Journal of Lasers in Medical Sciences

J Lasers Med Sci 2022;13:e70

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2022.70

Identification of Targeted Central Genes (IGF1 and HMOX1) by Indirect Cold Physical Plasma in Human Melanocytes

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Received: December 10, 2022
Accepted: December 17, 2022
Published online December 18, 2022

Abstract

Introduction: Cold physical plasma is a growing tool in medicine which is applied for the treatment of different cancers. In the present study, the gene profiles of human melanocytes exposed to indirect cold physical plasma versus control individuals are analyzed via protein-protein interaction (PPI) network analysis.

Methods: The gene expression profiles were derived from Gene Expression Omnibus (GEO), and the significant differentially expressed genes (DEGs) were decoded via "Expression Atlas". PPI network analysis was applied to find the targeted central genes by indirect cold physical plasma.

Results: The main connected component of the constructed network including 74 queried DEGs and 50 added first neighbors was analyzed. Considering degree value, betweenness centrality, closeness centrality, and stress, IGF1 and HMOX1 were introduced as the central nodes.

Conclusion: The finding of this study indicates that the down-regulation of IGF1 and the up-regulation of HMOX are the prominent events in response to indirect cold physical plasma treatment at the cellular level. Detection of related biological terms via gene ontology is suggested.

Keywords: Cold physical plasma; PPI network; Human melanocytes; Central genes; Gene expression change.

Introduction

Cold physical plasma including light radiation, electric fields, reactive oxygen and nitrogen species, and ions and electrons is a partly ionized gas state of matter which is produced at body temperature.[1] A pioneering and developing field that is concerned with clinical medicine, plasma physics, and life science is called "plasma medicine". Two modes of plasma applications are pointed out by experts; the first one is the "indirect" mode and the second one is applying plasm for therapeutic purposes on or in the human body.[2] Investigations indicate that several parameters such as temperature, feeding gas flow rate, air humidity, voltage frequency, and atmospheric gas composition are involved in the complex mechanism which is induced by plasma.[3] Recently, considerable achievements have occurred in the technological development of cold atmospheric pressure plasma technology in various fields such as agriculture, medicine, and industry. Due to the essential benefits of cold plasma technology, it has attracted the attention of many specialists in the fields of biomedical and technological applications.[4] Today cold plasma therapy is a known therapeutic method in medicine. Targeting head and neck squamous cell carcinoma cells, cancer therapy, and other treated diseases by cold plasma therapy have been pointed out in the medicine literature.[5-7]

High-throughput methods such as metabolomics, proteomics, and genomics are applied widely to explore the molecular mechanism of many biological and clinical events. In such investigations, a large number of dysregulated genes, proteins, or metabolites are determined and discussed. Cancer is a disease that is investigated widely via these high-throughput methods.[8-10] Bioinformatics which is tied strongly to high-throughput methods is an attractive tool to evaluate the complex findings by these approaches.[11] Protein-protein interaction (PPI) is a field that is concerned with proteomics and bioinformatics.[12]

Please cite this article as follows: Vafaee R, Arjmand B, Hamzeloo-Moghadam M, Razzaghi Z, Rezaei Tavirani M, Rezaei Tavirani S. Identification of targeted central genes (IGF1 and HMOX1) by indirect cold physical plasma in human melanocytes. *J Lasers Med Sci.* 2022;13:e70. doi:10.34172/jlms.2022.70.

Based on graph theory, PPI network analysis can be applied to characterize individual proteins via analysis of their properties in the network. PPI network analysis is applied to explore the molecular mechanism of many diseases and therapeutic protocols. Central proteins such as hubs and bottlenecks that control the elements of a PPI network are considered important individuals that affect the function of the investigated organism.[13-15] In the present study, the gene expression profiles of human melanocyte cells which are treated by "indirect cold physical plasma" were extracted from literature and were investigated via PPI network analysis to find the core of gene expression alteration.

Materials and Methods

Gene expression profiles of 6 human primary melanocytes which were radiated by indirect cold physical plasma were extracted from GSE157824 that is published in Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157824). The 5 control samples were also chosen to explore the related differentially expressed genes (DEGs). Data were analyzed by using GEO2R and the gene expression profiles were compared via boxplot analysis.

Considering P value < 0.05 and fold change > 2, the significant DEGs were selected for more analysis. Since the DEGs were presented by spot ID, the names of the genes were extracted from "Expression Atlas" (https://www.ebi.ac.uk/gxa/home).

The decoded genes were included in a network by STRING "Protein Query" via Cytoscape software version 3.7.2.[16] To maximize connections between the queried DEGs, we added 50 and 100 first neighbor genes from the STRING database to the DEGs in two steps. The optimized numbers of the added first neighbors were determined. The nodes were connected to the neighbors via undirected edges. The confidence cutoff (score) was 0.4.

The network was analyzed by the "Network Analyzer" application of Cytoscape software[17] to find the central nodes. The main connected component of the analyzed network was laid out by degree value and visualized. About 10% of the top queried nodes which were involved in the main connected component based on degree value, betweenness centrality, closeness centrality, and stress were selected as the central nodes. Common central nodes were identified as the crucial central nodes. The crucial central nodes are the hub-bottlenecks which were pointed as the top nodes based on closeness centrality and stress.

Results

The box plot as a result of GEO2R analysis is presented in Figure 1. Six gene expression profiles of the cells which were exposed to indirect cold physical plasma versus 5 profiles of the control cells are shown in Figure 1. As it is depicted in Figure 1 (box plot), the mid-points of the

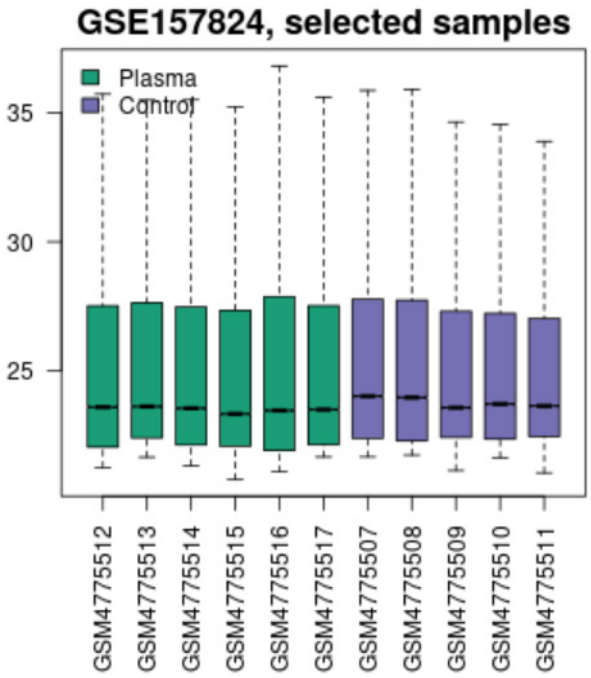

Figure 1. Box Plot Presentation of Gene Expression Profiles of Treated Cells With Indirect Cold Physical Plasma Versus Gene Expression Profiles of The Control Cells.

profiles are similar, and therefore, the gene expression pattern of the exposed cell to plasma is comparable with the profiles of the control cells. However, the gene expression pattern is similar for the two compared cells, and there are covered differences in the profiles which discriminate the details of gene expressions for the treated and control cells.

Among the 250 extracted DEGs, 98 individuals were decoded via "Expression Atlas". The 98 queried DEGs were included in the PPI network analysis. 74 DEGs among the 98 queried genes were recognized by STRING. The network was formed while 55 nodes were isolated. The network was renewed by adding 50 first neighbors. In this case, 32 queried DEGs remained as isolated nodes. When 100 first neighbors were added to the queried DEGs, 29 isolated nodes appeared. Therefore, the network including 50 added first neighbors and 74 recognized queried DEGs were considered for more analysis. This network was analyzed by "Network Analyzer". The main connected component of this network which is visualized by degree value is shown in Figure 2.

IGF1 and HMOX1 were branded as hub-bottlenecks. The analysis revealed that the hub-bottlenecks are common with the top nodes based on closeness centrality and stress. Thus, the hub-bottlenecks were introduced as the crucial central genes. The centrality properties of IGF1 and HMOX1 are tabulated in Table 1.

Discussion

PPI network analysis as a useful tool is applied to evaluate biological alteration following radiation at the cellular

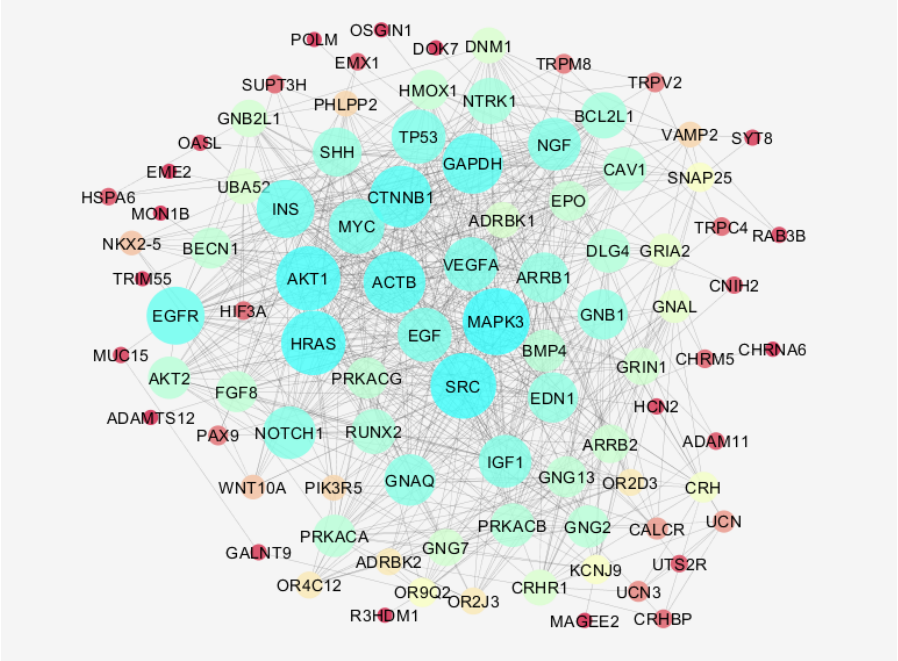

Figure 2. Main Connected Component of the PPI Network of the Treated Cells With Indirect Cold Physical Plasma Cells Versus the Control Cells. Red to blue refers to the increment of degree value.

Table 1. Crucial Central Queried Genes of the Main Connected Component

Gene Name	Degree	Betweenness Centrality	Closeness Centrality	Stress
IGF1	31	0.013	0.555	1182
HMOX1	21	0.025	0.497	2118

and organism levels.[18] In the present study, the molecular mechanism of indirect cold physical plasm was assessed by PPI network analysis. As it is shown in Figure 1, the gene expression profiles of the treated cells and the control individuals presented a similar pattern. Box plot presentation is a suitable method for showing comparable gene expression profiles.[19] Adding the first neighbor genes provided a network with minimized isolated nodes. However, the added first neighbors were not considered for centrality analysis, and they helped to form an informative network of the queried DEGs (such as the main connected component that is shown in Figure 2). As it is represented in Table 1 two potent central genes are introduced as the key nodes of the network: IGF1 and HMOX1.

Insulin-like growth factor 1 (IGF1) was recognized in 1957. Due to its facility to combine sulphate into rat cartilage, it was nominated "sulphation factor".[20] As it is reported by Hu et al, the binding of growth hormone to its receptor leads to the activation of IGF1 synthesis by the JAK2/STAT% pathway. It is explored that this axis (growth hormone-growth hormone receptor-IDF1 axis) is involved prominently in cell proliferation, cell division and differentiation, and cell survival (the processes which promote somatic growth).[21] Log (fold change) for IGF1 in the studied gene expression profiles is reported as -1.38. Hao et al published data about the effect of IGF1 down-regulation on the promotion of Schwann cell de-differentiation and de-myelination.[22] IGF1 appeared as the potent (top) hub gene (see Table 1). The role of IGF1 as a hub gene is highlighted in several cancers which are investigated by PPI network analysis.[23,24]

Amounts of degree value and betweenness centrality for heme oxygenase-1 (HMOX1) are 21 and 0.025 respectively (see Table 1). It is a weaker hub and stronger bottleneck relative to IGF1. Log (fold change) for HMOX1 is recorded as 1.04. It means that HMOX1 is upregulated after applying indirect cold physical plasma. Bekeschus et al have published a document about the effects of low-dose, tumor-static plasma treated medium on 8 human cancer cell lines. In this investigation, 22 redox-related genes were evaluated to find immune-modulatory properties, cellular activity, and transcriptional level changes. Based on this report, a moderate reduction of metabolic activity was detected in the treated cells. Besides this event, the modest modulation of the markers of immunogenic cell death and chemokine/cytokine pattern was reported in this published data. It is expressed that HMOX1 is up-regulated in all treated cell lines.[1] This finding is consistent with the results from analyzed GSE157824.

However, many genes are dysregulated by indirect cold physical plasma in the exposed cell. Our finding shows that IGF1 and HMOX1 are more important targets for plasm. In the literature, it is pointed out that IGF1 and HMOX1 are anti-inflammatory genes. The up-regulation of these genes at the same time is reported in Qiburi and colleagues' investigation.[25] In the original data from GSE157824, an inverse correlation is reported for the

regulation mode of IGF1 and HMOX1 that may reflect the complex process of plasma effect of cell function.

Conclusion

In conclusion, among the many dysregulated genes resulting from the application of indirect cold physical plasma, IGF1 and HMOX1 were pointed as the two critical affected genes. This finding provides a new perspective on the molecular mechanism of cold physical plasma effects at the cellular level.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Considerations

Not applicable.

Funding

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

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