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Assessment of Cytokine-Mediated Signaling Pathway Dysregulation in Arm Skin After CO2 Laser Therapy

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Abstract

Introduction: Laser therapy is known as an efficient approach in dermatology surgery. CO2 laser therapy is a gold standard treatment in skin surgery. This study aimed to evaluate the interferons change after CO2 laser surgery

Methods: Significant differentially-expressed genes (DEGs) of arm skin after 7 days of treatment by the CO2 laser relative to the controls are downloaded from Gene Expression Omnibus (GEO) and are included in the protein-protein interaction network via a STRING database (an application of Cytoscape software). The central DEGs were identified and enriched via gene ontology by using Clue GO software.

Results: A network including 78 DEGs and 100 neighbors was constructed and STAT1, MX1, ISG15, OAS1, IFIT1, IRF8, OASL, OAS2, and RSAD2 as hubs and STAT1, PTPRC, MX1, IRF8, ISG15, IL6, RORC, SAMSN1, and IFIT1 as bottlenecks were introduced. The cytokine-mediated signaling pathway, interferon gamma signaling, hepatitis C, interferon alpha/beta signaling, and the type I interferon signaling pathway were identified as 5 clusters of biological terms which are related to the central nodes.

Conclusion: It can be concluded that the cytokine-mediated signaling pathway is the major pathway that is dysregulated after laser application in the treated skin.

Keywords: Laser CO2; Skin; Interferon; Gene; Network.

Introduction

There is a wide variety of laser applications in biomedical sciences. Skin-care laser therapy is a basic clinical approach in dermatology. Photo aging and facial skin damage therapy are known as useful and safe tasks in medicine.[1] In this regard, CO2 laser therapy has attracted more attention and has become a gold standard treatment for improving skin and mucosal lesions.[2] Different aspects of skin response to laser therapy are investigated. Tatmatsu-Rocha et al have reported that low-level laser therapy (904 nm) elevates collagen and decreases oxidative and nitrosative stresses in the skin of the diabetic wounded mice.[3] The importance of laser therapy in wound healing implies doing wide 12 in vivo investigations to understand the mechanism and efficacy of the applied method.[4] Furthermore, the safety of laser therapy as an important approach, especially for such an individual at risk as a pregnant woman, has attracted the scientists' attention.[5]

The molecular mechanism determination of the clinical methods and body response to treatments require the application of system biology analysis. The creation of a large number of data, complicated analysis, screening of a huge number of data to find crucial ones, and finding

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biomarkers are set of activities in system biology, which can be used as diagnostic tools, drug targets, and also agents in follow up of patients. Network analysis is an efficient method in this regard.[6-8] PPI network analysis provides critical information about central differentially-expressed genes (DEGs) that are involved in a biological or pathological phenomenon. In this approach, proteins, genes, or metabolites that discriminate 2 conditions such as before and after treatment are interacted to create a network. The elements that play a crucial role in the integrity of the constructed network (called central nodes) have been identified and their biological roles will investigate.[9-11] In the present study, gene expression changes in an arm skin biopsy after 7 days of laser therapy versus an untreated condition were investigated via network analysis to find significant events.

Materials and Methods

The microarray results of a document entitled "Noncoding dsRNA induces retinoic acid synthesis to stimulate hair follicle regeneration via TLR3"[12] were downloaded from Gene Expression Omnibus (GEO). The Data are presented as GSE131789/GPL15207 for 16 women that are treated to improve photo aging. The gene expression pattern of the arm skin biopsies of the samples after 7 days of treatment with a 1550 nm non-ablative fractional CO2 laser was compared with similar biopsies before the treatment as controls. The Data were analyzed by GEO2R to statistical matching. The top 250 significant DEGs were selected. Considering *P* value <0.05 and fold change >1.5, the significant DEGs were identified. The identified DEGs plus 100 neighbors from a STRING database were included in interactome by Cytoscape software v3.7.1.[1] The main connected component of the constructed network was analyzed by a Network analyzer application of Cytoscape. Ten percent of the top nodes based on the degree value were selected as hub nodes. The bottlenecks were determined in a similar manner based on betweenness centrality values. The descriptions of the central nodes were extracted from a STRING database and were summarized. The connections between the central nodes were shown via a subnetwork.

Gene ontology analysis was applied to determine the biological processes and biochemical pathways for the central nodes via Clue GO v2.5.4. The biological terms were clustered and represented as a network and tables (kappa score = 1 and *P* value <0.05).

Results

The gene expression distribution of the 16 patient samples and the controls was matched via boxplot presentation. As it is shown in Figure 1, the samples could be compared statistically. Since a large number of genes were dysregulated, the top significant 250 genes were selected for more analysis; considering fold change more than 1.5 and *P* value less than 0.05, 82 DEGs were identified. These selected DEGs were imported into STRING database via Cytoscape software. Among them, 78 individuals were recognized by STRING and the others were not found. The PPI network including 78 DEGs was created and due to poor interactions between the DEGs, 100 neighbors from a STRING database were added to the queried DEGs.

The constructed network included a main connected component and 9 isolated nodes (DEGs). The main connected component, namely the network contained 169 interacted genes (see Figure 2). The network was a scale-free network and therefore it was possible to introduce a limited number of genes that were important relative to the others. These crucial genes can be identified via the centrality analysis of the network. Based on the degree value, 10% of the top queried DEGs including

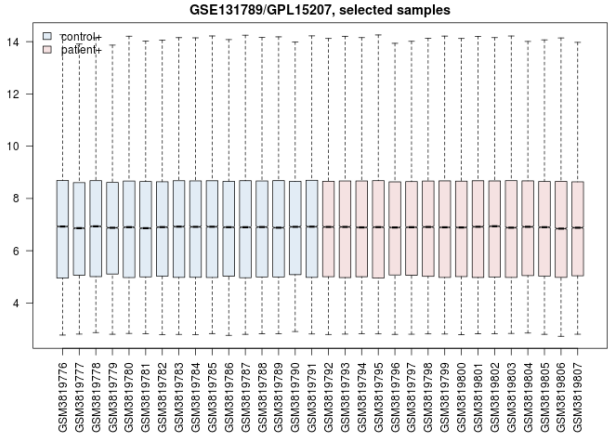

Figure 1. The Box-Plot Representation of Gene Expression Distribution of 16 Patient Samples Versus Controls.

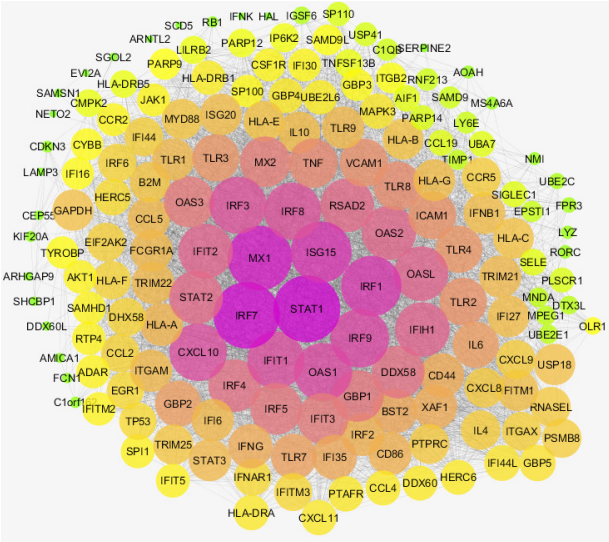

Figure 2. The Main Connected Component Including the Recognized Queried Genes (Without the Isolated Ones) and 100 Neighbors. The nodes are laid out based on the degree value.

STAT1, MX1, ISG15, OAS1, IFIT1, IRF8, and OASL were determined as hubs. Two DEGs, namely OAS2 and RSAD2 which had a degree value near the last hubs, were also added to the hub nodes (see Table 1). Similar to the hubs, the bottlenecks were identified based on betweenness centrality and are tabulated in Table 2. The connections between the hubs and the bottlenecks are shown in the illustrated sub-network in Figure 3. The number of links and kinds of neighbors in this sub-network are different. Since gene ontology can provide useful information about the roles of the central nodes, the related biological terms were determined for the central genes. As it is shown in Figure 4, except SAMSN1, the other hubs and bottlenecks were related to the relevant biological terms. The biological terms are shown in Figure 5 and Table 3 in more details. The terms were extracted from KEGG, REACTOME_Pathways, GO_Cellular Component-EBI-UniProt-GOA, and GO_Biological PROCESS-EBI-UniProt-GOA (27.02.2019) via Clue GO v2.5.4.

Discussion

There are different documents about the molecular aspects of laser therapy, which explain waste alterations in the gene expression pattern of the treated samples. The problems such as side effects and also the accurate molecular mechanism of laser therapy have attracted the attention of experts and scientists. In the present study, 13 critical genes which were affected by laser therapy were introduced. It was expected that biological processes related to these genes set be affected following laser therapy. STAT1, MX1, ISG15, OAS1, IFIT1, IRF8, OASL OAS2, RSAD2, IL6, PTPRC, PORC, and SAMSN1 were the 13 central DEGs. As it is shown in Tables 1 and 2, it seems the central genes were mostly related to the interferons and therefore the immune system. The finding of gene ontology provided useful information about the correlation between central genes and biological terms. Except SAMSN1, the other 12 critical genes were related to the 5 clusters of biological terms. The cytokine-mediated signaling pathway, interferon gamma signaling, hepatitis C, interferon alpha/beta signaling, and the type I interferon signaling pathway were the 5 classes of biological terms which included 1, 4, 5, 12, and 21 terms respectively. As it is shown in Figures 4 and 5 and Table

Table 1. The Hub Nodes of the Main Connected Component Sub-network

Name	Description	Degree	BC	CC
STAT1	Signal transducer and activator of transcription 1-alpha/beta; it mediates cellular responses to interferons (IFNs), cytokine KITLG/SCF and other cytokines and other growth factors. The phosphorylated STATs dimerize and are associated with ISGF3G/IRF-9 to form a complex termed ISGF3 transcription factor that enters the nucleus. ISGF3 binds to the IFN stimulated response element (ISRE) to activate the transcription of IFN-stimulated genes (ISG), which drive the cell in an antiviral state.	119	1.00	0.76
MX1	Interferon-regulated resistance GTP-binding protein MxA; Interferon-induced dynamin-like GTPase with antiviral activity against a wide range of RNA viruses and some DNA viruses.	114	0.50	0.73
ISG15	Interferon-induced 15 kDa protein; Ubiquitin-like protein which plays a key role in the innate immune response to viral infection via either its conjugation to a target protein (ISGylation) or its action as a free or unconjugated protein. The secreted form of ISG15 can induce natural killer cell proliferation, act as a chemotactic factor for neutrophils and act as an IFN-gamma-inducing cytokine playing an essential role in antimycobacterial immunity.	107	0.25	0.70
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46 kDa; Interferon-induced, a dsRNA-activated antiviral enzyme which plays a critical role in the cellular innate antiviral response. In addition, it may play a role in other cellular processes such as apoptosis, cell growth, differentiation and gene regulation. It synthesizes higher oligomers of 2'-5'-oligoadenylates (2-5A) from ATP, which then bind to the inactive monomeric form of ribonuclease L (RNase L) leading to its dimerization and subsequent activation. The activation of RNase L leads to the degradation of cellular as well as viral RNA, resulting in the inhibition of protein synthesis, thus terminating viral replication.	101	0.25	0.68
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1; Interferon-induced antiviral RNA-binding protein that specifically binds single-stranded RNA bearing a 5'-triphosphate group (PPP-RNA), thereby acting as a sensor of viral single-stranded RNAs and inhibiting the expression of viral messenger RNAs.	100	0.25	0.67
IRF8	Interferon consensus sequence-binding protein; it plays a role as a transcriptional activator or repressor. Specifically, it binds to the upstream regulatory region of type I IFN and IFN-inducible MHC class I genes (the interferon consensus sequence (ICS)). It plays a negative regulatory role in the cells of the immune system.	99	0.50	0.69
OASL	59 kDa 2'-5'-oligoadenylate synthase-like protein; it can bind double-stranded RNA. It displays antiviral activity against the encephalomyocarditis virus (EMCV) and the hepatitis C virus (HCV).	94	0.25	0.66
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa; Interferon-induced, a dsRNA-activated antiviral enzyme which plays a critical role in the cellular innate antiviral response. In addition, it may also play a role in other cellular processes such as apoptosis, cell growth, differentiation and gene regulation.	93	0.25	0.65
RSAD2	Virus inhibitory protein, endoplasmic reticulum-associated, interferon-inducible; Interferon-inducible iron-sulfur (4FE-4S) cluster-binding antiviral protein which plays a major role in the cell antiviral state induced by type I and type II interferon.	92	0.25	0.65

BC and CC refer to betweenness centrality and closeness centrality respectively. BC values are normalized.

Table 2. The Bottlenecks of the Main Connected Component Sub-network

Name	Description	Degree	BC	CC
STAT1	It is described in Table 1.	119	1.00	0.76
PTPRC	Protein tyrosine phosphatase, receptor type, C; it is required for T-cell activation through the antigen receptor. It acts as a positive regulator of T-cell coactivation upon binding to DPP4.	64	0.50	0.61
MX1	It is described in Table 1.	114	0.50	0.73
IRF8	It is described in Table 1.	99	0.50	0.69
ISG15	It is described in Table 1.	107	0.25	0.70
IL6	B-cell stimulatory factor 2; Cytokine with a wide variety of biological functions. It is a potent inducer of the acute phase response. It plays an essential role in the final differentiation of B-cells into Ig-secreting cells Involved in lymphocyte and monocyte differentiation. It acts on B-cells, T-cells, hepatocytes, hematopoietic progenitor cells and cells of the CNS. It is required for the generation of T(H)17 cells. It also acts as a myokine. It is discharged into the bloodstream after muscle contraction and it acts to increase the breakdown of fats and to improve insulin resistance. It induces myeloma and plasmacytoma growth and induces nerve cells differentiation; Interferons	77	0.25	0.64
RORC	Nuclear receptor subfamily 1 group F member 3; Key regulator of cellular differentiation, immunity, peripheral circadian rhythm as well as lipid, steroid, xenobiotics and glucose metabolism. It plays an indispensable role in the induction of IFN-gamma dependent on anti-mycobacterial systemic immunity.	13	0.25	0.49
SAMSN1	SAM domain, SH3 domain and nuclear localization signal protein 1; Negative regulator of B-cell activation. It down-regulates cell proliferation (in vitro). It promotes RAC1-dependent membrane ruffle formation and reorganization of the actin cytoskeleton.	4	0.25	0.39
IFIT1	It is described in Table 1.	100	0.25	0.67

BC values are normalized.

3, the type I interferon signaling pathway was the largest cluster that had an overlap with the other classes. On the other hand, the cytokine-mediated signaling pathway was highlighted as a term that included all 12 central DEGs (see Figure 5). As it is shown in Figure 4, the 12 genes accounted for only about 1.2% of the total involved genes in this term. The total numbers of genes that were related to the cytokine-mediated signaling pathway were calculated at about 1000 genes.

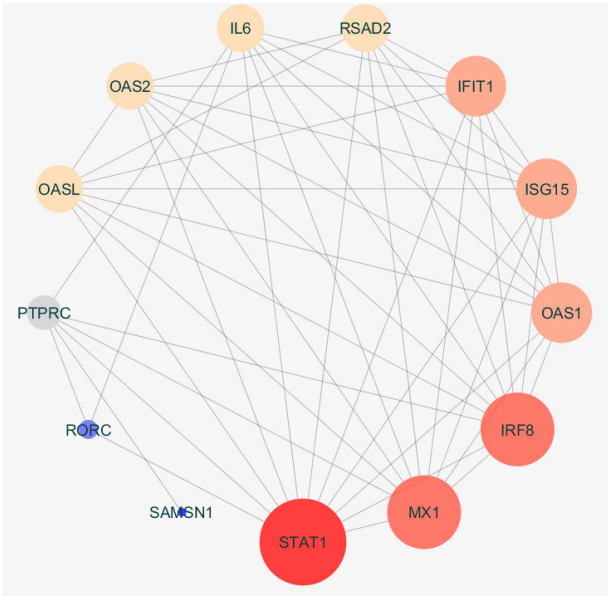

Figure 3. A Sub-network Including the Hubs and Bottlenecks. The nodes are laid out based on the degree value.

Cytokines are well-known as low molecular weight protein mediators which are involved in different biological processes such as inflammation, cell growth and differentiation, immune system, and tissue repair.[13,14]

The roles of most central nodes in inflammation and immune-deficiency diseases have been identified by researchers. STAT1 that is targeted in anti-inflammatory treatment is a well-known member of elements that promote CD.[15-17] As it is illustrated in Figure 4, STAT1 was related to all 5 clusters of the biological terms. Based on the results shown in Tables 1 and 2, STAT1 was a top hub, a bottleneck and also a hub-bottleneck. As it can be seen in Figure 3, among all central genes, only STAT1 was connected to the other 11 central genes. The mediator of the cellular responses to interferons (IFNs), cytokine KITLG/SCF and other cytokines and other growth factors are mentioned as the description of STAT1 in Table 1.

The second top hub-bottleneck was MX1, an interferon-regulated resistance GTP-binding protein. Like STAT1, this gene was involved in all 5 clusters of the biological processes (see Figure 4). This protein and its corresponding family are known as key mediators of interferon-regulated host resistance to intracellular pathogens.[18] Antiviral activity against a wide range of RNA viruses and some DNA viruses is highlighted as the description of MX1 in Table 1.

IRF8 was the third hub-bottleneck determined in this study. The role of IRF8 in the inflammation process in neuro-degenerative diseases is investigated vastly.[19-21] Graham et al reported that each of 5 loci, including NCF2, IKZF1, IRF8, IFIH1, and TYK2, can be mapped

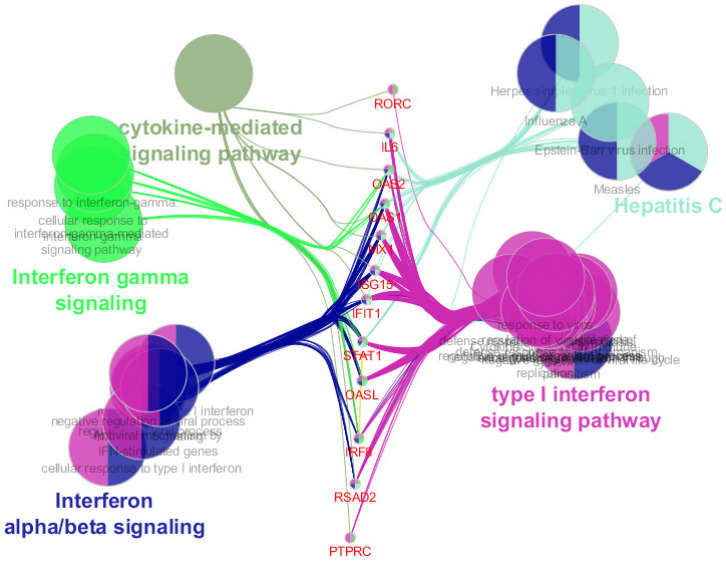

Figure 4. Five clustered biological terms and the related hubs and bottlenecks are presented. The terms titles are displayd as background and the names of the clusters are highlighted. The central DEGs are shown in red.

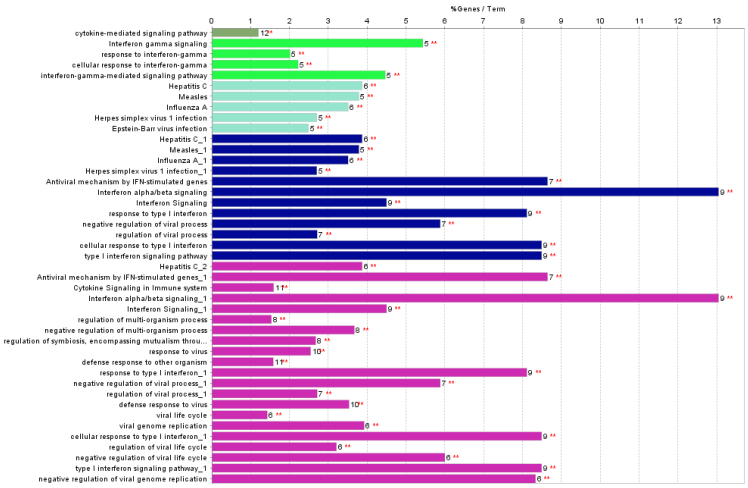

Figure 5. The biological terms that are organized in Figure 4 are shown. The names of terms are represented in Table 3. The numbers beside the terms refer to the hubs or bottlenecks which are involved in the terms. % genes/Term indicates the percentage of the ratio of the hub or bottleneck genes number per the total number of genes that are involved in the term.

into interferon signalling pathways and are involved in the promotion of systemic lupus erythematosus.[22] Like MX1, IRF8 except RORC was connected to the other central DEGs and related to all 5 classes of the biological processes.

Based on the description in Table 1, interferon-induced 15 kDa protein (ISG15) plays a crucial role in the innate immune response to viral infection. It induces natural killer cell proliferation, acts as a chemotactic factor for neutrophils, and acts as an IFN-gamma-inducing cytokine which plays an important role in anti-mycobacterial immunity. The antiviral function of ISG15 is reported and discussed in more detail.[23,24]

The last hub-bottleneck was IFIT1, Interferon-induced protein with tetratricopeptide repeats 1. Pei et al reported the role of IFIT1 in the limitation of hepatitis B virus replication, while Danish et al introduced IFIT1 as a prognostic marker for local control in T1-2 N0 breast cancer treated with breast-conserving surgery and radiation therapy.[25,26] Like the other central DEGs, IFIT1 is connected to most of the biological processes (see Figure 4).

Conclusion

It can be concluded that the activation of the immune system, especially the cytokine-mediated signaling

Table 3. The Name of the Biological Terms That Are Shown in Figure 5

Cytokine-mediated signaling pathway
Interferon gamma signaling
Response to interferon-gamma
Cellular response to interferon-gamma
Interferon-gamma-mediated signaling pathway
Hepatitis C
Measles
Influenza A
Herpes simplex virus 1 infection
Epstein-Barr virus infection
Hepatitis C
Measles
Influenza A
Herpes simplex virus 1 infection
Antiviral mechanism by IFN-stimulated genes
Interferon alpha/beta signaling
Interferon Signaling
Response to type I interferon
Negative regulation of the viral process
Regulation of the viral process
Cellular response to type I interferon
Type I interferon signaling pathway
Hepatitis C
Antiviral mechanism by IFN-stimulated genes
Cytokine Signaling in the Immune system
Interferon alpha/beta signaling
Interferon Signaling
Regulation of the multi-organism process
Negative regulation of the multi-organism process
Regulation of symbiosis, encompassing mutualism through parasitism
Response to virus
Defense response to other organisms
Response to type I interferon
Negative regulation of the viral process
Regulation of the viral process
Defense response to the virus
Viral life cycle
Viral genome replication
Cellular response to type I interferon
Regulation of viral life cycle
Negative regulation of the viral life cycle
Type I interferon signaling pathway
Negative regulation of viral genome replication

The terms are displayed in the same color in Figure 5 and Table 3.

pathway is the common feature of laser application in the treated skin. It seems that interferons play a significant role in response to laser therapy which can be considered as a natural response of the body to stress.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare no conflict of interest.

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