Chronological Gene Expression of Human Gingival Fibroblasts with Low Reactive Level Laser (LLL) Irradiation

Yuki Wada 1,*, Asami Suzuki 2,*, Hitomi Ishiguro 1,3, Etsuko Murakashi 1 and Yukihiro Numabe 1,3

Abstract: Though previously studies have reported that Low reactive Level Laser Therapy (LLLT) promotes wound healing, molecular level evidence was uncleared. The purpose of this study is to examine the temporal molecular processes of human immortalized gingival fibroblasts (HGF) by LLLT by the comprehensive analysis of gene expression. HGF was seeded, cultured for 24 h, and then irradiated with a Nd: YAG laser at 0.5 W for 30 s. After that, gene differential expression analysis and functional analysis were performed with DNA microarray at 1, 3, 6 and 12 h after the irradiation. The number of genes with up- and downregulated differentially expression genes (DEGs) compared to the nonirradiated group was large at 6 and 12 h after the irradiation. From the functional analysis results of DEGs, Biological Process (BP) based Gene Ontology (GO), BP ‘the defense response’ is considered to be an important process with DAVID. Additionally, the results of PPI analysis of DEGs involved in the defense response with STRING, we found that the upregulated DEGs such as CXCL8 and NFKB1, and the downregulated DEGs such as NFKBIA and STAT1 were correlated with multiple genes. We estimate that these genes are key genes on the defense response after LLLT.

Keywords: Low reactive Level Laser Therapy (LLLT); human gingival fibroblasts (HGF); microarray; differentially gene expression (DEGs); gene ontology; biological processes (BP); protein–protein interaction (PPI)

1. Introduction

Periodontal disease is a chronic multifactorial inflammatory disease caused by genetic, immune, environmental, microbial factors and lifestyles, with anaerobic bacteria in the oral cavity as the main causative organism. Periodontal disease is generally treated with nonsurgical therapy, that is performed with hand or power-driven instrumentation. In recent years, combination therapies with scaler and laser, or laser alone have attracted attention [1–3].

The laser therapy methods currently used for treatment of periodontal diseases can be broadly divided into two types: High reactive Level Laser Therapy (HLLT) and Low reactive Level Laser Therapy (LLLT).

HLLT is an application of laser intensity that produces an irreversible reaction (photobiological destruction reaction) beyond the cell survival region and is used for tissue incision and transpiration [4–6].

On the other hand, LLLT is a treatment that applies laser intensity to generate a reversible reaction (photobiologically active reaction) within the cell survival threshold. LLLT are expected to have anti-inflammatory effects [7–12]; pain relief [13], improvement/promotion of blood flow [14], activation of cells in tissues, wound healing by proliferation [15] and tissue regeneration without causing tissue degeneration with low-power...
laser irradiation conditions [16–18]. In recent years, the promotion of wound healing with LLLT has been one of the highlights.

Wound healing is thought to progress in the process of hemorrhagic coagulation phase, inflammatory phase, proliferative phase, reconstruction phase after injury by external stimulus. During the hemorrhagic coagulation phase, blood is coagulated by platelets, which is one of the coagulation factors, and growth factors such as platelet-derived growth factor (PDGF) and cytokines are released from the platelets. During the inflammatory phase, factors such as Nuclear Factor-κB (NF-κB) cause infiltration of inflammatory cells such as neutrophils and macrophages. Then, the release of growth factors and cytokines such as transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) is observed [19]. During the proliferative phase, it promotes the migration and proliferation of fibroblasts and keratinocytes [20]. Extracellular matrix is synthesized from fibroblasts and serves as a scaffold for cell migration and adhesion. During maturity, scar tissue formation occurs.

In the study of LLLT, TGF-β1 is closely involved in cell differentiation, migration, and adhesion by LLLT. In addition, it is thought to be involved in a wide range of areas such as ontogeny, tissue reconstruction, wound healing, inflammation/immunity, and cancer infiltration/metastasis. It has also been reported that the expression of NF-κB is increased [21].

Previous studies reported that the effects of low-reaction level laser irradiation on periodontium-derived cultured cells have been mainly on cell proliferation and cell transport ability related to wound healing. However, the elucidation of the mechanism at the molecular level leading to the promotion of wound healing by laser irradiation has been insufficient. It is considered that there may be a series of processes related to various wound healing by LLLT by the approach from biological processes (BP). There are few studies on mechanism analysis at the gene level using microarrays by LLLT for HGF, and only a limited number of studies have analyzed gene expression over time [22,23]. In order to clarify the effect of laser irradiation by LLLT, it is important to analyze and consider changes in gene expression and changes in BP of differentially expression genes (DEGs) over time in order to understand the mechanism at the molecular level.

In this study, HGF was irradiated with LLLT, and gene expression fluctuations at 1, 3, 6, and 12 h after irradiation were analyzed using a DNA microarray. In addition, we focused on the defense response, which showed remarkable changes in gene expression over time in relation to wound healing obtained from the results of vast amounts of analytical data, and to investigate for the mechanism from the expression change genes and BP due to the photobiological effects of laser. The aim of this study was to elucidate the changes of gene expression on the wound healing, especially defense response, over time after irradiation.

2. Materials and Methods

2.1. Cell Culture

Human immortalized gingival fibroblasts (HGF; Applied Biological Material, Richmond, BC, Canada) were used, and 10% Fetal Bovine Serum (Moregate, Bulimba, Australia), 50 U/mL Penicillin G, 50 μg/mL Amphotericin. The study was carried out by culturing in D-MEM/F-12 medium (Life Technologies Corporation, Grand Island, NY, USA) under 37 °C, and 5% CO₂ conditions. The HGF at the time of irradiation was in the logarithmic growth phase.

2.2. Dental Laser Device and Laser Irradiation Stent

A dental Nd: YAG laser: impulse dental laser (Incisive Japan Co., Ltd., Tokyo, Japan) was used as a dental laser device, and an ultrafine fiber with a diameter of 320 nm was used for the laser light guide tip. Stents (Gikousha, Kanagawa, Japan) were prepared to uniformly irradiate cells with laser, attached to a handpiece, and used for research. Irradiation conditions were found to be significantly different in cell proliferation curvature in previous studies, irradiation output conditions 0.5 W (100 mJ, 5 pps), irradiation time
30 s, irradiation distance from the tip of the fiber guide to each well plate. Laser irradiation was performed with a distance of 20 mm to the bottom [24].

2.3. Microarray Analysis

Total RNA was extracted from HGF with RNase® Plus Micro Kit (QIAGEN, Valencia, CA, USA) before laser irradiation 1, 3, 6 and 12 h after irradiation on a 96-well plate. cDNA was synthesized from total RNA using the SuperScript® VILO® cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). After synthesis, the cDNA was fragmented and biotin labeled. Biotin-labeled cDNA was added to the GeneChipTM Human Gene 2.0 ST Array (Thermo Fisher Scientific Inc., Waltham, MA, USA) and hybridized with a probe (GeneChipTM Hybridization, Wash, and Stain Kit; Thermo Fisher Scientific Inc., Waltham, MA, USA). Phycoerythrin staining was performed, and the fluorescence signal was measured with a GeneChip scanner (Scanner 3000 7 G; Thermo Fisher Scientific Inc., Waltham, MA, USA). After normalization, Expression Gene was analyzed by SST-RMA algorithm.

2.4. Data Analysis of Differentially Expressed Genes (DEGs)

DEGs were extracted with Affymetrix® Expression ConsoleTM (Thermo Fisher Scientific Inc. Waltham, MA, USA). The cutoff values were fold change (FC) ≥ |1.5| and \( p \)-value < 0.05. The nonirradiated group (control), the irradiated group (test) were defined as upregulated DEGs with significantly increased expression, and downregulated DEGs with significantly decreased expression with respect to the control group.

2.5. Functional Analysis of Differentially Expressed Genes

A functional analysis of DEGs was performed based on Gene Ontology (GO) with the database for annotation, visualization and integrated discovery (DAVID). Enrichment analysis on the BP was performed on the DEGs at 1, 3, 6, and 12 h after irradiation. The cutoff value was a modified Fisher exact \( p \)-value < 0.1, total count ≤ 2. The DEGs contained in the upregulated and the downregulated regions at each irradiation time were analyzed.

2.6. Protein–Protein Interaction (PPI) of Up- or Downregulated DEGs

We focused on the defense response which is an important response on the wound healing process. PPI analysis of up- or downregulated DEGs, which were involved in defense response and continuously observed as up- or downregulated at 6 and 12 h, and related expression fluctuations were observed at all times after irradiation and Search Tool for the Retrieval of Interacting Genes (STRING) was performed. Furthermore, from the analysis of PPI, variation in the expression of genes correlated with other DEGs over time was analyzed.

3. Results

3.1. Extraction of DEGs

In order to compare the gene expression after laser irradiation on time course, the DEGs were extracted. DEGs were extracted under the condition that the cutoff value was FC ≥ |1.5| and \( p \)-value < 0.05. Control and test were defined as upregulated DEGs with significantly increased expression and downregulated DEGs with significantly decreased expression with respect to the control group. At 1 h after the irradiation, 83 upregulated and 50 downregulated genes were extracted (Supplementary Tables S1 and S2). At 3 h after, 46 upregulated genes and 32 downregulated genes (Supplementary Tables S3 and S4), at 6 h after, 362 upregulated and 549 downregulated genes (Supplementary Tables S5 and S6) and at 12 h after, 253 upregulated genes and 413 downregulated were extracted (Supplementary Tables S7 and S8).

The number of DEGs was large 6 and 12 h after the irradiation. At 6 h, the number of DEGs was the highest in both the upregulated gene and downregulated gene groups.
3.2. Functional Analysis on GO

From the results of functional analysis with DAVID, the number of BP related with each DEG after the irradiation was 13 BP on upregulated and 35 BP on downregulated DEGs at 1 h after, 6 BP on upregulated and 68 BP on downregulated DEGs at 3 h after, 212 BP on upregulated and 288 BP on downregulated DEGs at 6 h after, and 84 BP on upregulated and 425 BP on downregulated DEGs at 12 h after. The number of BP was small at 1 and 3 h, and the number of BP was large at 6 and 12 h. Tables 1–8 show the top BPs for each irradiation time.

BPs on upregulated DEGs at 1 h after irradiation are, for example, GO:0050867 ~ positive regulation of cell activation, GO:0050865 ~ regulation of cell activation (Table 1), BPs on downregulated DEGs are, for example, GO:0032774 ~ RNA biosynthetic process, GO:0007267 ~ cell–cell signaling (Table 2).

At 3 h after the irradiation, BPs on upregulated DEGs are, for example, GO:0055085 ~ transmembrane transport, GO:0006820 ~ anion transport (Table 3), BPs on down-regulated DEGs are, for example, GO:0032774 ~ RNA biosynthetic process, GO:0016070 ~ RNA metabolic process (Table 4).

At 6 h, BPs on upregulated DEGs are, for example, GO:0008283 ~ cell proliferation, GO:0007155 ~ cell adhesion GO:0022610 ~ biological adhesion, GO:0042127 ~ regulation of cell proliferation, GO:0006952 ~ defense response, GO:0060429 ~ epithelium development (Table 5), BP on downregulated DEGs are, for example, GO:0034645 ~ cellular macromolecule biosynthetic process, GO:019438 ~ aromatic compound biosynthetic process, GO:0006325 ~ chromatin organization, GO:0007049 ~ cell cycle (Table 6).

At 12 h, BPs on upregulated DEGs are, for example, GO:0006955 ~ immune response, GO:0006952 ~ defense response, GO:0009605 ~ response to external stimulus, GO:0048584 ~ positive regulation of response to stimulus (Table 7), BPs on downregulated DEGs are, for example, GO:0010468 BP such as ~ regulation of gene expression, GO:0007155 ~ cell adhesion, GO:0022610 ~ biological adhesion (Table 8).

Table 1. The functional analysis of the upregulated genes at 1 h after Low Reactive Level Laser (LLL) irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %   | p-value          |
|----------------------------------------------------------|-------|-----|------------------|
| GO:0061024~membrane organization                          | 6     | 6.67| 4.45 × 10⁻²      |
| GO:002696~positive regulation of leukocyte activation      | 4     | 4.44| 2.15 × 10⁻²      |
| GO:0050867~positive regulation of cell activation          | 4     | 4.44| 2.31 × 10⁻²      |
| GO:0002694~regulation of leukocyte activation              | 4     | 4.44| 6.00 × 10⁻²      |
| GO:0072657~protein localization to membrane               | 4     | 4.44| 6.66 × 10⁻²      |
| GO:0050865~regulation of cell activation                   | 4     | 4.44| 7.06 × 10⁻²      |
| GO:0008037~cell recognition                                | 3     | 3.33| 3.60 × 10⁻²      |
| GO:0072659~protein localization to plasma membrane         | 3     | 3.33| 5.98 × 10⁻²      |
| GO:1990778~protein localization to cell periphery          | 3     | 3.33| 6.97 × 10⁻²      |
| GO:0007009~plasma membrane organization                    | 3     | 3.33| 9.88 × 10⁻²      |
| GO:0006910~phagocytosis, recognition                       | 2     | 2.22| 6.52 × 10⁻²      |
| GO:2000243~positive regulation of reproductive process     | 2     | 2.22| 8.66 × 10⁻²      |
| GO:0006911~phagocytosis, engulfment                        | 2     | 2.22| 9.18 × 10⁻²      |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.
Table 2. The functional analysis of the downregulated genes at 1 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %    | p-Value       |
|-----------------------------------------------------------|-------|------|---------------|
| GO:0032774--RNA biosynthetic process                       | 10    | 18.52| $1.27 \times 10^{-2}$ |
| GO:0034654--nucleobase-containing compound biosynthetic process | 10    | 18.52| $2.59 \times 10^{-2}$ |
| GO:0018130--heterocyte biosynthetic process                | 10    | 18.52| $2.79 \times 10^{-2}$ |
| GO:0019438--aromatic compound biosynthetic process         | 10    | 18.52| $2.85 \times 10^{-2}$ |
| GO:0016070--RNA metabolic process                          | 10    | 18.52| $3.87 \times 10^{-2}$ |
| GO:0034645--cellular macromolecule biosynthetic process    | 10    | 18.52| $6.07 \times 10^{-2}$ |
| GO:0010467--gene expression                                | 10    | 18.52| $8.40 \times 10^{-2}$ |
| GO:007267--cell–cell signaling                             | 5     | 9.26 | $7.48 \times 10^{-2}$ |
| GO:0006614--SRP-dependent cotranslational protein targeting to membrane | 3     | 5.56 | $4.74 \times 10^{-3}$ |
| GO:0006613--cotranslational protein targeting to membrane  | 3     | 5.56 | $5.44 \times 10^{-3}$ |
| GO:0045047--protein targeting to ER                       | 3     | 5.56 | $5.54 \times 10^{-3}$ |
| GO:0000184--nuclear-transcribed mRNA catabolic process, nonsense-mediated decay | 3     | 5.56 | $7.67 \times 10^{-3}$ |
| GO:0072599--establishment of protein localization to endoplasmic reticulum | 3     | 5.56 | $7.96 \times 10^{-3}$ |
| GO:000184--nuclear-transcribed mRNA catabolic process      | 3     | 5.56 | $8.03 \times 10^{-3}$ |
| GO:0070972--protein localization to endoplasmic reticulum | 3     | 5.56 | $8.28 \times 10^{-3}$ |
| GO:0019083--viral transcription                            | 3     | 5.56 | $1.60 \times 10^{-2}$ |
| GO:0006612--protein targeting to membrane                  | 3     | 5.56 | $1.74 \times 10^{-2}$ |
| GO:0010467--gene expression                                | 3     | 5.56 | $1.74 \times 10^{-2}$ |
| GO:000956--nuclear-transcribed mRNA catabolic process      | 3     | 5.56 | $1.79 \times 10^{-2}$ |
| GO:004033--multigorganism metabolic process                | 3     | 5.56 | $2.05 \times 10^{-2}$ |
| GO:0006402--mRNA catabolic process                         | 3     | 5.56 | $2.33 \times 10^{-2}$ |
| GO:0006401--RNA catabolic process                          | 3     | 5.56 | $2.91 \times 10^{-2}$ |
| GO:0016072--rRNA metabolic process                         | 3     | 5.56 | $3.36 \times 10^{-2}$ |
| GO:0042254--ribosome biogenesis                            | 3     | 5.56 | $3.52 \times 10^{-2}$ |
| GO:0090150--establishment of protein localization to membrane | 3     | 5.56 | $5.00 \times 10^{-2}$ |
| GO:0034655--nucleobase-containing compound catabolic process | 3     | 5.56 | $5.91 \times 10^{-2}$ |
| GO:0034470--ncRNA processing                               | 3     | 5.56 | $6.25 \times 10^{-2}$ |
| GO:0047000--heterocycle catabolic process                  | 3     | 5.56 | $7.17 \times 10^{-2}$ |
| GO:004270--cellular nitrogen compound catabolic process    | 3     | 5.56 | $7.17 \times 10^{-2}$ |
| GO:0019439--aromatic compound catabolic process            | 3     | 5.56 | $7.38 \times 10^{-2}$ |
| GO:1901361--organic cyclic compound catabolic process      | 3     | 5.56 | $7.54 \times 10^{-2}$ |
| GO:0019058--viral life cycle                               | 3     | 5.56 | $8.23 \times 10^{-2}$ |
| GO:0022613--ribonucleoprotein complex biogenesis           | 3     | 5.56 | $8.55 \times 10^{-2}$ |
| GO:0072657--protein localization to membrane               | 3     | 5.56 | $9.67 \times 10^{-2}$ |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.

Table 3. The functional analysis of the upregulated genes at 3 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %    | p-Value       |
|-----------------------------------------------------------|-------|------|---------------|
| GO:0055085--transmembrane transport                        | 5     | 0.30 | $5.87 \times 10^{-2}$ |
| GO:1901615--organic hydroxy compound metabolic process     | 3     | 0.18 | $7.85 \times 10^{-2}$ |
| GO:000620--anion transport                                | 3     | 0.18 | $9.85 \times 10^{-2}$ |
| GO:005180--vitamin transport                              | 2     | 0.12 | $3.81 \times 10^{-2}$ |
| GO:006767--water-soluble vitamin metabolic process         | 2     | 0.12 | $9.58 \times 10^{-2}$ |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.
Table 4. The functional analysis of the downregulated genes at 3 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP)                            | Count | %      | p-Value       |
|-------------------------------------------------------------------------------------|-------|--------|---------------|
| GO:0032774--RNA biosynthetic process                                                | 6     | 15.79  | 3.8 × 10⁻²    |
| GO:0034654--nucleobase-containing compound biosynthetic process                    | 6     | 15.79  | 5.85 × 10⁻²   |
| GO:0018130--heterocycle biosynthetic process                                       | 6     | 15.79  | 6.14 × 10⁻²   |
| GO:0019438--aromatic compound biosynthetic process                                 | 6     | 15.79  | 6.21 × 10⁻²   |
| GO:0016070--RNA metabolic process                                                  | 6     | 15.79  | 7.52 × 10⁻²   |
| GO:0044085--cellular component biogenesis                                          | 5     | 13.16  | 5.62 × 10⁻²   |
| GO:0006614--SRP-dependent cotranslational protein targeting to membrane           | 4     | 10.53  | 1.52 × 10⁻⁵   |
| GO:0006613--cotranslational protein targeting to membrane                           | 4     | 10.53  | 1.88 × 10⁻⁵   |
| GO:0045047--protein targeting to ER                                                | 4     | 10.53  | 1.94 × 10⁻⁵   |
| GO:0072599--establishment of protein localization to endoplasmic reticulum        | 4     | 10.53  | 2.17 × 10⁻⁵   |
| GO:000184--nuclear-transcribed mRNA catabolic process, nonsense-mediated decay     | 4     | 10.53  | 3.20 × 10⁻⁵   |
| GO:0070972--protein localization to endoplasmic reticulum                         | 4     | 10.53  | 3.60 × 10⁻⁵   |
| GO:0019083--viral transcription                                                     | 4     | 10.53  | 1.01 × 10⁻⁴   |
| GO:0006413--protein targeting to membrane                                          | 4     | 10.53  | 1.17 × 10⁻⁴   |
| GO:0019080--viral gene expression                                                  | 4     | 10.53  | 1.20 × 10⁻⁴   |
| GO:000956--nucleobase-containing compound biosynthetic process                    | 4     | 10.53  | 1.48 × 10⁻⁴   |
| GO:0044033--multiorganism metabolic process                                       | 4     | 10.53  | 1.66 × 10⁻⁴   |
| GO:0006402--RNA metabolic process                                                 | 4     | 10.53  | 1.83 × 10⁻⁴   |
| GO:0006401--RNA catabolic process                                                 | 4     | 10.53  | 2.60 × 10⁻⁴   |
| GO:0006412--translation                                                           | 4     | 10.53  | 3.26 × 10⁻⁴   |
| GO:0016072--RNA catabolic process                                                 | 4     | 10.53  | 3.51 × 10⁻⁴   |
| GO:0042254--ribosome biogenesis                                                    | 4     | 10.53  | 6.20 × 10⁻⁴   |
| GO:0090150--establishment of protein localization to membrane                     | 4     | 10.53  | 8.15 × 10⁻⁴   |
| GO:0034655--nucleobase-containing compound biosynthetic process                  | 4     | 10.53  | 8.94 × 10⁻⁴   |
| GO:0034470--ncRNA processing                                                       | 4     | 10.53  | 1.12 × 10⁻³   |
| GO:0046700--heterocycle catalytic process                                         | 4     | 10.53  | 1.12 × 10⁻³   |
| GO:0044270--cellular nitrogen compound catalytic process                           | 4     | 10.53  | 1.18 × 10⁻³   |
| GO:0019439--aromatic compound catalytic process                                   | 4     | 10.53  | 1.22 × 10⁻³   |
| GO:1901361--organic cyclic compound catalytic process                             | 4     | 10.53  | 1.41 × 10⁻³   |
| GO:0019058--viral life cycle                                                      | 4     | 10.53  | 1.51 × 10⁻³   |
| GO:0022613--ribonucleoprotein complex biogenesis                                  | 4     | 10.53  | 1.76 × 10⁻³   |
| GO:0072657--protein localization to membrane                                      | 4     | 10.53  | 1.85 × 10⁻³   |
| GO:0034660--ncRNA metabolic process                                               | 4     | 10.53  | 2.87 × 10⁻³   |
| GO:0006412--translation                                                           | 4     | 10.53  | 4.01 × 10⁻³   |
| GO:0072594--establishment of protein localization to organelle                    | 4     | 10.53  | 4.47 × 10⁻³   |
| GO:0043043--peptide biosynthetic process                                          | 4     | 10.53  | 4.49 × 10⁻³   |
| GO:0016071--mRNA metabolic process                                                | 4     | 10.53  | 4.58 × 10⁻³   |
| GO:1902582--single-organism intracellular transport                               | 4     | 10.53  | 5.04 × 10⁻³   |
| GO:0006605--protein targeting                                                     | 4     | 10.53  | 5.16 × 10⁻³   |
| GO:0043604--amide biosynthetic process                                            | 4     | 10.53  | 5.92 × 10⁻³   |
| GO:0006518--peptide metabolic process                                            | 4     | 10.53  | 7.94 × 10⁻³   |
| GO:0044802--single-organism membrane organization                                | 4     | 10.53  | 9.30 × 10⁻³   |
| GO:0033365--protein localization to organelle                                      | 4     | 10.53  | 1.04 × 10⁻²   |
| GO:0006396--RNA processing                                                        | 4     | 10.53  | 1.07 × 10⁻²   |
| GO:0016032--viral process                                                          | 4     | 10.53  | 1.26 × 10⁻²   |
| GO:0044764--multiorganism cellular process                                        | 4     | 10.53  | 1.28 × 10⁻²   |
| GO:004265--cellular macromolecule catalytic process                                | 4     | 10.53  | 1.29 × 10⁻²   |
| GO:004303--cellular amide metabolic process                                       | 4     | 10.53  | 1.36 × 10⁻²   |
| GO:004403--symbiosis, encompassing mutualism through parasitism                   | 4     | 10.53  | 1.37 × 10⁻²   |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.
Table 5. The functional analysis of the upregulated genes at 6 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %  | p-Value  |
|----------------------------------------------------------|-------|----|----------|
| GO:00034645–cellular macromolecule biosynthetic process | 72    | 19.20 | 4.50 x 10^-3 |
| GO:0010467–gene expression                              | 67    | 17.87 | 9.63 x 10^-2 |
| GO:0016070–RNA metabolic process                        | 65    | 17.33 | 1.49 x 10^-2 |
| GO:0010468–regulation of gene expression                | 64    | 17.07 | 4.90 x 10^-3 |
| GO:0051171–regulation of nitrogen compound metabolic process | 64    | 17.07 | 6.75 x 10^-3 |
| GO:0019219–regulation of nucleobase-containing compound metabolic process | 61    | 16.27 | 5.24 x 10^-3 |
| GO:0010556–regulation of macromolecule biosynthetic process | 60    | 16.00 | 8.42 x 10^-3 |
| GO:0034654–nucleobase-containing compound biosynthetic process | 59    | 15.73 | 7.09 x 10^-3 |
| GO:0018130–heterocycle biosynthetic process             | 59    | 15.73 | 4.99 x 10^-2 |
| GO:0019438–aromatic compound biosynthetic process       | 59    | 15.73 | 5.42 x 10^-2 |
| GO:0015125–regulation of RNA metabolic process          | 58    | 15.47 | 4.04 x 10^-3 |
| GO:0097659–nucleic acid-templated transcription          | 57    | 15.20 | 5.01 x 10^-3 |
| GO:0032774–RNA biosynthetic process                     | 57    | 15.20 | 1.01 x 10^-2 |
| GO:0006355–regulation of transcription, DNA-templated   | 54    | 14.40 | 8.34 x 10^-3 |
| GO:1903506–regulation of nucleic acid-templated process | 54    | 14.40 | 9.41 x 10^-3 |
| GO:2001141–regulation of RNA biosynthetic process       | 54    | 14.40 | 1.03 x 10^-3 |
| GO:0006351–transcription, DNA-templated                 | 53    | 14.13 | 1.32 x 10^-3 |
| GO:0010646–regulation of cellular macromolecule biosynthetic process | 47    | 12.53 | 7.96 x 10^-3 |
| GO:0023051–regulation of signaling                      | 47    | 12.53 | 1.07 x 10^-2 |
| GO:0009893–positive regulation of metabolic process     | 46    | 12.27 | 1.53 x 10^-2 |
| GO:0010604–positive regulation of macromolecule metabolic process | 43    | 11.47 | 2.02 x 10^-2 |
| GO:0009966–regulation of signal transduction            | 42    | 11.20 | 1.56 x 10^-2 |
| GO:009892–negative regulation of metabolic process      | 40    | 10.67 | 1.19 x 10^-2 |
| GO:0007166–cell surface receptor signaling pathway      | 40    | 10.67 | 2.78 x 10^-2 |
| GO:0031325–positive regulation of cellular metabolic process | 40    | 10.67 | 6.31 x 10^-3 |
| GO:0010608–negative regulation of macromolecule metabolic process | 39    | 10.40 | 5.48 x 10^-3 |
| GO:0031324–negative regulation of cellular metabolic process | 37    | 9.87  | 1.76 x 10^-2 |
| GO:0006366–transcription from RNA polymerase II promoter | 35    | 9.33  | 1.02 x 10^-2 |
| GO:0006357–regulation of transcription from RNA polymerase II promoter | 35    | 9.33  | 1.17 x 10^-2 |
| GO:0010628–positive regulation of gene expression       | 33    | 8.80  | 1.19 x 10^-2 |
| GO:0071310–cellular response to organic substance       | 32    | 8.53  | 6.76 x 10^-3 |
| GO:0051173–positive regulation of nitrogen compound metabolic process | 31    | 8.27  | 8.31 x 10^-3 |
| GO:0048584–positive regulation of response to stimulus  | 31    | 8.27  | 6.42 x 10^-2 |
| GO:0008981–positive regulation of biosynthetic process  | 30    | 8.00  | 1.39 x 10^-2 |
| GO:0031328–positive regulation of cellular biosynthetic process | 29    | 7.73  | 1.93 x 10^-2 |
| GO:0006468–protein phosphorylation                      | 29    | 7.73  | 4.20 x 10^-2 |
| GO:0008283–cell proliferation                           | 29    | 7.73  | 4.43 x 10^-2 |
| GO:0008219–cell death                                  | 29    | 7.73  | 7.43 x 10^-2 |
| GO:0010557–positive regulation of macromolecule biosynthetic process | 28    | 7.47  | 1.29 x 10^-2 |
| GO:0045935–positive regulation of nucleobase-containing compound metabolic process | 28    | 7.47  | 2.02 x 10^-2 |
| GO:0012501–programmed cell death                       | 28    | 7.47  | 6.56 x 10^-2 |
| GO:0007155–cell adhesion                               | 27    | 7.20  | 4.37 x 10^-2 |
| GO:0022610–biological adhesion                        | 27    | 7.20  | 4.53 x 10^-2 |
| GO:200026–regulation of multicellular organismal development | 27    | 7.20  | 4.97 x 10^-2 |
| GO:0006915–apoptotic process                           | 27    | 7.20  | 5.76 x 10^-2 |
| GO:0051254–positive regulation of RNA metabolic process | 26    | 6.93  | 9.98 x 10^-3 |
| GO:1902531–regulation of intracellular signal transduction | 26    | 6.93  | 7.47 x 10^-2 |
| GO:0048585–negative regulation of response to stimulus | 25    | 6.67  | 1.26 x 10^-2 |
| GO:0009890–negative regulation of biosynthetic process | 25    | 6.67  | 3.44 x 10^-2 |
| GO:0042127–regulation of cell proliferation            | 25    | 6.67  | 4.68 x 10^-2 |
| GO:0048646–anatomical structure formation involved in morphogenesis | 24    | 6.40  | 3.17 x 10^-3 |
| GO:0010558–negative regulation of macromolecule biosynthetic process | 24    | 6.40  | 3.25 x 10^-2 |
### Table 5. Cont.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %   | p-Value  |
|----------------------------------------------------------|-------|-----|----------|
| GO:0031327--negative regulation of cellular biosynthetic process | 24    | 6.40 | 4.89 × 10⁻² |
| GO:0010629--negative regulation of gene expression | 24    | 6.40 | 5.04 × 10⁻² |
| GO:0045893--positive regulation of transcription, DNA-templated | 23    | 6.13 | 3.43 × 10⁻² |
| GO:1903508--positive regulation of nucleic acid-templated transcription | 23    | 6.13 | 3.43 × 10⁻² |
| GO:1902680--positive regulation of RNA biosynthetic process | 23    | 6.13 | 3.94 × 10⁻² |
| GO:0002682--regulation of immune system process | 23    | 6.13 | 4.19 × 10⁻² |
| GO:0006952--defense response | 23    | 6.13 | 9.61 × 10⁻² |
| GO:0010648--negative regulation of cell communication | 22    | 5.87 | 1.69 × 10⁻² |
| GO:0023057--negative regulation of signaling | 22    | 5.87 | 1.74 × 10⁻² |
| GO:0080134--regulation of response to stress | 22    | 5.87 | 3.76 × 10⁻² |
| GO:2000113--negative regulation of cellular macromolecule biosynthetic process | 22    | 5.87 | 4.90 × 10⁻² |
| GO:0051240--positive regulation of multicellular organismal process | 22    | 5.87 | 9.32 × 10⁻² |
| GO:0051094--positive regulation of developmental process | 21    | 5.60 | 1.41 × 10⁻² |

**Count:** genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.

### Table 6. The functional analysis of the downregulated genes at 6 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %   | p-Value  |
|----------------------------------------------------------|-------|-----|----------|
| GO:0034645--cellular macromolecule biosynthetic process | 158   | 0.26 | 1.20 × 10⁻⁶ |
| GO:0010467--gene expression | 147   | 0.24 | 2.91 × 10⁻³ |
| GO:0019438--aromatic compound biosynthetic process | 141   | 0.23 | 4.17 × 10⁻⁶ |
| GO:0018130--heterocycle biosynthetic process | 138   | 0.23 | 1.59 × 10⁻⁵ |
| GO:0034654--nucleobase-containing compound biosynthetic process | 135   | 0.22 | 3.51 × 10⁻⁵ |
| GO:0016070--RNA metabolic process | 132   | 0.22 | 2.23 × 10⁻³ |
| GO:0051171--regulation of nitrogen compound metabolic process | 128   | 0.21 | 1.10 × 10⁻³ |
| GO:0010648--regulation of gene expression | 126   | 0.21 | 1.36 × 10⁻³ |
| GO:0032774--RNA biosynthetic process | 121   | 0.20 | 1.21 × 10⁻⁴ |
| GO:00200112--regulation of cellular macromolecule biosynthetic process | 121   | 0.20 | 3.10 × 10⁻⁴ |
| GO:0010556--regulation of macromolecule biosynthetic process | 121   | 0.20 | 9.90 × 10⁻⁴ |
| GO:0019219--regulation of nucleobase-containing compound metabolic process | 118   | 0.20 | 2.92 × 10⁻³ |
| GO:0097659--nucleic acid-templated transcription | 113   | 0.19 | 8.72 × 10⁻⁴ |
| GO:0006351--transcription, DNA-templated | 109   | 0.18 | 8.23 × 10⁻⁴ |
| GO:0006355--regulation of transcription, DNA-templated | 107   | 0.18 | 1.77 × 10⁻³ |
| GO:1903506--regulation of nucleic acid-templated transcription | 107   | 0.18 | 2.18 × 10⁻³ |
| GO:2001141--regulation of RNA biosynthetic process | 107   | 0.18 | 2.60 × 10⁻³ |
| GO:0051252--regulation of RNA metabolic process | 107   | 0.18 | 7.16 × 10⁻³ |
| GO:0009892--negative regulation of metabolic process | 90    | 0.15 | 9.30 × 10⁻⁶ |
| GO:0010605--negative regulation of macromolecule metabolic process | 86    | 0.14 | 4.06 × 10⁻⁶ |
| GO:0031324--negative regulation of cellular metabolic process | 85    | 0.14 | 1.02 × 10⁻⁵ |
| GO:0044085--cellular component biogenesis | 85    | 0.14 | 1.03 × 10⁻² |
| GO:0043933--macromolecular complex subunit organization | 83    | 0.14 | 1.72 × 10⁻⁴ |
| GO:0009893--positive regulation of metabolic process | 83    | 0.14 | 5.25 × 10⁻² |
| GO:0010604--positive regulation of macromolecule metabolic process | 81    | 0.13 | 2.37 × 10⁻² |
| GO:0022607--cellular component assembly | 79    | 0.13 | 6.13 × 10⁻³ |
| GO:0051276--chromosome organization | 67    | 0.11 | 7.48 E-12 |
| GO:0033554--cellular response to stress | 66    | 0.11 | 6.62 × 10⁻⁵ |
| GO:0032268--regulation of cellular protein metabolic process | 65    | 0.11 | 6.56 × 10⁻² |
| GO:0031327--negative regulation of cellular biosynthetic process | 63    | 0.10 | 1.62 × 10⁻⁶ |
| GO:0009890--negative regulation of biosynthetic process | 63    | 0.10 | 2.75 × 10⁻⁶ |
| GO:0051172--negative regulation of nitrogen compound metabolic process | 63    | 0.10 | 2.91 × 10⁻⁶ |
| GO:0010558--negative regulation of macromolecule biosynthetic process | 61    | 0.10 | 1.79 × 10⁻⁶ |
| GO:0010629--negative regulation of gene expression | 61    | 0.10 | 7.71 × 10⁻⁶ |
| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | % | p-Value |
|----------------------------------------------------------|-------|---|---------|
| GO:0001113--negative regulation of cellular macromolecule biosynthetic process | 60 | 0.10 | $4.10 \times 10^{-7}$ |
| GO:0071822--protein complex subunit organization | 60 | 0.10 | $4.18 \times 10^{-4}$ |
| GO:0045934--negative regulation of nucleobase-containing compound metabolic process | 59 | 0.10 | $2.74 \times 10^{-6}$ |
| GO:0065003--macromolecular complex assembly | 59 | 0.10 | $1.21 \times 10^{-3}$ |
| GO:0006461--protein complex assembly | 56 | 0.09 | $9.86 \times 10^{-5}$ |
| GO:0070271--protein complex biogenesis | 56 | 0.09 | $1.00 \times 10^{-4}$ |
| GO:0007049--cell cycle | 56 | 0.09 | $3.16 \times 10^{-3}$ |
| GO:006325--chromatin organization | 54 | 0.09 | $4.01 \times 10^{-13}$ |
| GO:1903507--negative regulation of nucleic acid-templated transcription | 53 | 0.09 | $2.84 \times 10^{-6}$ |
| GO:1902679--negative regulation of RNA biosynthetic process | 53 | 0.09 | $4.28 \times 10^{-6}$ |
| GO:001253--negative regulation of RNA metabolic process | 53 | 0.09 | $1.24 \times 10^{-5}$ |
| GO:0045892--negative regulation of transcription, DNA-templated | 52 | 0.09 | $1.93 \times 10^{-6}$ |
| GO:0034622--cellular macromolecular complex assembly | 47 | 0.08 | $4.38 \times 10^{-6}$ |
| GO:0031399--regulation of protein modification process | 47 | 0.08 | $9.08 \times 10^{-2}$ |
| GO:0044248--cellular catabolic process | 46 | 0.08 | $8.76 \times 10^{-2}$ |
| GO:0022402--cell cycle process | 44 | 0.07 | $1.65 \times 10^{-2}$ |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.

Table 6. Cont.

Table 7. The functional analysis of the upregulated genes at 12 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | % | p-Value |
|----------------------------------------------------------|-------|---|---------|
| GO:0007166--cell surface receptor signaling pathway | 28 | 10.73 | $2.37 \times 10^{-2}$ |
| GO:0006955--immune response | 24 | 9.20 | $3.39 \times 10^{-4}$ |
| GO:0006952--defense response | 23 | 8.81 | $6.37 \times 10^{-4}$ |
| GO:0009605--response to external stimulus | 23 | 8.81 | $2.57 \times 10^{-2}$ |
| GO:0048584--positive regulation of response to stimulus | 22 | 8.43 | $3.73 \times 10^{-2}$ |
| GO:0003008--system process | 21 | 8.05 | $5.96 \times 10^{-2}$ |
| GO:0002682--regulation of immune system process | 19 | 7.28 | $6.30 \times 10^{-3}$ |
| GO:0050776--regulation of immune response | 18 | 6.90 | $1.70 \times 10^{-4}$ |
| GO:0016192--vesicle-mediated transport | 17 | 6.51 | $5.14 \times 10^{-2}$ |
| GO:0045087--innate immune response | 16 | 6.13 | $6.31 \times 10^{-4}$ |
| GO:0007186--G-protein coupled receptor signaling pathway | 16 | 6.13 | $2.69 \times 10^{-2}$ |
| GO:0051707--response to other organism | 15 | 5.75 | $1.72 \times 10^{-3}$ |
| GO:0043207--response to external biotic stimulus | 15 | 5.75 | $1.72 \times 10^{-3}$ |
| GO:0009607--response to biotic stimulus | 15 | 5.75 | $2.76 \times 10^{-3}$ |
| GO:0050877--neurological system process | 15 | 5.75 | $6.02 \times 10^{-2}$ |
| GO:0006897--endocytosis | 14 | 5.36 | $6.77 \times 10^{-4}$ |
| GO:0002252--immune effector process | 14 | 5.36 | $1.83 \times 10^{-3}$ |
| GO:0001775--cell activation | 14 | 5.36 | $1.00 \times 10^{-2}$ |
| GO:0002684--positive regulation of immune system process | 14 | 5.36 | $1.51 \times 10^{-2}$ |
| GO:0009617--response to bacterium | 13 | 4.98 | $3.80 \times 10^{-4}$ |
| GO:0050778--positive regulation of immune response | 13 | 4.98 | $2.71 \times 10^{-3}$ |
| GO:0045321--leukocyte activation | 13 | 4.98 | $5.47 \times 10^{-3}$ |
| GO:005104--positive regulation of developmental process | 13 | 4.98 | $7.29 \times 10^{-2}$ |
| GO:0002768--immune response-regulating cell surface receptor signaling pathway | 12 | 4.60 | $9.96 \times 10^{-5}$ |
| GO:0002764--immune response-regulating signaling pathway | 12 | 4.60 | $1.12 \times 10^{-3}$ |
| GO:0046649--lymphocyte activation | 12 | 4.60 | $4.91 \times 10^{-3}$ |
| GO:0002449--lymphocyte mediated immunity | 11 | 4.21 | $2.83 \times 10^{-5}$ |
| GO:0002443--leukocyte mediated immunity | 11 | 4.21 | $1.79 \times 10^{-4}$ |
| GO:0002250--adaptive immune response | 11 | 4.21 | $6.62 \times 10^{-4}$ |
| GO:0098542--defense response to other organism | 11 | 4.21 | $2.23 \times 10^{-3}$ |
| GO:0042742--defense response to bacterium | 10 | 3.83 | $9.53 \times 10^{-5}$ |
| GO:0002460--adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 10 | 3.83 | $1.65 \times 10^{-4}$ |
Table 7. Cont.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %   | p-Value |
|----------------------------------------------------------|-------|-----|---------|
| GO:0002459--immune response-activating cell surface receptor signaling pathway | 10    | 3.83| $1.01 \times 10^{-3}$ |
| GO:0002756--immune response-activating signal transduction | 10    | 3.83| $7.63 \times 10^{-3}$ |
| GO:0002253--activation of immune response | 10    | 3.83| $1.40 \times 10^{-2}$ |
| GO:0016064--immunoglobulin mediated immune response | 9     | 3.45| $2.44 \times 10^{-5}$ |
| GO:0019724--B cell mediated immunity | 9     | 3.45| $2.66 \times 10^{-5}$ |
| GO:0042113--B cell activation | 9     | 3.45| $3.08 \times 10^{-4}$ |
| GO:0006959--humoral immune response | 9     | 3.45| $3.25 \times 10^{-4}$ |
| GO:0051251--positive regulation of lymphocyte activation | 9     | 3.45| $8.51 \times 10^{-4}$ |
| GO:0002696--positive regulation of leukocyte activation | 9     | 3.45| $1.42 \times 10^{-3}$ |
| GO:0050867--positive regulation of cell activation | 9     | 3.45| $1.70 \times 10^{-3}$ |
| GO:0051249--regulation of lymphocyte activation | 9     | 3.45| $7.68 \times 10^{-3}$ |
| GO:0002694--regulation of leukocyte activation | 9     | 3.45| $1.59 \times 10^{-2}$ |
| GO:0050865--regulation of cell activation | 9     | 3.45| $2.28 \times 10^{-2}$ |
| GO:0006958--complement activation, classical pathway | 8     | 3.07| $5.50 \times 10^{-6}$ |
| GO:0002455--humoral immune response mediated by circulating immunoglobulin | 8     | 3.07| $1.24 \times 10^{-5}$ |
| GO:0006956--complement activation | 8     | 3.07| $1.65 \times 10^{-5}$ |
| GO:0006959--humoral immune response | 8     | 3.07| $6.86 \times 10^{-5}$ |
| GO:0006909--phagocytosis | 8     | 3.07| $3.11 \times 10^{-3}$ |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.

Table 8. The functional analysis of the downregulated genes at 12 h after LLL irradiation.

| Gene Ontology (GO) ID and Terms on Biological Process (BP) | Count | %   | p-Value |
|----------------------------------------------------------|-------|-----|---------|
| GO:0034645--cellular macromolecule biosynthetic process | 102   | 23.83| $6.82 \times 10^{-2}$ |
| GO:0051171--regulation of nitrogen compound metabolic process | 94    | 21.96| $2.85 \times 10^{-2}$ |
| GO:0010556--regulation of macromolecule biosynthetic process | 90    | 21.03| $1.75 \times 10^{-2}$ |
| GO:0010468--regulation of gene expression | 90    | 21.03| $6.33 \times 10^{-2}$ |
| GO:0018130--heterocycle biosynthetic process | 90    | 21.03| $8.55 \times 10^{-2}$ |
| GO:0034654--nucleobase-containing compound biosynthetic process | 89    | 20.79| $8.55 \times 10^{-2}$ |
| GO:0019219--regulation of nucleobase-containing compound metabolic process | 88    | 20.56| $3.19 \times 10^{-2}$ |
| GO:2000112--regulation of cellular macromolecule biosynthetic process | 87    | 20.33| $2.26 \times 10^{-2}$ |
| GO:0097659--nucleic acid-templated transcription | 82    | 19.16| $2.93 \times 10^{-2}$ |
| GO:0032774--RNA biosynthetic process | 82    | 19.16| $6.02 \times 10^{-2}$ |
| GO:0051252--regulation of RNA metabolic process | 81    | 18.93| $3.76 \times 10^{-2}$ |
| GO:0006351--transcription, DNA-templated | 80    | 18.69| $2.03 \times 10^{-2}$ |
| GO:0035006--regulation of nucleic acid-templated transcription | 80    | 18.69| $2.30 \times 10^{-2}$ |
| GO:2001141--regulation of RNA biosynthetic process | 80    | 18.69| $2.58 \times 10^{-2}$ |
| GO:0006355--regulation of transcription, DNA-templated | 79    | 18.46| $2.76 \times 10^{-2}$ |
| GO:0023051--regulation of signaling | 77    | 17.99| $1.04 \times 10^{-3}$ |
| GO:0010646--regulation of cell communication | 76    | 17.76| $1.04 \times 10^{-3}$ |
| GO:0009966--regulation of signal transduction | 71    | 16.59| $6.57 \times 10^{-4}$ |
| GO:0065009--regulation of molecular function | 71    | 16.59| $2.42 \times 10^{-3}$ |
| GO:0035556--intracellular signal transduction | 69    | 16.12| $8.62 \times 10^{-4}$ |
| GO:0008993--positive regulation of metabolic process | 68    | 15.89| $3.21 \times 10^{-2}$ |
| GO:0010694--positive regulation of macromolecule metabolic process | 67    | 15.65| $1.20 \times 10^{-2}$ |
| GO:0006796--phosphate-containing compound metabolic process | 67    | 15.65| $5.25 \times 10^{-2}$ |
| GO:0006793--phosphorus metabolic process | 67    | 15.65| $5.42 \times 10^{-2}$ |
| GO:0031325--positive regulation of cellular metabolic process | 66    | 15.42| $1.53 \times 10^{-2}$ |
| GO:0044085--cellular component biogenesis | 65    | 15.19| $3.58 \times 10^{-2}$ |
| GO:0050790--regulation of catalytic activity | 63    | 14.72| $7.74 \times 10^{-4}$ |
| GO:0022507--cellular component assembly | 63    | 14.72| $9.06 \times 10^{-3}$ |
| GO:0071666--cell surface receptor signaling pathway | 59    | 13.79| $5.38 \times 10^{-2}$ |
| GO:0051218--regulation of cellular component organization | 57    | 13.32| $9.42 \times 10^{-3}$ |
Table 8. Cont.

| Gene Ontology (GO) ID and Terms on Biological Process (BP)                          | Count | %      | p-value  |
|----------------------------------------------------------------------------------|-------|--------|----------|
| GO:0016310--phosphorylation                                                      | 56    | 13.08  | $6.37 \times 10^{-3}$ |
| GO:0051246--regulation of protein metabolic process                               | 55    | 12.85  | $7.26 \times 10^{-2}$ |
| GO:0033554--cellular response to stress                                           | 53    | 12.38  | $1.80 \times 10^{-4}$ |
| GO:0007399--nervous system development                                           | 52    | 12.15  | $2.06 \times 10^{-2}$ |
| GO:0031324--negative regulation of cellular metabolic process                    | 52    | 12.15  | $6.82 \times 10^{-2}$ |
| GO:0032268--regulation of cellular protein metabolic process                      | 51    | 11.92  | $9.24 \times 10^{-2}$ |
| GO:1902531--regulation of intracellular signal transduction                      | 50    | 11.68  | $5.12 \times 10^{-4}$ |
| GO:0044093--positive regulation of molecular function                             | 50    | 11.68  | $2.35 \times 10^{-3}$ |
| GO:0006928--movement of cell or subcellular component                            | 48    | 11.21  | $4.02 \times 10^{-3}$ |
| GO:0009605--response to external stimulus                                         | 47    | 10.98  | $6.26 \times 10^{-2}$ |
| GO:0043085--positive regulation of catalytic activity                             | 46    | 10.75  | $7.29 \times 10^{-4}$ |
| GO:0007049--cell cycle                                                           | 46    | 10.75  | $2.94 \times 10^{-3}$ |
| GO:0006357--regulation of transcription from RNA polymerase II promoter           | 46    | 10.75  | $1.51 \times 10^{-2}$ |
| GO:1902589--single-organism organelle organization                                | 45    | 10.51  | $2.52 \times 10^{-3}$ |
| GO:0006366--transcription from RNA polymerase II promoter                         | 45    | 10.51  | $2.03 \times 10^{-2}$ |
| GO:0008219--cell death                                                           | 44    | 10.28  | $8.02 \times 10^{-2}$ |
| GO:0031399--regulation of protein modification process                            | 43    | 10.05  | $1.20 \times 10^{-2}$ |
| GO:0048585--negative regulation of response to stimulus                          | 41    | 9.58   | $1.17 \times 10^{-3}$ |
| GO:0007155--cell adhesion                                                        | 41    | 9.58   | $3.80 \times 10^{-2}$ |
| GO:0006366--transcription from RNA polymerase II promoter                         | 41    | 9.58   | $3.98 \times 10^{-2}$ |

Count: genes involved in the term; percentage (%): involved genes/total genes; p-value: modified Fisher exact p-value.

3.3. PPI of Up- or Downregulated DEGs

As for the upregulated DEGs, there were DEGs involved in the defense response at all irradiation times. From the functional analysis of DAVID, we focused on the defense response related to wound healing, which is the BP of the DEGs of the upregulated DEGs at 6 and 12 h after the irradiation. Among the genes involved in the defense response, HCP5, DAPK3, IGLC2 and IGHV1 OR21-1 at 1 h after the irradiation for the upregulated DEGs, HP, IGHE and TPSAB1 at 3 h, CAKM2B, IGHM, SEMA7A, CXCL8 and TNFAIP6 at 6 h. IL34, ITGA2, SERPINE1, INHBA, PRDM1, LYZL4, PVR, BMP6, NFKB1, FOXP1, PER1, IGHC1, IGHA1, IGHA2, LDLR, SLC25A6, PF4 and TGM2. At 12 h, it was IGHM, ITIH4, SEMA7A, EDN1, HIST1 H2 BJ, KCNJ8, TNFAIP6, CCL21, CARD9, IFI6, SERPINB9, LYZL2, DEFB108B, ECSIT, IGHC1, IGKC, IGHD, CHRFAM7A and SLAMF6.

As the results of PPI analysis of DEGs involved in the defense response with STRING, CXCL8 was a gene associated with multiple genes in the upregulated DEGs of the defense response. The relationships with SERPINE1, PF4, NFKB1, TNFAIP6, EDN1, CCL21, ITIH4 and HP were confirmed. In addition, HP, ITIH4, TNFAIP6 and NFKB1 were also associated with multiple genes (Figure 1). In the downregulated DEGs, STAT1 was a gene associated with multiple genes. In particular, it was associated with SMAD3, IL15, CCL22, NAI5P, NRIH3, LY96, NFKBIA, PSMB8, PSMB9, PSMB10, OSA2, OAS3, IFI2, BCL6 and DDX58. In addition, NFKBIA, TLR3, IL15 and IRF2 were genes associated with multiple genes (Figure 2).

Figure 3 shows the changes in the expression of major genes related to other genes over time.
DEGs, HP, IGHE and TPSAB1 at 3 h, CAKM2 B, IGHM, SEMA7 A, CXCL8 and TNFAIP6 were confirmed. In addition, HP, ITIH4, TNFAIP6 and NFKB1 were also associated with multiple genes (Figure 1). In the downregulated DEGs, STAT1 was a gene associated with multiple genes (Figure 2). In the upregulated DEGs, NAIP, NRIH3, LY96, NFKBIA, PSMB8, PSMB9, PSMB10, OSA2, OAS3, IRF2, BCL6 and HP were confirmed. In addition, HP, ITIH4, TNFAIP6 and NFKB1 were also associated with multiple genes. In particular, it was associated with SMAD3, IL15, CCL22, and HP were confirmed. In addition, HP, ITIH4, TNFAIP6 and NFKB1 were also associated with multiple genes.

**Figure 1.** PPI of upregulated DEGs. Search tool STRING analysis of interacting genes and proteins reveals a protein–protein interaction PPI network in defense response by LLLT. PPI of upregulated DEGs related to ‘defense response’ Related genes after the irradiation at 3 h; ②, 6 h; ⑥ 12 h; ④ Red line: indicates the presence of fusion evidence; green line: neighborhood evidence; blue line: cooccurrence evidence; purple line: experimental evidence; yellow line: text mining evidence; light blue line: database evidence; black line: coexpression evidence.

**Figure 2.** PPI of downregulated DEGs. Search tool STRING analysis of interacting genes and proteins reveals a protein–protein interaction network between proteins in defense response by LLLT. PPI of downregulated DEGs related to ‘defense response.’ Related genes after the irradiation at 3 h; ②, 6 h; ⑥ 12 h; ④.
4. Discussion

Laser treatment has been studied for clinical application in many fields including medical and dentistry. In the field of dentistry, lasers are used for various purposes such as promoting wound healing, gingival incision, caries removal and sterilization/disinfection effect [25]. It has been reported that LLLT promotes wound healing and bone formation in the oral cavity by utilizing the bioactivating effect of chronic periodontitis.

In addition, at the cellular and tissue levels, there are research reports related to promotion of wound healing, such as cell proliferation of fibroblasts [15,20], osteoblasts [22,26] and vascular endothelial cells [18,27] by LLLT. However, clinical application in medical and dental treatments has not been carried out much. This is because the molecular biological findings are not clear.

Intracellular biological effects of LLLT include physiological activity by photoreceptors, changes in intracellular signal cascades, and changes in genes. The intracellular photoreceptor of LLLT is cytochrome c oxidase, which is a transmembrane protein complex that is an electron transport chain enzyme found in mitochondria. Promotion of cell proliferation by increasing cytochrome c oxidase activity and ATP is one of the representative mechanisms in LLLT research [28–31].

As a method for elucidating the mechanism of wound healing by LLLT, DNA microarrays are considered to be useful because they can examine thousands to tens of thousands of gene expressions at a time. From the obtained gene expression data, DEGs are extracted using bioinformatics analysis tools [32,33]. Furthermore, based on GO, by analyzing the biological process (BP) of DEGs, it is possible to estimate what is happening at each time by knowing the known functions of the gene contained in DEGs. It is also possible to infer what is about to happen at that time by performing chronological analysis. In addition, by searching for protein–protein interaction (PPI), it is possible to search for relationships at the molecular level [34,35]. The method plays a major role in elucidating the effects of proteins controlled by LLLT-stimulated genes at the molecular level.

In this study, we focused on the defense response from a huge amount of microarray data and analyzed the chronological changes in gene expression and the function of the genes after LLLT to HGF and the function of the genes.

The gene expression reactions related to wound healing with LLLT were remarkable 6–12 h after the irradiation. Analysis of the gene expression changes within these times were considered important for investigating the molecular mechanism of effects on HGF.
with LLLT. In particular, among the DEGs, those that are common over time and those with a significantly large expression fluctuation amount were considered largely affected by LLLT.

Analysis of BP over time revealed that upregulated were activated from 1 to 3 h after the initial irradiation, and downregulated BP was involved in RNA metabolism and activity. In both cases, the number of applicable DEGs for BP was 10 or less. It was suggested that there was no cohesive expression fluctuation as a function.

At 6 h after the irradiation, upregulated DEGs were observed related to cell proliferation, adhesion and defense reaction. Additionally, downregulated DEGs were observed in many BPs involved in RNA metabolism, activity, cell polymer production, and metabolism.

At 12 h after the irradiation, upregulated DEGs were observed with many BPs involved in defense reaction, immune reaction and response to external stimuli, and downregulated DEGs were observed with many BPs involved in RNA metabolism, activity, cell polymer production and metabolism. In the upregulated group, many BPs associated with wound healing were observed at 6–12 h after irradiation. Additionally, in downregulated, similar BP such as RNA metabolism were observed from 1 to 12 h after irradiation.

The BP ‘the defense response’ focused on in this study belongs to the BP ‘the response to stress of response to stimulus’ in GO. The response to stimulus is the process by which the state or activity of a cell or organism changes as a result of stimulation. The response to stress also causes motility, secretion, enzyme production, gene expression, etc., as a result of impaired homeostasis of the organism or cell due to extrinsic factors (temperature, humidity, ionizing radiation). The defense response, which belongs to response to stress, is a reaction caused in response to the presence of foreign substances or the occurrence of injuries, and is an important BP involved in the restriction, prevention/recovery of damage to living organisms.

In the BP ‘defense response’, the protein encoded by CXCL8, which is a downregulated DEG, is called interleukin-8 (IL-8). IL-8 is secreted by mononuclear macrophages, neutrophils, eosinophils, T lymphocytes, epithelial cells, and fibroblasts. IL-8 is also known as a neutrophil chemotactic factor with two major functions. It induces chemotaxis of target cells to the infected site. IL-8 is also known as a strong promoter of angiogenesis. IL-8 expression is regulated by the transcription factor NF-κB [36–42].

In particular, the upregulated DEGs NFKB1 and the downregulated DEGs NFKBIA are genes involved in NF-κB. These are one of the genes involved in the existing mechanism. NFKB1 is a transcriptional regulator that is activated by various intracellular and extracellular stimuli such as cytokines, oxidant free radicals, UV irradiation and bacterial or viral products. Activated NFKB stimulates the expression of genes involved in biological functions associated with many biological processes such as inflammation, immunity, differentiation and cells [43]. NFKBIA is a member of a family of cellular proteins that function to inhibit NF-κB transcription factors and IκBα masks the nuclear localization signals (NLS) of NF-κB proteins and inactivates them in the cytoplasm. It inhibits NF-κB by isolating it into a state [44]. From this result, it can be seen that NFKB1 increases and NFKBIA decreases when irradiation is performed, so that the activity of the pathway containing NF-κB occurs. Additionally, the defense response of BP is considered to be strongly related to the NF-κB pathway.

In a study by Chen et al., NF-κB activity was observed 1–10 h after irradiation [21].

In this study as well, changes in the expression of NFKB1 and NFKBIA were observed 6 h after irradiation, and the results of analysis from the viewpoint of BP also suggest that the movement of NF-κB due to irradiation is an important process in the mechanism of LLLT. In this study, we focused on the defense response. Further, we also will need to focus on wound healing-related processes such as BP ‘the immune response’.

By adding analysis more time points, it is possible to analyze detailed time-series processes after LLL irradiation. As future developments, we plan to study other BPs, collect LLLT microarray data at different time points, and analyze the effects of laser irradiation on fibroblasts and molecular-level processes during the healing process. We will lead to
the elucidation of molecular evidence in ‘the response to stress of response to stimulus’ by LLLT.

5. Conclusions

The time points of 1, 3, 6 and 12 h after LLL irradiation were compared over time. The most DEGs after the LLL irradiation on HGF were showed at 6 h upregulated gene. The number of DEGs peaked 6 h after irradiation and slightly decreased at 12 h after irradiation. From the time-dependent functional analysis, the upregulated DEGs were involved in BPs of cell proliferation, adhesion, and defense response related to wound healing from 6 h. In addition, defense response is one of the important mechanisms in BP after the irradiation. We found that the upregulated DEGs such as CXCL8 and NFKB1, and the downregulated DEGs such as NFKBIA and STAT1 were correlated with multiple genes from these PPI. From these results, irradiation of LLLT showed fluctuations in the expression of genes related to BP defense response.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jcm10091952/s1, Table S1: DEGs of the up-regulated genes at 1 h after LLL irradiation, Table S2: DEGs of the down-regulated genes at 1 h after LLL irradiation, Table S3: DEGs of the up-regulated genes at 3 h after LLL irradiation, Table S4: DEGs of the down-regulated genes at 3 h after LLL irradiation, Table S5: DEGs of the up-regulated genes at 6 h after LLL irradiation, Table S6: DEGs of the down-regulated genes at 6 h after LLL irradiation, Table S7: DEGs of the up-regulated genes at 12 h after LLL irradiation, Table S8: DEGs of the down-regulated genes at 12 h after LLL irradiation.

Author Contributions: Conceptualization, Y.W., A.S., H.I., E.M. and Y.N.; methodology, Y.W., A.S., H.I., E.M. and E.M.; software, Y.W. and A.S.; validation, Y.W., A.S., H.I., and E.M.; formal analysis, Y.W., A.S. and H.I.; investigation, Y.W., A.S., H.I., and E.M.; resources, Y.W., A.S., H.I., and E.M.; data curation, Y.W., A.S., H.I., and E.M.; writing—original draft preparation, Y.W., A.S., H.I., E.M. and Y.N.; writing—review and editing, Y.W., A.S., H.I., E.M. and Y.N.; visualization, Y.W. and A.S.; supervision, Y.N.; project administration, A.S., H.I., and E.M.; funding acquisition, H.I. and E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by grants from the JSPS KAKENHI (grant numbers: JP18 K09585).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lopes, B.M.V.; Marcantonio, R.A.C.; Thompson, G.M.A.; Neves, L.H.M.; Theodoro, L.H. Short-Term Clinical and Immunologic Effects of Scaling and Root Planing With Er:YAG Laser in Chronic Periodontitis. *J. Periodontol.* 2008, 79, 1158–1167. [CrossRef] [PubMed]
2. Schwarz, F.; Sculean, A.; Georg, T.; Reich, E. Periodontal Treatment With an Er:YAG Laser Compared to Scaling and Root Planing, A Controlled Clinical Study. *J. Periodontal*. 2001, 72, 361–367. [CrossRef]
3. Crespi, R.; Cappare, P.; Toscanelli, I.; Gherlone, E.; Romanos, G.E. Effects of Er:YAG Laser Compared to Ultrasonic Scaler in Periodontal Treatment: A 2-Year Follow-Up Split-Mouth Clinical Study. *J. Periodontal*. 2007, 78, 1195–1200. [CrossRef]
4. White, J.M.; Goodis, H.E.; Rose, C.L. Use of the Pulsed Nd:YAG Laser for Intraoral Soft Tissue Surgery. *Lasers Surg. Med.* 1991, 11, 455–461. [CrossRef]
5. Lauritano, D.; Lucchese, A.; Gabbrone, F.; Di Stasio, D.; Silvestre Rangil, J.; Carinci, F. The Effectiveness of Laser-Assisted Surgical Excision of Leukoplakias and Hyperkeratosis of Oral Mucosa: A Case Series in A Group of Patients. *Int. J. Environ. Res. Public. Health* 2019, 16, 210. [CrossRef]
6. Pick, R.M.; Colvard, M.D. Current Status of Lasers in Soft Tissue Dental Surgery. *J. Periodontol.* 1993, 64, 589–602. [CrossRef]
7. Aimbire, F.; Santos, F.V.; Albertini, R.; Castro-Faria-Neto, H.C.; Mittmann, J.; Pacheco-Soares, C. Low-Level Laser Therapy Decreases Levels of Lung Neutrophils Anti-Apoptotic Factors by a NF-KappaB Dependent Mechanism. *Int. Immunopharmacol*. 2008, 8, 603–605. [CrossRef]
8. Aimbire, F.; Ligeiro de Oliveira, A.P.; Albertini, R.; Corrêa, J.C.; Ladeira de Campos, C.B.; Lyon, J.P.; Silva, J.A.; Costa, M.S. Low Level Laser Therapy (LLLT) Decreases Pulmonary Microvascular Leakage, Neutrophil Influx and IL-1bta Levels in Airway and Lung from Rat Subjected to LPS-Induced Inflammation. *Inflammation* 2008, 31, 189–197. [CrossRef]
9. Albertini, R.; Aimbire, F.S.C.; Correa, F.I.; Ribeiro, W.; Cogo, J.C.; Antunes, E.; Teixeira, S.A.; De Nucci, G.; Castro-Faria-Neto, H.C.; Zängaro, R.A.; et al. Effects of Different Protocoll Doses of Low Power Gallium-Aluminum-Arsenete (Ga-Al-As) Laser Radiation (650 Nm) on Carrageenan Induced Rat Paw Oedema. J. Photochem. Photobiol. B 2004, 74, 101–107. [CrossRef] [PubMed]

10. Albertini, R.; Villaverde, A.B.; Aimbire, F.; Bjordal, J.; Brugnera, A.; Mittmann, J.; Silva, J.A.; Costa, M. Cytokine MRNA Expression Is Decreased in the Subplantar Muscle of Rat Paw Subjected to Carrageenan-Induced Inflammation after Low-Level Laser Therapy. Photomed. Laser Surg. 2008, 26, 19–24. [CrossRef] [PubMed]

11. Albertini, R.; Aimbire, F.; Villaverde, A.B.; Silva, J.A.; Costa, M.S. CO2-2 MRNA Expression Decreases in the Subplantar Muscle of Rat Paw Subjected to Carrageenan-Induced Inflammation after Low Level Laser Therapy. Inflamm. Res. Off. J. Eur. Histamine Res. Soc. Al 2007, 56, 228–229. [CrossRef]

12. Albertini, R.; Villaverde, A.B.; Aimbire, F.; Salgado, M.A.C.; Bjordal, J.M.; Alves, L.P.; Munin, E.; Costa, M.S. Anti-Inflammatory Effects of Low-Level Laser Therapy (LLLT) with Two Different Red Wavelengths (660 Nm and 684 Nm) in Carrageenan-Induced Rat Paw Edema. J. Photochem. Photobiol. B 2007, 89, 50–55. [CrossRef] [PubMed]

13. Aoki, A.; Mizutani, K.; Schwarz, F.; Sculean, A.; Yikna, R.A.; Takasaki, A.A.; Romanos, G.E.; Taniguchi, Y.; Sasaki, K.M.; Zeredo, J.L.; et al. Periodontal and Peri-Implant Wound Healing Following Laser Therapy. Periodontol. 2000 2015, 68, 217–269. [PubMed]

14. Kami, T.; Yoshimura, Y.; Nakajima, T.; Ohshiro, T.; Fujino, T. Effects of Low-Power Diode Lasers on Flap Survival. Ann. Plast. Surg. 1985, 14, 278–283. [CrossRef]

15. Ogita, M.; Tsuchida, S.; Aoki, A.; Satoh, M.; Kado, S.; Sawabe, M.; Nanbara, H.; Kobayashi, H.; Takeuchi, Y.; Mizutani, K.; et al. Increased Cell Proliferation and Differential Protein Expression Induced by Low-Level Er:YAG Laser Irradiation in Human Gingival Fibroblasts: Proteome Analysis. Lasers Med. Sci. 2015, 30, 1855–1866. [CrossRef] [PubMed]

16. Kipshidze, N.; Nikolaychik, V.; Keelan, M.H.; Shankar, L.R.; Khanna, A.; Kornowski, R.; Leon, M.; Moses, J. Low-Power Helium: Neon Laser Irradiation Enhances Production of Vascular Endothelial Growth Factor and Promotes Growth of Endothelial Cells in Vitro. Lasers Surg. Med. 2001, 28, 355–364. [CrossRef] [PubMed]

17. Yu, H.S.; Chang, K.L.; Yu, C.L.; Chen, J.W.; Chen, G.S. Low-Energy Helium-Neon Laser Irradiation Stimulates Interleukin-1 Alpha and Interleukin-8 Release from Cultured Human Keratinocytes. J. Investig. Dermatol. 1996, 107, 593–596. [CrossRef]

18. Khanna, A.; Shankar, L.R.; Keelan, M.H.; Kornowski, R.; Leon, M.; Moses, J.; Kipshidze, N. Augmentation of the Expression of Proangiogenic Genes in Cardiomyocytes with Low Dose Laser Irradiation in Vitro. Cardiovasc. Radiat. Med. 1999, 1, 265–269. [CrossRef]

19. Gkogkos, A.S.; Karoussis, I.K.; Prevezanos, I.D.; Marcopoulou, K.E.; Kyriakidou, K.; Vrotsos, I.A. Effect of Nd:YAG Low Level Laser Therapy on Human Gingival Fibroblasts. Int. J. Dent. 2015. [CrossRef]

20. Misa, O.; Etsuko, M.; Hitomi, I.; Hiroko, T.-I.; Yukihiro, N. Effect of Low-Level Nd: YAG Laser Irradiation for Wound Healing on Human Gingival Fibroblasts. Ph.D. Thesis, The Nippon Dental University, Tokyo, Japan, 2016.

21. Chen, A.C.-H.; Arany, P.R.; Huang, Y.-Y.; Tomkinson, E.M.; Sharma, S.K.; Kharkwal, G.B.; Saleem, T.; Mooney, D.; Yull, F.E.; Blackwell, T.S.; et al. Low-Level Laser Therapy Activates NF-KB via Generation of Reactive Oxygen Species in Mouse Embryonic Fibroblasts. PLoS ONE 2011, 6, e22453. [CrossRef]

22. Obsugi, Y.; Aoki, A.; Mizutani, K.; Katagiri, S.; Komaki, M.; Noda, M.; Takagi, T.; Kakizaki, S.; Meinerz, W.; Izumi, Y. Evaluation of Bone Healing Following Er:YAG Laser Ablation in Rat Calvaria Compared with Bur Drilling. J. Biophotonics 2019, 12, e201800245. [CrossRef] [PubMed]

23. Kong, S.; Aoki, A.; Iwasaki, K.; Mizutani, K.; Katagiri, S.; Suda, T.; Ichinose, S.; Ogita, M.; Pavlic, V.; Izumi, Y. Biological Effects of Er:YAG Laser Irradiation on the Proliferation of Primary Human Gingival Fibroblasts. J. Biophotonics 2018, 11. [CrossRef] [PubMed]

24. Naoya, Y.; Etsuko, M.; Hiroko, I.; Yukihiro, N. The Effect of Nd:YAG Laser Irradiation on Human Gingival Fibroblasts—A Study of the Irradiation Output and Distance. J. Jpn. Soc. Laser Med. 2013, 24, 72–82. (In Japanese) [CrossRef]

25. Akiyama, F.; Aoki, A.; Miura-Uchiyama, M.; Sasaki, K.M.; Ichinose, S.; Umeda, M.; Ishikawa, I.; Izumi, Y. In Vitro Studies of the Ablation Mechanism of Periodontopathic Bacteria and Decontamination Effect on Periodontally Diseased Root Surfaces by Erbium:Yttrium-Aluminum-Garnet Laser. Lasers Med. Sci. 2011, 26, 193–204. [CrossRef]

26. Pyo, S.-J.; Song, W.-W.; Kim, I.-R.; Park, B.-S.; Kim, C.-H.; Shin, S.-H.; Chung, I.-K.; Kim, Y.-D. Low-Level Laser Therapy Induces the Expressions of BMP-2, Osteocalcin, and TGF-B1 in Hypoxic-Cultured Human Osteoblasts. Lasers Med. Sci. 2013, 28, 543–550. [CrossRef] [PubMed]

27. Li, Y.; Xu, Q.; Shi, M.; Gan, P.; Huang, Q.; Wang, A.; Tan, G.; Fang, Y.; Liao, H. Low-Level Laser Therapy Induces Human Umbilical Vascular Endothelial Cell Proliferation, Migration and Tube Formation through Activating the PI3K/Akt Signaling Pathway. Microvasc. Res. 2020, 129, 103959. [CrossRef]

28. Karu, T.I.; Pyatibrat, L.V.; Afanasyeva, N.I. Cellular Effects of Low Power Laser Therapy Can Be Mediated by Nitric Oxide. Lasers Surg. Med. 2005, 36, 307–314. [CrossRef] [PubMed]

29. Smith, K.C. The Photobiological Basis of Low Level Laser Radiation Therapy. Laser Ther. 1991, 3, 19–24. [CrossRef]

30. Silveira, P.C.L.; Streck, E.L.; Pinho, R.A. Evaluation of Mitochondrial Respiratory Chain Activity in Wound Healing by Low-Level Laser Therapy. J. Photochem. Photobiol. B 2007, 86, 279–282. [CrossRef]

31. Lim, J.; Sanders, R.A.; Snyder, A.C.; Eells, J.T.; Henshel, D.S.; Watkins, J.B. Effects of Low-Level Light Therapy on Streptozotocin-Induced Diabetic Kidney. J. Photochem. Photobiol. B 2010, 99, 105–110. [CrossRef]
32. Huang, D.W.; Sherman, B.T.; Lempicki, R.A. Bioinformatics Enrichment Tools: Paths toward the Comprehensive Functional Analysis of Large Gene Lists. *Nucleic Acids Res.* 2009, 37, 1–13. [CrossRef] [PubMed]

33. Huang, D.W.; Sherman, B.T.; Lempicki, R.A. Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources. *Nat. Protoc.* 2009, 4, 44–57. [CrossRef] [PubMed]

34. Zeidán-Chuliá, F.; Gursoy, M.; de Oliveira, B.-H.N.; Gelain, D.P.; Könönen, E.; Gursoy, U.K.; Moreira, J.C.F.; Uitto, V.-J. Focussed Microarray Analysis of Apoptosis in Periodontitis and Its Potential Pharmacological Targeting by Carvacrol. *Arch. Oral Biol.* 2014, 59, 461–469. [CrossRef]

35. Zeidán-Chuliá, F.; Rybarczyk-Filho, J.L.; Gursoy, M.; Könönen, E.; Uitto, V.-J.; Gursoy, O.V.; Cakmakci, L.; Moreira, J.C.F.; Gursoy, U.K. Bioinformatical and in Vitro Approaches to Essential Oil-Induced Matrix Metalloproteinase Inhibition. *Pharm. Biol.* 2012, 50, 675–686. [CrossRef]

36. Van Damme, J.; Rampart, M.; Conings, R.; Decock, B.; Van Osselaer, N.; Willems, J.; Billiau, A. The Neutrophil-Activating Proteins Interleukin 8 and Beta-Thromboglobulin: In Vitro and in Vivo Comparison of NH2-Terminally Processed Forms. *Eur. J. Immunol.* 1990, 20, 2113–2118. [CrossRef]

37. Schutyser, E.; Struyf, S.; Proost, P.; Opdenakker, G.; Laureys, G.; Verhasselt, B.; Peperstraete, L.; Van de Putte, I.; Saccani, A.; Allavena, P.; et al. Identification of Biologically Active Chemokine Isoforms from Ascitic Fluid and Elevated Levels of CCL18/Pulmonary and Activation-Regulated Chemokine in Ovarian Carcinoma. *J. Biol. Chem.* 2002, 277, 24584–24593. [CrossRef] [PubMed]

38. Hébert, C.A.; Luscinskas, F.W.; Kiely, J.M.; Luis, E.A.; Darbonne, W.C.; Bennett, G.L.; Liu, C.C.; Obin, M.S.; Gimbrone, M.A.; Baker, J.B. Endothelial and Leukocyte Forms of IL-8. Conversion by Thrombin and Interactions with Neutrophils. *J. Immunol.* 1990, 145, 3033–3040.

39. Ungureanu, D.; Vanhatupa, S.; Kotaja, N.; Yang, J.; Aittomaki, S.; Jänne, O.A.; Palvimo, J.J.; Silvernoinen, O. PIAS Proteins Promote SUMO-1 Conjugation to STAT1. *Blood* 2003, 102, 3311–3313. [CrossRef]

40. Zhang, Y.; Mao, D.; Roswit, W.T.; Jin, X.; Patel, A.C.; Patel, D.A.; Agapov, E.; Wang, Z.; Tidwell, R.M.; Atkinson, J.J.; et al. PARP9-DTX3L Ubiquitin Ligase Targets Host Histone H2B and Viral 3C Protease to Enhance Interferon Signaling and Control Viral Infection. *Nat. Immunol.* 2015, 16, 1215–1227. [CrossRef]

41. Chen, K.; Liu, J.; Liu, S.; Xia, M.; Zhang, X.; Han, D.; Jiang, Y.; Wang, C.; Cao, X. Methyltransferase SETD2-Mediated Methylation of STAT1 Is Critical for Interferon Antiviral Activity. *Cell* 2017, 170, 492–506.e14. [CrossRef]

42. Liu, B.; Liao, J.; Rao, X.; Kushner, S.A.; Chung, C.D.; Chang, D.D.; Shuai, K. Inhibition of Stat1-Mediated Gene Activation by PIAS. *Proc. Natl. Acad. Sci. USA* 1998, 95, 10626–10631. [CrossRef] [PubMed]

43. Beinke, S.; Robinson, M.J.; Hugunin, M.; Ley, S.C. Lipopolysaccharide Activation of the TPL-2/MEK/Extracellular Signal-Regulated Kinase Mitogen-Activated Protein Kinase Cascade Is Regulated by IkappaB Kinase-Induced Proteolysis of NF-KappaB1 P105. *Mol. Cell. Biol.* 2004, 24, 9658–9667. [CrossRef] [PubMed]

44. Scherer, D.C.; Brockman, J.A.; Chen, Z.; Maniatis, T; Ballard, D.W. Signal-Induced Degradation of I Kappa B Alpha Requires Site-Specific Ubiquitination. *Proc. Natl. Acad. Sci. USA* 1995, 92, 11259–11263. [CrossRef] [PubMed]