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Assessment of the Response of Human Umbilical Vein Endothelial Cells to Photodynamic Therapy: Highlighting the Role of Il-17 Signaling Pathway

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Abstract

Introduction: Photodynamic therapy (PDT) is a method based on the application of a photosensitive agent and the administration of light irradiation on the treated samples. PDT is applied as an effective tool with minimal side effects against tumor tissues. This study aimed to assess the targets of critical genes by PDT at the cellular level of cancer to provide a new perspective on its molecular mechanism.

Methods: To assess the effect of PDT, we extracted the differentially expressed genes (DEGs) from the gene expression profiles of human umbilical vein endothelial cells (HUVECs) treated with PDT from Gene Expression Omnibus (GEO) databases. The queried DEGs were evaluated via a regulatory network and gene ontology enrichment to find the critical targets.

Results: Among 76 queried significant DEGs, 27 individuals were interacted by activation, inhibition, and co-expression actions. Thirty DEGs were related to the five classes of biological terms. The IL-17 signaling pathway and PTGS2, CXCL8, FOS, JUN, CXCL1, ZFP36, and FOSB were identified as the crucial targets of PDT.

Conclusion: PDT as a stimulator of gene expression and an activator of gene activity overexpressed and hyper-activated many genes. It seems that PDT introduces a number of genes and pathways that can be regulated by anticancer drugs to fight against cancers.

Keywords: Cell; Pathway; Gene; Photodynamic therapy, Human.

Introduction

Photodynamic therapy (PDT) is a method which is applied to fight against tumor tissues by using a tumor-localizing photosensitizing agent and then activating the agent via the exposure of a specific light wavelength. PDT effects including photochemical and photobiologic processes lead to irreversible photodamage to tumor tissues.[1] This method is approved as a therapeutic procedure with minimal side effects and an almost non-invasive technique in clinics. It can be applied against malignant cells via a selective cytotoxic activity.[2] The importance of PDT in the prevention and treatment of skin cancer has been pointed out by researchers.[3]

Recently, immunogenic cell death (ICD) as a most promising way against tumor cells has attracted experts' attention. PDT like other anticancer treatment modalities can trigger ICD. Findings indicate that induced ICD by PDT in combination with nanotechnology that provides a new generation of photosensitizers plays a critical role in the inhibition of tumor tissues in future. It seems that with the elimination of PDT methods such as hypoxic tumor microenvironment, the efficacy of PDT will increase significantly in future. Research to achieve oxygen-boosted PDT and nanoparticles will make gross advances in PDT as an efficient treatment of cancers.[4,5] High efficacy associated with minimal side effects and negligible invasiveness of PDT in comparison with the other methods of cancer treatment such as radiotherapy

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and chemotherapy has satisfied scientists to apply PDT as a therapeutical method in the field of brain tumors. It appears PDT is a suitable method for treating central nervous system cancers such as glioblastoma multiforme.[6]

The alteration of the gene expression profiles of the treated sample via PDT has been investigated by researchers. Aniogo et al have assessed gene expression alteration in a multidrug resistant breast cancer sample after treatment via PDT.[7] Investigations indicate that network analysis is a useful tool to evaluate the alteration of gene expression profiles. In such an assessment of a large number of differentially expressed genes (DEGs), the critical individuals will be introduced as the core of the affected genes. The regulatory network focuses on the effects of genes on the activity and expression of the neighbor genes. The studied genes can be screened via regulatory network to find the crucial individuals.[8,9] Mansouri et al. pointed out regulatory relationships between EGFR, CDH1, and JUN as a consequence of PDT. The regulation of cellular survival, differentiation, and proliferation was introduced as the outcome of PDT application.[10] In the present study, gene expression profiles of human umbilical vein endothelial cells (HUVECs) which were treated via PDT were extracted from the Gene Expression Omnibus (GEO) database and assessed via system biology to find the core of molecular events.

Methods

GSE84758 (GPL10558) is recorded as "Transcriptomic, (phospho)proteomic, and metabolomic analysis of tumor-comprising cells treated by photodynamic therapy" in the GEO database. GSM2249747, GSM2249769, and GSM2249777are related to the HUVEC cells that are incubated with LC50 concentration of photosensitizer zinc phthalocyanine and irradiated with a 671-nm diode laser (CNI, Changchun, China) at a laser power of 500 mW with a fluence of 15 J/cm^2. These profiles are compared with the control gene expression profiles (GSM2249752, GSM2249770, and GSM2249776). The full description of the methods is presented in the report of Weijer et al.[11]

Analysis was done via GEO2R program to find the significant DEGs that discriminate the treated cells from controls. The Venn diagram and the UMAP plot were extracted to detect the number of significant DEGs and the pattern of profile separation by the DEGs. The significant DEGs were imported in the CluePedia application of Cytoscape software to find the regulatory relationships (including activation, inhibition, and coexpression) between the recognized DEGs. The pathways related to the significant DEGs were searched from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database via the ClueGO application of Cytoscape software. Findings of regulatory relationships and pathway analysis were screened and the prominent individuals were assessed based on fold change (FC) and relationships between genes and pathways.

Results

Results of GEO2R analysis are shown in Figures 1-2. As it is depicted in Figure 1, among the 47 243 studied genes, 80 individuals are significant DEGs. This finding indicates that the expression of about 0.2% of the dysregulated genes is altered significantly. The UMAP plot indicates that the studied samples are separated into two groups. Four DEGs were deleted due to incomplete characterizations. Among the 76 imported significant DEGs in CluePedia, 65 individuals including 36 isolated genes, 2 paired DEGs, and a main connected component of 27 nodes were recognized. The main connected component is shown in Figure 3. As depicted in Figure 3, there is only a single inhibition relationship between DUSP1 and HSPA1A while most connections are activation type of links between the presented nodes. The co-expression bond appears as a dominant bridge between the elements of the network. Since pathway analysis can provide useful information about the characterization of the studied

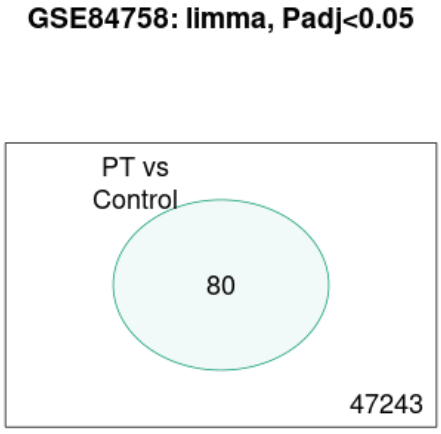

Figure 1. Venn Diagram; 80 significant DEGs among 47243 studied genes discriminate the studied groups

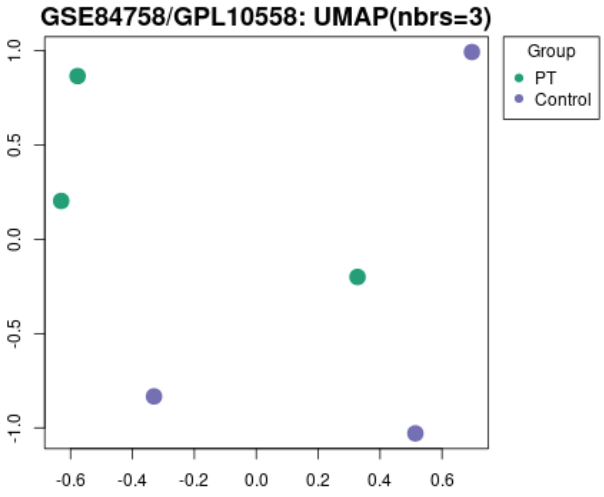

Figure 2. UMAP Plot; the two studied groups are separated by the introduced DEGs

genes, the results of the enrichment of the 76 significant DEGs via the KEGG database are presented in Figure 4. The determined five classes of biochemical pathways and the involved DEGs are shown in this figure. The IL-17 signaling pathway including five pathways appears as the main class of biological terms. The other four classes of the pathway ("Transcriptional misregulation in Cancer", PI3K-AKT signaling pathway", "MicroRNAs in cancers", and "MAPK signaling pathway") include a single term. To explore more details, we identified the common DEGs of two analyses (pathway analysis and regulatory examination), as shown in Table 1. The fold change and the related biochemical pathways of the common genes are also presented in Table 1. As it is depicted in Figure 5, RCAN1 which is a common gene is isolated from the other critical genes. Therefore, RCAN1 was ignored from the common genes and the remaining 15 DEGs were selected for more assessment.

Discussion

Evaluation of PDT efficacy in the treatment of cancers has attracted experts' attention.[12] Network analysis is a suitable method for discovering the molecular mechanism of the applied therapeutic procedures.[13] As it is shown in Figure 1, 80 genes are significantly affected by PDT in the treated cells. The affected genes can discriminate the treated cells from the control cells (see Figure 2). The present study aimed to determine the critical affected genes. Thus, among the 80 significant DEGs, 27 individuals were highlighted as the genes which are linked to each other

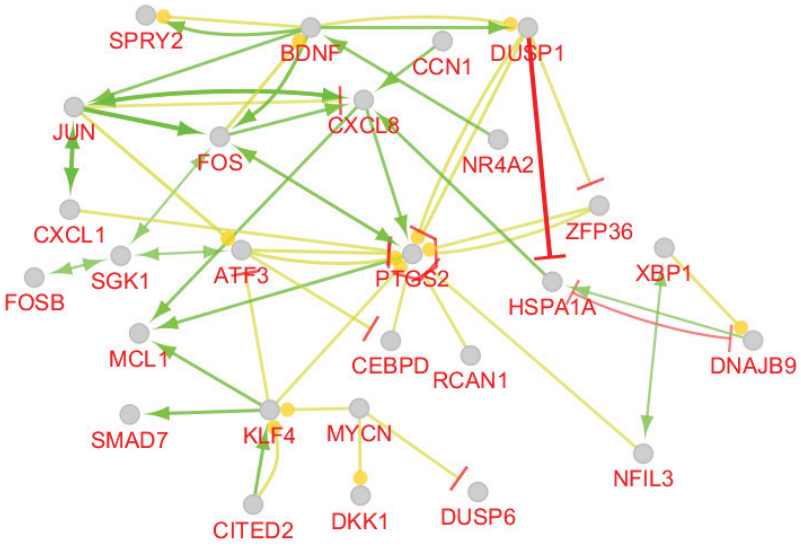

Figure 3. Regulatory Relationship Between the Main Connected Component of the Significant DEGs Network. Green, Red, and yellow refer to activation, inhibition, and coexpression relationships

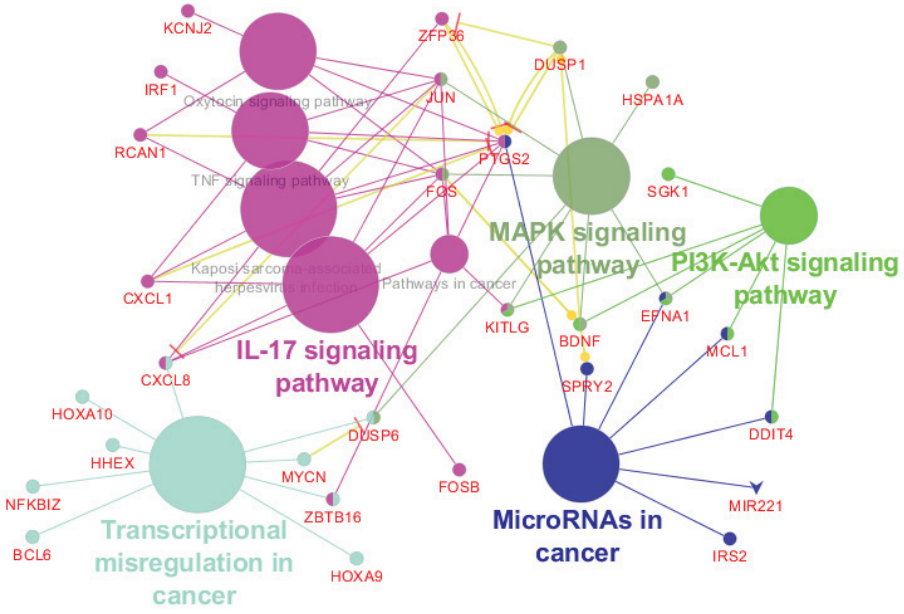

Figure 4. Pathways and the Related Significant DEGs. The identified DEGs are labeled with red

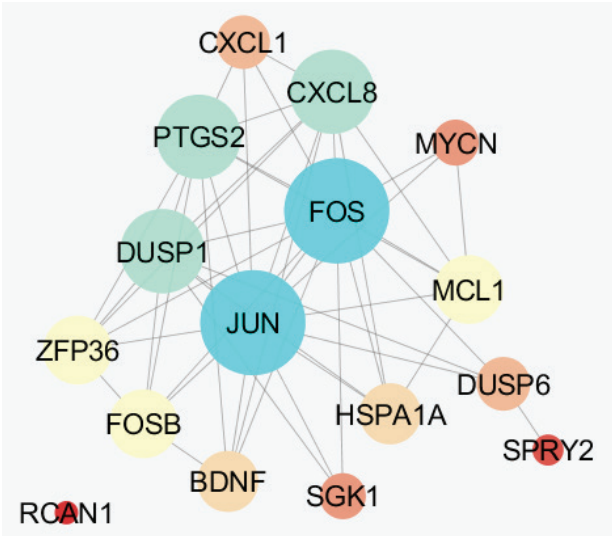

Figure 5. A Network Including the Common DEGs of Pathway Analysis and Regulatory Examination

Table 1. The Common Elements of Regulatory Analysis and Pathway Enrichment

Gene	LogFC	Related Pathways
SPRY2	1.784	MicroRNAs in cancers
MCL1	0.873	MicroRNAs in cancers PI3K-Akt signaling pathway
FOSB	1.616	IL-17 signaling pathway
BDNF	1.416	MAPK signaling pathway PI3K-Akt signaling pathway
DUSP1	1.984	MAPK signaling pathway
MYCN	0.924	Transcriptional misregulation in cancer
SGK1	2.009	PI3K-Akt signaling pathway
HSPA1A	1.342	MAPK signaling pathway
PTGS2	1.424	MicroRNAs in cancers IL-17 signaling pathway
DUSP6	0.875	Transcriptional misregulation in cancer MAPK signaling pathway
FOS	2.211	IL-17 signaling pathway MAPK signaling pathway
JUN	1.411	IL-17 signaling pathway MAPK signaling pathway
ZFP36	1.349	IL-17 signaling pathway
CXCL1	1.580	IL-17 signaling pathway
CXCL8	3.230	IL-17 signaling pathway Transcriptional misregulation in cancer
RCAN1	1.450	IL-17 signaling pathway

with regulatory links. Using regulatory relationships between genes has been reported in cancer studies by researchers to detect more details of the disease.[14] As it is shown in Figure 3, co-expression and activation are the two prominent actions between the studied genes. This finding refers to the positive relationship between the functions of the affected genes in PDT.

The introduced DEGs were assessed via gene ontology enrichment. The application of gene ontology, especially pathway analysis, to the assessment of PDT effects on cellular function has been reported by researchers.[15] As it is presented in Figure 4, 30 queried DEGs are related to the introduced biological terms. The IL-17 signaling pathway, including 5 biological terms, appears as the prominent class of the identified pathways. It has been reported that because of the early inflammatory response induced by PDT treatment, IL-17 expression increases.[16] A comparison between gene ontology findings and regulatory network analysis led to the introduction of 16 common DEGs. The connection between the 16 highlighted DEGs (see Figure 5) showed that, except for RCAN1, the other 15 genes were connected to each other. Since the IL-16 signaling pathway was introduced as the critical class of pathways, the genes which were connected to this pathway class were selected from among the 15 common DEGs and were determined as critical genes. As shown in Table 1, FOSB, PTGS2, FOS, JUN, ZFP36, CXCL1, and CXCL8 can be considered the critical genes in response to PDT. The role of HSPA1A can be important because it activates CXCL1. As shown in Figure 3, PTGS2, CXCL8, FOS, JUN, CXCL1, ZFP36, and FOSB are connected to 4, 3, 3, 3, 2, 1, and 0 of the critical genes respectively. It seems that prostaglandin-endoperoxide synthase 2 (PTGS2) is a crucial gene that is affected by PDT. The role of PTGS2 in promoting the growth and metastasis of cancers such as colorectal cancer and lung cancer is reported by researchers.[17,18] Investigations indicate that PDT causes alteration in the expression of PTGS2.[19] The co-expression of PTGS2, CXCL8, and FOS in coronary heart disease has been investigated and reported by Yang et al.[20] Considering the high value of fold change for the FOS gene and its activation by four neighbors, it can be concluded that the hyper-activation of FOS is a key point in the effect of PDT. CXCL8 is up-regulated extremely, and similar to FOS, it is activated by three neighbors. The role of FOS in the induction of apoptosis has been a well-known fact since many years ago.[21] The effect of PDT on the expression of JUN and FOS has been confirmed in the primary study about the molecular mechanism of PDT.[22]

Conclusion

In conclusion, PDT acts as a stimulator of gene expression and an activator of gene activity. Findings indicated that many genes are overexpressed and hyper-activated in treated cells by PDT. Analyses revealed that PDT induces more activity in PTGS2, CXCL8, FOS, JUN, CXCL1, ZFP36, and FOSB. The IL-17 signaling pathway appeared as the prominent chemical pathway in response to the application of PDT. The findings open new windows about the molecular mechanism of PDT. It can be suggested that, like PDT, the stimulation of the introduced critical gene by drugs be investigated to find a new possible protocol to fight against cancers which are not accessible to PDT.

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Authors' Contribution

Conceptualization: Babak Arjmand, Mostafa Rezaei Tavirani, Majid Rezaei Tavirani.
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Writing–review & editing: Babak Arjmand, Zahra Razzaghi, Majid Rezaei Tavirani.

Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

This project was approved by the ethical committee Shahid Beheshti University of Medical Sciences (ethical code: IR.SBMU.LASER.REC.1402.011).

References

1. Dougherty TJ, Gomer CJ, Henderson BW, Jori G, Kessel D, Korbelik M, et al. Photodynamic therapy. J Natl Cancer Inst. 1998;90(12):889-905. doi: 10.1093/jnci/90.12.889.
2. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, et al. Photodynamic therapy of cancer: an update. CA Cancer J Clin. 2011;61(4):250-81. doi: 10.3322/caac.20114.
3. Zhao B, He YY. Recent advances in the prevention and treatment of skin cancer using photodynamic therapy. Expert Rev Anticancer Ther. 2010;10(11):1797-809. doi: 10.1586/era.10.154.
4. Alzeibak R, Mishchenko TA, Shilyagina NY, Balalaeva IV, Vedunova MV, Krysko DV. Targeting immunogenic cancer cell death by photodynamic therapy: past, present and future. J Immunother Cancer. 2021;9(1):e001926. doi: 10.1136/jitc-2020-001926.
5. Ahmed A, Tait SWG. Targeting immunogenic cell death in cancer. Mol Oncol. 2020;14(12):2994-3006. doi: 10.1002/1878-0261.12851.
6. Bartusik-Aebisher D, Żołyniak A, Barnaś E, Machorowska-Pieniążek A, Oleś P, Kawczyk-Krupka A, et al. The use of photodynamic therapy in the treatment of brain tumors-a review of the literature. Molecules. 2022;27(20):6847. doi: 10.3390/molecules27206847.
7. Aniogo EC, Plackal Adimuriyil George B, Abrahamse H. The role of photodynamic therapy on multidrug resistant breast cancer. Cancer Cell Int. 2019;19:91. doi: 10.1186/s12935-019-0815-0.
8. Arjmand B, Safari-Alighiarloo N, Razzaghi M, Rezaei Tavirani M, Rostami Nejad M, Rezaei Tavirani M. Assessment of post-radiation time effect on gene expression profiles of *Saccharomyces cerevisiae* samples after appling a UV laser. J Lasers Med Sci. 2021;12:e91. doi: 10.34172/jlms.2021.91.
9. Rezaei-Tavirani M, Rezaei Tavirani M, Zamanian Azodi M, Moravvej Farshi H, Razzaghi M. Evaluation of skin response after erbium:yttrium-aluminum-garnet laser irradiation: a network analysis approach. J Lasers Med Sci. 2019;10(3):194-9. doi: 10.15171/jlms.2019.31.
10. Mansouri V, Arjmand B, Hamzeloo-Moghadam M, Rezaei Tavirani M, Razzaghi Z, Ahmadzadeh A, et al. Introducing critical genes in response to photodynamic therapy: a network analysis. J Lasers Med Sci. 2023;14:e25. doi: 10.34172/jlms.2023.25.
11. Weijer R, Clavier S, Zaal EA, Pijls MM, van Kooten RT, Vermaas K, et al. Multi-OMIC profiling of survival and metabolic signaling networks in cells subjected to photodynamic therapy. Cell Mol Life Sci. 2017;74(6):1133-51. doi: 10.1007/s00018-016-2401-0.
12. Shen Z, Ma Q, Zhou X, Zhang G, Hao G, Sun Y, et al. Strategies to improve photodynamic therapy efficacy by relieving the tumor hypoxia environment. NPG Asia Mater. 2021;13(1):39. doi: 10.1038/s41427-021-00303-1.
13. Kudatarkar N, Jalalpure S, Patil VS, Kurangi B. System biology and chemoinformatics approaches to decode the molecular mechanisms of chrysin against colon cancer. J Appl Pharm Sci. 2021;11(9):57-65. doi: 10.7324/japs.2021.110907.
14. Adabor ES, Acquaah-Mensah GK. Restricted-derestricted dynamic Bayesian network inference of transcriptional regulatory relationships among genes in cancer. Comput Biol Chem. 2019;79:155-64. doi: 10.1016/j.compbiolchem.2019.02.006.
15. Fang S, Wu Y, Zhang H, Zeng Q, Wang P, Zhang L, et al. Molecular characterization of gene expression changes in murine cutaneous squamous cell carcinoma after 5-aminolevulinic acid photodynamic therapy. Photodiagnosis Photodyn Ther. 2022;39:102907. doi: 10.1016/j.pdpdt.2022.102907.
16. Bernardini N, Skroza N, Tolino E, Mambrin A, Anzalone A, Balduzzi V, et al. IL-17 and its role in inflammatory, autoimmune, and oncological skin diseases: state of art. Int J Dermatol. 2020;59(4):406-11. doi: 10.1111/ijd.14695.
17. Wang D, Xia D, Dubois RN. The crosstalk of PTGS2 and EGF signaling pathways in colorectal cancer. Cancers (Basel). 2011;3(4):3894-908. doi: 10.3390/cancers3043894.
18. Rausch SM, Gonzalez BD, Clark MM, Patten C, Felten S, Liu H, et al. SNPs in PTGS2 and LTA predict pain and quality of life in long term lung cancer survivors. Lung Cancer. 2012;77(1):217-23. doi: 10.1016/j.lungcan.2012.02.017.
19. Broekgaarden M, Weijer R, Krekorian M, van den Ijssel B, Kos M, Alles LK, et al. Inhibition of hypoxia-inducible factor 1 with acriflavine sensitizes hypoxic tumor cells to photodynamic therapy with zinc phthalocyanine-encapsulating cationic liposomes. Nano Res. 2016;9(6):1639-62. doi: 10.1007/s12274-016-1059-0.
20. Yang G, Zhou S, He H, Shen Z, Liu Y, Hu J, et al. Exploring the "gene-protein-metabolite" network of coronary heart disease with phlegm and blood stasis syndrome by integrated multi-omics strategy. Front Pharmacol. 2022;13:1022627. doi: 10.3389/fphar.2022.1022627.
21. Preston GA, Lyon TT, Yin Y, Lang JE, Solomon G, Annab L, et al. Induction of apoptosis by c-Fos protein. Mol Cell Biol. 1996;16(1):211-8. doi: 10.1128/mcb.16.1.211.
22. Kick G, Messer G, Plewig G, Kind P, Goetz AE. Strong and prolonged induction of c-Jun and c-Fos proto-oncogenes by photodynamic therapy. Br J Cancer. 1996;74(1):30-6. doi: 10.1038/bjc.1996.311.