



# Gene Ontology Assessment of Indirect Cold Physical Plasma and UV-Radiation Molecular Mechanism at the Cellular Level

Zahra Razzaghi<sup>1</sup>, Babak Arjmand<sup>2,3</sup>, Maryam Hamzeloo-Moghadam<sup>4</sup>, Mostafa Rezaei Tavarani<sup>5</sup>

<sup>1</sup>Laser Application in Medical Sciences Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>2</sup>Cell Therapy and Regenerative Medicine Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

<sup>3</sup>Iranian Cancer Control Center (MACSA), Tehran, Iran

<sup>4</sup>Traditional Medicine and Materia Medica Research Center, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>5</sup>Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

\*Correspondence to  
Mostafa Rezaei Tavarani,  
Email: [tavarany@yahoo.com](mailto:tavarany@yahoo.com)

Received: December 12, 2022

Accepted: February 12, 2023

Published online April 4, 2023

## Abstract

**Introduction:** The development of therapeutic methods implies an understanding of the molecular mechanism of the applied methods. Due to the widespread use of UV radiation and cold physical plasma in medicine, the molecular mechanism of these two methods is compared via gene ontology.

**Methods:** Data were derived from Gene Expression Omnibus (GEO). The differentially expressed genes (DEGs) which discriminate the cells treated with UV radiation versus indirect cold physical plasma were analyzed via gene ontology enrichment. The related biochemical pathways were extracted from the "Kyoto Encyclopedia of Genes and Genomes" (KEGG).

**Results:** Among the 152 queried DEGs, 18 critical genes including SOC1, LDLR, ALO5, PTGS2, TNF, JUNB, TNFRSF1A, CD40, SMAD7, ID1, SMAD6, SERPINE1, PMAIP1, MDM2, CREB5, GADD45A, E2F3, and ETV5 were highlighted as the genes that victimize the two methods.

**Conclusion:** NOTCH1 and TNF as the main genes plus SERPINE1, KLF, and BDNF were introduced as the significant genes that are involved in the processes which discriminate cold physical plasma administration and UV-radiation as the two evaluated therapeutic methods.

**Keywords:** Chemical pathway analysis; Cold physical plasma; UV-radiation; Gene expression change; Gene ontology.



## Introduction

The promotion of therapeutical methods in medicine, especially in fighting against cancers, is the main aim of experts. Radiation is a field that has developed and progressed quickly in recent years. Using different sources of radiation in medicine has been raised, so the varieties of lasers, UV producers, and cold physical plasma sources are common tools in clinics. In this regard, therapeutic procedures have become more efficient.<sup>1-5</sup>

Environmental ultraviolet radiation is tied to human life. It is counted as a risk factor for skin cancer, probably via the oxidative stress mechanism.<sup>6</sup> On the other hand, it is a therapeutic tool that has attracted experts' attention. Laikova et al have published a document which pointed to anticancer drugs such as mutations, UV-radiation, and antisense oligonucleotides.<sup>7</sup>

Non-thermal atmospheric pressure plasma is a partly ionized gas comprising moderately slow ions and fast

electrons.<sup>8</sup> Tabares and Junkar published a review about the application of cold physical plasma in medicine. The authors emphasized the application of cold physical plasma for surface treatments.<sup>9</sup> Also, Harley et al have discussed the challenges of physical plasma application as an anticancer agent.<sup>10</sup> As it has been reported, reactive oxygen and nitrogen species is introduced as the key suppliers for the efficacy of cold atmospheric pressure plasma against cancer cells.<sup>11</sup>

The molecular mechanism detection of anticancer drugs is an important activity that is performed continuously. Therefore, there are many reports about the genomic response of living organisms to anticancer drugs and the applied radiations. The findings are presented as a large set of differentially expressed genes (DEGs) which discriminate the irradiated samples from the control individuals.<sup>12,13</sup> Gene ontology and pathway analysis can be applied to explore the prominent roles of

the assessed genes. Onik et al have used gene ontology to find the mechanism by which UV-radiation causes the quality promotion of early ripening apple fruit.<sup>14</sup> The Kyoto Encyclopedia of Genes and Genomes” (KEGG) database (<https://www.kegg.jp>) integrates various biological items branded into system, genomic, chemical and health information. KEGG is a suitable source of biochemical pathways.<sup>15</sup> In the present study, the gene expression profiles of human melanocytes exposed to indirect cold physical plasma versus the cells irradiated by UV radiation were downloaded from GEO and analyzed via gene ontology to find the different molecular mechanisms of the two compared methods.

## Methods

Genomics data were downloaded from Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157824>). As it is described in the recorded data, the gene expression changes of human skin melanocytes under indirect cold physical plasma and UV radiation were investigated to find the impact of cold atmospheric plasma on the treated cells. Microarray analysis was done after 2-hour incubation. GSM4775512-GSM4775517 as the samples exposed to the indirect cold physical plasma and GSM4775518-GSM4775521 as the UV-irradiated cells were selected to be examined.

The 250 top significant DEGs were downloaded by the GEO2R program. Since all gene expression amounts were characterized by  $P$  value  $< 0.001$  and fold-change  $> 2$ , the selected 250 DEGs were considered for more analysis. The detected spots were characterized by spot ID, and then the name of genes was mined from “Expression Atlas” (<https://www.ebi.ac.uk/gxa/home>). Among the queried 250 DEGs, 152 individuals were deciphered.

To evaluate the relationship between the  $P$  value and the fold change, we assessed the gene expression profiles of the cells which were exposed to indirect cold physical plasma versus the UV-irradiated cells via volcano plot analysis.

Since the CluePedia application of Cytoscape software enables researchers to provide a regulatory relationship between the queried DEGs, the decoded genes were included in CluePedia. Activation, inhibition, and expression actions were selected as the directed edges between the evaluated nodes of the constructed network. The isolated DEGs were deleted and the remained DEGs that were distributed in different clusters were analyzed.

Related biochemical pathways for the decoded genes were searched via ClueGO version 2.5.7 from KEGG\_08.05.2020. The biochemical pathways were grouped based on the kappa score.

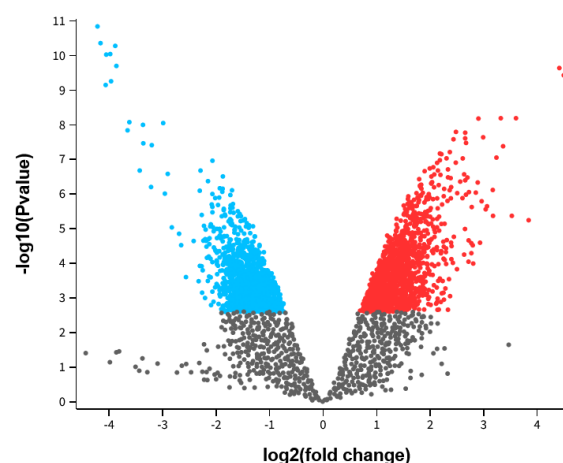
## Results

To assess the pattern of gene expression, we evaluated the data by the volcano plot (see Figure 1). Displaying

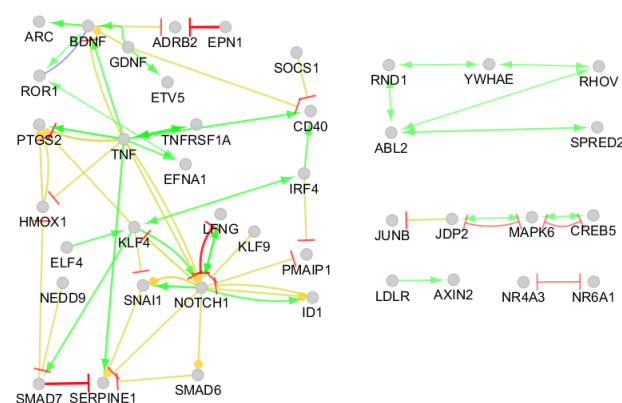
an unstandardized signal such as the log (fold change) versus the noise-adjusted/standardized signal is known as the volcano plot.<sup>16</sup> 152 DEGs including 39 down-regulated and 113 up-regulated genes were decoded. The 152 queried DEGs were included in the CluePedia application of Cytoscape software to determine regulatory relationships between the assessed genes. 148 DEGs were recognized by CluePedia. Among the recognized DEGs, 108 genes remained isolated and 4 individuals were paired. All the clusters of the DEGs without the isolated individuals are shown in Figure 2. Inhibition, activation, and expression relationships between the evaluated genes are shown in this figure.

To access the related biochemical pathways, we included 152 decoded DEGs in ClueGO. Under the  $P$  value  $< 0.05$  and “Medium Network Specificity” conditions, 5 groups

**Volcano plot**  
**GSE157824: Comparison of gene expression**  
**signatures in human...**  
**Plasma vs UV, Padj<0.05**



**Figure 1.** The Volcano Plot of the Gene Expression Profiles of the Exposed Cells to Indirect Cold Physical Plasma Versus UV-Irradiated Cells. The colored dots refer to up- and down-regulated genes



**Figure 2.** Regulatory Relationships Between the Queried 152 Decoded Genes (148 Recognized Genes). 108 isolated DEGs are deleted. Green, red, and yellow refer to activation, inhibition, and expression actions

of biological groups were detected. The introduced biochemical pathways and the related DEGs are shown in Figure 3. As it is depicted in Figure 3, 18 DEGs were connected to the identified biological terms.

### Discussion

Developing the application of cold atmospheric plasma due to the noninvasive nature of this method implies more investigations into its molecular mechanism.<sup>17</sup> The “Box plot” as a statistical technique is used to determine the patterns that may be concealed in a group of numbers. By using this statistical method, comparing the data groups via visually summarizing the data is possible. The highest and lowest levels of data, the approximate quartiles, and the median of point data are presented in the illustrated “box plot”. The probable outlier data will appear in the box plot. Experiments have revealed that the application of the “box plot” makes the interpretation of data easy.<sup>18</sup> The gene expression profiles of the compared cells (the exposed cells to indirect cold physical plasma versus the irradiated cells by UV radiation) in Figure 1 indicate that there are many up- and down-regulated genes. It can be concluded that the two assessed methods are discriminated by many dysregulated genes. This finding points to the different molecular mechanism which is governed by the two methods.

Singh et al published a document about the application of differential gene regulatory networks to evaluate disease conditions. The promotion of this method is highlighted in this investigation.<sup>19</sup> Regulatory relationships between the queried DEGs are shown in Figure 2. As it is depicted in this figure, among the 148 recognized genes, 40 individuals participated in the 5 clusters and the other genes were isolated. The main connected component is constructed from 27 DEGs and the second sub-network is formed from five genes. Four DEGs are organized in a cluster and the two paired ones also appear. Activation and expression are the two prominent relationships between the evaluated genes.

Pathway analysis is a common way to detect the molecular mechanism of diseases.<sup>20</sup> Pathway analysis

revealed the differences between both methods in signaling pathway activation. Five groups of pathways were identified as the related biological terms for the 148 queried genes. However, 18 genes including SOC1, LDLR, ALO5, PTGS2, TNF, JUNB, TNFRSF1A, CD40, SMAD7, ID1, SMAD6, SERPINE1, PMAIP1, MDM2, CREB5, GADD45A, E2F3, and ETV5 among the queried DEGs were involved in the determined pathways. The combination of data from Figures 2 and 3 indicates that among the 18 introduced genes, 14 individuals (78%) are common genes. 11 common DEGs (79%) including SOC1, PTGS2, TNF, TNFRSF1A, CD40, SMAD7, ID1, SMAD6, SERPINE1, PMAIP1, and ETV5 are organized in the main connected component. It seems that this cluster is the core of genes, discriminating the induced molecular mechanism of “indirect cold physical plasma” from “UV-radiation” at the cellular level. As it is depicted in Figure 2, NOTCH1, TNF, SERPINE1, KLF, and BDNF are characterized by 9, 8, 5, 5, and 5 first neighbors, respectively.

Neurogenic locus notch homolog protein 1 (Notch 1) is a member of the NOTCH family (the highly conserved transmembrane receptors). This family is involved in many important physiological events such as growth, differentiation, and apoptosis in normal cells. The dysregulation of NOTCH1 in many cancers such as leukemias, breast cancer, and brain cancer is reported by investigators.<sup>21</sup> Tumor necrosis factor (TNF) which is characterized by a connection to 8 first neighbors in Figure 2 is a critical cytokine. It is pointed out that TNF is involved in both physiological and pathological processes.<sup>22</sup> The dual role of TNF in breast cancer is highlighted by Cruceriu et al.<sup>23</sup> The expression relationship between TNF and NOTCH 1 is presented in Figure 2. TNF in the dual administration up- and down-regulates NOTCH1. This complex relationship between TNF and NOTCH1 depends on the body condition.

### Conclusion

There are essential differences between the molecular mechanism of UV radiation and cold physical plasma treatment. It seems that the top discriminators of the mentioned differences are the function of NOTCH1 and TNF. However, SERPINE1, KLF, and BDNF also appear as the prominent elements which can discriminate the molecular mechanism of the two assessed methods.

### Acknowledgments

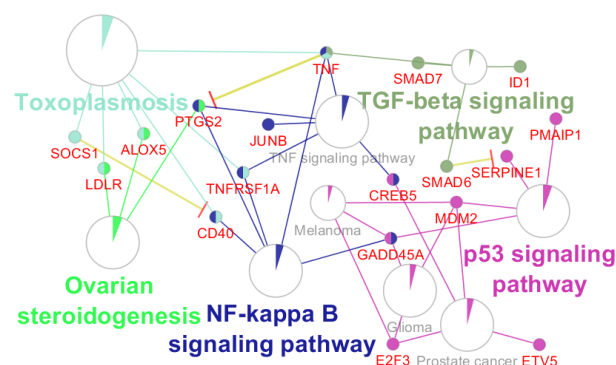
This project was supported by Shahid Beheshti University of Medical Sciences.

### Competing Interests

The authors declare that they have no conflict of interest.

### Ethical Approval

Not applicable.



**Figure 3.** The Related Biochemical Pathways to the Queried 152 Decoded Genes (148 Recognized Genes). The red-labeled circles refer to the DEGs

## References

- Urruticoechea A, Alemany R, Balart J, Villanueva A, Viñals F, Capellá G. Recent advances in cancer therapy: an overview. *Curr Pharm Des*. 2010;16(1):3-10. doi: [10.2174/138161210789941847](https://doi.org/10.2174/138161210789941847).
- Smith GL, Ganz PA, Bekelman JE, Chmura SJ, Dignam JJ, Efstathiou JA, et al. Promoting the appropriate use of advanced radiation technologies in oncology: summary of a national cancer policy forum workshop. *Int J Radiat Oncol Biol Phys*. 2017;97(3):450-61. doi: [10.1016/j.ijrobp.2016.10.042](https://doi.org/10.1016/j.ijrobp.2016.10.042).
- Kumar Dubey S, Dabholkar N, Narayan Pal U, Singhvi G, Kumar Sharma N, Puri A, et al. Emerging innovations in cold plasma therapy against cancer: a paradigm shift. *Drug Discov Today*. 2022;27(9):2425-39. doi: [10.1016/j.drudis.2022.05.014](https://doi.org/10.1016/j.drudis.2022.05.014).
- Wang C, Cheng L, Liu Z. Upconversion nanoparticles for photodynamic therapy and other cancer therapeutics. *Theranostics*. 2013;3(5):317-30. doi: [10.7150/thno.5284](https://doi.org/10.7150/thno.5284).
- Shirkavand A, Sarkar S, Ataie Fashtami L, Mohammadreza H. Detection of melanoma skin cancer by elastic scattering spectra: a proposed classification method. *Iran J Med Phys*. 2017;14(3):162-6.
- Allegra A, Pioggia G, Tonacci A, Musolino C, Gangemi S. Oxidative stress and photodynamic therapy of skin cancers: mechanisms, challenges and promising developments. *Antioxidants (Basel)*. 2020;9(5):448. doi: [10.3390/antiox9050448](https://doi.org/10.3390/antiox9050448).
- Laikova KV, Oberemok VV, Krasnodubets AM, Gal'chinsky NV, Useinov RZ, Novikov IA, et al. Advances in the understanding of skin cancer: ultraviolet radiation, mutations, and antisense oligonucleotides as anticancer drugs. *Molecules*. 2019;24(8):1516. doi: [10.3390/molecules24081516](https://doi.org/10.3390/molecules24081516).
- Shojaei E, Zare S, Shirkavand A, Eslami E, Fathollah S, Mansouri P. Biophysical evaluation of treating adipose tissue-derived stem cells using non-thermal atmospheric pressure plasma. *Sci Rep*. 2022;12(1):11127. doi: [10.1038/s41598-022-14763-0](https://doi.org/10.1038/s41598-022-14763-0).
- Tabares FL, Junkar I. Cold plasma systems and their application in surface treatments for medicine. *Molecules*. 2021;26(7):1903. doi: [10.3390/molecules26071903](https://doi.org/10.3390/molecules26071903).
- Harley JC, Suchowerska N, McKenzie DR. Cancer treatment with gas plasma and with gas plasma-activated liquid: positives, potentials and problems of clinical translation. *Biophys Rev*. 2020;12(4):989-1006. doi: [10.1007/s12551-020-00743-z](https://doi.org/10.1007/s12551-020-00743-z).
- Semmler ML, Bekeschus S, Schäfer M, Bernhardt T, Fischer T, Witzke K, et al. Molecular mechanisms of the efficacy of cold atmospheric pressure plasma (CAP) in cancer treatment. *Cancers (Basel)*. 2020;12(2):269. doi: [10.3390/cancers12020269](https://doi.org/10.3390/cancers12020269).
- Guéguen Y, Bontemps A, Ebrahimian TG. Adaptive responses to low doses of radiation or chemicals: their cellular and molecular mechanisms. *Cell Mol Life Sci*. 2019;76(7):1255-73. doi: [10.1007/s00018-018-2987-5](https://doi.org/10.1007/s00018-018-2987-5).
- Marques DA, Jones FC, Di Palma F, Kingsley DM, Reimchen TE. Experimental evidence for rapid genomic adaptation to a new niche in an adaptive radiation. *Nat Ecol Evol*. 2018;2(7):1128-38. doi: [10.1038/s41559-018-0581-8](https://doi.org/10.1038/s41559-018-0581-8).
- Onik JC, Xie Y, Duan Y, Hu X, Wang Z, Lin Q. UV-C treatment promotes quality of early ripening apple fruit by regulating malate metabolizing genes during postharvest storage. *PLoS One*. 2019;14(4):e0215472. doi: [10.1371/journal.pone.0215472](https://doi.org/10.1371/journal.pone.0215472).
- Kanehisa M, Furumichi M, Sato Y, Kawashima M, Ishiguro-Watanabe M. KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Res*. 2023;51(D1):D587-D92. doi: [10.1093/nar/gkac963](https://doi.org/10.1093/nar/gkac963).
- Li W. Volcano plots in analyzing differential expressions with mRNA microarrays. *J Bioinform Comput Biol*. 2012;10(6):1231003. doi: [10.1142/s0219720012310038](https://doi.org/10.1142/s0219720012310038).
- Mariachiara DA, Anna V, Alessandra G, Edoardo GP, Stefania B, Mariateresa R, et al. Cold atmospheric plasma (CAP) as a promising therapeutic option for mild to moderate acne vulgaris: clinical and non-invasive evaluation of two cases. *Clin Plasma Med*. 2020;19-20:100110. doi: [10.1016/j.cpm.2020.100110](https://doi.org/10.1016/j.cpm.2020.100110).
- Williamson DF, Parker RA, Kendrick JS. The box plot: a simple visual method to interpret data. *Ann Intern Med*. 1989;110(11):916-21. doi: [10.7326/0003-4819-110-11-916](https://doi.org/10.7326/0003-4819-110-11-916).
- Singh AJ, Ramsey SA, Filtz TM, Kioussi C. Differential gene regulatory networks in development and disease. *Cell Mol Life Sci*. 2018;75(6):1013-25. doi: [10.1007/s00018-017-2679-6](https://doi.org/10.1007/s00018-017-2679-6).
- Rezaei Tavirani M, Zamanian Azodi M, Rostami-Nejad M, Morravej H, Razzaghi Z, Okhovatian F, et al. Introducing serine as cardiovascular disease biomarker candidate via pathway analysis. *Galen Med J*. 2020;9:e1696. doi: [10.31661/gmj.v9i0.1696](https://doi.org/10.31661/gmj.v9i0.1696).
- Gharaibeh L, Elmadany N, Alwosaiab K, Alshaer W. Notch1 in cancer therapy: possible clinical implications and challenges. *Mol Pharmacol*. 2020;98(5):559-76. doi: [10.1124/molpharm.120.000006](https://doi.org/10.1124/molpharm.120.000006).
- Chu WM. Tumor necrosis factor. *Cancer Lett*. 2013;328(2):222-5. doi: [10.1016/j.canlet.2012.10.014](https://doi.org/10.1016/j.canlet.2012.10.014).
- Cruceriu D, Baldasici O, Balacescu O, Berindan-Neagoe I. The dual role of tumor necrosis factor-alpha (TNF-α) in breast cancer: molecular insights and therapeutic approaches. *Cell Oncol (Dordr)*. 2020;43(1):1-18. doi: [10.1007/s13402-019-00489-1](https://doi.org/10.1007/s13402-019-00489-1).