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Evaluation of the Cellular Resistance Process in Treated Cells Via Extracorporeal Photopheresis

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Abstract

Introduction: Extracorporeal photopheresis (ECP) is a therapeutic method applied against some diseases such as cancers. Using 8-methoxypsoralen (8-MOP) and UVA radiation in ECP is associated with achievement in the treatment of patients with leukemic cutaneous T-cell lymphoma (CTCL). Evaluation of cellular resistance versus ECP is the aim of this study.

Methods: Data were downloaded from the Gene Expression Omnibus (GEO) database and were analyzed via the GEO2R program. The significant DEGs were assessed via protein-protein interaction (PPI) network analysis by using the STRING database and Cytoscape software. The critical genes were evaluated via gene ontology by using the ClueGO application of Cytoscape software. The identified biological processes were determined and analyzed.

Results: Fifty-seven significant DEGs were determined. The main connected component of the PPI network including 32 queried significant DEGs plus 50 first neighbors was constructed. Nineteen histones as critical nodes were assessed via gene ontology, and "nucleosome organization" was pointed out as the crucial biological process. Finally, 15 histones from H2A, H2B, and H3 histone families were identified as the key genes that are involved in the resistance property of the treated cells.

Conclusion: In conclusion, 15 members of H2A, H2B, and H3 families (especially H2A family) were considered as the origin of resistance versus ECP treatment. It is concluded that sensitivity to ECP treatment depends on gross molecular events which are involved in the functions of histones.

Keywords: Extracorporeal photopheresis; Network analysis; Gene; Cell; Human.

Introduction

Therapeutic methods as "apheresis procedures" use a variety of techniques to separate different compounds of blood of patients and elimination of the etiological factor of disease. The processed blood does re-infusion into the patient. In this method, a replacement fluid or volume is often added to the re-infused components.[1] As pointed out in extracorporeal photopheresis (ECP), leukocytes are treated with 8-methoxypsoralen (8-MOP) and UVA radiation ex vivo. Then, these cells are reinfused into patients with leukemic cutaneous T-cell lymphoma (CTCL).[2] An investigation indicates that 8-MOP and UVA treatment have a strong effect on platelet activation, but red blood cells remain stable.[3] Cyclosporine A is introduced as an efficient treatment associated with considerable side effects for severe atopic dermatitis. Another investigation indicates that ECP is a suitable alternative to cyclosporine.[4] An improvement in ECP for the advanced treatment of diseases is the experts' aim.[5]

The analysis of the gene expression profiles of diseases has a strong impact on the evaluation and approvement of treatment protocols. There are many documents about the evaluation of laser therapy and its related methods that are concerned with the analysis of the gene expression alterations of the studied samples.[6,7] The close relationship between genomics and bioinformatics

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provides a facility to interpret the alteration of genes function. Since the number of dysregulated genes in a genomic study is considerably large, bioinformatics is a useful complementary technique to understand the core of molecular events.[8,9] One of the bioinformatic techniques is network analysis. Many elements join each other to form a network. The protein-protein interaction (PPI) network is an interactome including genes, proteins, or metabolites as nodes which are connected by edges. The nodes of a network play a different role in comparison with their neighbors. PPI network analysis is a useful method for screening a set of genes. Many diseases and therapeutic methods are evaluated via PPI network analysis.[10-12]

Gene ontology enrichment of genes can provide suitable information about biochemical pathways, molecular functions, and biological processes, which are related to the studied genes. Gene ontology is applied widely to assess diseases such as cancers.[13] In the present study, the cellular response to ECP in sensitive and resistant cells was studied via the network analysis and gene ontology technique by using data from the GEO database. It can be expected that a few genes and biological processes are the key to this difference between the two study cells.

Methods

GSE114891 recorded in Gene Expression Omnibus (GEO) included the gene expression profiles of human peripheral blood mononuclear cells (PBMCs). The cells were derived from patients with leukemic CTCL. Two groups of the cells were marked as "responsive to ECP treatment" and "Non-responsive to ECP treatment" cells, two days after the treatment. Five profiles of the responsive cells (Responsive D2) versus two profiles of the non-responsive cells (Non-responsive D2) were selected to be analyzed.

The selected gene expression profiles were analyzed via the GEO2R program to find the top 250 affected genes. According to the uniform manifold approximation and projection (UMAP) plot, the separation of the two studied groups was assessed via clustering analysis. The significant DEGs were pointed out based on P value < 0.01 and (fold change) > 1.5.

The significant differentially expressed genes (DEGs) were included in the "Protein query" of STRING via Cytoscape software. To maximize interaction between the interacted genes, 50 first neighbors were added to the queried DEGs and the PPI network was constructed. The nodes were connected by undirected edges to form the PPI network. The statistical parameter of the confidence score (cutoff) was 0.4. The network was analyzed by a "Network analyzer" to assess the centrality of the nodes. The queried nodes were evaluated based on degree value, and the important DEGs were introduced as the key targeted genes via ECP treatment in the "Responsive D2" cells versus the "Non-responsive D2" cells. The key genes were assessed via gene ontology enrichment to find the related biological processes and molecular functions via ClueGO. Inhibition, activation, and reaction between the key elements of the network were determined via CluePedia.

Results

UMAP plot analysis indicated that the two classes of the studied cell lines were separable by gene expression profile evaluation (see Figure 1). As it is shown in Figure 1, the two clusters of the cells are located in the two zones of the plot. Fifty-seven significant DEGs, including 24 up-regulated and 33 down-regulated genes, which discriminate the "Responsive D2" cells from the "Non-responsive D2" cells were identified.

To screen data, PPI network analysis was selected as the first analytical method. Among the 57 queried DEGs, 49 individuals were recognized by the STRING database. The main connected component of the PPI network including the 49 recognized DEGs and the 50 added first neighbors is shown in Figure 2. The network includes 15 isolated genes, two paired DEGs, and a main connected component of 82 nodes. Analyses revealed the 32 queried DEGs which were included in the main connected component and were categorized into two groups: the first class of nodes including 13 genes with a degree value less than 10 and the second group of nodes with degree values of 50-70. Considering the degree value, the second group of DEGs was selected as the crucial targeted genes by ECP treatment. The second class of nodes was identified as HIST2H2AC, HIST1H2AH, HIST1H2AJ, HIST1H4F, HIST1H4H, HIST1H4I, HIST2H4B, HIST1H3B, HIST1H3D, HIST1H2AB, HIST1H2AE, HIST1H2BH, HIST1H2BO, HIST1H2AG, HIST1H2AK, HIST1H2AM, HIST1H2BB, HIST3H2A, and HIST3H2BB.

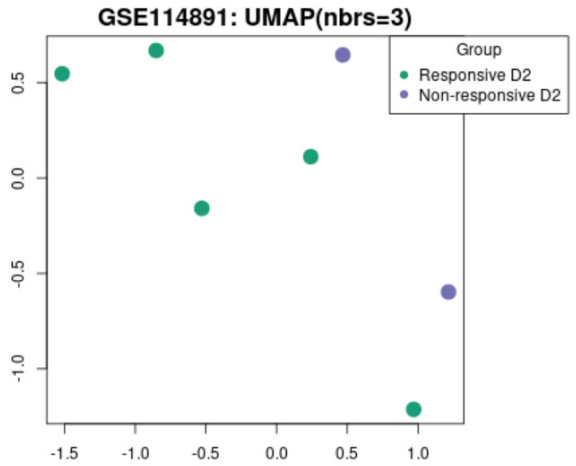

Figure 1. Uniform Manifold Approximation and Projection Plot of the Analyzed Gene Expression Profiles of "Responsive D2" and "Non-responsive D2" Human Peripheral Blood Mononuclear Cells

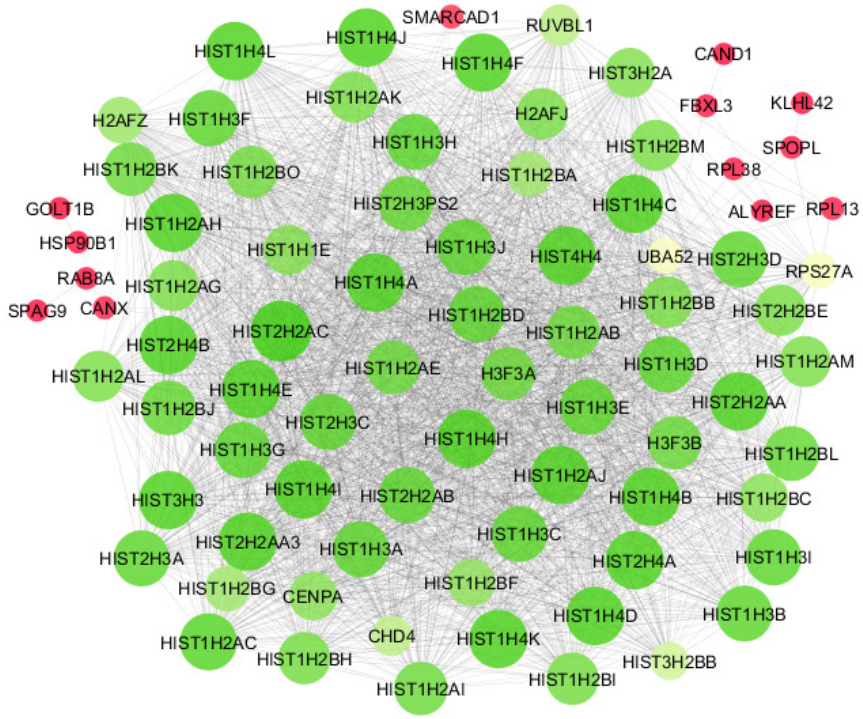

Figure 2. Main Connected Component of the PPI Network of the Query DEGs Plus 50 Added First Neighbors. Red to green and increment of size refer to the increase of degree value

To find the main affected biological processes, gene ontology was applied as the related method. The 19 genes of the second cluster were enriched via gene ontology and the findings are presented in Figures 3 and 4. Thirty-two biological processes and molecular functions which were clustered in four classes of biological terms were related to the 19 crucial DEGs. The four groups of biological terms were presented as "Nucleosome organization", "Negative regulation of gene expression epigenetic", "Chromatin silencing", and "Protein heterodimerization activity". Activation, inhibition, and reaction relationship between the 19 analyzed DEGs are illustrated in Figure 4. As it is shown in Figure 4, there is no inhibition relationship between the assessed genes. Different types of histones are organized in this set of elements.

Disscusion

Efficacy approvement of apheresis procedures is a challenge point for experts to apply apheresis as an effective therapeutic method.[14] In the present study, resistance to the apheresis procedure was investigated to explore the possible problems which restrict the use of this technique in a clinic. As it is shown in Figure 1, the sensitive and resistant samples are divided into two classes by the administrated analysis. This separation is induced by the introduced 57 significant DEGs. PPI network analysis was selected to screen the identified significant DEGs. Ying et al published a document about the molecular mechanism of ECP by using leukemic CTCL. They used whole human genome microarray technology and pathway analysis to discriminate five clinically responsive patients and five clinically resistant patients. Their finding refers to the role of cell adhesion and suppression of IL-1β, which underlie the efficacy of ECP in patients.[15]

PPI network analysis revealed that 32 genes among the 57 queried significant DEGs interacted in the main connected component of the formed PPI network. Interpretation of PPI networks is always a challenge for scientists. Identification of a set of genes which are involved in a specific function is possible for studied networks.[16] As mentioned in the results section, there is a distinguishable set of queried significant DEGs that are characterized by a high value of degree.

Gene ontology (including pathway analysis, biological process, and molecular function) as a useful tool is used in bioinformatics to explore more details of the function of the studied genes.[17] Pathway analysis (see Figure 3) showed that "Nucleosome organization" is the main biological process which is related to the 19 elements of the gene set. The nucleosome is introduced as a key modulator of DNA accessibility to other cellular issues. It is stated that the regulation of recombination or DNA repair, DNA replication, and transcription depend strongly on nucleosome positioning.[18] The role of nucleosome organization in gene regulation is highlighted by researchers. Multiple factors such as the action of chromatin remodelers are involved in the determination of nucleosome organization.[19] The second important biological process is the "Negative regulation

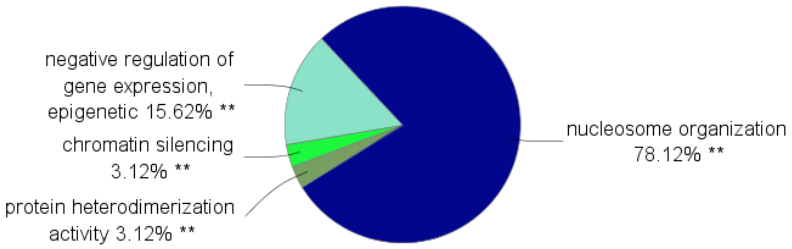

Figure 3. Term Percent Per Group of Biological Processes and Molecular Functions Related to the 19 Critical Genes. "Nucleosome organization", "Negative regulation of gene expression epigenetic", "Chromatin silencing", and "Protein heterodimerization activity" groups include 25, 5, 1, and 1 biological terms respectively

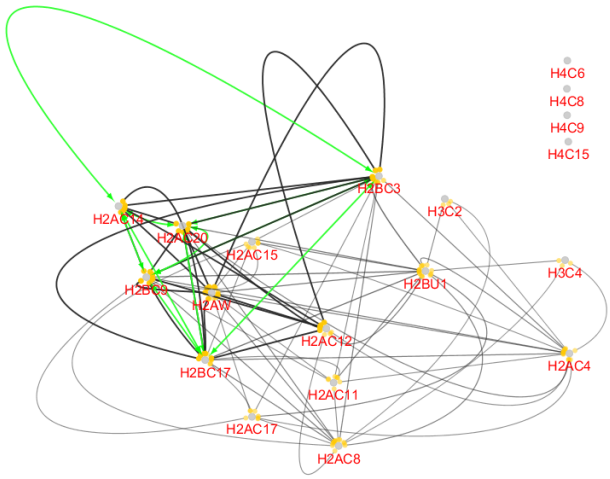

Figure 4. Activation (Green), Inhibition (Red), and Reaction (Black) Relationship Between 19 Crucial DEGs. Inhibition links did not appear as edges between the evaluated nodes

of gene expression epigenetic". Waddington et al. have described epigenetics as "the branch of biology that studies the causal interactions between genes and their products which bring the phenotype into being".[20]

The findings indicate that there are essential differences between Responsive D2 cells and Non-responsive D2 cells. Application of the regulatory network to find the relationship between the studied genes is a suitable tool to determine the importance rate of the function of each gene. This method is used by researchers to explore the details of the molecular mechanism of diseases.[21] Relationships between the 19 critical genes are presented in Figure 4. Two kinds of links are identified: activation and reaction. H4C6, H4C8, H4C9, and H4C15 remain isolated while the other 15 genes participate in the formation of the regulatory network. The network is constructed by 9 members of the H2A family, 4 members of the H2B family, and 2 members of the H3 family. It can be concluded that the H2A family plays a critical role in the separation of the studied cells into sensitive and resistant cells to ECP.

Conclusion

Findings indicate that the different responses of the examined cells originate from differences in the function of three histone families including H2A, H2B, and H3. Analyses showed a prominent role of H2A family relative to the H2B and H3 histone families. It seems that "nucleosome organization" is the central biological process that differentiates the sensitive cells from resistant individuals during treatment with ECP. Experimental validation can provide useful information about our findings. In conclusion, there is sensitivity to ECP treatment, which depends on gross molecular events considering the functions of histones. The findings highlight the main differences between the sensitive and resistant cells in response to ECP.

Authors' Contribution

Conceptualization: Babak Arjmand, Mostafa Rezaei Tavirani, Majid Rezaei Tavirani.
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Funding acquisition: Babak Arjmand.
Investigation: Somayeh Jahani Sherafat, Mitra Rezaei.
Methodology: Masoumeh Farahani.
Project administration: Babak Arjmand, Majid Rezaei Tavirani.
Resources: Somayeh Jahani Sherafat, Mitra Rezaei, Masoumeh Farahani.
Software: Mostafa Rezaei Tavirani.
Supervision: Babak Arjmand.
Validation: Masoumeh Farahani.
Visualization: Masoumeh Farahani.
Writing–original draft: Mostafa Rezaei Tavirani.
Writing–review editing: Babak Arjmand, Mostafa Rezaei Tavirani, Somayeh Jahani Sherafat, Mitra Rezaei, Masoumeh Farahani, Majid Rezaei Tavirani.

Competing Interests

The authors declare that they have no conflict of interest.

Ethical Approval

This project is approved by ethical code: IR.SBMU.LASER.REC.1402.011.

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