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# Introducing Critical Genes in Response to Photodynamic Therapy: A Network Analysis

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**Abstract**

**Introduction:** Photodynamic therapy (PDT) is applied as an efficient method for preventing the progress of cancers. Light and a photosensitive compound which is known as photosensitizer (PS) are the main parts of PDT. In the present study, molecular events after using PDT in the presence of a super lethal dose of a PS were assessed via protein-protein interaction (PPI) analysis.

**Methods:** Data were extracted from Gene Expression Omnibus (GEO). The gene expression profiles of the treated human Sk-Cha1 cells via PDT were compared with the control cells. Expressed change analysis and PPI network analysis were administrated via Cytoscape software v 3.7.2 to find the critical differentially expressed genes (DEGs). Regulatory relationships between the central DEGs were evaluated and the highlighted genes were identified.

**Results:** The significant amounts of gene expression values were grouped and a few DEGs characterized by tremendously expressed values were identified. EGFR, CANX, HSPA5, MYC, JUN, ITGB1, APP, and CDH1 were highlighted as hub-bottleneck DEGs. EGFR, CDH1, and JUN appeared as a set of SEGs, which play a crucial role in response to PDT in the treated Sk-Cha1 cells.

**Conclusion:** In conclusion, regulatory relationships between EGFR, CDH1, and JUN, which have an effect on the regulation of cellular survival, differentiation, and proliferation, were highlighted in the present investigation.

**Keywords:** PPI network analysis; Photodynamic therapy; Gene expression change; Human; EGFR.

## Introduction

Photodynamic therapy (PDT) is a combined method including light radiation and a photosensitizer (PS) which is activated in response to light radiation. PSs do not have cytotoxicity properties alone. PDT is applied to treat several cancers, and the outcome has satisfied investigators and clinicians. Like the other methods, the efficacy of PDT depends on several parameters. A suitable PS with optimum concentration plays a critical role in the effectiveness of PDT. However, although much research has been conducted on the molecular mechanism of PDT, more evaluation is required to detect the details of molecular events in response to PDT.[1-4]

Gene expression analysis is a common approach to exploring the molecular mechanism of diseases and the effect of therapeutic methods. Using radiation to inhibit cancers is tied to two challenges: increment of treatment efficacy and decrement of the side effect of treatment. Therefore, gene expression analysis as an effective method has attracted researchers' attention to follow and progress therapeutic methods. PDT, like the other methods, is assessed via gene expression analysis. Based on the literature, the expression of many genes is altered in response to PDT.[5-7]

Bioinformatics in combination with genomics and other high throughput methods has provided valuable information about molecular events in living systems. Screening a large number of genes to find crucial individuals is a critical task in research. Bioinformatic methods such as network analysis are applied to evaluate genomics and proteomics data. Protein-protein interaction (PPI) network analysis is a method that,

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based on graph theory, investigates genes and proteins in an interacted unit that is called an interactome.[8,9] PPI network analysis is applied to detect targeted genes by laser radiation in many investigations. Analysis of the constructed PPI network leads to the differentiation of the elements of the network based on the centrality properties of the nodes of the network. One important type of node of a network is known as a hub. Hub nodes make many connections with the first neighbor nodes. The second key nodes of a network are known as bottlenecks, the nodes that participate frequently in the shortest paths. Common hubs and bottlenecks are named hub-bottlenecks. Hub-bottlenecks are critical elements of a network.[10-12] In the present study, the molecular mechanism of human cells response to PDT was investigated by data from GEO via a bioinformatics approach to open a new window about the main targets of the applied therapeutic method.

## Methods

To assess the effect of super lethal dose of PS on PDT of Sk-Cha1 cell line, the gene expression profiles of the treated cells were compared with control cells. Data were extracted from GSE84758 (Platform GPL10558) derived from GEO. The gene expression profiles were evaluated by GEO2R program. Analyses revealed that the presence of a PS without PDT did not have a significant effect on the gene expression profiles of the investigated cells. To evaluate the feasibility of analysis, the gene expression profiles were assessed via mean-difference plot analysis.

The top 250 differentially expressed genes (DEGs) were selected to be analyzed and screened. Considering *P* value ≤ 0.001 and fold change (FC) ≥ 1.5, the characterized DEGs were screened. Down- and Up-regulation values were categorized in suitable groups to find the frequency of the dysregulated DEGs in detail. The significant DEGs were included in the "Protein query" of the STRING database by Cytoscape software v 3.7.2 to form the PPI network. The recognized nodes were connected via undirected links. The main connected component of the created PPI network was analyzed by a "network analyzer" to find the topological parameters of the network. The 10 top nodes based on degree value were identified as hubs, and the common hubs and bottlenecks were pointed out as hub-bottlenecks. Closeness centrality and stress were considered as the other centrality parameters. The hub-bottleneck genes were connected by directional links to find a regulatory relationship between the central nodes via CluePedia.

## Results

The result of gene expression profile evaluation by GEO2R program as a mean-difference plot is shown in Figure 1. The up- and down-regulated DEGs are shown in Figure 1. 232 significant DEGs were identified and candidates for more analysis. The significant DEGs were grouped based on the expression rate (see Figure 2). As it is shown in Figure 2, 166 DEGs (about 72% of the significant DEGs) are down-regulated and the most range of down-regulation is (-2) ≤ logFC < (-1).

PPI network analysis revealed that 188 DEGs among the queried 232 individuals were recognized by the STRING database. The created network included 35 isolated genes, six paired DEGs, and a main connected component of 147 nodes. The main connected component of the analyzed network is presented in Figure 3.

Ten top nodes based on degree value were determined as hubs. Eight hubs were highlighted as hub-bottlenecks. The hubs and the related centrality parameters are presented in Table 1. To discriminate the hub-bottleneck nodes, these DEGs were assessed via action map analysis. Expression, activation, inhibition, binding, catalysis, reaction, and post-translational modification relationships between the evaluated hub-bottlenecks are shown in Figure 4.

## Discussion

A PS is a photosensitive compound that accumulates in

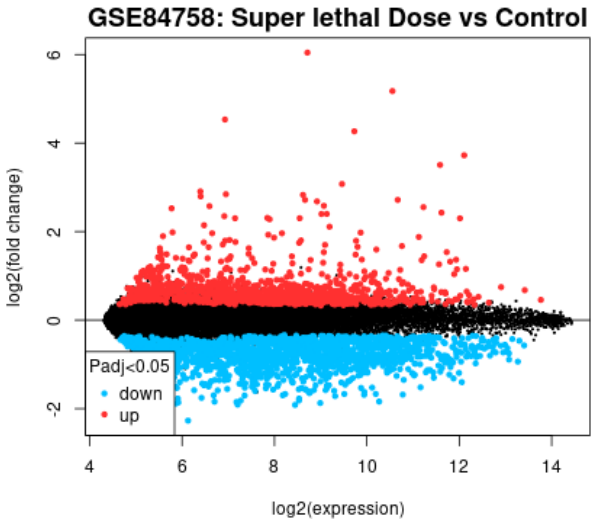


**Figure 1.** Mean-Difference Plot of the Gene Expression Profiles of the Treated Sk-Cha1 Cells by Photodynamic Therapy in the Presence of a Super Lethal Dose of a Photosensitizer Versus Control Cells.

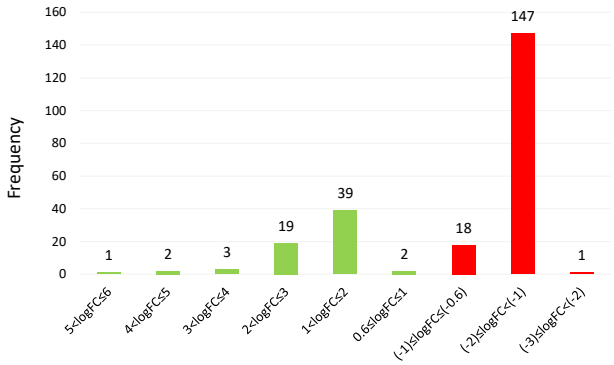


**Figure 2.** Frequency of DEGs sets which are characterized by a range of gene expression alteration. The green and red colors refer to up- and down-regulation.

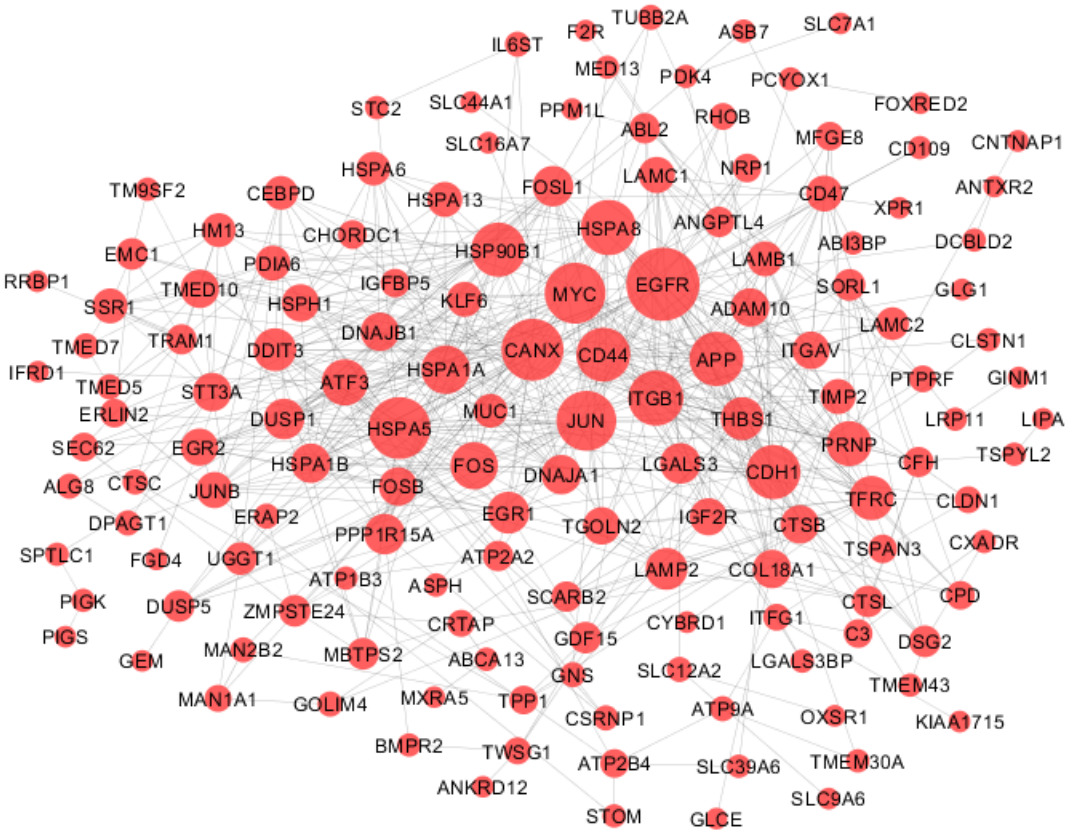


**Figure 3.** Main Connected Component of the Analyzed PPI Network. The nodes are laid out based on degree value. The bigger size of the node is consistent with the increase in degree. The confidence score was considered to be 0.4.

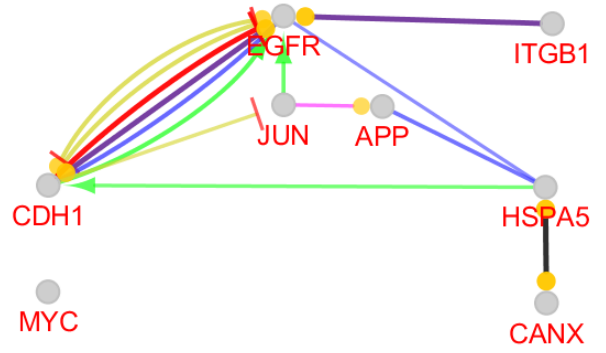


**Figure 4.** Regulatory Relationship Between 8 Hub-Bottlenecks. Yellow, green, red, blue, purple, black, and pink colors refer to expression, activation, inhibition, binding, catalysis, reaction, and post-translational modification respectively.

the pathological tissues. A PS is activated in the presence of a certain wavelength and initiates the selective destruction of the inapt cells. Since a PS distributes in a specific area, its cytotoxic reactions appear only within the pathological tissues.[4] There are many documents about using super lethal doses of radiation to achieve efficient radiation against cancer cells.[13] As it is depicted in Figure 1, the gene expression of the irradiated cells is altered considerably and many significant up- and down-regulated genes appear after applied PDT. Considerable gene expression changes after PDT are reported for the treated samples.[14] The analysis of gene expression profiles via network analysis regarding hubs and bottleneck nodes has been pointed out by previous studies.[15] The distribution of gene expression amounts for the up- and down-regulated genes is presented in Figure 2. Down-regulation is a prominent process relative to up-regulation. A few genes are characterized by extreme dysregulation. FOS, HSPA6, and EGR1 are the genes that are up-regulated extremely while UGGT1 is down-regulated tremendously.

PPI network analysis as a suitable probe is applied to determine the key genes among the large numbers of the studied individuals.[16] As it is shown in Figure 3, most of the recognized significant DEGs participate in the interactome. The analysis led to the introduction of EGFR, CANX, HSPA5, MYC, JUN, ITGB1, APP, and CDH1 as hub-bottleneck DEGs. The introduced hubs and hub-bottlenecks were also characterized by top values of closeness centrality and stress. The importance of hub-bottleneck nodes in control of function in the studied samples is emphasized by researchers.[17] Since there is not a common gene between the results of network analysis (see Table 1) and the tremendously dysregulated genes, it can be concluded that the role of extremely up- and down-regulated DEGs in control of molecular changes after PDT is not considerable.

It has been expressed that the detection of regulatory relationships between genes is a suitable tool to analyze

**Table 1.** The List of Hub Nodes of the Analyzed PPI

| R | Display Name | Degree | BtwC | Cc | Stress | Ranked as Bottleneck | LogFC |
|---|---|---|---|---|---|---|---|
| 1 | EGFR | 44 | 0.16 | 0.51 | 21328 | 1 | -1.217 |
| 2 | CANX | 34 | 0.13 | 0.49 | 15716 | 2 | -1.357 |
| 3 | HSPA5 | 34 | 0.10 | 0.49 | 15920 | 4 | -1.41 |
| 4 | MYC | 33 | 0.09 | 0.48 | 13118 | 5 | 1.8 |
| 5 | JUN | 32 | 0.07 | 0.48 | 11570 | 8 | 2.716 |
| 6 | HSPA8* | 28 | 0.05 | 0.46 | 7060 | - | 1.362 |
| 7 | ITGB1 | 28 | 0.08 | 0.48 | 9068 | 6 | -1.519 |
| 8 | APP | 27 | 0.12 | 0.48 | 13498 | 3 | -1.688 |
| 9 | CD44* | 27 | 0.04 | 0.45 | 5992 | - | -1.129 |
| 10 | CDH1 | 27 | 0.07 | 0.46 | 8806 | 7 | -1.261 |

Abbreviations: BtwC, betweenness centrality; Cc, closeness centrality.
The hub-bottlenecks are labeled by a star

molecular events in the studied systems.[18,19] Regulatory relationships between the introduced hub-bottlenecks are displayed in Figure 4. MYC remains isolated and has no connection with the other genes. CANX is only related to HSPA5 by reaction action, and ITGB1 is connected to EGFR via catalysis action. HSPA5 has binding connections with APP and EGFR and also activates CDH1. The post-translational modification relationship is presented as a connection between APP and JUN. Complex connections are seen between EGFR and CDH1. It seems that the control of EGFR is the critical process in this presented map.

The epidermal growth factor receptor is a transmembrane glycoprotein. The binding of EGF to EGFR initiates signaling pathways that are involved in the regulation of cellular functions such as proliferation, differentiation, and cell survival. The up-regulation of EGFR in tumor cells is reported by several researchers. There is a document about the activation of EGFR in resistance to chemotherapy and radiation treatment in tumor cells.[20] Liu et al published data about the relationship between EGFR and CDH1. Based on their report, silencing EGFR leads to considerable regulation of lung cancer progression via the TGFBR1–EGFR–CTNNB1–CDH1 axis.[21]

## Conclusion

In conclusion, the expression of many genes is affected by PDT in the treated cells. Complex relationships between EGFR, CDH1, and JUN appeared as the core of molecular events in response to PDT. Cellular survival, differentiation, and proliferation were pointed out as the main relatively regulated process in the studied cells after PDT. It can be recommended that the role of EGFR, CDH1, and JUN in response to PDT be assessed via the synergic effects of the related drugs.

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### Authors' Contribution

**Data curation:** Zahra Razzaghi.
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**Project administration:** Babak Arjmand, Alireza Ahmadzadeh.
**Supervision:** Vahid Mansouri, Reza Mohamoud Robati.
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### Competing Interests

The authors declare that they have no conflict of interest.

### Ethical Approval

This project is approved by Shahid Beheshti University of Medical Sciences (code: IR.SBMU.RETECH.REC.1401.483).

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