

# Assessment of Dysregulation of HERC6 and Essential Biological Processes in Response to Laser Therapy of Human Arm Skin



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## Abstract

**Introduction:** The widespread application of lasers in medicine, especially in the treatment of diseases implies more investigations to understand the precious molecular mechanism of the laser effect on the human body. In the present study, the prominent role of HERC6 in response to CO<sub>2</sub> Laser therapy of human skin is investigated.

**Methods:** The numbers of 16 gene expression profiles before and after the treatment with the CO<sub>2</sub> laser are downloaded from Gene Expression Omnibus (GEO), and differentially-expressed genes (DEGs) are analyzed to find the significant DEGs. Gene ontology analysis revealed that HERC6 and a set of its neighbors played a significant role in response to laser application.

**Results:** The expression changes of 52 significant DEGs were compared via heat map analysis and 27 significant DEGs were introduced as the critical genes which are involved in response to laser irradiation. "Thymidylate kinase activity" among 9 clusters of biological terms was highlighted as an important biological process related to the identified DEGs. HERC2 was proposed as a critical DEG which was related to several essential cellular processes in response to laser application.

**Conclusion:** The findings from the present study indicate that HERC6 and the numbers of its first neighbors are involved in the essential cellular response to laser therapy of human skin.

**Keywords:** Laser therapy; Skin; HERC6; Gene Expression; Gene ontology.



## Introduction

Various types of laser are applied in medicine and this process is rising progressively. Instrumental development besides the improvement of procedures has provided the possibility of the widespread usage of lasers in the treatment of many diseases.<sup>1</sup> However, although the safety of laser therapy is agreed, more pieces of evidence are needed to understand the accurate molecular mechanism of laser therapy.<sup>2</sup> The understanding of the molecular mechanism of low-intensity laser radiation which has been applied for therapeutic purposes is not a new effort. Several documents refer to the researches conducted about three decades ago on molecular bases of laser therapy.<sup>3,4</sup> Recently, high throughput methods such as proteomics have been presented as suitable tools for monitoring a molecular event during medical interventions. There are several proteomic investigations into the biological effects of therapeutic lasers.<sup>5,6</sup> In such researches, large numbers

of dysregulated proteins or genes due to laser irradiation have been determined, which can be considered as differentially expressed genes or proteins.<sup>7</sup>

Considerable efforts are needed to find suitable interpretive skills for the analysis of proteomics or genomics findings. Network analysis is one of the suitable system biology methods which are used to screen large numbers of data to find the limited numbers of the significant differentially markers. In this approach, the genes or proteins are interacted to construct an interactome. The limited numbers of critical genes which are related to the studied condition identify via topological analysis of the network.<sup>8,9</sup> Gene ontology is another method for the assessment of the role of differentially expressed genes in the body related to the studied condition. This approach identifies molecular functions, cellular components, biological processes, and biochemical pathways related to the studied genes.<sup>10</sup> Physical and chemical properties of

genes and their regulatory effects on each other provide useful information to screen and rank a set of genes to find the crucial ones. Activation, inhibition, catalysis, binding, reaction, and post-translation modification actions can be determined when an action map is plotted.<sup>11</sup> In the present study, the significant differentially-expressed genes (DEGs) which discriminate laser-treated human arm skin from non-treated samples are selected from a gene expression profile to analyze via gene ontology. The findings are limited to a few genes which probably play a critical role in the mechanism of laser therapy.

## Materials and Methods

In the previous report<sup>12</sup> entitled “Assessment of Cytokine-Mediated Signaling Pathway Dysregulation in Arm Skin after CO<sub>2</sub> Laser Therapy”, we downloaded GSE131789/GPL15207 for 16 women from the Gene Expression Omnibus (GEO). The samples were prepared from arm skins before (as controls) and 7 days after the treatment with a 1550 nm non-ablative fractional CO<sub>2</sub> laser (as treated). The original data were published by Kim et al as “Noncoding dsRNA induces retinoic acid synthesis to stimulate hair follicle regeneration via TLR3”<sup>13</sup> in *Nature Communications*. We determined and discussed the role of the central nodes of the protein-protein interaction network of the DEGs to highlight “cytokine-mediated signaling pathway” as a major affected individual by laser irradiation. Here based on expression value, the significant DEGs after laser irradiation are determined and via gene ontology, the key gene is introduced.

Among downloaded top 250 DEGs based on *P* value, 52 significant DEGs by applying *P* value <0.001 and fold change >2 were determined. The amounts of expression for each sample were obtained from the related 32 GSMs. The heat map for the expression amounts of 52 significant DEGs was plotted in the red-green style via heatmap. ca as an online program.<sup>14</sup> The isoforms were determined and the gene with more than one isoform was considered as a family and regarded as an individual in the later analysis.

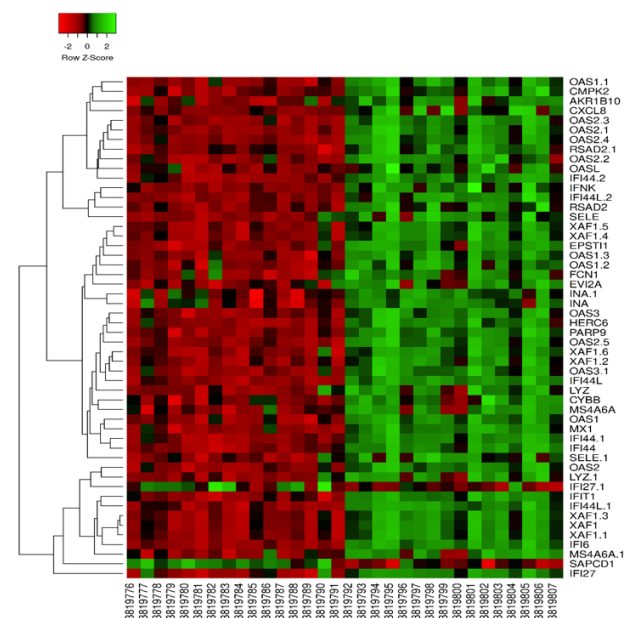
Gene ontology for the candidate DEGs was done via the CleGO v2.5.4 application of Cytoscape software v 3.7.1.<sup>7</sup> Biological terms were searched in all databases which are related to the ClueGO. The terms were clustered based on *P* < 0.05. The Cellular involvement of the significant DEGs was identified by CluePedia which is a plugin of Cytoscape software. The relationship between the significant DEGs was unwavering by CluePedia as an action map. The key element in the action map and its 20 first neighbors were investigated in the STRING database. The findings were discussed and the key element related to the effect of laser irradiation was introduced.

## Results

The gene expression profiles of the 16 treated samples by the non-ablative fractional CO<sub>2</sub> laser from women

were compared with the 16 biopsy samples which were prepared before the treatment as controls. The samples were matched via GEO2R statistically. Since the distribution of gene expression amounts was a median center, the samples were suitable for more analysis. To find the significant and characterized DEGs, the top 250 genes based on fewer *p*-value quantities were identified by GEO2R analysis. After applying fold change >2 and *P* value <0.001, 52 DEGs were determined as significant differentially expressed genes. The heat map presentation of expressed amounts of each DEG in the 32 samples is shown in Figure 1. GSM3819777-GSM3819791 belongs to the control samples while GSM3819792-GSM3819807 refers to the treated skins. The heat map plot separated the control and treated samples clearly based on gene expression changes. As it is shown in Figure 1, there are several isoforms for 11 DEGs. These 11 DEGs and numbers of their isoforms are presented in Table 1. Regarding a set of isomers as a gene, there are 27 significant DEGs that are related to the application of the laser.

GEO2R analysis revealed that all DEGs are up-regulated except for the SAPCD1 gene. The logarithm of fold change for this gene is equal to (-1.034). As it is shown in Table 1, SAPCD1 has no additional isoform. The heat map as a suitable tool separated the samples in the two the control and treated groups. The GSM3819777-GSM3819791 is presented in the red area of the map which refers to the down-regulation process and the treated GSMs shown in the green part of Figure 1 correspond to the up-regulation manner.



**Figure 1.** Heat Map Presentation of the Expression Amounts of 52 Significant DEGs in the 16 Control Arm Skin (GSM3819777-GSM3819791) and the Treated Arm Skin With Laser Irradiation (GSM3819792-GSM3819807). GSMs are shown in the horizontal axis and the dendrogram is presented in the left vertical axis. DEGs are shown at the right-hand vertical side.

**Table 1.** Different Isoforms of 11 Significant DEGs Related to the Effect of Laser Irradiation on Human Arm Skin

| Gene Name | No. of Isoforms |
|-----------|-----------------|
| XAF1      | 7               |
| OAS2      | 6               |
| IFI44     | 6               |
| OAS1      | 4               |
| MS4A6A    | 2               |
| OAS3      | 2               |
| IFI27     | 2               |
| INA       | 2               |
| LYZ       | 2               |
| RSAD2     | 2               |
| SELE      | 2               |
| Total     | 37              |

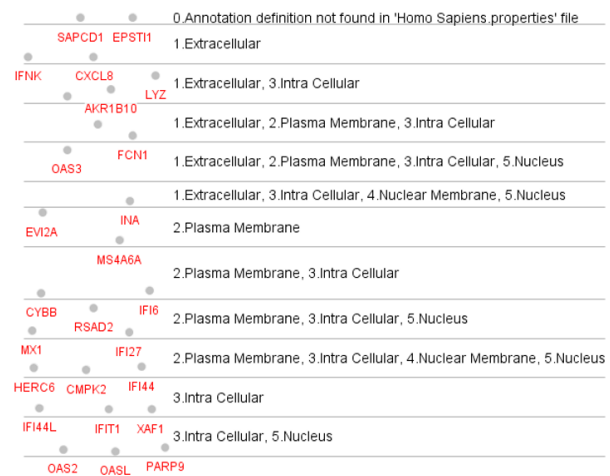
Gene ontology results for the 27 significant DEGs are displayed in Figure 2. Nine clusters including 25 biological terms are determined as related to the effects of the laser on arm skin via the highlighted significant DEGs. “Thymidylate kinase activity” including 9 biological terms (36% of all biological terms) is the largest cluster, while there are three single clustered terms. Surprisingly, annotation definitions for Homo sapiens properties for SAPCD1 (the only down-regulated DEG) and EPSTI1 were not found in CluePedia (see Figure 3). The distribution of the other 25 significant DEGs in the related cellular compartments, including the extracellular, plasma membrane, intracellular, nuclear membrane, and nucleus locations is presented in Figure 3.

The action map of the 27 significant DEGs including binding, expression, catalysis, reaction, post-translational modification, and inhibition is shown in Figure 4. As illustrated in Figure 4, only reaction, binding, and catalysis actions were found for 11 connected DEGs and the other isolated ones. Since HERC6 plays a crucial role

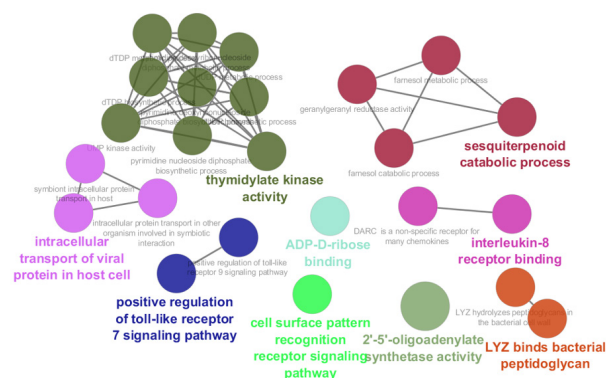
in the action map, it seems that the first neighbors of this gene are involved in the effect of the laser on the skin. Therefore, the 10 first neighbors of the HERC6 gene were determined via the STRING database and linked to each other by Cytoscape software (see Figure 5).

## Discussion

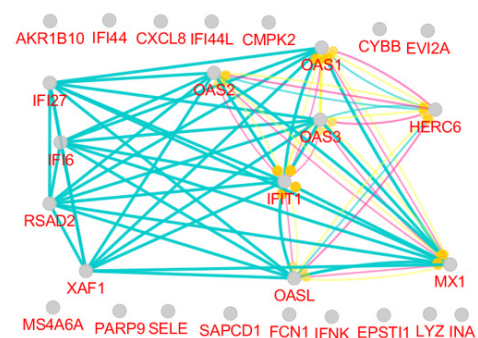
Laser application in medicine has attracted many clinicians' attention in different fields.<sup>15,16</sup> Dermatologists by using different types of lasers solve diverse kinds of patients' problems.<sup>17</sup> In spite of the widespread applications of lasers in medicine, more investigation is needed to understand the molecular mechanism of laser irradiation on the human body. The high throughput methods such as genomics and proteomics have provided a new perspective of the laser effect on the body.<sup>18</sup> In the present study, the findings from microarray investigation into the effect of the laser on human arm skin were analyzed to



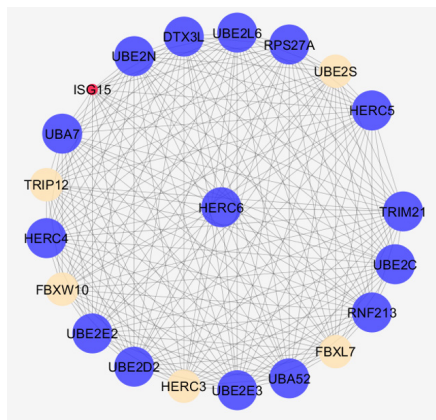
**Figure 3.** Cellular components including the extracellular, plasma membrane, intracellular, nuclear membrane, and nucleus locations for the 27 significant DEGs were determined via ClueGO. The gray spots refer to the genes.



**Figure 2.** Nine Clusters Including 25 Biological Terms Related to the 27 Significant DEGs. All databases related to the ClueGO were searched and  $P < 0.05$  was considered.



**Figure 4.** The action map for the 27 DEGs including 11 connected genes and 16 isolated individuals is plotted via CluePedia. Blue, yellow, and purple colors refer to reaction, binding, and catalysis respectively.



**Figure 5.** HERC6 in Connection With the 20 First Neighbors by 205 Edges. The network was constructed by Cytoscape software by using the STRING database. The nodes are laid out based on degree value; red to blue refers to the increment of degree.

find the possible key element in this regard. As it is shown in Figure 1, the screened and selected DEGs separate the samples in the two groups, including before and after laser irradiation classes. Therefore, it can be concluded that the mentioned DEGs can be considered as important affected genes by the laser. Since there are several isoforms of numerous DEGs, the limited individuals remain to be analyzed by the complementary methods. Gene ontology analysis leads to introducing biological terms which are different from the terms that were presented for the central DEGs in the previous report.<sup>12</sup> It seems that considering the expression properties of the significant DEGs is a suitable way to find complementary information about the critical genes beside the central genes. There are several documents about different parts of cells which are affected by laser irradiation.<sup>19,20</sup> As it is shown in Figure 3, all cellular compartments are exaggerated by laser therapy. From the extracellular region to the nucleus are treated by laser irradiation. Since there is not sufficient regulatory information about the relationship between the introduced DEGs in the databases, the action map is plotted in a limited style and only three types of actions are formed between the several numbers of significant DEGs.

HERC6 is a highlighted element in the action map that was not considered as a central node in the previous report. It is linked directly and indirectly to the other nodes of the action map to promote its important role in the body. As it is shown in Figure 3, HERC 6 is an intracellular individual that affects the plasma membrane, the intracellular region, the nuclear membrane, and the nucleus. One of the first neighbors of HERC6 is ISG15 which was introduced as a central DEG in the previous report.<sup>12</sup> Interferon-stimulated gene 15 (ISG15) is an ubiquitin-like modifier (UBL); it is reported that ISG15 modification has an essential role in the antiviral defense of the body.<sup>21</sup> In the other document, it is discussed that

HERC6 is the main E3 ligase for ISG15 conjugation.<sup>22</sup> HERC3, HERC4, and HERC5 are the three first neighbors of HERC6 that are illustrated in Figure 5. It is reported that 6 HERC genes in the human genome encode two different types of proteins. Investigations have revealed that these proteins are involved in membrane-trafficking events with the role of ubiquitin ligases.<sup>23</sup>

Numbers of 7 ubiquitin-conjugating enzymes including UBE2E3, UBE2D2, UBE2E2, UBE2N, UBE2L6, UBE2S, and UBE2C are connected to HERC6 directly. Researchers have shown that several types of cellular processes including stress response, protein turnover, organelle biosynthesis, cell cycle regulation, and cellular homeostasis are regulated by the ubiquitylation system.<sup>24</sup> Connections between this important family and HERC6 refer to the crucial role of HERC6 in the regulation of important cellular processes and functions.

## Conclusion

The findings indicate that extracellular, plasma membrane, intracellular, nuclear membrane, and nucleus parts are affected by laser irradiation via the expression change of large numbers of significant DEGs. HERC6 and a set of its first neighbors which are involved in the regulation of several essential biological processes such as organelle biosynthesis, stress response, cellular homeostasis, cell cycle regulation, and protein turnover appeared as key elements in response to laser irradiation. It seems that the application of the laser in the treatment of diseases and also for other medical purposes requires more safety considerations.

## Ethical Considerations

Not applicable.

## Conflict of Interests

The authors declare no conflict of interest.

## Acknowledgment

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