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Assessment of Immunological Effects of Low-Level Er: YAG Laser Radiation

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Abstract

Introduction: Low-level laser radiation has a significant effect on cell proliferation. Various investigations into the effect of Er: YAG laser on the treated cell lines have been published. Determining core targeted proteins is an attractive subject. This research aimed at identifying the critical targeted protein by a low-level Er: YAG laser in primary osteoblast-like cells.

Methods: Data were extracted from the literature about proteomic assessment of 3.3 J/cm^2 of low-level Er: YAG laser radiation on osteoblast-like cells of rat calvaria. The significant differentially expressed proteins plus 100 first neighbors were analyzed via network analysis and gene ontology enrichment.

Results: Nine differentially expressed proteins among the 12 queried proteins were included in the main connected component. Analysis revealed that Cxcl1 was a key targeted protein in response to laser radiation. The presence of Cxcl1 in the significant cellular pathways indicated that cell growth and proliferation were affected.

Conclusion: It can be concluded that the immune system is affected by the laser to activate cellular defense against stress.

Keywords: Laser; Proteomics; Cxcl1; Protein; Cell proliferation.

Introduction

The application of low-level lasers in medical fields and also other aspects of human life has attracted researchers' attention to an exploration into useful and possible unfavorable properties of lasers. The promotion of laser equipment and clinical protocols requires extensive investigations into the efficacy of the used lasers in the fields.[1,2] The beneficial effects of the low-level Er: YAG laser in cell growth and development of other biological processes have been reported by several researchers.[3,4]

High throughput methods are used to analyze the molecular mechanism of low-level laser therapy (LLLT) by the treatment of various cell lines. Fibroblast cells are the suitable candidate to assess protein expression changes after LLLT. Ogita et al reported a document about the low-level Er: YAG laser effect on increment cell proliferation in human gingival fibroblast cells via the proteomics approach. The differentially expressed proteins due to laser radiation were analyzed and the possible affected biological processes were discussed.[5]

As it is known, proteomics is a powerful method to produce a large number of dysregulated proteins after inducing stress in the biological media. Results of proteomic investigation lead to introduce significant dysregulated proteins.[6] The combination of proteomics and bioinformatics is a well-known approach in the evaluation of the treated biological samples. In such researches, proteomic data are analyzed via bioinformatic methods to screen the data and find the critical individuals.[7,8] Network analysis is a common method to

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analyze proteomics data. In this approach, large numbers of proteins include in a network to be analyzed based on their contribution in constructing the network. Based on network topology, the central nodes of the network will be accessible to researchers. It has been suggested that the central proteins are the same proteins that are involved in the main dysregulated biological processes.[9,10]

Several parameters are presented to determine the central nodes of a network. The degree which refers to the numbers of the first neighbors of a node is applied to identify the hub nodes. Nodes with a higher value of degree are known as hubs. The hub nodes are considered the critical nodes of an analyzed network.[11] The other important central node is the bottleneck node. The bottleneck nodes determine based on the betweenness centrality parameter. The common hubs and bottlenecks are known as hub-bottlenecks that are considered the crucial central proteins.[12,13]

Osteoblasts are known as multi-functional cells, and the critical roles of this type of cells in rapid periosteal bone formation and angiogenesis following the fracture are confirmed.[14] In the present study, data from a proteomics study by Niimi et al, entitled "Effects of low-level Er: YAG laser irradiation on proliferation and calcification of primary osteoblast-like cells isolated from rat calvaria",[15] were analyzed via network analysis to explore the possible critical proteins, especially the central dysregulated genes and biological terms.

Materials and Methods

Data Collection

Data were extracted from the report by Niimi et al.[15] The content of this document was about the assessment of laser radiation on the osteoblast-like cells of rat calvaria. In the original source of the data, it was described that the studied cells were exposed to 3.3 J/cm^2 of a low-level Er: YAG laser and they were harvested after 6 hours. The gene expression alteration of irradiated cells was investigated versus the control sample via Microarray analysis. More details of methods are described by the authors in the published document.[15] Among numerous gens, the significant differentially expressed genes (DEGs) based on *P* value < 0.05 and fold change (FC) > 1.5 were screened and selected to be analyzed.

Network Analysis

The characterized DEGs were included in a network via the STRING database by Cytoscape software.[16,17] The nodes were connected by undirected edges to form an interactome. Since there were not enough links between the interacted nodes to construct a scale-free network, 100 first neighbor genes from the STRING database were added to the queried DEGs and the network was created again. The network was analyzed to determine the central nodes regarding the degree value and betweenness centrality. To find the hub nodes, 10% of the top nodes based on the degree value were introduced as hubs. Bottleneck nodes, like the hubs, were identified regarding higher values of betweenness centrality.

Gene Ontology Analysis

Due to the distribution of the significant DEGs into the main connected component and isolated individuals, the nodes included in the main connected component were enriched to explore the related biological terms via gene ontology by ClueGO application of Cytoscape. The determined relative biological terms were categorized and discussed.

Results

Twelve significant DEGs were determined as the targeted genes by the radiated laser. As it is shown in Table 1, TREML4 and TRD5213 are characterized by the highest and lowest amounts of FC respectively. All DEGs were recognized by the STRING database. The result indicates that all queried DEGs almost remained isolated (one paired DEG was identified) (see Figure 1).

Table 1. Significant DEGs of Osteoblast-Like Cells Targeted by the Radiated Laser

No.	Gene Symbol	logFC
1	Treml4	1.102
2	C2cd4b	1.050
3	Cox6b2	0.900
4	Serpina4	0.880
5	Cxcl3	0.856
6	Jsrp1	0.828
7	Tbx10	0.790
8	Fuz	0.743
9	Casz1	0.689
10	Cxcl1	0.678
11	Elp5	0.624
12	Tpd52l3	0.607

The genes are arranged by the amounts of logFC. *P* value < 0.05 and FC > 1.5 were considered.

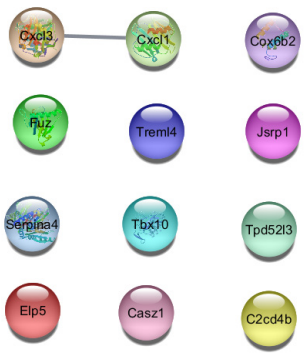

Figure 1. The 12 Significant DEGs related to the laser radiation on the treated cells which are recognized by the STRING database. Only one edge appeared between the nodes.

To make more connections between the queried DEGs and explore more related genes, 100 first neighbors were added to the examined DEGs. The network including 2 isolated nodes and a triple unit plus a main connected component were formed with 112 nodes and 2057 edges. The isolated nodes and the triple unit plus a part of the main connected component are represented in Figure 2. The other part of the network which included the other share of the main connected component is illustrated in Figure 3. The 11 bottlenecks and also 11 hub nodes are represented in Tables 2 and 3. For a better understanding of queried DEGs properties, the degree value, betweenness centrality, and logFC of these genes are tabulated in Table 4. Since the DEGs that were included in the main connected component were prominent to construct the network, the nine queried DEGs including Treml4,

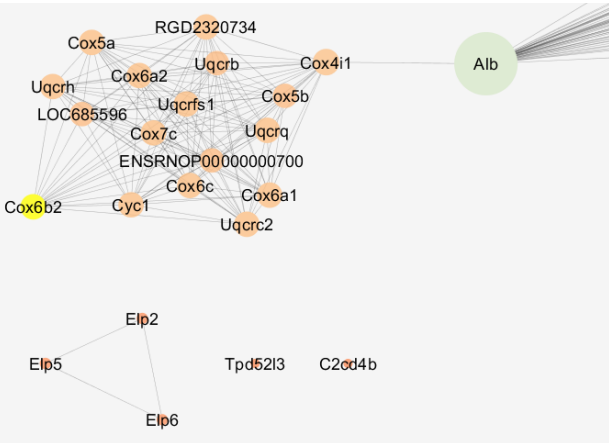

Figure 2. A Part of the Network Including Isolated Nodes and the Triple Unit Plus a Part of the Main Connected Component. The highlighted node is a queried DEG which is included in the left domain of the main connected component of the network. ELP5 as an element of the triple unit is a queried DEG.

Table 2. Bottleneck Nodes of the Analyzed Network

No.	Display Name	Query Term	Betweenness Centrality	Degree
1	Alb		0.292781	51
2	Cox4i1		0.258784	17
3	Prickle1		0.060613	7
4	Crebbp		0.05756	20
5	Gli1		0.051706	10
6	Ptgs2		0.035967	42
7	Mmp9		0.027398	50
8	Cxcl1	Cxcl1	0.022972	76
9	Jun		0.021819	45
10	Cxcl2		0.021475	75
11	Cxcl10		0.020422	73

The nodes are picked based on the betweenness centrality value.

Table 3. Hub Nodes of the Network

No.	Display Name	Query Term	Degree	Betweenness Centrality
1	Cxcl1	Cxcl1	76	0.022972
2	Cxcl2		75	0.021475
3	Cxcl10		73	0.020422
4	Cxcl3	Cxcl3	73	0.01472
5	Cxcl6		72	0.018254
6	Ccl5		71	0.01232
7	Cd4		69	0.00888
8	Tnf		68	0.013877
9	Cxcl9		68	0.008798
10	Ccl2		67	0.010717
11	Il6		66	0.011874

The nodes are picked based on the degree value.

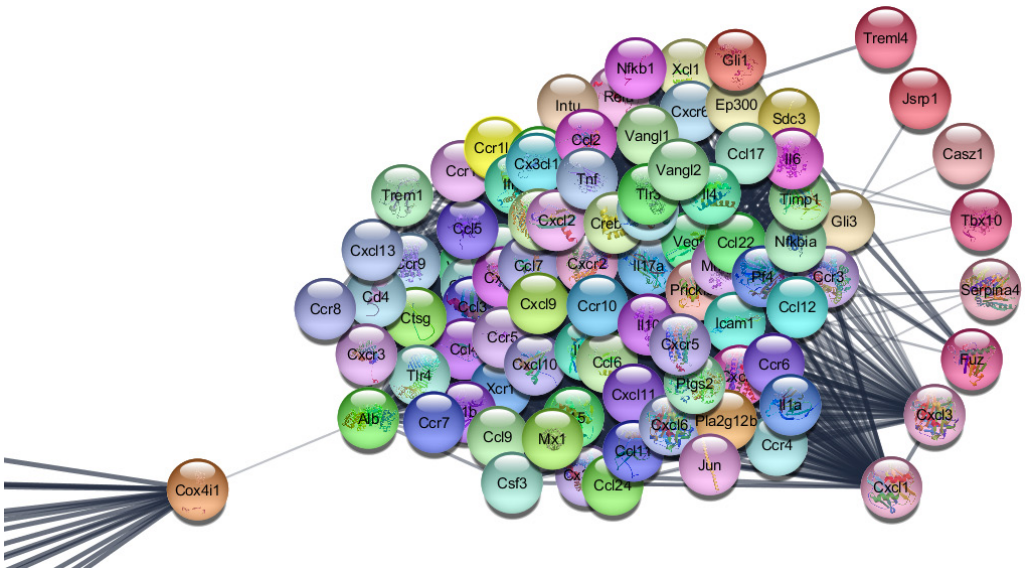

Figure 3. The other part of the network that includes the remained part of the main connected component (the queried DEGs are picked on the right side of the figure).

Cox6b2, Serpina4, Cxcl3, Jsrp1, Tbx10, Fuz, Casz1, and Cxcl1 were enriched via gene ontology. The results of gene ontology are presented in Figure 4.

Discussion

Using the gene and protein expression change to assess laser effects on cell growth and proliferation is highlighted in many reports.[18,19] As it is depicted in Table 1, the FC of "triggering receptor expressed on myeloid cells like 4" (TREML4) is the highest value in Table 1. It seems that TREML4 is the main dysregulated protein in response of the radiated cells to the laser. However, the presented results in Figure 1 do not correspond to this finding. TREML4 like the other proteins remained isolated and did not have any connection with the other proteins.

Table 4. Two centrality parameters including degree and betweenness centrality and logFC of the 12 queried DEGs

No.	Query Term	Degree	Betweenness Centrality	logFC
1	Cxcl1	76	0.023	0.678
2	Cxcl3	73	0.015	0.856
3	Cox6b2	16	0.000	0.900
4	Fuz	6	0.013	0.743
5	Serpina4	3	0.004	0.880
6	Tbx10	3	0.000	0.790
7	Elp5	2	0.000	0.624
8	Casz1	1	0.000	0.689
9	Jsrp1	1	0.000	0.828
10	Treml4	1	0.000	1.102
11	C2cd4b	0	0.000	1.050
12	Tpd52l3	0	0.000	0.607

Network analysis by using some added first neighbors has shown that the isolated node can be connected by the first neighbors. On the other hand, adding some related nodes to the network increases network connectivity.[20] Here, 100 first neighbors were added to the isolated 12 queried proteins. Results showed that the queried proteins were distributed in the three categories of the nodes; (a) two remained isolated proteins, (b) ELP5 that is connected to the two first neighbors, and (c) the other 9 proteins which are involved in the main connected component of the network. It seems the network is characterized by the central nodes. The importance of central nodes in the analysis of the network is a well-known concept in system biology.[21]

PPI network analysis has shown that the networks contain the dense core as clusters.[22] As it is shown in Figures 2 and 3, the main connected component contains two left and right domains or clusters. The distribution of the 9 queried proteins among the main connected component indicates that Cox6b2 is included in the left domain while the other 8 proteins are presented in the right domain. The two clusters of the main connected component are connected by the Cox4i1-Alb bridge. The two elements of this bridge are the added first neighbors. As it is shown in Table 2, Alb and Cox4i1 are the first and second ranked bottlenecks. It is pointed out that the bottleneck deletion of the network leads to distortion in the network, which refers to the important role of the bottleneck nods.[23]

Based on Tables 2 and 3, Cxcl1 and Cxcl3 are the queried proteins that play roles as hub nodes, and the single queried bottleneck is Cxcl1. Since the common hubs and bottlenecks are known as hub-bottleneck,[24]

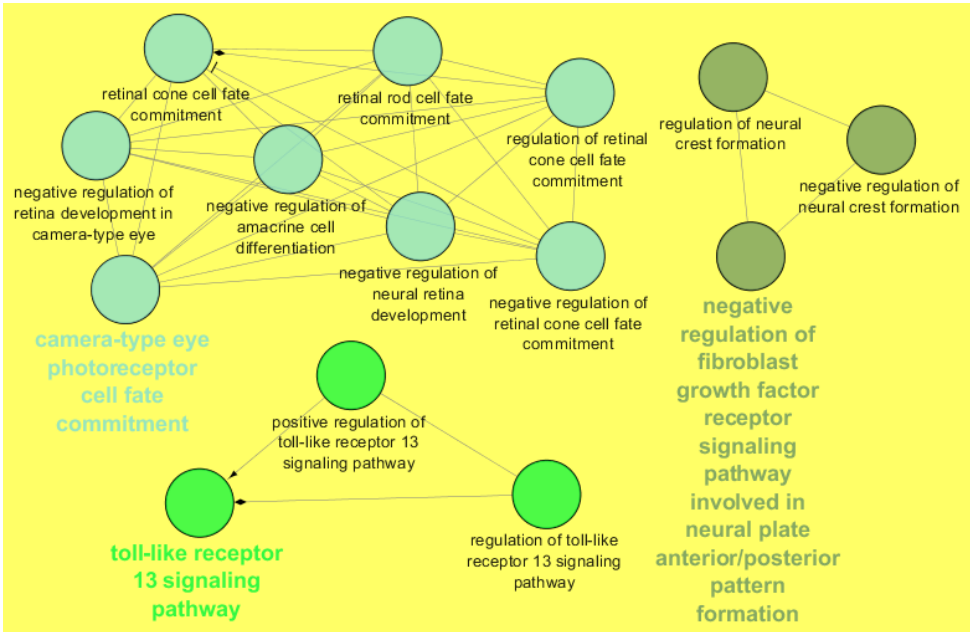

Figure 4. Results of Enrichment of 9 Queried DEGs Included in the Main Connected Component. The term which represents the related group is shown as a colored individual.

Cxcl1 is a single hub-bottleneck protein among the 12 queried individuals. Central properties and FCs of the queried 12 proteins are tabulated in Table 4. Based on the results of Table 4, TERML4 is characterized by the approximately lowest values of centrality parameters. Cxcl1 is a hub-bottleneck that appears with the highest amounts of centrality parameters; however, its FC is low.

As it was described, among the 12 queried proteins, 9 individuals were included in the main connected component. The results of the enrichment of these 9 proteins are shown in Figure 4. "Camera-type eye photoreceptor cell fate commitment" as the major cluster of biological terms was identified. This cluster is related to the restriction of the fate developmental of a cell to produce a photoreceptor cell.[25] The second terms are determined as "Negative regulation of fibroblast growth factor receptor signaling pathway involved in plate anterior/posterior pattern formation". "Toll-like receptor 13 signaling pathway" is the last appeared pathway that is related to the enriched proteins. It is explored that toll-like receptors are involved in the increment of cytokines expression by inflammatory genes.[26] Prominent roles of the 9 enriched proteins in cell proliferation are reported by Niimi et al.[15]

The Finding of the present study led to the introduction of Cxcl1 as a central targeted protein by low-level Er: YAG laser radiation. As it has been reported, Cxcl1 plays a role in the angiogenesis of human endothelial cells, hyperglycemia, obesity, impaired islet function, and cancer development.[27-29]

Conclusion

Findings indicate that Cxcl1 is a critical protein that is targeted by low-level Er: YAG laser radiation. A wide range of activities that are related to Cxcl1 imply more investigations into the possibly useful and harmful effects of laser application. It seems that the immune system is the main targeted subjects in the treated cells. Immune response to laser radiation can be considered a defense process against stress which can activate cellular proliferation and also metabolic pathways.

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Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare they have no conflicts of interest.

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