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Assessment of Recovery Time Effects on Human Primary Neonatal Dermal Fibroblasts After Exposure to Solar-Simulated Ultraviolet Radiation

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Abstract

Introduction: Photoaging that is accompanied by gene expression alteration is known as early aging of the skin due to overexposure to natural and/or artificial ultraviolet radiation (UVR). The assessment of gene expression alteration in human primary neonatal dermal fibroblasts depending on recovery time after exposure to solar simulated ultraviolet radiation (ssUVR) is the main aim of this bioinformatic study.

Methods: Data are extracted from Gene Expression Omnibus (GEO). The pre-evaluation is done via the GEO2R program. The Significant differentially expressed genes (DEGs) were assessed via protein-protein interaction (PPI) network analysis, and the central genes were identified. The central genes were enriched via gene ontology assessment.

Results: Among 224 significant DEGs, 20 central genes including TOP2A, MKI67, BRCA1, HELLS, MAD2L1, ANLN, KIF11, MSH2, KRAS, NCAPG, RFC3, PLK4, WDHD1, BLM, CDKN3, KIF15, SMARCA5, and ATAD2 as hub genes and TOP2A, MKI67, BRCA1, ANLN, KRAS, PLK4, SMARCA5, MMP2, and TLR4 as bottleneck genes were determined. Eight central genes were associated with 16 biological terms.

Conclusion: In conclusion, significant differences appeared between gene expression conditions of the cells after 1-day and 5-day recovery. Molecular events include the repair and continuation of photodamages. It is possible to introduce drug targets to prevent the progress of induced damages.

Keywords: Fibroblast Ultraviolet radiation; Recovering time; Network analysis; Photoaging.

Introduction

Photoaging is attributed to the early aging of skin from over experience of natural and/or artificial ultraviolet radiation (UVR).[1] As it is reported, skin aging is a complex process that is concerned with intrinsic and extrinsic processes. UVR is a well-known extrinsic factor that promotes skin aging [2]. Understanding the molecular mechanism and prevention methods for skin photoaging has attracted the attention of many researchers. Hyperpigmentation, deep wrinkles, and deterioration of skin are counted as clinical characterizations of photoaging.[3,4] There are several pieces of evidence about gene expression change investigations related to photoaging and the role of UVR in the progress of this process.[5-7]

Gene expression change assessment is a useful tool to evaluate diseases and biological phenomena. Extrinsic factors such as radiation or drugs affect the gene expression profiles of the biological system such as organisms or cultured cells. Experiments have shown that the expression of large numbers of genes changes

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in response to external stimulating issues.[8,9] Bustamante et al published data about the effects of dose and time of solar-simulated ultraviolet radiation (*ssUVR*) on the transcriptome of human skin.[10] Hudson et al have studied damage biomarkers in human skin cells after exposure to combined or individual visible, infrared, and UV of solar radiation. The dysregulation of genes related to apoptosis, inflammation, keratinization, cytoskeleton organization, immunoreactivity, transcription/translation, and hyaluronan biosynthesis are highlighted in this research.[11]

The combination of genomics and bioinformatics has provided an efficient method for analyzing the results of genomic studies.[12] Protein-protein interaction (PPI) network analysis as a bioinformatics approach is used to assess many gene sets related to diseases and stress conditions. Heidari et al have studied the effect of the microbiome role on the protection of exposed skin to UV irradiation via PPI network analysis.[13] Mansouri et al have investigated the effect of interval time between two laser sessions applied to treat human skin via PPI network analysis. They found that collagen synthesis is a prominent process in the recovery time between the two laser sessions.[14]

Gene ontology is a well-known method for the evaluation of biochemical pathways, molecular function, and biological processes related to the studied genes. Many diseases are studied via gene ontology to understand the molecular mechanism and details of molecular events.[15] In the present study, the gene expression profiles of human primary neonatal dermal fibroblasts which are exposed to 12 J/cm^2 ssUVR after 5-day recovery versus control cells (the radiated cells with the same intensity of radiation and 1-day recovery) were extracted from the GEO database. The differentially expressed genes (DEGs) were studied via PPI network analysis and gene ontology assessment to explore the critical molecular events.

Methods

The gene expression profiles of human primary neonatal dermal fibroblasts which are exposed to 12 J/cm^2 ssUVR on a modified Hand Foot II phototherapy instrument (National Biological Corporation) that emits 95% UVA and 5% UVB were extracted from GEO (GSE60796). The details of laser radiation and total RNA extraction and the full methods of data gathering are described in GSE60796 for all samples (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60796). The gene expression profiles of the cell samples that experienced 5-day recovery (12J 5d R) were compared with the gene expression outlines of the cells with 1-day recovery (12J 1day R).

Pre-evaluation Analysis

The GEO2R program was applied to evaluate the selected dataset. Gene expression profiles were assessed via the volcano plot to visualize significant up and downregulated genes. The numbers of significant DEGs relative to the dysregulated genes were plotted via the Venn diagram.

PPI Network Analysis

The significant DEGs were inserted in the STRING database via Cytoscape software v 3.7.2. The queried DEGs were included in a PPI network via undirected edges. The network was analyzed by using the "Network analyzer" application of Cytoscape. The main connected component of the PPI network was visualized based on degree value. The top 10% of nodes based on degree value and 5% of them considering the betweenness centrality parameter were determined as hubs and bottlenecks, respectively. The common hubs and bottlenecks were introduced as hub-bottlenecks.

Gene Ontology Enrichment

The central nodes including hubs and bottlenecks were assessed via gene ontology enrichment. Genes were enriched by using the ClueGO application of Cytoscape software.

Statistical Analysis

The significant DEGs were identified based on adjusted *P* value (padj) less than 0.05. The PPI network was formed considering a confidence score = 0.2. The gene ontology terms were identified in view of term *P* value, term *P* value corrected with Bonferroni step-down, group *P* value, and group *P* value corrected with Bonferroni step-down less than 0.05. "Network specificity" was selected near detailed. The significant terms were pointed out based on the applied statistical parameters.

Results

According to pre-evaluation analysis, the volcano plot schema for 12J 5-day R samples versus 12J 1-day R is shown in Figure 1. As presented in the volcano plot, there are many significant DEGs that separate both 12J

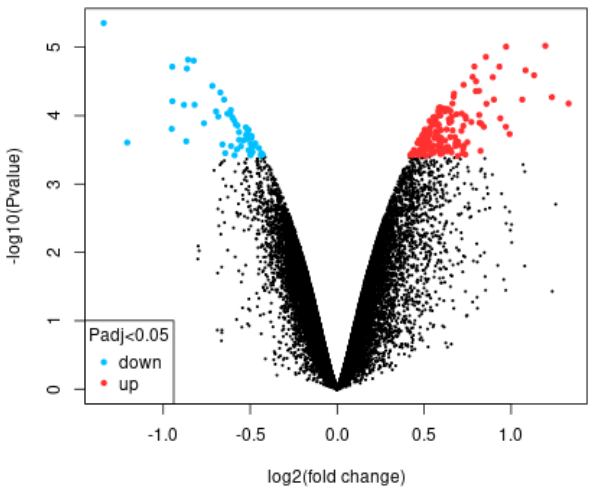

Figure 1. Volcano Plot Schema for 12J 5-Day Recovery Time Samples Versus 12J 1-Day Recovery Time Individuals

5-day recovery time samples versus 12J 1-day recovery time individuals. The results of the Venn diagram (see Figure 2) indicate that there are 224 significant DEGs among the 28 605 dysregulated genes.

To form a PPI network, we recognized 193 genes of the 224 significant DEGs by the STRING database. A PPI network including 13 isolated DEGs and a main connected component of 180 nodes and 1447 edges was constructed. The main connected component of the PPI network is illustrated in Figure 3. As depicted in Table 1, TOP2A, MKI67, BRCA1, HELLS, MAD2L1, ANLN, KIF11, MSH2, KRAS, NCAPG, RFC3, PLK4, WDHD1, BLM, CDKN3, KIF15, SMARCA5, and ATAD2 appear as hub genes, while TOP2A, MKI67, BRCA1, ANLN, KRAS, PLK4, SMARCA5, MMP2, and TLR4 are marked as bottlenecks. Analysis revealed that TOP2A, MKI67, BRCA1, ANLN, KRAS, PLK4, and SMARCA5 are hub-bottleneck genes of the PPI network.

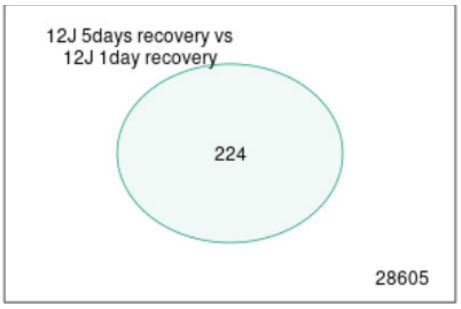

Figure 2. Venn Diagram for 12J 5-Day Recovery Time Samples Versus 12J 1-Day Recovery Time Individuals

The results of gene ontology enrichment are shown in Figure 4. The identified biological processes, molecular function, and biochemical pathways and the associated genes are presented in this figure. As in Figure 4, eight central genes are associated with 16 biological terms.

Discussion

Recovery time is an important parameter in radiation.[16] In the present study, the effect of 5-day recovery time on the gene expression profiles of human primary neonatal dermal fibroblasts was compared with the effect of 1-day recovery time. The pre-evaluation analysis indicates that there are significant differences between the gene expression profiles of the studied samples. In the present study, the crucial differences which were associated with long recovery time were explored. As shown in Figure 1, the significant DEGs discriminate two groups of irradiated cells. 224 significant DEGs were identified as the discriminators of samples. Vafaee R et al. published a document about reversible molecular events after UV radiation via network analysis.[17] Zhang et al. have studied skin photodamage via dynamic network biomarker analysis. Thirteen genes such as COL7A1 and CTNNB1 have been pointed out as biomarkers for skin photoaging.[18]

The enrichment of the central DEGs indicates that eight genes are associated with the identified biological terms.

As presented in Figure 3, There are several DEGs that play roles as central nodes. Twenty central genes are listed

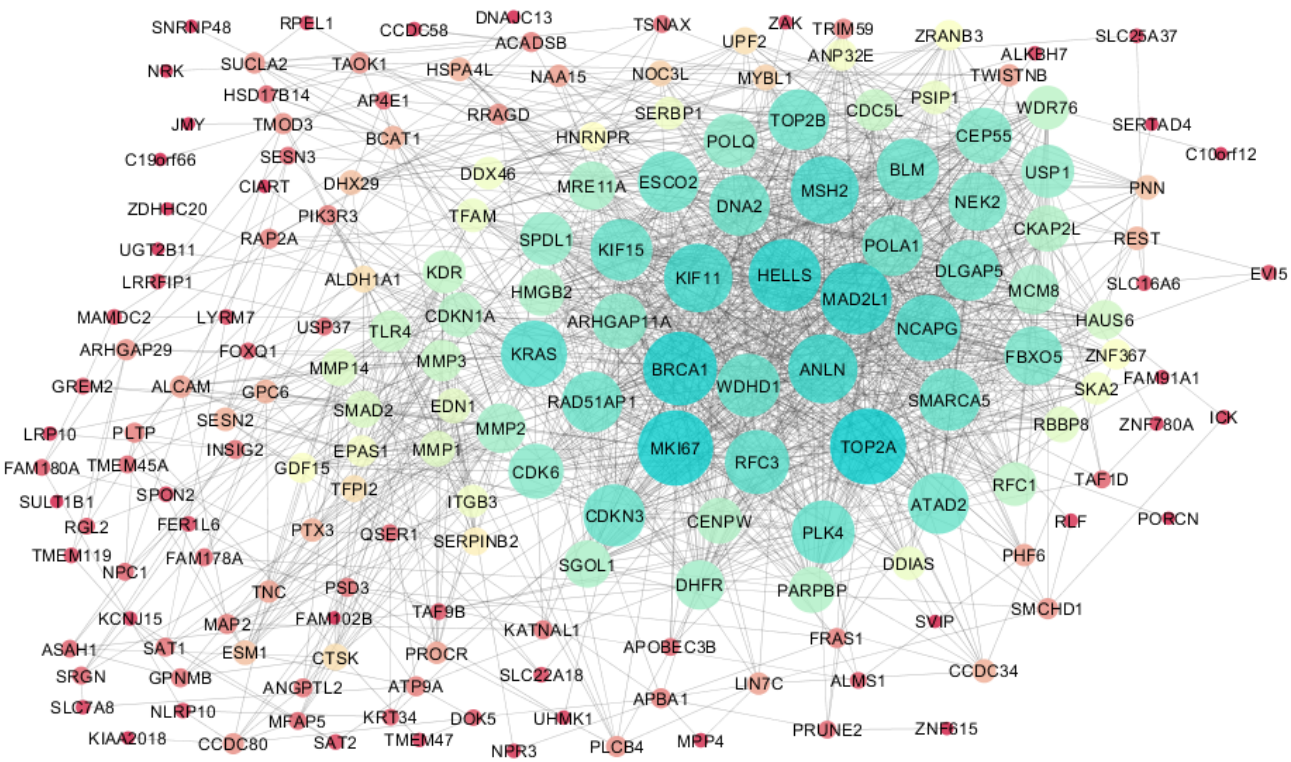

Figure 3. Main Connected Component of the PPI Network for 12J 5-Day Recovery Time Samples Versus 12J 1-Day Recovery Time Individuals. Red to green and small to large refer to the increment of degree value. A confidence score = 0.2 is applied

Table 1. List of the Central Nodes of the Main Connected Component of the PPI Network

No.	Display name	Gene description	Degree	BC	Centrality	LogFC
1	TOP2A	Topoisomerase (DNA) II alpha	61	0.042	Hub-bottleneck	0.581
2	MKI67	Marker of proliferation Ki-67	60	0.093	Hub-bottleneck	0.420
3	BRCA1	BRCA1, DNA repair associated	58	0.053	Hub-bottleneck	0.645
4	HELLS	Helicase, lymphoid-specific	56	0.021	Hub	0.686
5	MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)	55	0.023	Hub	0.569
6	ANLN	Anillin actin binding protein	52	0.055	Hub-bottleneck	0.779
7	KIF11	Kinesin family member 11	51	0.015	Hub	0.798
8	MSH2	MutS homolog 2	50	0.021	Hub	0.503
9	KRAS	KRAS proto-oncogene, GTPase	48	0.114	Hub-bottleneck	0.463
10	NCAPG	Non-SMC condensin I complex subunit G	48	0.007	Hub	0.652
11	RFC3	Replication factor C subunit 3	46	0.016	Hub	0.467
12	PLK4	Polo like kinase 4	45	0.040	Hub-bottleneck	0.900
13	WDHD1	WD repeat and HMG-box DNA binding protein 1	45	0.004	Hub	0.642
14	BLM	Bloom syndrome RecQ like helicase	44	0.012	Hub	0.659
15	CDKN3	Cyclin-dependent kinase inhibitor 3	44	0.024	Hub	0.564
16	KIF15	Kinesin family member 15	44	0.011	Hub	0.593
17	SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5	44	0.036	Hub-bottleneck	0.477
18	ATAD2	ATPase family, AAA domain containing 2	43	0.018	Hub	0.541
19	MMP2	Matrix metallopeptidase 2	33	0.054	Bottleneck	-0.573
20	TLR4	Toll like receptor 4	26	0.047	Bottleneck	0.677

Abbreviations: BC, betweenness centrality; FC, fold change.

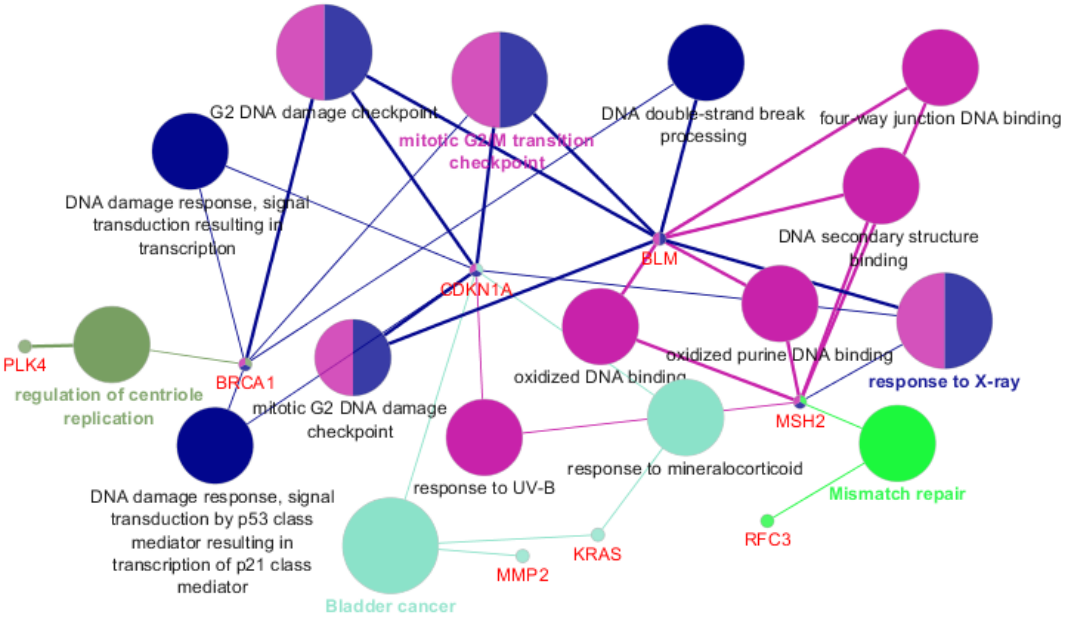

Figure 4. Gene Ontology Result of the Enrichment of the Hubs and Bottlenecks. The associated genes are labeled in red. Term *P* -value, term *P* value corrected with Bonferroni step-down, group *P* value, and group *P* value corrected with Bonferroni step-down less than 0.05. "Network specificity" was selected near detailed

in Table 1 which represents differences between 5-day and 1-day recovery times. Here, the crucial central nodes are discussed. Bloom syndrome RecQ like helicase (BLM) is connected to the nine biological terms which are mostly related to the DNA function and mitotic process. BLM overexpression is associated with DNA damages.[19] The upregulation of BLM in a 5-day recovery time versus a 1-day recovery time indicates that DNA damages and the worthless effect of UV radiation experience a progressive trend in a 5-day recovery time. It can be concluded that the repair process implies more expression of BLM to fix damages.

Cyclin-dependent kinase inhibitor 3 (CDKN3) is another central gene that is upregulated in a 5-day recovery time. The connections of CDKN1A are presented in Figure 4. Like BLM, it is involved in DNA damage processes. Investigations have revealed that CDKN3 promotes cell proliferation, invasion, and migration.[20] This finding is consistent with the condition of BLM overexpression.

MutS homolog 2 (MSH2) is another central DEG that is involved in seven biological terms. Lv L et al. published a document about the role of mismatch repair protein MSH2 in the regulation of translation DNA synthesis in cells which were exposed to UV radiation.[21] Eso and colleagues' investigation indicates that NF-κB signaling downregulates MSH2 in human hepatocytes.[22] Based on Kaplan and colleagues' report, TOP2A and MSH2 expression levels decrease in chemotherapeutic resistance to etoposide in breast cancer. This process may play a significant role in the progress of chemotherapeutic resistance.[23] As depicted in Table 1, topoisomerase (DNA) II alpha (TOP2A) is an upregulated central DEG similar to MSH2. It can be concluded that the upregulation of TOP2A and MSH2 in cells with 5-day recovery is involved in the repair process. BRCA1 is highlighted as an upregulated central gene. Several research studies have confirmed the significant role of BRCA1 in DNA repair after exposure to UV radiation.[24,25] The only downregulated central DEG is matrix metallopeptidase 2 (MMP2). Dang L et al.'s research indicates that the application of low-dose UVB irradiation inhibits inflammation and reactive oxygen species (ROS) production. This inhibition process leads to the prevention of MMP2-induced skin hyperplasia.[26] On the basis of the results, repair and DNA damage are two crucial processes that depend on long recovery time.

Conclusion

In conclusion, ssUVR induces wide gene expression alterations in human primary neonatal dermal fibroblasts. There are many differences between the gene expression condition of cells after 1-day and 5-day recovery. It seems that two principal phenomena occur at the interval between both recovery times; the first one is the repair process, and the second one is the progress of damages. The crucial central genes can be divided into two groups: the genes that are involved in repair courses like BRCA1, TOP2A and MSH2, and others that promote damages like BLM and CDKN1A (the possible target to prevent photoaging damages).

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Authors' Contribution

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Software: Mostafa Rezaei-Tavirani.
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Validation: Somayeh Jahani Sherafat.
Visualization: Fatemeh Bandarian.
Writing–original draft: Mostafa Rezaei-Tavirani, Somayeh Jahani Sherafat.
Writing–review & editing: Babak Arjmand, Farideh Razi, Fatemeh Bandarian.

Competing Interests

The authors declare they have no conflicts of interest

Ethical Approval

This project was approved by Shahid Beheshti University of Medical Sciences with the ethical code of IR.SBMU.RETECH.REC.1402.453.

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