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The dual role of cold atmospheric plasma as a promising modulator in melanoma

Melanomada umut verici bir düzenleyici olarak soğuk atmosferik plazmanın ikili rolü

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ABSTRACT

Objective: Cold Atmospheric Plasma (CAP),known for its antibacterial properties and potential therapeutic benefits, is emerging as a promising alternativeto traditional chemical disinfectants. This study aims to investigate the dual role of CAP in skin disinfection and its potential effects on melanoma. Specifically, the study examines how CAP affects gene expression and the potential implications of these effects on both skin health and cancer progression.

Methods: In this study, gene expression analysis was performed on human skin epithelial keratinocytes (HaCaT) post-treatment with Cold Atmospheric Plasma (CAP) and melanoma cell lines. Raw gene expression data were obtained from Array Express (E-GEOD-46343) and Gene Expression Omnibus (GSE22301) databases. The data were normalized using the Robust Multichip Average (RMA) method. For the melanoma cell lines, normal human melanocytes were used as the control group to ensure a comparative analysis of gene expression changes between cancerous and non-

ÖZET

Amaç: Antibakteriyel özellikleri ve potansiyel terapötik faydalarıyla bilinen Soğuk Atmosferik Plazma (SAP), geleneksel kimyasal dezenfektanlara umut verici bir alternatif olarak ortaya çıkmaktadır. Bu çalışma, SAP'nın cilt dezenfeksiyonundaki çift rolünü ve melanom üzerindeki potansiyel etkilerini araştırmayı amaçlamaktadır. Özellikle, SAP'nın gen ekspresyonunu nasıl etkilediği ve bu etkilerin hem cilt sağlığı hem de kanser progresyonu üzerindeki potansiyel sonuçları incelenmiştir.

Yöntem: Bu çalışmada, insan deri epitelyal keratinositlerinin (HaCaT) Soğuk Atmosferik Plazma (SAP) tedavisi sonrası ve melanom hücre hatlarının gen ekspresyonu analiz edilmiştir. Ham gen ekspresyon verileri Array Express (E-GEOD-46343) ve Gene Expression Omnibus (GSE22301) veritabanlarından alınmıştır. Veriler Robust Multichip Average (RMA) yöntemiyle normalize edilmiştir. Melanom hücre hatlarında, kanserli ve kanser olmayan hücreler arasındaki gen ekspresyonu değişimlerinin karşılaştırmalı analizini sağlamak amacıyla kontrol grubu olarak normal insan melanositleri kullanılmıştır.

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cancerous cells. Differentially expressed genes (DEGs) were identified using R packages Limma, affy, and edgeR. Common genes were identified with Venny 2.1.0, and hierarchical clustering analysis was performed using Gene Cluster v3.0. Pathway analysis was conducted using DAVID and KEGG, DAVID provides functional annotation clustering, while KEGG offers pathway mapping to identify significant biological processes. In addition, network analysis was performed using GeneMANIA and Cytoscape software.

Results: This study revealed significant differential gene expressions highlighting CAP's impact on both antimicrobial activity and cancer-related gene regulation. Key genes involved in oxidative stress responses, including ARID1A, NIPBL, BPTF, and OTUB1, showed notable changes. Downregulation of ARID1A, NIPBL, and BPTF is linked to impaired DNA repair and increased genomic instability, critical in melanoma progression. In contrast, upregulation of OTUB1 promotes cell survival and resistance to apoptosis. These findings suggest CAP's dual role in enhancing antimicrobial defenses and influencing cancer-related pathways, particularly through the modulation of oxidative stress responses.

Conclusion: Our findings provide a foundation for understanding the molecular effectsof CAP and its potential in developing novel therapeutic strategies for melanoma.These studies clarify CAP's beneficial impacts on skin health and potential carcinogenic risks, providing robust scientific data for future applications.

Key words: CAP, gene expression, disinfection, melanoma, oxidative stress, DEG

Diferansiyel olarak eksprese edilen genler (DEG) R paketleri Limma, affy ve edgeR kullanılarak belirlenmiştir. Ortak genler Venny 2.1.0 ile tanımlanmış, hiyerarşik kümeleme analizi Gene Cluster v3.0 ile yapılmıştır. Yolak analizi DAVID ve KEGG kullanılarak yapılmıştır, DAVID fonksiyonel anotasyon kümelemesi sağlarken, KEGG önemli biyolojik süreçleri tanımlamak için yolak haritalaması sunmaktadır. Ek olarak, ağ analizi GeneMANIA ve Cytoscape yazılımları kullanılarak gerçekleştirilmiştir.

Bulgular: Bu çalışma, SAP'nın hem antimikrobiyal aktivite hem de kanserle ilişkili gen düzenlemesi üzerindeki etkisini vurgulayan önemli farklı gen ifadelerini ortaya koymaktadır. ARID1A, NIPBL, BPTF ve OTUB1 dahil olmak üzere oksidatif stres tepkilerinde yer alan anahtar genler, dikkate değer değişiklikler göstermiştir. ARID1A, NIPBL ve BPTF'nin aşağı regülasyonu, melanom ilerlemesinde kritik olan bozulmuş DNA onarımı ve artan genomik instabilite ile bağlantılıdır. Buna karşılık, OTUB1'in yukarı regülasyonu hücrenin hayatta kalmasını ve apoptoza direncini desteklemektedir. Bu bulgular, SAP'nın antimikrobiyal savunmaları geliştirmede ve özellikle oksidatif stres tepkilerinin modülasyonu yoluyla kanserle ilişkili yolakları etkilemede ikili bir rol oynadığını göstermektedir.

Sonuç: Bulgularımız, SAP'nın moleküler etkilerinin anlaşılması ve melanom için yeni tedavi stratejilerinin geliştirilmesindeki potansiyeli için bir temel oluşturmaktadır. Bu çalışmalar, CAP'nin cilt sağlığı üzerindeki olumlu etkilerini ve olası kanserojen risklerini netleştirerek, gelecekteki uygulamalar için sağlam bilimsel veriler sunmaktadır.

Anahtar Kelimeler: SAP, gen ekspresyonu, dezenfeksiyon, melanoma, oksidatif stres, DEG

INTRODUCTION

Disinfectants are effective in reducing and preventing disease transmission, but their potential adverse effects on humans and the environment, such as aquatic ecosystem pollution and resistant populations, are increasing. Disinfectants must be strictly controlled to protect human and environmental health. Because chemical disinfectants can irritate the skin, trigger allergies and disrupt the microbiota (1). Given these challenges, CAP offers a safer alternative due to its non-thermal properties

and minimal environmental impact.

Gaseous partially ionized Cold Atmosphere Plasma (CAP), also known as Non-Thermal atmospheric pressure plasma (NTAPP), has reactive species that do not require high temperatures and has antibacterial abilities (2). Comprehensive antibacterial activities prevent diseases associated with multidrug-resistant bacteria (3). Cell growth and tissue regeneration can improve wound healing using cold atmospheric plasma (CAP). CAP helps manage permanent wounds and surgeries that require infection control and good healing (4, 5).

Melanoma is spreading worldwide, and people with fair skin are often more exposed to the harmful effects of the sun. Additionally, mutations and family histories may also increase the risk of disease. The risk of melanoma increases significantly due to genetic changes. For example, mutations in the melanocortin 1 receptor (MC1R) and other genes increase the risk of melanoma (6, 7).

Melanoma has also been associated with disinfectant exposure, especially frequent and persistent exposure. These elements damage skin cells and structure (8). Genetics, UV light, and disinfectants contribute to melanoma (9).

In this study, we used advanced computational methods and comprehensive analyzes to identify genes that show significant changes after exposure to CAP in human skin epithelial keratinocytes. By discovering how CAP exposure affects molecular pathways in keratinocytes, we have gained valuable insight into cancer biology and believe it will shed light on future studies that may reveal potential biomarkers or treatment targets for melanoma.

MATERIAL and METHOD

Obtaining and Normalizing Whole-Genome Expression Data

Whole genome expression data of human skin epithelial keratinocytes were obtained from Array Express (E-GEOD-46343) (10). Gene expression data of Melanoma cell lines and melanocyte controls were obtained from the Gene Expression Omnibus (GSE22301). Affymetrix Human Genome U133A 2.0 Array is used for the analysis (11).

In order to address technical differences across the arrays and eliminate background noise, the raw sample data undergo normalization using the Robust Multichip Average (RMA) approach, implemented using the R packages Limma and affy. (12, 13). Additionally, to account for potential batch effects between the datasets (E-GEOD-46343 and GSE22301), the “ComBat” function from the sva package was applied. This method adjusts for systematic biases arising from batch effects, ensuring comparability across datasets. However, we acknowledge that while batch correction minimizes technical variance, some residual effects may persist, which could potentially influence the interpretation of subtle gene expression differences.

Identification of Differentially Expressed Genes and Commonly Altered Genes

Differentially Expressed Genes (DEGs) analysis is a molecular biology approach that compares the levels of gene expression across distinct sample groups, such as healthy and diseased tissues or cells treated with different substances (14, 15).

The main goal of DEGs analysis is to identify genes that are expressed differently in the circumstances.

By doing DEG analysis using the R package edgeR, we identified statistically significant genes (p-value < 0.05) with a high expression change ratio in human skin epithelial keratinocytes (HaCaT) after 60 seconds of cold plasma treatment. We compared these results to an untreated control group and also compared them to a melanoma cell line in comparison to normal human melanocytes.

A Venn diagram was produced using the web application Venny 2.1.0 to identify the common genes that exhibit substantial changes in expression in both cold plasma-treated epithelial cells and melanoma cells, as compared to their respective control groups

(16, 17).

Hierarchical Cluster Analysis

To demonstrate the capacity of common genes with the highest variation between groups to distinguish between the compared groups, hierarchical cluster analysis was conducted.

Genes identified were grouped hierarchically based on their mean standardized expression values (Euclidean Gene Cluster 3.0). After clustering, the data was standardized and visualized with Treeview. The hierarchical clustering enabled the visualization of distinct gene expression patterns, thereby highlighting the differential expression profiles and helped in the identification of key genes involved in the response to CAP exposure as well as melanoma (18).

Pathway Analysis

The pathways associated with our genes were identified by Database for Annotation, Visualization and Integrated Discovery (DAVID) which is an online tool to elucidate the biological pathways associated with the common differentially expressed genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) database within DAVID is used to map the genes by using known pathways that may be implicated in the response to exposure to CAP in human skin epithelial keratinocytes (19, 20).

Network Analysis

GeneMANIA software was used and genes in similar pathways were identified with Cytoscape version 3.10.2 to explore the network and pathway relationships between the selected differentially expressed genes and other genes. The corresponding network provided valuable insights into the functional associations and putative regulatory mechanisms of the discovered genes. The identification of important genes and crucial pathways that may have major roles in the response to NTAPP exposure and melanoma development was achieved via the visualization of the network (21, 22).

Statistical analysis

The statistical analysis was performed using the R programming environment with packages such as Limma, affy, and edgeR. Data were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test was applied to determine the data's suitability for normal distribution (Data not shown). For normally distributed data, comparisons between groups were made using a t-test. Non-parametric data were analyzed using the Mann-Whitney U test. Statistical significance was set at a p-value < 0.05. To show the clustering of selected genes, the pathways they are involved in, and their network relations, Euclidean Gene Cluster, DAVID, and GeneMANIA software were used.

This study does not require Ethics Committee Approval.

RESULTS

Differential Expression Analysis and Volcano Plot Visualization

The volcano plot shown in Figure 1A shows the comparative study of gene expression between human skin epithelial keratinocytes treated with CAP and the control group. Every data point corresponds to a specific gene, where the x-axis represents the log2 fold change (LFC) and the y-axis indicates the negative logarithm (base 10) of the adjusted p-value. Genes that exhibit a substantial increase in expression ($p<0.01$) in response to plasma therapy are visually emphasized in red, while genes that show a significant decrease in expression ($p<0.01$) are visually represented in green. The gray dots represent genes that do not show statistically significant changes in expression. The horizontal blue dashed line depicts the significance threshold, which denotes the critical level at which p-values are deemed significant ($p<0.01$). This image clearly illustrates a noticeable difference between the treated and control groups, with a large number of genes displaying substantial

differences in expression. This emphasizes the influence of plasma therapy on the regulation of genes in human skin epithelial keratinocytes. A total of 407 genes exhibited substantial up-regulation after NTAPP therapy, while 10868 genes showed down-regulation.

Heat Map Visualization of Differentially Expressed Genes

The heat map in Figure 1B provides a complete display of the genes that are expressed differently between the control group and human skin epithelial keratinocytes treated with CAP. Each row represents a gene, and each column represents samples from either the control or treatment groups. The color red represents an increase in gene expression, whereas the color green represents a decrease. The intensity of the color reflects the degree of gene expression. The heat map clearly displays distinct clustering patterns that demonstrate substantial variations in gene expression profiles between the treated and non-treated groups. Differentiation emphasizes the substantial impact of plasma treatment on the gene expression pattern of human skin epithelial keratinocytes, indicating how this intervention might regulate certain biological processes.

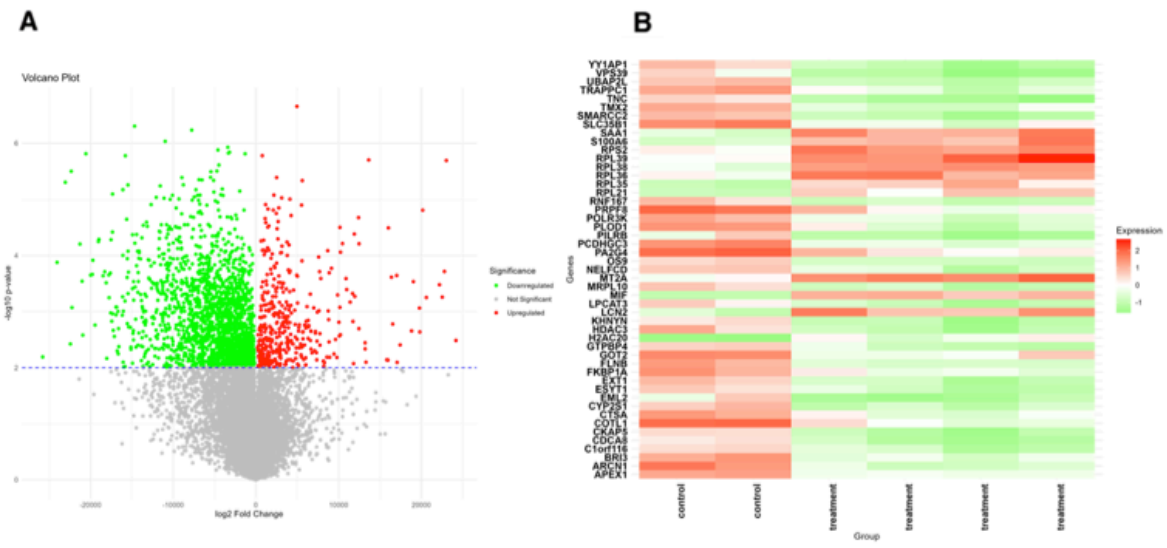

Figure 1. (A) Differential gene expression analysis showing genes that are statistically significantly up- or down-expressed in Non-Thermal Atmospheric Pressure Plasma (NTAPP)-treated human skin epithelial keratinocytes compared to the control group. (B) Heat map analysis demonstrating that the differentially expressed genes can significantly distinguish between treated and non-treated groups.

Identifying Molecular Distinctions and Therapeutic Target Genes

Figure 2A illustrates the comparison of gene expression between melanoma cell lines and normal human melanocyte cell lines. The x-axis of the graph indicates the log2 fold change (LFC) of each gene, while the y-axis represents the -log10 adjusted p-value. Each dot on the graph represents an individual gene. The red dots indicate genes that are considerably elevated in melanoma cell lines

($p<0.01$), whereas the green dots reflect genes that are strongly downregulated ($p<0.01$). The gray dots represent genes that have not shown any substantial change in their expression levels. The horizontal dashed lines represent the levels used to determine statistical significance. Disparities in gene expression patterns between the two cell line groupings, emphasizing significant changes in gene regulation linked to melanoma. This investigation highlights the molecular distinctions between malignant melanoma cells and normal melanocytes, offering valuable insights into possible routes and targets for therapeutic intervention. A total of 83 genes exhibited substantial up-regulation, whilst 154 genes showed down-regulation in melanoma cell lines as compared to normal human melanocyte cell lines. Furthermore, the use of hierarchical cluster analysis revealed that these genes had the ability to differentiate between melanoma and healthy groups, as seen in Figure 2B.

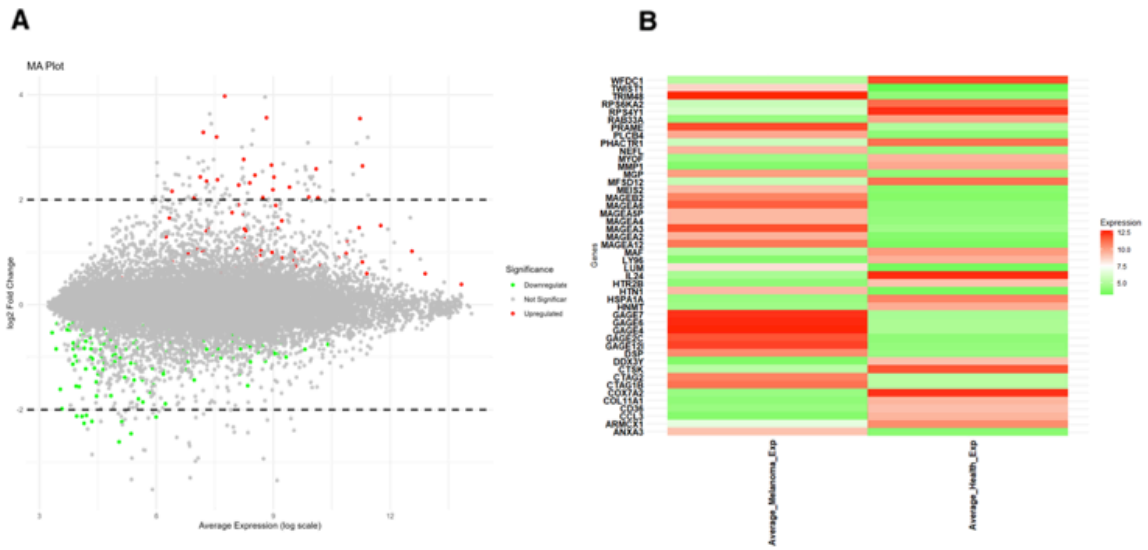

Figure 2. (A) A scatter plot illustrating the significantly up- and down-regulated genes between melanoma cells and healthy skin cells. The data reveal distinct gene expression patterns, indicating substantial alterations in gene regulation associated with melanoma. (B) A heat map of gene expression profiles differentiating between melanoma and healthy skin cells. The expression data highlights clear distinctions, underscoring the potential of these genes to serve as biomarkers for melanoma detection and characterization.

The Venn diagram (Figure 3) shows the intersection of genes that are highly regulated in both CAP-treated human skin epithelial keratinocytes and melanoma cell lines. More precisely, the data reveals that there are 12 genes that are noticeably reduced in expression in both scenarios, as seen in Figure 3A. Additionally, there are 3 genes that exhibit increased expression, as shown in Figure 3B. A comprehensive inventory of these genes that have been downregulated and elevated is shown in Table 1.

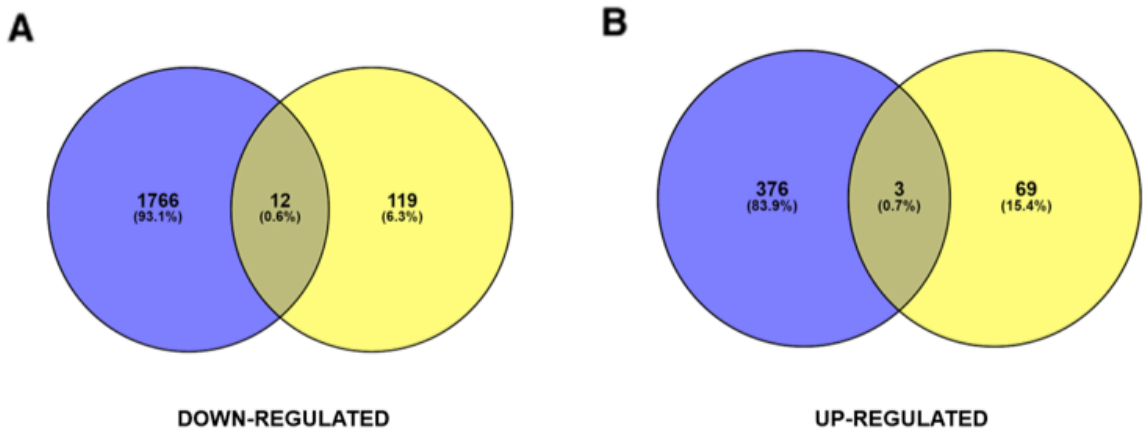

Figure 3. The Venn diagrams illustrate the overlap of significantly regulated genes between NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. (A) The left diagram shows 12 genes that are significantly downregulated in both NTAPP-treated keratinocytes and melanoma cell lines compared to their respective controls. (B) The right diagram shows 3 genes that are significantly upregulated in both NTAPP-treated keratinocytes and melanoma cell lines. These overlaps highlight potential common pathways and molecular mechanisms affected by NTAPP treatment and associated with melanoma progression.

Table 1. Differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. The table categorizes the genes into up-regulated and down-regulated groups

up-regulated genes	down-regulated genes
CARMIL1	SLC7A1
OTUB1	SMG7
GUK1	EXOC7
	NUP54
	POU2F1
	DST
	NIPBL
	ARID1A
	MRPL57
	DCK
	ZBTB39
	BPTF

Biological Processes and Molecular Functions

The functional annotation clustering study yielded five separate groups, as shown in Table 2. Melanoma cell lines and CAP-treated human epithelial keratinocytes share a set of 15 genes with differential expression. Clusters of genes have been shown to be more abundant in relation to certain biological processes, cellular components, or molecular activities. An example instance is Cluster 1, which comprises genes associated with the nucleoplasm,

nucleus, and phosphoproteins, and has an enrichment value of 1.42. Cluster 2 consists of genes involved in chromatin remodeling, transcription control, and RNA polymerase II activity. This cluster has an enrichment value of 0.84. The genes related to cytoplasmic components and coiled coil domains are emphasized in Cluster 3, with an enrichment value of 0.58. Cluster 4, which has an enrichment value of 0.2, consists of genes associated with the cell membrane and plasma membrane. Cluster 5, which has an enrichment value of 0.17, specifically targets genes related to isopeptide bonds, ubiquitin-like conjugation, and metal-binding. for their regulation in response to CAP therapy and the advancement of melanoma.

Table 2. Functional annotation clustering of differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines

Annotation Cluster 1	**Enrichment Score: 1.42**	**Count**	**P-Value**	**Benjamini**
GOTERM_CC_DIRECT	Nucleoplasm	9	1.90E-03	1.30E-01
GOTERM_CC_DIRECT	Nucleus	8	8.70E-02	1.00E+00
UP_KW_PTM	Phosphoprotein	10	3.30E-01	1.00E+00
Annotation cluster 2	**Enrichment Score: 0.84**	**Count**	**P-Value**	**Benjamini**
GOTERM_BP_DIRECT	Chromatin remodeling	3	2.40E-02	1.00E+00
GOTERM_CC_DIRECT	Chromatin	4	3.90E-02	8.10E-01
UP_KW_CELLULAR_COMPONENT	Nucleus	5	5.10E-02	6.70E-01
UP_KW_BIOLOGICAL_PROCESS	Transcription regulation	5	1.40E-01	1.00E+00
GOTERM_BP_DIRECT	Negative regulation of transcription by RNA polymerase II	3	1.50E-01	1.00E+00
UP_KW_BIOLOGICAL_PROCESS	Transcription	3	1.50E-01	1.00E+00
UP_KW_DISEASE	Intellectual disability	3	1.80E-01	1.00E+00
GOTERM_BP_DIRECT	Positive regulation of transcription by RNA polymerase II	3	2.20E-01	1.00E+00
GOTERM_MF_DIRECT	RNA polymerase II cis-regulatory region sequence-specific DNA binding	3	2.40E-01	1.00E+00
GOTERM_BP_DIRECT	Regulation of transcription by RNA polymerase II	3	3.20E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Pro residues	3	3.80E-01	1.00E+00
UP_KW_MOLECULAR_FUNCTION	DNA-binding	3	5.50E-01	1.00E+00
Annotation cluster 3	**Enrichment Score: 0.58**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	4	2.30E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Basic and acidic residues	6	2.70E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cytoplasm	7	2.80E-01	1.00E+00
Annotation Cluster 4	**Enrichment Score:0.2**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	4	2.30E-01	1.00E+00
UP_SEQ_FEATURE	COMPBIAS:Basic and acidic residues	4	6.40E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cytoplasma	4	7.70E-01	1.00E+00
Annotation Cluster 5	**Enrichment Score: 0.17**	**Count**	**P-Value**	**Benjamini**
UP_KW_DOMAIN	Coiled coil	3	8.90E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	Cell membrane	5	9.50E-01	1.00E+00
GOTERM_CC_DIRECT	Plasma membrane	3	5.30E-01	1.00E+00
GOTERM_CC_DIRECT	Membrane	3	7.40E-01	1.00E+00
UP_KW_CELLULAR_COMPONENT	mMembrane	3	8.10E-01	1.00E+00

Elucidating Key Interactions and Regulatory Mechanisms

In Figure 4, a network analysis of 15 genes that showed differential expression in human skin epithelial keratinocytes and melanoma cell lines that were constructed using Cytoscape is shown. In this network, each node stands for a gene, and the connections between them show several kinds of interactions, such physical interactions, common pathways, or co-expression. Larger nodes signify genes with higher degrees of connectivity, highlighting their potential central roles in the network. Key genes, such as NUP54, CAD, and DST, show extensive interactions, suggesting their importance in the regulatory processes affected by CAP treatment and melanoma.

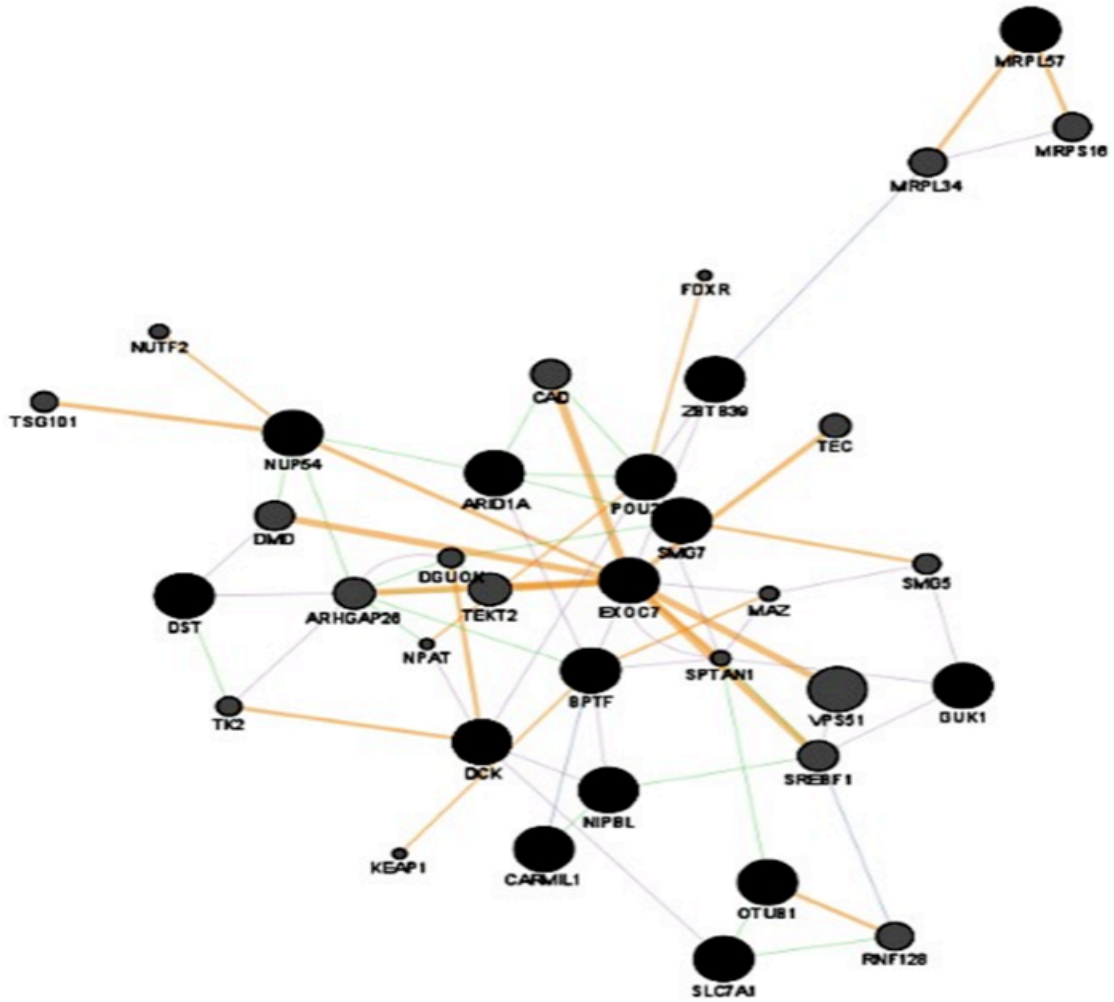

Figure 4. Network analysis of 15 differentially expressed genes common to NTAPP-treated human skin epithelial keratinocytes and melanoma cell lines. Each node represents a gene, with larger nodes indicating higher degrees of connectivity. The edges between nodes represent various types of interactions, such as co-expression, physical interactions, or shared pathways. Key hub genes, such as NUP54, CAD, and DST, exhibit extensive interactions, suggesting their central roles in the regulatory processes affected by NTAPP treatment and melanoma progression.

DISCUSSION

Cold atmospheric plasma has important roles in the medical field in treatments such as wound healing, bacterial sterilization, cleaning and tissue coagulation. However, the molecular mechanisms underlying these roles are complex as they involve many physical and chemical activities. Although studies reveal that CAP produces ROS and RNS, it is predicted that the presence of these reagents may cause oxidative stress, which may cause genetic abnormalities or disruptions in signaling systems, and this may facilitate the development of cancer. High ROS production of melanoma cells has the ability to damage DNA, accelerate tumor growth, and increase the possibility of metastasis (23, 24). Elevated amounts of ROS in melanoma cells may trigger the activation of oncogenic signaling pathways, such as the often mutant MAPK pathway. Consequently, blocking these pathways is a fundamental approach for treatment. ROS and RNS also regulate the tumor microenvironment, stimulate angiogenesis, and suppress immune responses, therefore facilitating melanoma proliferation. Melanoma cells are susceptible to ROS, so therapeutic strategies are focusing on targeting oxidative stress pathways.

We identified 15 genes that were expressed differently in CAP-treated melanoma cells than in healthy skin cells. 12 of these genes were downregulated in both CAP-treated cells and melanoma cells (SLC7A1, SMG7, EXOC7, NUP54, POU2F1, DST, NIPBL, ARID1A, MRPL57, DCK, ZBTB39 and BPTF). Three of them were upregulated in both CAP-treated cells and melanoma cells (CARMIL1, OTUB1 and GUK1). This result suggests a potential link between differential expression of these genes in response to CAP treatment and their role in melanoma progression.

ARID1A, NIPBL, and BPTF has a significant impact on ROS and RNS production in various cancer cells, including melanoma.

Downregulation of ARID1A facilitates cancer progression by causing impairments in the cell's response to DNA damage (25). Additionally, a decrease in NIPBL expression has been associated with an increase in oxidative stress and genomic instability. This encourages the growth of melanoma cells (26). Similarly, downregulation of BPTF increases ROS levels and causes DNA damage. The results highlight the important functions of these genes in controlling oxidative stress and influencing the behavior of cancer cells (27). Conversely, increased expression of OTUB1 has been associated with elevated levels of ROS in cancer cells, which may cause oxidative stress and affect the proliferation and survival of cancer cells (28).

Research suggests that the development of melanoma cancer is strongly dependent on the growth, movement, and creation of blood vessels in cells. Melanoma cells exhibit increased rates of proliferation and migration, which promote the growth and spread of tumors. Additionally, they display accelerated growth, resulting in the development of larger tumors. The process is influenced by several molecular processes and factors, one of which is the overexpression of astrocyte increased gene-1 (AEG-1). Studies suggest that AEG-1 promotes the growth and movement of melanoma cells, hence accelerating the development of tumors (29). Tissue inhibitor of metalloproteinases-3 (TIMP3) influences tumor growth and blood vessel formation by regulating the movement of endothelial cells, which is crucial for the spread of melanoma (30).

Furthermore, suppression of ARID1A, NIPBL, BPTF and ZBTB39 genes may greatly contribute to the aggressive behavior of malignancies. Downregulation or mutation of ARID1A, a component of the SWI/SNF chromatin remodeling complex, has been associated with increased cell proliferation and migration. ARID1A dysfunction may lead to structural disruption of chromatin and changes in gene expression, contributing to the development of aggressive cancer behavior (31). NIPBL is essential for regulating gene expression and maintaining the

connection between sister chromatids. Therefore, its downregulation may lead to increased susceptibility to genetic instability and accelerated tumor development (32). Also, BPTF, which stands for bromodomain PHD finger transcription factor, is a constituent of the NURF chromatin remodeling complex. The decrease in BPTF expression has been associated with enhanced movement and infiltration of melanoma cells, hence promoting metastasis (33). While there is a lack of extensive research on ZBTB39 in melanoma, its decrease in activity is linked to changes in gene expression that might potentially promote the advancement of cancer via several signaling pathways (34).

Other studies and investigations have shown that the up-regulation of OTUB1 has a major impact on cell proliferation, migration, and angiogenesis in melanoma cells. OTUB1, also known as OTU deubiquitinase and ubiquitin aldehyde binding 1, has a role in controlling cellular responses to oxidative stress and repairing DNA damage. The upregulation of this gene has been associated with increased cell proliferation and migration.

According to the functional annotation clustering findings of our work, out of the 12 genes that were downregulated, a number of them have previously been linked to important biological functions.

Accordingly, ARID1A is required to control the activation or deactivation of genes. When ARID1A is suppressed, it is often associated with increased cell growth and progression of tumors, leading to the aggressive nature of malignancies such as melanoma (35). If NIPBL is reduced, it can cause genomic instability, which is a hallmark of cancer progression. When downregulated, BPTF, an essential component of NURF, inhibits cell cycle control and processes involved in DNA repair (36). If ZBTB39 is downregulated, it can lead to uncontrolled cell proliferation and survival, which promotes cancer development (37). Additional downregulated genes, such as *SLC7A1*, *SMG7*, *EXOC7*, *NUP54*, *POU2F1*, *DST*, *MRPL57*, and *DCK*, have diverse functions in cellular processes including transport, RNA surveillance, vesicle traffic, nuclear pore complex function, transcriptional regulation, structural integrity, and other factors. These genes have not been extensively investigated in the context of cancer. The simultaneous decrease in the expression of these genes disrupts the balance in the cells, leading to the development of cancer features seen in melanoma. Conversely, increased activity of *CARMIL1*, *OTUB1* and *GUK1* genes also has important consequences. CARMIL1 increases the ability of melanoma cells to migrate and invade (38). OTUB1 affects protein degradation and signaling cascades. Its increased expression has been associated with improved cell survival and resistance to apoptosis, thus promoting cancer cell survival (39). GUK1 facilitates rapid growth of cancer cells by providing a consistent source of nucleotides for DNA replication (40).

Overall, the differential expression of these genes underscores the complex molecular interaction affected by CAP treatment. Downregulation of genes associated with cell cycle regulation, chromatin restructuring, and genomic stability, combined with upregulation of genes that promote cell motility and survival, appears to drive the aggressive behavior of melanoma.

Further functional studies are needed to confirm these findings and explore the therapeutic potential of targeting these genes in melanoma treatment.

In conclusion, despite its promising applications, CAP therapy leads to changes in gene expression that may have implications for melanoma development. Although CAP is effective in antimicrobial action and enhancing wound healing, long-term effects on gene expression and its potential to promote oxidative stress and genetic mutations require further investigation.

Our findings suggest that CAP could be a double-edged sword in clinical settings, offering therapeutic benefits in wound healing and antimicrobial defense, while also potentially modulating gene pathways that influence cancer progression. This highlights the need for careful clinical application, particularly in patients

with a predisposition to melanoma or other cancers.

These insights into CAP-induced gene expression changes may guide future experimental and clinical studies aimed at optimizing CAP use for melanoma treatment. For example, understanding which genes are differentially regulated by CAP can inform targeted therapeutic strategies that either mitigate cancer-promoting effects or enhance CAP's beneficial actions. Additional research, particularly clinical trials and in vitro and animal studies, is essential to confirm the safety and effectiveness of CAP in humans and to explore its full potential as an adjunctive therapy in melanoma treatment.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Hashemi F, Hoepner L, Hamidinejad FS, Haluza D, Afrashteh S, Abbasi A, et al. A comprehensive health effects assessment of the use of sanitizers and disinfectants during COVID-19 pandemic: a global survey. Environ Sci Pollut Res Int, 2023;30(28):72368-88.
2. Hara H, Adachi T. Molecular mechanisms of non-thermal atmospheric pressure plasma-induced cellular responses. Jpn J Appl Phys, 2021;60(2):020501.
3. Haertel B, Von Woedtke T, Weltmann K-D, Lindequist U. Non-thermal atmospheric-pressure plasma possible application in wound healing. Biomol Ther, 2014;22(6):477.
4. Bernhardt T, Semmler ML, Schäfer M, Bekeschus S, Emmert S, Boeckmann L. Plasma medicine: Applications of cold atmospheric pressure plasma in dermatology. Oxid Med Cell Longev, 2019;2019(1):3873928.
5. Chatraie M, Torkaman G, Khani M, Salehi H, Shokri B. In vivo study of non-invasive effects of non-thermal plasma in pressure ulcer treatment. Sci Rep, 2018;8(1):1-11.
6. D'Orazio JA, Marsch A, Veith J. Skin pigmentation and melanoma risk. Advances in malignant melanoma-clinical research perspective. InTech: 2011.

7. Hawkes JE, Truong A, Meyer LJ. Genetic predisposition to melanoma. Semin Oncol, 2016;43(5):591-7.

8. Fusaro RM, Lynch HT. The FAMMM syndrome: epidemiology and surveillance strategies. Cancer Invest, 2000;18(7):670-80.

9. Rastrelli M, Tropea S, Rossi CR, Alaibac M. Melanoma: epidemiology, risk factors, pathogenesis, diagnosis and classification. In Vivo. 2014;28(6):1005-11.

10. Schmidt A, Bekeschus S, Jablonowski H, Barton A, Weltmann K-D, Wende K. Role of ambient gas composition on cold physical plasma-elicited cell signaling in keratinocytes. Biophys J, 2017;112(11):2397-407.

11. Rose AE, Poliseno L, Wang J, Clark M, Pearlman A, Wang G, et al. Integrative genomics identifies molecular alterations that challenge the linear model of melanoma progression. Cancer Res, 2011;71(7):2561-71.

12. McCall MN, Bolstad BM, Irizarry RA. Frozen robust multiarray analysis (fRMA). Biostatistics. 2010;11(2):242-53.

13. McGee M, Chen Z. Parameter estimation for the exponential-normal convolution model for background correction of affymetrix GeneChip data. Stat Appl Genet Mol Biol, 2006;5(1).

14. Costa-Silva J, Domingues D, Lopes FM. RNA-Seq differential expression analysis: An extended review and a software tool. PloS one. 2017;12(12):e0190152.

15. Kontou PI, Pavlopoulou A, Bagos PG. Methods of analysis and meta-analysis for identifying differentially expressed genes. Genetic Epidemiology: Methods and Protocols. 1st ed. Humana, 2018.

16. Dong H, Li M, Chen H, Tian L, Wei W, Wang S, et al. Using network pharmacological analysis and molecular docking to investigate the mechanism of action of quercetin's suppression of oral cancer. J Cancer Res Clin Oncol, 2023;149(16):15055-67.

17. Oliveros JC. Venny. An interactive tool for comparing lists with Venn's diagrams. 2007-2015 [Available from: https://bioinfogp.cnb.csic.es/tools/venny/index.html.

18. Abu-Jamous B, Kelly S. Clust: automatic extraction of optimal co-expressed gene clusters from gene expression data. Genome Biol, 2018;19(1):172.

19. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protocs, 2009;4(1):44-57.

20. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res, 2022;50(W1):W216-w21.

21. Franz M, Rodriguez H, Lopes C, Zuberi K, Montojo J, Bader GD, et al. GeneMANIA update 2018. Nucleic Acids Res, 2018;46(W1):W60-W4.

22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res, 2003;13(11):2498- 504.

23. Ibañez IL, Notcovich C, Policastro LL, Durán H, Tanaka Y. Reactive oxygen species in the biology of melanoma. Breakthroughs in Melanoma Research, InTech. 2011:1-30.

24. Kamiński K, Kazimierczak U, Kolenda T. Oxidative stress in melanogenesis and melanoma development. Contemporary Oncology/Współczesna Onkologia. 2022;26(1):1-7.

25. Kwan SY. Regulation Of The Oxidative Stress Response By Arid1A. Doktora Tezi, The University of Texas Graduate School of Biomedical Sciences 2015.

26. Guarrera L. Multi-omic computational analysis to develop a new strategy for pharmacological treatment of Cornelia de Lange syndrome. Yüksek Lisans Tezi, Politecnico di Milano, 2018.

27. Roberts MG. Investigating the effects of nucleosome remodeling factor knockdown on antitumor immunity. Yüksek Lisans Tezi, Virginia Commonwealth University, 2016.

28. Li D, Wang Y, Dong C, Chen T, Dong A, Ren J, et al. CST1 inhibits ferroptosis and promotes gastric cancer metastasis by regulating GPX4 protein stability via OTUB1. Oncogene, 2023;42(2):83-98.

29. Zhang Y, Peng G, Wang Y, Cui L, Wu W, Wang L, et al. Silencing of astrocyte elevated gene-1 inhibits proliferation and migration of melanoma cells and induces apoptosis. Clin Exp Pharmacol Physiol, 2017;44(7):815-26.

30. Das AM, Seynhaeve AL, Rens JA, Vermeulen CE, Koning GA, Eggermont AM, et al. Differential TIMP3 expression affects tumor progression and angiogenesis in melanomas through regulation of directionally persistent endothelial cell migration. Angiogenesis. 2014;17:163-77.

31. Saqcena M, Leandro-Garcia LJ, Maag JL, Tchekmedyian V, Krishnamoorthy GP, Tamarapu PP, et al. SWI/SNF complex mutations promote thyroid tumor progression and insensitivity to redifferentiation therapies. Cancer Discov, 2021;11(5):1158-75.

32. Xu W, Ying Y, Shan L, Feng J, Zhang S, Gao Y, et al. Enhanced expression of cohesin loading factor NIPBL confers poor prognosis and chemotherapy resistance in non-small cell lung cancer. J Transl Med, 2015;13:1-12.

33. Dar AA, Nosrati M, Bezrookove V, de Semir D, Majid S, Thummala S, et al. The role of BPTF in melanoma progression and in response to BRAF-targeted therapy. J Natl Cancer Inst, 2015;107(5):djv034.

34. Perez-Torrado R, Yamada D, Defossez PA. Born to bind: the BTB protein-protein interaction domain. Bioessays, 2006;28(12):1194-202.

35. Xu S, Tang C. The role of ARID1A in tumors: tumor initiation or tumor suppression? Front Oncol, 2021; 11:745187.

36. Mazzola M, Deflorian G, Pezzotta A, Ferrari L, Fazio G, Bresciani E, et al. NIPBL: a new player in myeloid cell differentiation. Haematologica, 2019;104(7):1332.

37. Maeda T. Regulation of hematopoietic development by ZBTB transcription factors. Int J Hematol, 2016; 104:310-23.

38. Ge Y, Xiao B, Zhao R, Li B, Yang S, He KF, et al. CARMIL1 regulates liver cancer cell proliferation by activating the ERK/mTOR pathway through the TRIM27/p53 axis. Int Immunopharmacol, 2024;134:112139.

39. Herhaus L, Al-Salihi M, Macartney T, Weidlich S, Sapkota GP. OTUB1 enhances TGFß signalling by inhibiting the ubiquitylation and degradation of active SMAD2/3. Nat Commun, 2013;4(1):2519.

40. Wolfe K, Kofuji S, Yoshino H, Sasaki M, Okumura K, Sasaki AT. Dynamic compartmentalization of purine nucleotide metabolic enzymes at leading edge in highly motile renal cell carcinoma. Biochem Biophys Res Commun, 2019;516(1):50-6.