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Assessment of Photosensitizer Concentration Effects on the Efficacy of Photodynamic Therapy

Reza Vafaee[1*], Babak Arjmand[2], Maryam Hamzeloo-Moghadam[3], Mostafa Rezaei Tavirani[4], Zahra Razzaghi[1], Reza M Robati[5], Mitra Rezaei[6,7], Fatemeh Montazer[8]

[1]Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[3]Traditional Medicine and Materia Medica Research Center, School of Traditional Medicine Shahid, Beheshti University of Medical Sciences, Tehran, Iran
[4]Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[5]Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[6]Genomic Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[7]Clinical Tuberculosis and Epidemiology Research Center. National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
[8]Department of Pathology, Firoozabadi Hospital, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

***Correspondence to**
Reza Vafaee,
Email: vafaeereza@gmail.com

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Abstract

Introduction: Photodynamic therapy (PDT) is an attractive approach in medicine. Due to its noninvasive nature and low side effects, PDT has been developed quickly. In the present study, the gene expression profiles of the human cell line that was treated via PDT in the sub-lethal concentration (LC50) and super-lethal concentration (LC90) of a photosensitizer (PS) from Gene Expression Omnibus (GEO) were extracted and the common differentially expressed genes (DEGs) were investigated.

Methods: The gene expression profiles of the treated cells were compared with a control, and the common DEGs were determined. The common DEGs were assessed via protein-protein interaction (PPI) network analysis, and gene ontology enrichment was evaluated. The related biological terms for the common genes were identified.

Results: Ninety-four common DEGs were selected to be analyzed. It appeared that the activation and increment of gene expression were prominent processes. Jun, Dusp1, Atf4, and Atf3 as four critical genes were highlighted. "Chromosomal and microsatellite instability in colorectal cancer" was identified as the main class of biological terms related to the assessed DEGs.

Conclusion: The major molecular events which happened in both analyses indicated that PDT, independent from the concentration of PS, induced gross molecular changes such as the upregulation of Jun and Dusp1.

Keywords: Photodynamic therapy; Pathway analysis; Gene expression change; Gene ontology; Network analysis.

Introduction

The photochemical-based treatment approach is known as photodynamic therapy (PDT). Light and photosensitizer (PS) (a light activated chemical compound) are two elements of PDT. The applied PSs are the photosensitizing chemicals that specially accumulate in target tumor cells relative to the normal cells.[1] Due to its low side effects, high medication rate, and noninvasive nature, PDT can be a suitable substitution for conventional cancer treatment.[2] It is suggested that PDT is a useful treatment modality in various medical fields such as dentistry, oncology, pneumology, immunology, cardiology, urology, ophthalmology, dermatology, and ophthalmology.[3] Investigations emphasize the efficacy of PDT against cancers. It is reported that PDT is used effectively in unworkable cutaneous squamous cell carcinoma treatment.[4]

Gene expression analysis is a usual method for evaluating the molecular mechanism of the therapeutic approaches. During treatment expression, many genes alter in a certain pattern. The gene expression approach is applied to assess PDT.[5] The genomic investigations

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produce some informative findings about a large number of genes that are differentially expressed. Such numerous genes should be evaluated via proper methods to explore the critical individuals.[6,7]

Protein-protein interaction (PPI) network analysis is a well-known method that is used to evaluate the gene sets or proteomes. In this approach, the genes participate in network formation. Each gene can be characterized based on the roles it plays in the network. The molecular mechanism of the effect of many diseases and drugs on the body has been investigated via PPI network analysis by experts.[8-10] Gene ontology is a widespread tool to find the terms which are associated with the gene. Gene ontology is used to explore the results of PDT effects on human tumor cells.[11,12] Pathway analysis is an approach which is applied to detect the affected pathway in the investigated biological and medical samples. Tim et al investigated the effects of low-level laser therapy on the treated rat and reported the related pathways via pathway analysis.[13] In the present study, the gene expression profiles of the cells which were treated via PDT in the presence of sublethal dose (LC50) and super lethal dose (LC90) concentrations of PS were downloaded from GEO and evaluated by PPI network analysis and gene ontology to detect the critical genes and pathways.

Methods

The gene expression profiles of the RAW 264.7 cell line were extracted from GSE84758 (Platform GPL6887) of GEO. GSM2249783-GSM2249794 were selected to be analyzed by GEO2R. The studied cells were distributed in 4 groups including control, dark toxicity (DT), sublethal dose (LC50), and super lethal dose (LC90). The cells receiving zinc phthalocyanine PS that was encapsulated in cationic liposomes (ZPCLs) without laser radiation were nominated as the DT group. The cells that received LC50 and LC90 concentrations of ZPCLs and were 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 were pointed as LC50 and LC90 groups respectively.[14] Since there were no differences between control and DT groups, the differentially expressed genes (DEGs) that discriminate LC50 and LC90 groups from control cells were identified separately. The common DEGs of the two analyses were determined as the DEGs which are independent of ZPCLs concentrations.

The common DEGs were evaluated via PPI network analysis by using the STRING database and Cytoscape software v 3.7.2. The network was analyzed by a "Network Analyzer", and the main connected component of the network was selected for more investigation. Elements of the main connected component were assessed via CluePedia v1.5.7 application of Cytoscape to find regulatory relationships between the queried genes. The related biological terms for the nodes of the main connected component were derived from ClueGO 2.5.7. The biological processes, molecular functions, and biochemical pathways were extracted from GO_BiologicalProcess-EBI-UniProt-GOA-ACAP-ARAP,GO_MolecularFunction-EBI-UniProt-GOA-ACAP-ARAP , WikiPathways, and KEGG updated in 08.05.2020. The statistical analysis of the examination was based on the following criteria:

Term p-value: 0.01

Group p-value: 0.01

Correction test: Bonferroni step-down

Results

The analysis of the gene expression profiles of the DT group and the control group revealed that there were no differences between the two assessed groups. The box plot presentation of the gene expression profiles of LC50 versus the control group and LC90 against the control group are presented in Figure 1. As depicted in Figure 1, there are many significant DEGs that discriminate the

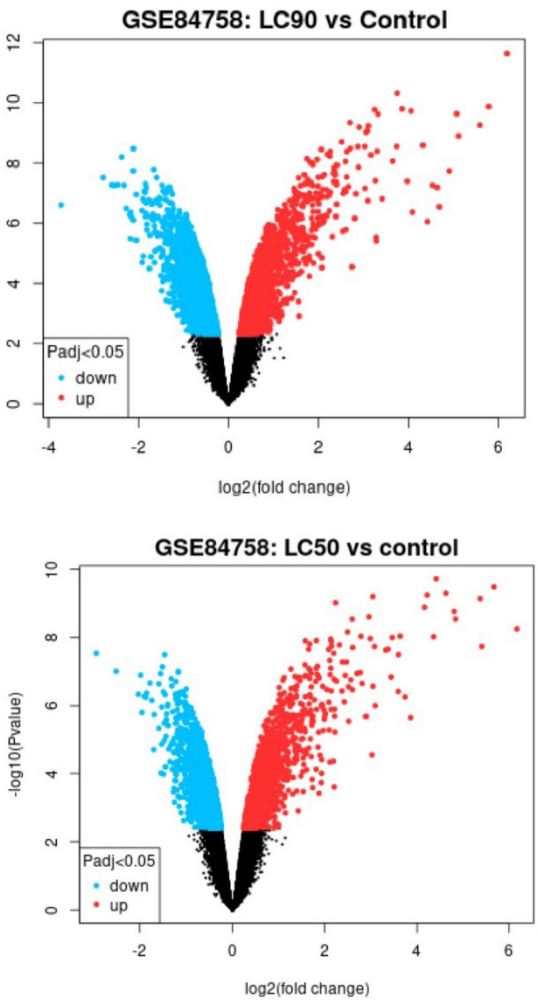

Figure 1. Box Plot Presentation of the Compared Gene Expression Profiles; up): LC90 versus the control group and down): LC50 against the control group. The up and down-regulated genes are colored in blue and red respectively

irradiated cells by a laser in the presence of ZPCLs (two LC50 and LC90 concentrations) from the control cells. The gene expression profiles of LC50 and LC90 groups are discriminated from controls by 211 and 206 DEGs, respectively. Ninety-four common DEGs were determined for the two analyses.

Among the 94 queried common DEGs, 90 individuals were recognized by Cytoscape. The PPI network including 31 isolated nodes, a triple sub-network, and a main connected component was constructed. The 56 nodes of the main connected component were included in CluePedia to find expression, activation, and inhibition relationships between the genes. Twenty-five genes were isolated and two individuals were paired. Two triple and tetrad sub-networks were also formed. The main connected component is shown in Figure 2.

The elements of the main connected component were assessed via gene ontology enrichment. As shown in Table 1, 124 biological terms, which are classified into 10 groups, are determined. The groups of 1-10 contains 1, 1, 2, 2, 2, 3, 6, 14, 14, and 79 biological terms, respectively. The percentage of terms per group of biological terms is shown in Figure 3. To find the critical genes that are related to at least 10 neighbors are shown in Figure 3. Connections of the largest group of biological terms with the associated genes are presented in Figure 3.

Discussion

There are many investigations about PDT that are related to the GEO database. Each research activity led to the exploration of a small part of a puzzle that can be named related molecular events of PDT.[15] As shown in Figure 1, there are many significant DEGs that discriminate the treated cells by PDT (in LC50 and LC90 groups) from control individuals. However, both analyses were characterized by about 200 DEGs, but the common DEGs which appeared in the two performed assessments were 94.

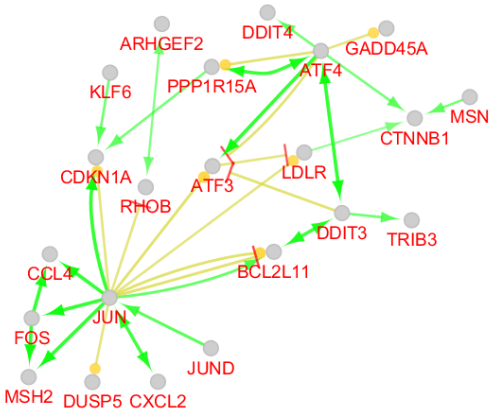

Figure 2. The Main Sub-network of the Action Map Related to the Elements of the Main Connected Component of the PPI Network. Green and yellow refer to activation and expression. Round and bar tips of expression edges refer to up and down-regulation respectively. A Kappa score of 0.4 is considered

It can be concluded that about 50% of DEGs are common in both analyses. This finding indicates that about 50% of PDT effects are independent of PS concentration. Although there are many documents about the role of PS concentrations in the efficacy of PDT,[16] the common property of PSs is a less studied subject. The top five up and down-regulated genes for LC90 analysis are (Dusp1, Cxcl2, Jun, Atf3, and Fos) and (Dido1, Arhgef2, Itga4, Tpm4, and Fdps) respectively. The similar individuals for LC50 analysis are (Cxcl2, Dusp1, Jun, and Rgs1, and Atf3) and (Tmem219, Lpl, Ldlr, Tpm4, Fdps). It can be concluded that Dusp1, Cxcl2, and Jun are the top common up-regulated genes in both analyses. Tpm4 and Fdps appear as the top common down-regulated genes. Jun and Dusp1 are ranked as the first and seventh hubs. The involvement of c-Jun in PDT was discussed in 1996.[17] The up-regulation of Dusp1 due to PDT was reported by Wild et al.[18] Jun is highlighted as an important member of the action map (see Figure 2). Jun activates directly 6 genes and controls the expression of 6 individuals. As depicted in the figure, relationships between genes are mostly of the activation type and no inhibition is seen. It has been pointed out that after PDT treatment, the gene expression of the assessed genes increases.[19]

The results of gene ontology enrichment indicate that there are 10 groups of biological terms that are related to the selected DEGs (see Table 1 and Figure 3). The largest group of biological terms is "Chromosomal and microsatellite instability in colorectal cancer". This group contains 63.71% of the introduced biological terms. Three main groups of molecular alterations which occur in colorectal cancer are introduced as microsatellite instability, chromosomal instability, and CpG island methylator phenotype.[20] It seems that "Chromosomal and microsatellite instability in colorectal cancer" is the main affected cluster after application of PDT in presence of both concentrations of PS.

The prominent genes that were associated with the more biological term (especially with the term of group 10) are presented in Figure 4. 16 DEGs are shown in this figure while Jun and Dusp1 are highlighted. The down-regulation of Dusp1 in advanced carcinomas has been reported by researchers.[21] Atf4 is another pointed gen in the action map which is in regulatory contact with several genes. Atf3 is a bridge between Atf4 and Jun. Atf4 is introduced as a principal regulator to control the transcription of key essential genes which are responsible for adaptative functions.[22] It can be concluded that the core genes in PDT are common and are independent of PS concentration. However, the concentration of PS is required for treatment and it should be determined by experts.[23]

Conclusion

In conclusion, significant molecular events occur in PDT

Table 1. Clusters of Biological Terms Related to Nodes of Main Connected Component Sub-network

Group	Gene Ontology Terms	Associated Genes Found
1	Regulation of the extrinsic apoptotic signaling pathway via death domain receptors	[ARHGEF2, ATF3, PMAIP1]
2	Spinal cord injury	[CXCL2, FOS, GADD45A, RHOB, ZFP36]
3	Negative regulation of viral transcription	[CCL4, JUN, ZFP36]
	Regulation of viral transcription	[CCL4, JUN, ZFP36]
4	Endothelial cell development	[CTNNB1, MSN, S1PR2]
	Establishment of endothelial barrier	[CTNNB1, MSN, S1PR2]
5	Cellular response to corticosteroid stimulus	[BCL2L11, DDIT4, ZFP36]
	Cellular response to glucocorticoid stimulus	[BCL2L11, DDIT4, ZFP36]
6	Amphetamine addiction	[ARC, ATF4, FOS, JUN]
	Myometrial relaxation and contraction pathways	[ATF3, ATF4, FOS, JUN, MAFF, RGS1, RGS2]
	Skeletal muscle cell differentiation	[ATF3, FOS, MAFF]
7	p53 signaling pathway	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	miRNA regulation of DNA damage response	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	TP53 network	[CDKN1A, GADD45A, PMAIP1]
	DNA damage response	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	Intrinsic apoptotic signaling pathway by p53 class mediator	[CDKN1A, DDIT4, MSH2, PMAIP1]
	Intrinsic apoptotic signaling pathway in response to DNA damage by p53 class mediator	[CDKN1A, DDIT4, MSH2]
8	Glioma	[CDKN1A, GADD45A, GADD45G]
	Melanoma	[CDKN1A, GADD45A, GADD45G]
	Non-small cell lung cancer	[CDKN1A, GADD45A, GADD45G]
	TP53 network	[CDKN1A, GADD45A, PMAIP1]
	ATM signaling pathway	[CDKN1A, GADD45A, JUN]
	Non-small cell lung cancer	[CDKN1A, GADD45A, GADD45G]
	Genotoxicity pathway	[CDKN1A, GADD45A, PLK3]
	Mitotic cell cycle arrest	[CDKN1A, DUSP1, GADD45A]
	Regulation of cyclin-dependent protein kinase activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Regulation Of Cyclin-Dependent Protein Serine/Threonine Kinase Activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Kinase inhibitor activity	[CDKN1A, HEXIM1, TRIB3]
	Protein kinase inhibitor activity	[CDKN1A, HEXIM1, TRIB3]
	Cyclin-dependent protein serine/threonine kinase regulator activity	[CCNO, CCNT2, CDKN1A, HEXIM1]
	Negative regulation of protein serine/threonine kinase activity	[CDKN1A, DUSP1, DUSP16, DUSP5, GADD45A, HEXIM1, RGS2]
9	Inactivation of MAPK activity	[DUSP1, DUSP16, DUSP5]
	MAP kinase phosphatase activity	[DUSP1, DUSP16, DUSP5]
	Endoderm development	[ARC, CTNNB1, DUSP1, DUSP5]
	Mitotic cell cycle arrest	[CDKN1A, DUSP1, GADD45A]
	Endoderm formation	[CTNNB1, DUSP1, DUSP5]
	Mitogen-activated protein kinase binding	[DUSP1, DUSP16, DUSP5]
	Regulation of cyclin-dependent protein kinase activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Peptidyl-threonine dephosphorylation	[DUSP1, DUSP16, DUSP5]
	Regulation of cyclin-dependent protein serine/threonine kinase activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Protein tyrosine/serine/threonine phosphatase activity	[DUSP1, DUSP16, DUSP5]
	Protein tyrosine/threonine phosphatase activity	[DUSP1, DUSP16, DUSP5]
	Negative regulation of MAP kinase activity	[DUSP1, DUSP16, DUSP5, RGS2]
	Negative regulation of protein serine/threonine kinase activity	[CDKN1A, DUSP1, DUSP16, DUSP5, GADD45A, HEXIM1, RGS2]
	Peptidyl-tyrosine dephosphorylation involved in the inactivation of protein kinase activity	[DUSP1, DUSP16, DUSP5]

Table 1. Continued

Group	Gene Ontology Terms	Associated Genes Found
	FoxO signaling pathway	[BCL2L11, CDKN1A, GADD45A, GADD45G, PLK2, PLK3]
	p53 signaling pathway	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	Apoptosis	[ATF4, BCL2L11, DDIT3, FOS, GADD45A, GADD45G, JUN, PMAIP1]
	IL-17 signaling pathway	[CXCL2, FOS, JUN, JUND]
	Amphetamine addiction	[ARC, ATF4, FOS, JUN]
	Colorectal cancer	[BCL2L11, CDKN1A, CTNNB1, FOS, GADD45A, GADD45G, JUN, MSH2, PMAIP1]
	Endometrial cancer	[CDKN1A, CTNNB1, GADD45A, GADD45G]
	Glioma	[CDKN1A, GADD45A, GADD45G]
	Thyroid cancer	[CDKN1A, CTNNB1, GADD45A, GADD45G]
	Basal cell carcinoma	[CDKN1A, CTNNB1, GADD45A, GADD45G]
	Melanoma	[CDKN1A, GADD45A, GADD45G]
	Non-small cell lung cancer	[CDKN1A, GADD45A, GADD45G]
	Breast cancer	[CDKN1A, CTNNB1, FOS, GADD45A, GADD45G, JUN]
	Androgen receptor signaling pathway	[CDKN1A, CTNNB1, JUN, RHOB]
	miRNA regulation of DNA damage response	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	TP53 network	[CDKN1A, GADD45A, PMAIP1]
	Apoptosis modulation and signaling	[BCL2L11, FOS, JUN, PMAIP1]
	Adipogenesis	[CDKN1A, CTNNB1, DDIT3, GADD45A, KLF6, TRIB3]
	Oncostatin M signaling pathway	[FOS, JUND, LDLR]
	ATM signaling pathway	[CDKN1A, GADD45A, JUN]
	Myometrial relaxation and contraction pathways	[ATF3, ATF4, FOS, JUN, MAFF, RGS1, RGS2]
	Photodynamic therapy-induced AP-1 survival signaling.	[BCL2L11, CDKN1A, FOS, JUN]
	Photodynamic therapy-induced unfolded protein response	[ATF3, ATF4, BCL2L11, DDIT3, DNAJB9, PPP1R15A, TRIB3]
	TGF-beta Signaling Pathway	[ATF3, CDKN1A, FOS, JUN, JUND, KLF6]
	Association between physico-chemical features and toxicity associated pathways	[CDKN1A, CTNNB1, JUN]
	Endometrial cancer	[CDKN1A, CTNNB1, FOS, GADD45A, GADD45G]
	Chromosomal and microsatellite instability in colorectal cancer	[BCL2L11, CDKN1A, CTNNB1, FOS, GADD45A, GADD45G, JUN, MSH2, PMAIP1]
	Non-small cell lung cancer	[CDKN1A, GADD45A, GADD45G]
	Genotoxicity pathway	[CDKN1A, GADD45A, PLK3]
	Gastrin Signaling Pathway	[CDKN1A, CTNNB1, FOS, JUN, RHOB]
	Melanoma	[CDKN1A, FOS, GADD45A, GADD45G]
	Host-pathogen interaction of human coronaviruses – ER stress	[ATF4, DDIT3, PPP1R15A]
	Host-pathogen interaction of human coronaviruses – MAPK signaling	[DDIT3, FOS, JUN]
	TGF-beta Receptor Signaling	[CTNNB1, FOS, JUN]
	DNA Damage Response	[CDKN1A, GADD45A, GADD45G, PMAIP1]
	DNA Damage Response (only ATM dependent)	[BCL2L11, CDKN1A, CTNNB1, JUN, LDLR, PMAIP1]
	Response to arsenic-containing substance	[ATF3, ATF4, CDKN1A]
	ER-nucleus signaling pathway	[ATF3, ATF4, BCL2L11, DDIT3, PPP1R15A]
10	Mitotic cell cycle checkpoint	[CDKN1A, DUSP1, GADD45A, INTS3, MSH2, PLK2, PLK3, TOPBP1]
	Endoplasmic reticulum unfolded protein response	[ATF3, ATF4, BCL2L11, DDIT3, DNAJB9, PPP1R15A]
	Signal transduction involved in cell cycle checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Positive regulation of neuron death	[ATF4, BCL2L11, CTNNB1, DDIT3, DDIT4, FOS, JUN]
	Regulation of response to endoplasmic reticulum stress	[BCL2L11, DDIT3, DNAJB9, PMAIP1, PPP1R15A]
	Positive regulation of response to endoplasmic reticulum stress	[BCL2L11, DDIT3, PMAIP1, PPP1R15A]
	Response to amino acid starvation	[ATF3, ATF4, CDKN1A]
	Release of cytochrome c from mitochondria	[BCL2L11, JUN, PMAIP1]
	Mitotic cell cycle arrest	[CDKN1A, DUSP1, GADD45A]
	RNA polymerase II activating transcription factor binding	[CTNNB1, FOS, JUN]
	DNA damage response, signal transduction by p53 class mediator	[CDKN1A, GADD45A, PLK2, PLK3, PMAIP1]
	Cellular response to amino acid starvation	[ATF3, ATF4, CDKN1A]
	PERK-mediated unfolded protein response	[ATF3, ATF4, DDIT3, PPP1R15A]
	Mitotic DNA integrity checkpoint	[CDKN1A, GADD45A, MSH2, PLK2, PLK3, TOPBP1]
	Intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	[ATF4, BCL2L11, DDIT3, PMAIP1, PPP1R15A, TRIB3]
	Positive regulation of cell cycle arrest	[CDKN1A, GADD45A, PLK2, PLK3]
	Intrinsic apoptotic signaling pathway by p53 class mediator	[CDKN1A, DDIT4, MSH2, PMAIP1]
	Signal transduction involved in the DNA integrity checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Signal transduction involved in the mitotic cell cycle checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Regulation of endoplasmic reticulum unfolded protein response	[BCL2L11, DNAJB9, PPP1R15A]
	Positive regulation of the neuron apoptotic process	[ATF4, BCL2L11, CTNNB1, DDIT3, JUN]
	Mitotic DNA damage checkpoint	[CDKN1A, GADD45A, MSH2, PLK2, PLK3, TOPBP1]
	G1 DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Mitotic G1/S transition checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Signal transduction involved in the DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Regulation of the endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway	[BCL2L11, DDIT3, PMAIP1]
	Positive regulation of the endoplasmic reticulum stress-induced intrinsic apoptotic signaling pathway	[BCL2L11, DDIT3, PMAIP1]
	Signal transduction involved in the mitotic DNA integrity checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Regulation of cyclin-dependent protein kinase activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Positive regulation of the intrinsic apoptotic signaling pathway	[BCL2L11, DDIT3, PMAIP1]
	Regulation of cyclin-dependent protein serine/threonine kinase activity	[CCNO, CCNT2, CDKN1A, GADD45A, HEXIM1]
	Mitotic G1 DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Intracellular signal transduction involved in the G1 DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Signal transduction involved in the mitotic DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	p38MAPK cascade	[DUSP1, GADD45A, GADD45G, ZFP36]
	DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	[CDKN1A, GADD45A, PLK2, PLK3]
	Signal transduction involved in the mitotic G1 DNA damage checkpoint	[CDKN1A, GADD45A, PLK2, PLK3]
	Regulation of the p38MAPK cascade	[DUSP1, GADD45A, GADD45G]
	Positive regulation of the transcription from RNA polymerase II promoter in response to stress	[ATF3, ATF4, DDIT3]
	NEGATIVE regulation of protein serine/threonine kinase activity	[CDKN1A, DUSP1, DUSP16, DUSP5, GADD45A, HEXIM1, RGS2]
	Positive regulation of transcription from RNA polymerase II promoter in response to endoplasmic reticulum stress	[ATF3, ATF4, DDIT3]

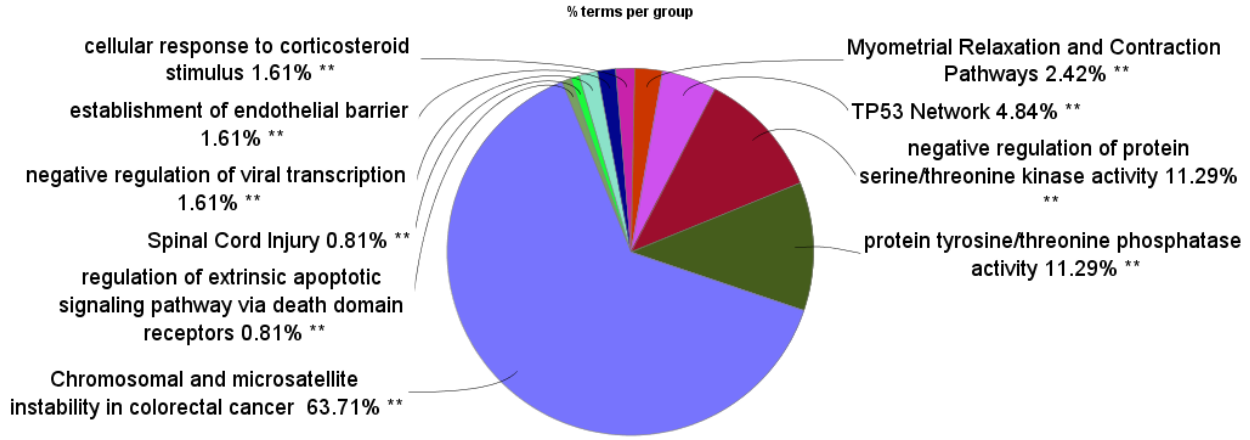

Figure 3. The Percentage of Terms Per Group of Terms for 9 Clusters of Related Biological Terms of the Elements of the Main Connected Component of the PPI Network

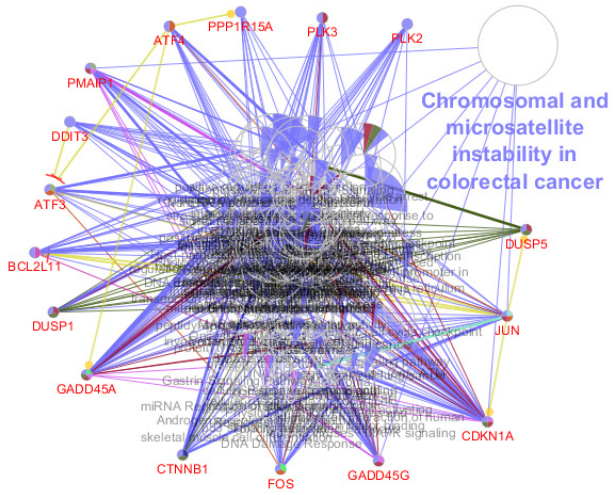

Figure 4. The main genes (labeled in red color) that are related to the biological terms. The genes with at least 10 connections are selected. The biological terms are backgrounded while the main class of terms (Chromosomal and microsatellite instability in colorectal cancer) is highlighted

in the presence of the LC50 and LC90 concentrations of PS. The up-regulation of Jun, Dusp1, Atf4, and Atf3 is a prominent process in PDT application. Gross molecular changes under the title of "Chromosomal and microsatellite instability in colorectal cancer" are presented. The findings provide a new perspective on the molecular mechanism of PDT. It can be suggested that the application of the LC50 concentration of PS in clinical approaches is a suitable manner, and more concentrations of PS are not needed. However, more investigations are required to find the optimum condition for applying PDT.

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Authors' Contribution

Conceptualization: Reza Vafaee, Mostafa Rezaei Tavirani.
Data curation: Babak Arjmand, Maryam Hamzeloo-Moghadam, Reza Vafaee.
Formal analysis: Maryam Hamzeloo-Moghadam, Zahra Razzaghi.
Funding acquisition: Reza Vafaee.
Investigation: Babak Arjmand, Maryam Hamzeloo-Moghadam, Fatemeh Montazer.
Methodology: Mostafa Rezaei Tavirani, Zahra Razzaghi.
Project administration: Babak Arjmand, Maryam Hamzeloo-Moghadam, Mitra Rezaei, Fatemeh Montazer.
Resources: Mitra Rezaei, Fatemeh Montazer.
Software: Babak Arjmand, Mostafa Rezaei Tavirani.
Supervision: Reza M Robati, Mitra Rezaei, Mostafa Rezaei Tavirani.
Validation: Zahra Razzaghi.
Visualization: Mostafa Rezaei Tavirani.
Writing–original draft: Reza Vafaee, Mostafa Rezaei Tavirani.
Writing–review & editing: Reza Vafaee, Babak Arjmand, Maryam Hamzeloo-Moghadam, Mostafa Rezaei Tavirani, Zahra Razzaghi, Reza M Robati, Mitra Rezaei, Fatemeh Montazer.

Competing Interests

The authors declare that they have no conflict of interest.

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