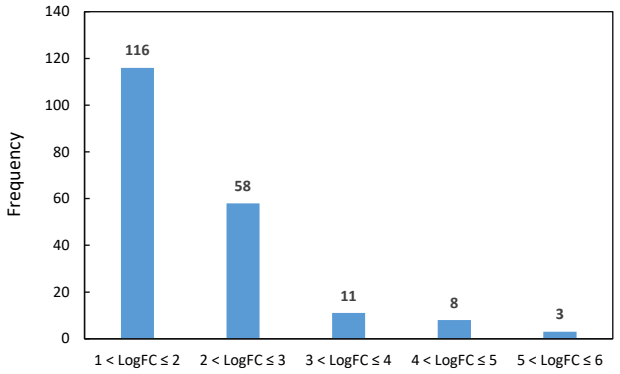

Figure 3. Grouping of 196 Significant DEGs Based on the Amounts of log|FC|. FC: fold change

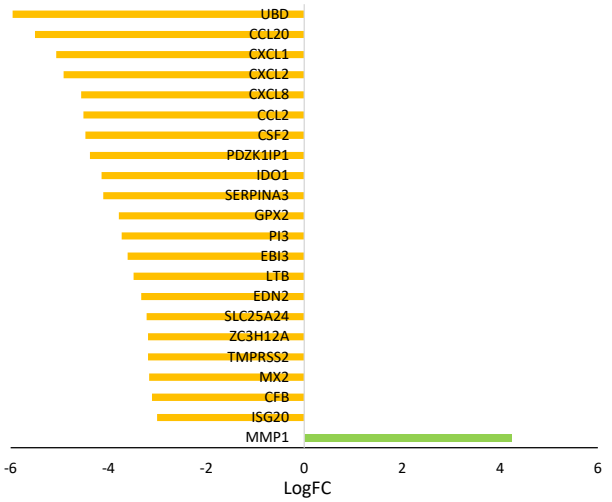

Figure 4. Presentation of logFC for the 22 Extremely Dysregulated DEGs. FC: fold change

the laser is associated with the dysregulation of 44825 genes including 2498 significant DEGs. Exploring the core of this molecular event is the aim of this study. As depicted in Figure 2, the regulation of most dysregulated genes is not significant. In the first step of the analysis, the significant DEGs were limited via (fold change) > 2. Based on expression change amounts, 196 genes are identified as the prominent dysregulated genes. Since gene expression change is a critical parameter which is considered to explore the molecular mechanism of biological events, the extremely dysregulated genes are presented in Figure 3. Based on the results, the other 21 extremely dysregulated genes, except for MMP1, are down-regulated. It can be implied that the higher intensity of the radiated laser is associated with the intense decrement of gene product in the treated cells.

PPI network analysis led to the introduction of 14 central genes. The evaluation showed there are no common genes between the extremely dysregulated genes and the introduced central individuals. This means the extremely dysregulated genes are not important players in response to higher radiation of the laser. The action map provided useful data to assess the central genes. As shown in Figure 5 and Table 1, STAT1, IRF7, IL1B, DDX58, ISG15, OASL, and STAT2 are common genes between the central DEGs and the elements of the action map. Except for CSF2, there is not any common gene between the top 10 elements of the action map and the extremely dysregulated genes (see Figure 3 and Table 1). This finding corresponds to PPI network analysis, and the extremely dysregulated genes are not powerful players to discriminate two assessed methods. As depicted in Table 1, the total degree value for genes that are ranked > 9 decreased considerably. Therefore, OASL and ISG15 were ignored for more analysis. Finally, STAT1, IRF7, IL1B, DDX58, and STAT2 were pointed out as the crucial genes which were targeted by the high-intensity laser in the treated cells.

LogFC amounts for STAT1, IRF7, IL1B, DDX58, and STAT2 are -1.58, -2.2, -1.74, -2.21, and -1.81, respectively. This finding indicates that all critical DEGs are down-regulated. STAT1 and STAT2 are members of a signal transducer and activator of transcription (STAT) family

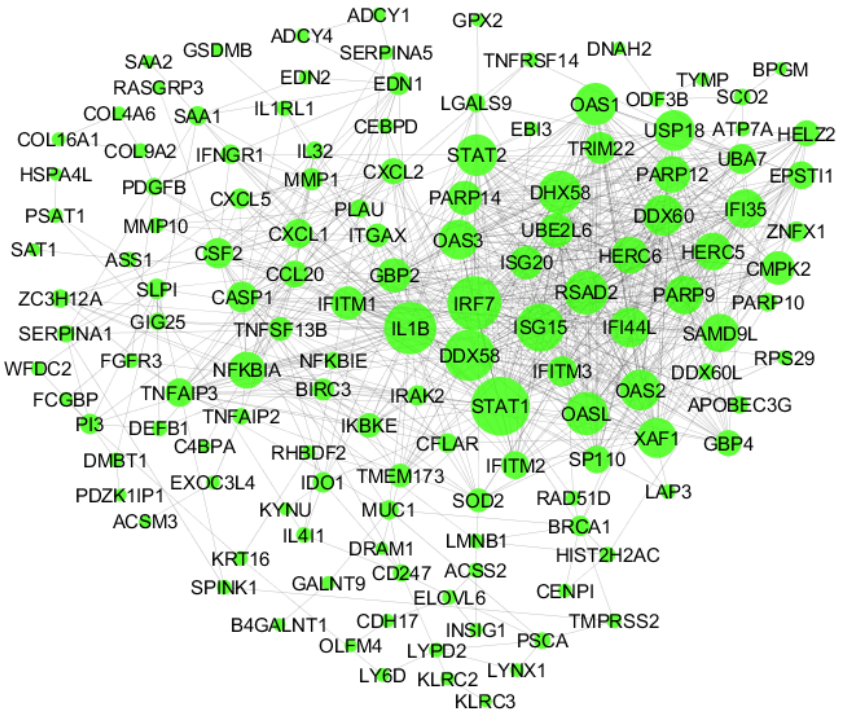

Figure 5. Main Connected Component of the PPI Network, Including 136 Nodes, Organized Via the STITCH–Protein/Compound Query Data Source Using Cytoscape Software. The bigger size of the nodes refers to the higher values of degree.

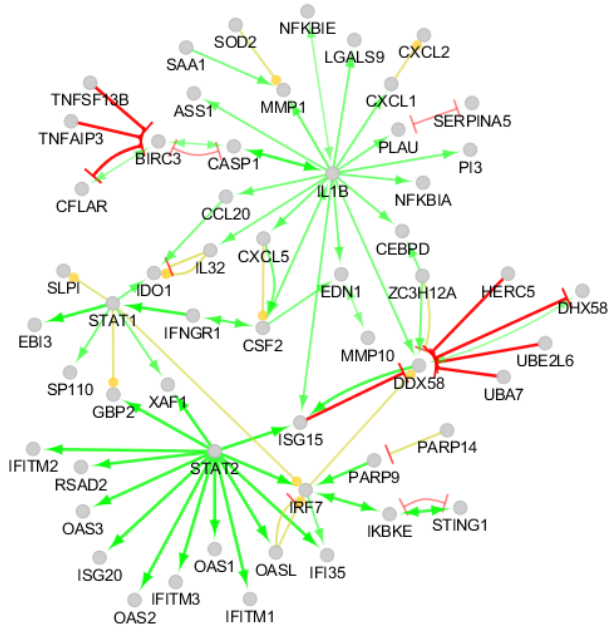

Figure 6. Action Map for the 56 Elements of the Main Connected Component of the PPI Network Participating in the Interactome Via CluePedia. Green, red, and yellow refer to activation, inhibition, and expression relationships. The round and bar tips correspond to positive co-expression and inhibition or negative co-expression respectively.

that is an imperative family of evolutionarily conserved transcription factors which are involved in various biological processes such as immune and blood cell function and development.[15] The key role of STAT proteins (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6) in apoptosis, differentiation, and cell proliferation besides angiogenesis has been investigated and confirmed by researchers.[16] The down-regulation of STAT1 and STAT2 in response to 500mW laser radiation compared with 50 mW intensity indicates that the higher intensity of laser radiation is more efficient than the 50 mW radiation.

Interleukin 1 beta (IL1B) is known as a powerful pro-inflammatory cytokine. Investigations indicate that IL1B is up-regulated via radiation and is involved in the regulation of other inflammation-related molecules.[17] Based on the literature, the application of polychromatic polarized light therapy leads to the down-regulation of IL1B in human monocyte cells.[18] IL1B is characterized as the first-ranked node of the analyzed action map (see Table 1). Considering $deg_{out} = 17$ for IL1B, it can be concluded that the down-regulation of this DEG has a gross effect on the regulation of the elements of the action map. This finding again confirms the effectiveness of 500mW laser radiation as a therapeutic tool.

Interferon regulatory factor 7 (IRF7) is highlighted as a main controller of type-I interferon-dependent immune responses.[19] The up-regulation of IRF7 during viral infection in the central nervous system is reported by Ousman et al.[20] Puthia et al reported that the inhibition of IRF7 is associated with the prevention of damaging innate immunity.[21] The down-regulation of IRF7 can be considered a positive point for the 500 mW laser as the improved method relative to 50 mW radiation.

The last critical DEG is DEXD/H-box helicase 58 (DDX58). It is reported that DDX58/RIG-I as a

Table 1. Out-degree (deg_{out}) and In-degree (deg_{in}) Values > 1 for the Nodes of the Action Map

No.	Gene	Deg_{in}	Deg_{out}	Total Degree
1	IL1B	3	17	20
2	STAT2	14	0	14
3	DDX58	10	3	13
4	BIRC3	6	4	10
5	IRF7	6	3	9
6	STAT1	1	6	8
7	CSF2	4	3	7
8	CASP1	3	3	6
9	IKBKE	3	3	6
10	ISG15	3	1	4
11	STING1	2	2	4
12	CFLAR	2	2	4
13	IDO1	4	0	4
14	EDN1	3	1	4
15	IL32	1	2	3
16	OASL	1	2	3
17	PLAU	2	1	3
18	MMP1	3	0	3
19	ZC3H12A	0	3	3
20	IFNGR1	1	2	3
21	CXCL5	1	2	3
22	MMP10	1	1	2
23	SERPINNA5	1	1	2
24	CCL20	1	1	2
25	CXCL1	1	1	2
26	NFKBIE	1	1	2
27	IFI35	2	0	2
28	XAF1	2	0	2
29	PARP9	1	1	2
30	GBP2	2	0	2
31	CEBPD	2	0	2

prominent element is involved in antiviral immunity and its posttranslational modifications and stability are strongly related to maintaining immune homeostasis.[22] There are documents about the significant role of DDX58 as an immune-related gene which is connected with ischemic heart failure. Like the other critical genes, DDX58 is upregulated in several types of cancers.[23] The down-regulation of DDX58 refers to the efficiency of the 500mW laser in treating cancer.

Conclusion

In conclusion, photodynamic therapy via the application of 500mW exposure power of a laser on the human SK-ChA-1 cell line is more effective compared to 50mW power. The down-regulation of STAT1, IRF7, IL1B, DDX58, and STAT2 is the key feature of molecular events. Biological processes such as apoptosis, differentiation, cell proliferation and angiogenesis, regulation of other inflammation-related molecules, innate immunity, and maintaining immune homeostasis are the targeted biological functions.

Authors' Contribution

Conceptualization: Mohammad Rostami Nejad, Fatemeh Bandarian, Reza M Robati,
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Formal analysis: Zahra Razzaghi, Maryam Hamzeloo-Moghadam.
Funding acquisition: Mostafa Rezaei-Tavirani.
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Methodology: Mostafa Rezaei-Tavirani, Babak Arjmand.
Project administration: Mohammad Rostami Nejad, Fatemeh Bandarian, Reza M Robati.
Resources: Farideh Razi, Mitra Rezaei.
Software: Zahra Razzaghi.
Supervision: Fatemeh Bandarian, Reza M Robati, Babak Arjmand.
Validation: Farideh Razi, Mitra Rezaei.
Visualization: Zahra Razzaghi.
Writing–original draft: Mohammad Rostami Nejad, Mostafa Rezaei-Tavirani.
Writing–review & editing: Mostafa Rezaei-Tavirani, Babak Arjmand.

Competing Interests

None declared.

Ethical Approval

This project was approved by the ethical committee of Shahid Beheshti University of Medical Sciences (ethical code No.: IR.SBMU.RETECH.REC.1403.105).

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