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Identification of molecular pathways and prognostic biomarkers with survival analysis associated with UV-mediated skin cancer by bioinformatics approaches

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Abstract

Skin cancer (SC) referred to as cutaneous carcinoma is a serious public health concern on a global scale particularly for those with fair skin. Ultraviolet (UV) radiation causes skin cancer, but the exact mechanism by which occurs and the most effective methods of intervention to prevent it are yet unknown. Using bioinformatic approaches several biomarkers have been identified to determine the severity of skin cancer. This study will use bioinformatics and pharmacology approaches to discover potential biomarkers of SC for early diagnosis, prevention of disease, and therapeutic target identification.

This study compared gene expression and protein levels in UV-mediated cultured keratinocytes and adjacent normal skin tissue using RNA sequencing data from the NCBI-GEO database. Then we employed GO and signalling pathway database, selection of hub genes from protein-protein interaction (PPI) network, survival and expression profile, and gene regulatory network analysis to screen potential clinical biomarkers.

In the study, we identified 32 shared differentially expressed genes (DEGs) including 19 upregulated genes and 13 downregulated genes by analyzing three different subsets of the GSE85443 dataset. Skin cancer development is related to control of several DEGs through cyclin-dependent protein serine/threonine kinase activity, cell cycle regulation and activation of the NIMA kinase pathways. The cytohubba plugin in Cytoscape identified twelve hub genes from PPI; among these three DEGs namely, AURKA, CDK4, and PLK1 were shown to be significantly associated with survival ($p < 0.05$) and highly expressed in SC tissues. Transcriptional, post-transcriptional, and protein-chemical also indicates the hub genes bound to several molecules. Further investigation and clinical experiments will be needed to evaluate the expression of these identified biomarkers regarding the prognosis of SC patients.

Keywords: Skin Cancer, Bioinformatics, Biomarker, Pathways, Hub Proteins

1. Introduction

The impacts of climate change on human health are ubiquitous and there is significant concern that the earth's atmosphere will continue to warm adversely. Since the middle of the twentieth century, the global incidence of skin cancer has increased at an alarming rate. Skin cancer also known as cutaneous carcinoma is a prominent global health issue especially the higher risk of the population with fair skin than darker skin [36]. Globally, in 2019 2.4 million squamous cell carcinoma (SCC) and 4.0 million basal cell carcinoma patients were identified. On the other hand, invasive melanoma is the most malignant and most serious type of skin cancer accounts for 80 percent of all skin cancer-related fatalities [20]. There is an annual increase in melanoma incidence of 4 to 6 percent among fair-skinned people in Northern Europe, North America, Australia, and New Zealand [67].

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However, several variables contribute to the disease including genetic factors, UV light exposure, and environmental factors [19]. Among those, UVR (a potent carcinogen) exposure is the root cause of skin cancer [17, 46, 12, 15] which is responsible for the mutation of a number of cancer-suppressor genes like p53 [65], and p21 [45], gelatinases [61] and galectin-3 has the potential to be utilized as a marker to assess both sun exposure and the risk of cancer [47]. In patients deficient in DNA repair enzymes such as xeroderma pigmentosa (XP), there is a significantly increased incidence of SCC [37]. EGFR is activated by UV light and upregulates several MAPKs [52, 30]. Phosphatidylinositol 3-kinase (PI3K)/AKT [24] signalling pathways whose deregulation is critical during tumor genesis to reduce cell death, increase cell proliferation, and enhance the process of epidermal hyperplasia. COX-2 is overexpressed in human skin actinic keratoses (pre-malignant UV induced skin lesions), SCCs, and BCCs, as well as in human epidermal cancer cell lines [1, 23].

Prognosis improves when lesions are found early including skin cancer that is melanoma and non-melanoma, early detection and treatment are required to reduce the number of deaths due to skin cancer [12]. However, given the difficulties of early detection and diagnosis, the overall survival rate of SC patients drops significantly. The above studies indicate that several biomarkers can explain the pathophysiology of SC and predict the consequences, but they still lack clinical studies. Therefore, more efficient biological markers should be investigated to fully understand SC's aetiology and guide treatment and prognosis.

The advancement of bioinformatics techniques, next-generation sequencing, proteomics, transcriptomics, and epigenetic research has enhanced our understanding of cancer growth and molecular processes and the design of appropriate drugs for the diseases. Recently, a promising technology called integrated system biology has been used to identify new molecular oncogenes and gene signatures utilizing Gene Expression Omnibus (GEO) gene expression profiles already in existence [32, 49, 40, 41]. Most existing bioinformatics studies focus on oncogene changes and neglect differences at the protein level, and molecular biology validation is seldom performed.

These protein-protein interactions are unique to the retrieval of putative hub genes, survival and expression level analysis of 12 hub genes, gene regulatory network and protein-drug-chemical interaction analysis of UV-mediated skin cancer. In this investigation, we have employed the bioinformatics and pharmacological approaches to explore the potential biomarker to understand better the molecular processes of skin cancer progression, early detection, and drug target identification through related signalling pathways. Our findings may serve as a clinical biomarker that helps us better understand the genesis of SC and predict its prognosis.

2. Materials and Methods

2.1. Data Retrieval and Preparation of SC Datasets

The RNA-sequence gene expression data sample used in this study was reclaimed from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus database. We used gene expression data from human skin cancers with accession number GSE85443. Total RNA was isolated from UV-irradiated human keratinocytes cells in different UVR conditions and non-irradiated control keratinocytes cells using the Illumina HiSeq 2000 platform. For analysis purposes, we made three datasets as groups N0, N1 and N2 for the separated three control groups to identify UV-responsive dysregulated genes in human SCC. Details information of GEO SC "patients" data were tabulated in Table 1. Then Statistical operations, including moderate t-statistic, B-statistics, and moderated F-statistics for all the pair-wise comparisons were performed on the datasets to determine the DEGs. First, the BioJupies (<https://maayanlab.cloud/biojupies/>) generator online server was used for RNA-Seq raw data analysis [64]. Additionally, the Benjamini-Hochberg false discovery rate approach is employed to balance the discovery of statistically significant genes and the limitation of false positives. In this study, genes with p-value < 0.05 and absolute log2 fold-change > 1 were considered DEGs. We also considered log2 fold-change ≥ 1 and log2 fold-change ≤ -1 criteria to explore up and down-regulated genes, respectively. Following that, we analyzed three SC sub-datasets using the Venny v2.1 web tool to identify shared DEGs [20]. Flow diagram shows on (Figure 1) system biology and bioinformatics approach on UV-mediated skin cancer.

2.2. Functional Enrichment Analysis

Pathway-based analysis is a recently established technique to understanding how complicated illnesses may be connected through their underlying biological processes [26]. On the other hand, gene ontology (GO) is a schema for describing gene activities and their connections to epigenetics [7]. We

Figure 1: A schematic diagram of integrative bioinformatics analytical approach applied in this study.

Figure 2: Volcano plot of the DEGs from datasets (A) GSE85443 N0, (B) GSE85443 N1, and (C) GSE85443 N2. X-axis: logFC, large-scale fold changes; y-axis: $-\log_{10}$ of the p-value is showing the statistical significance. The red points present the up-regulated genes and the blue points represent the down-regulated genes.

Figure 3: Heat map showing 32 DEGs between normal and skin cancer tissue in three groups of the GSE85443 datasets based on (A) log2 fold-change and (B) adjusted p-value. Identification of 32 common DEGs and co-expressed genes by comparing three subsets of datasets (GSE85443 N0, GSE85443 N1, and GSE85443 N2) through (C) Venn diagram software. Different color meant different datasets. These datasets have 19 up-regulated and 13 down-regulated genes.

Figure 4: Functional enrichment analyses of the differentially expressed genes (DEGs) in skin cancer. (A) The results of GO analysis for pathway associated with biological process (BP), (B) The results of GO analysis for pathway associated with cellular component (CC), (C) The results of GO analysis for pathway associated with molecular function (MF)

Figure 5: Pathway study of UV-mediated skin cancer using (A) KEGG, (B) BioCarta and (c) Reactome. The pathways generated from the DEGs by combined score was used to sort the term results (based on P-value) using R studio.

Figure 6: Visualization of the PPI network of skin cancer with 31 nodes and 58 edges. Nodes means proteins and the edges means the interaction of proteins.

Figure 7: The simplified PPI network is built with the significantly dysregulated genes common to Skin cancer. The highlighted 12 nodes represent the hub genes.

applied Enrichr [31] with Fisher’s exact test to run the functional enrichment analysis with the combined DEGs. Using a variety of databases, we evaluated the signaling pathways and gene ontologies associated with frequent DEGs. This over representation analysis identified a collection of enriched cell signalling pathways and functional GO keywords that show the biological importance of the identified DEGs. Molecular pathways were obtained from the databases such as Biocarta, Reactome and KEGG pathways, while gene ontologies were collected from biological process, cellular component, and molecular function databases. Only the significant pathways with a p-value less than 0.05 are examined after the duplicate pathways have been removed. The pathway and ontologies were showed in bubble plots that created using R Studio software.

2.3. Establishment of PPI and Selection of Hub Genes

The analysis of protein-protein interactions (PPI) provides information about the proteins and their connection. Considering the combined DEGs common among SC datasets, we created PPI networks to test our findings. Using the STRING [60] protein interactome database (provided 24,584,628 proteins from 5090 organisms), we were able to identify protein sub-networks. Then importing “.txt” to the “.csv” file into the Cytoscape [57], we generate a PPI network. In the PPI network, the number of nodes represents proteins while edges indicate interactions between proteins. PPI information was collected by mapping the hub genes, with high maximal clique centrality (MCC) values representing hub genes. To create the hub genes network, we used the cytohubba plugin of Cytoscape [5]. High-interaction proteins were discovered using topological metrics, such as an MCC algorithm is a topological metric, and its higher value indicates high interconnected proteins between PPI networks.

2.4. Survival and Expression Profile analysis of Hub Genes

Gene Expression Profiling Interactive Analysis (GEPIA2) enables quick and customized delivery of capabilities based on The Cancer Genome Atlas (TCGA) and genotype-tissue expression (GTEx) data to further study the influence of hub gene expression on patient clinical outcomes [62]. GEPIA2 analyzes DEGs in SC samples to determine the survival effect and to generate an expression profile. Using a median transcript per million (TPM), SC samples were classified into high and low expression hub gene groups. We calculated the log-rank p-value The overall survival effect of hub genes in SC was estimated by calculating the log-rank p-value < 0.05 and the HR (hazard ratio-95 percent confidence interval) for hypothesis test using GEPIA2 single-gene analysis. On the other hand, the relative expression levels of the hub genes were selected based on the Log_2FC cutoff value of < 1 , and the q-value Cutoff was < 0.01 . For the statistical significance of each hub gene, p-value < 0.05 was considered in our analysis. In our study, a p-value < 0.05 was considered significant for each hub gene.

Figure 8: Kaplan-Meier overall survival analysis of the core hub genes expressed in Skin cancer. Curves were generated using Gene Expression Profiling Interactive Analysis based on The Cancer Genome Atlas database ($p \leq 0.01$).

Figure 9: Relative expression levels of hub genes expressed in skin cancer. The GEPIA was used to verify the DEGs between normal and skin cancer tissues in the TCGA database. Red and black boxes represent the relative expression levels of genes in the tumor and normal samples, respectively.

Figure 10: : GRN study identify the potential TFs and miRNAs through Network analyst. (A) The network of DEGs and miRNA. (B) The network of TFs and miRNAs. (C) Highly enriched interrelated chemical agents.(D) The network of TFs and DEGs. Circle represent DEGs, and square represent miRNAs and TFs.

2.5. Identification of Regulatory Biomolecules

Transcriptional and post-transcriptional regulatory biomolecules like transcription factors (TFs) and microRNAs (miRNAs) may significantly alter how transcription and expression are processed. Using the Network Analyst platform, we can find DEGs-miRNAs and TFs-DEGs networks based on dysregulated genes that are shared among them. In order to explore DEGs-miRNAs interaction networks, the TarBase [42] database while the TFs-DEGs interaction network, the JASPAR [54] database is used. The DEGs-TFs network was screened for TFs and the miRNA were selected from the DEGs-miRNAs network with a degree of 10 and a betweenness centrality value of 100.

2.6. Protein-Chemical Compound Analysis

Analyses of protein-chemical compounds can be used to identify the chemical molecules responsible for the interaction of proteins in SC. Through the use of the enriched gene (common DEGs) among three groups of SC, the Comparative Toxicogenomics Database [9] was able to find protein-chemical interactions with the help of Network Analyst [69].

3. Result

3.1. Identification of DEGs in SC Datasets

The NCBI GEO database provided the SC RNA sequence data set GSE85443 for this study. Biojupies online server was used to find UV-responsive dysregulated gene of human skin squamous cell carcinoma (SCC). As a whole, the DEGs were found to meet the cutoff criteria ($\log_2 fc > 1$ and $p\text{-value} < 0.05$). We found 289, 620, and 649 DEGs in total through GSE85443 (N0), GSE85443 (N1), and GSE85443 (N2) subsets of GSE85443 dataset analysis, respectively (Table 1). The volcano plot in the Figure 2 was generated to compare the up and down-regulated genes. The number of shared DEGs between two groups of SC three datasets are presented in the Venn diagram (Figure 3C). Then the 32 common DEGs were identified by comparing three subsets of a dataset and among these 19 up-regulated genes (i.e., CDKN1A, CDK4, CLDN4, CLU, RNR1 and SEMA3B etc.) and 13 down-regulated genes (i.e. AURKA, ADAMTS3, CCNB1, CLMP, DOCK10, LMNB1 and PLK1 etc.). The heat maps display the 32 DEGs that were common throughout three groups of the GSE85443 dataset and their connections based on an adjusted p-value and a $\log_2 foldchange$ (Figure 3A and 3B).

3.2. Functional Enrichment Analysis Finds Significant GO Terms and Molecular Pathways

Pathways and gene ontology (GO) was discovered by doing a gene set enrichment analysis on the overlapping DEGs to understand SC development better. An enrichment test on DEGs using the Enrichr tool allowed us to discover cell signalling pathways and three functional GO terms significantly enhanced with DEGs. For classifying functional categories, we looked for DEGs that were excessively represented in three different SC sub-datasets. The Figure 5 showed the top ten pathways for each disease with a p-value less than 0.05 as a reference. Gene ontology (GO) methods were used to categorize improved standard DEG sets into biological importance and molecular functions. To narrow GO terms, we used a statistical method with a p-value below 0.05. Among 30 GO terms, progesterone-mediated oocyte maturation, cell cycle, phosphorylation of Emi1, activation of NIMA kinases NEK9, NEK6, NEK7, cyclin and cell cycle regulation and cell cycle: G2/M checkpoint were significant according to our analysis (Figure 4). The most significant molecular pathways in our study were found to the regulation of cyclin-dependent protein serine/threonine kinase activity, G2/M transition of the mitotic cell cycle, receptor-ligand activity, purine ribonucleoside triphosphate binding and cytoskeleton based on a $\log_{10}$ p-value (Figure 5).

3.3. Identification PPI and Hub Genes

We examined the STRING network and displayed it in Cytoscape using our gene sets for common illnesses to anticipate typical DEG linkages and adhesion pathways. A PPI network with 31 nodes and 58 edges was built using the interactions of common DEGs (Figure 6). As part of the organism's "interactomics," abnormal PPIs can lead to various health problems. If two diseases have one or more commonly linked in protein subnetworks, they have been connected. For the purpose of identifying the 12 central proteins including CDK4, CDKN1A, PLK1, CCND1, CDK2, AURKA, CCNA1, ESPL1, CCNB1, CCNE1, KIF23 and DLGAP5 from the PPI, we used the degree algorithm of the Cyto-Hubba plugin as shown in the Figure 7. Table 2 provided functional properties of the top 12 hub genes in the PPI network.

3.4. Survival and Expression Profile Analysis of Hub Genes

Skin cancer samples were divided into two groups based on their median expression level of hub genes. Twelve hub genes from both the up and down-regulated DEGs were examined for their overall relationship with survival using the GEPIA survival assessment (Figure 8). Among the twelve hub genes AURKA, CDK2, CDK4, CCNB1, ESPL1 and PLK1 showed significantly lower overall survival in the higher group for considering p-value < 0.05 and Hazard Ratio (HR). CDK4 and PLK1 showed a higher hazard ratio of about 1.6 which indicated the most survival proteins. However, based on the hub gene expression levels, we have identified that only one hub gene CDKN1A was up-regulated in normal cells and the other eleven hub genes were up-regulated in the tumor cells. Among 11 up-regulated genes AURKA, PLK1, CDK2, and DLGAP5 were higher expression levels in tumor tissue (Figure 9).

3.5. Gene regulatory network study identified DEG-miRNA and TF-DEG interaction networks

In all living organisms, TFs control transcription and gene expression levels. In addition, these proteins are essential for all aspects of cellular life [6]. Besides, small RNA molecules are known as microRNA (also known as miRNAs), control gene expression at the post-transcriptional level after transcription has taken place. A new class of molecular regulators known as microRNAs (miRNA) has been discovered that controls several genes and pathways essential in the pathogenesis of SC [38]. We used DEGs that are common to three datasets of SC. The DEGs-TFs linkage networks were examined using a network-based approach to uncover transcriptional and post-transcriptional regulatory networks for common DEGs. The network of DEGs-miRNAs, TFs-miRNAs and DEGs-TFs interactions are depicted in Figure10 A, Figure10 B, and Figure10 D. In these figures, circles represent DEGs whereas squares represent miRNAs and TFs. The biomolecules including TFAP2A, FOXC1, GATA2, MAX, MYC, FOXL1 and PPARG were the most significant TFs regulators of the identified common DEGs. Several miRNAs, including mir-1-3p, mir-7-5p, mir-147a, mir-34a-5p, and mir-124-3p were significant which provide deep knowledge of the DEGs at post-transcriptional regulators. AURKA and PLK1 are significant genes in a network that connect multiple edges. Biomolecules involved in regulating gene transcription and post-transcriptional processes are included in the Table 3.

Table 1: The differentially expressed gene (DEG) for all employed Datasets in the present study

Group	Number of samples	Disease state	Tissue	Cell type	Organism	Signature genes	Up	Down
2*GSE85443 N0	1 control 9 case	Non-irradiated control keratinocytes UV-irradiated human keratinocytes	2*Neonatal foreskin	2*Cultured primary keratinocytes	2*Homo sapiens	2*289	2*152	2*137
2*GSE85443 N1	1 control 6 case	Non-irradiated control keratinocytes UV-irradiated human keratinocytes	2*Neonatal foreskin	2*Cultured primary keratinocytes	2*Homo sapiens	2*620	2*345	2*275
2*GSE85443 N2	1 control 6 case	Non-irradiated control keratinocytes UV-irradiated human keratinocytes	2*Neonatal foreskin	2*Cultured primary keratinocytes	2*Homo sapiens	2*649	2*278	2*371

Table 2: Summary of hub genes identified by protein-protein interaction analysis encoded by DEGs that are common to three groups of UV-mediated skin cancer.

Hub genes name	Description	MCC value	Features	Uniprot ID
CDK2	Cyclin-dependent kinase-2	105960	Involved in the control of the cell cycle	P24941
CCNE1	G1/S-specific cyclin-E1	90720	Essential for the regulation of the cell cycle at the G1/S transition	P24864
CCND1	G1/S-specific cyclin-D1	80764	Regulates the cell cycle during G1/S transition	P24385
CDK4	Cyclin-dependent kinase-4	85680	Regulates the cell cycle during G1/S transition	P11802
CDKN1A	Cyclin-dependent kinase inhibitor 1	80643	Involved in p53/TP53 mediated inhibition of cellular proliferation in response to DNA damage	P38936
PLK1	Serine/threonine-protein kinase PLK1	71402	Regulating the centrosome maturation and spindle assembly	P53350
KIF23	Kinase-like protein KIF23	25202	Required for the myosin contractile ring formation during the cell cycle cytokinesis	Q02241
ESPL1	Separin	25920	Role in the chromosome segregation during the cell cycle process	Q14674
CCNB1		111726	Essential for the control of the cell cycle at the G2/M transition	P14635
CCNA1	Cyclin-A1	101522	Controlling of the mitotic cell cycle in some somatic cell.	P78396
DLGAP5	Disks-large-associated protein-5	20886	Cell cycle regulator that may play role in carcinogenesis of cancer cells	Q15398
AURKA	Aurora kinase A	111727	Contributes to the regulation of cell cycle progression	O14965

3.6. Analyzing Protein-Chemical Compounds Reveals Protein-Chemical Interactions

For the purpose of discovering new drugs, knowing how proteins underlie cellular and molecular processes are critical in the field of protein-chemical interaction research. Several proteins are interconnected to chemical interaction networks of SC which have found in our study. We discovered several potentially interrelated chemical compounds including valproic acid, coumestrol, estradiol, copper sulfate, calcitriol, cyclosporine and benzo (a) pyrene, among highly enriched chemical agents (Figure10 C). This network can also identify significant proteins such as AURKA, PLK1, CCNB1, CDKN1A, UHRF1, CLU, LMNB1, CLDN4, PSRC1, LRP8 and GDF15.

4. Discussion

The prognosis for early-stage SC is much better than for advanced-stage. Early skin cancer detection is essential and can prevent some cancers such as focal cell carcinoma and melanoma. Surgery, chemo-radiation, or a combination of treatments were effective in curing early-stage instances. Still CC patients in the advanced stages suffered more because of recurrences and the disease's incurable nature. Advanced-stage CC patients suffered the most because of recurrences and the incurable nature of the disease [3]. We employed several bioinformatics approaches to evaluate RNA-Seq datasets based on the NCBI-GEO database, comprising 21 UV- irradiated human keratinocytes tissue and three non-irradiated human keratinocytes tissue to find potential predictive biomarkers in skin cancer. Using the GEO2R online tool and Venn software, a set of 32 DEGs were found including 19 genes that were found to be up-regulated and 13 down-regulated genes. After that, GO and signalling pathway analysis was performed with the findings showed that they were highly enriched in a variety of cell systems. According to the pathway assessment, metabolic responses and cell cycle regulation accounted for most pathways responsible for pathogenesis and progression of UV-mediated skin cancer. After, further enrichment performed on the DEGs, we have discovered some noteworthy GOs. Regulation of cyclin-dependent protein serine/threonine kinase activity (GO: 0000079) and G2/M transition of the mitotic cell cycle (GO: 0000086) for biological process. Spindle microtubule (GO: 0005876) and cytoskeleton (GO: 0005856) for the cellular component. Receptor ligand activity (GO: 0048018) and cyclin-dependent protein serine/threonine kinase regulator activity (GO: 0016538) for respectively to molecular function. Regulation of cyclin-dependent protein serine/threonine kinase activity (GO: 0000079): Haidan Liu et al. suggested that inhibition of CDK4 and CDK2 in cell cycle regulator can be targeted as a molecular marker in skin cancer [35]. Cytoskeleton (GO: 0005856): Actin cytoskeleton was modulated by downregulating the AC-cAMP pathway; both collectively lead to skin carcinoma progression [2]. Receptor ligand activity (GO: 0048018): The result indicated that receptors (Eph) and ligand (ephrin) represent a new potential prognostic marker and therapeutic target in non-melanoma skin cancer [21]. From the molecular pathway analysis, we have found progesterone-mediated oocyte maturation and cell cycle for KEGG. Cell Cycle: G2/M checkpoint pathway and cell cycle regulation for biocarta analysis. Phosphorylation of Emi1 and activation of NIMA kinases NEK9, NEK6, NEK7 for Reactome. Progesterone-mediated oocyte maturation: Yumei Li et al. concluded that the progesterone-mediated oocyte maturation pathway is involved in melanoma metastasis through bioinformatics analysis [34]. Cell cycle: Thomas M. Runger et al. demonstrated that regulation of cell cycle checkpoints and DNA repair is responsible for UV mediated skin cancer [53][47]. Activation of NIMA Kinases like NEK9, NEK6, and NEK7 is responsible for CDK and PLK1 mediated phosphorylation [50]. Further, the string database was used to build the PPI network. The combined PPI analysis using Cytoscape revealed 12 hub genes based on degree value, clustering coefficient, betweenness centrality, and closeness centrality. These 12 hub genes CDK4, CDKN1A, PLK1, CCND1, CDK2, AURKA, CCNA1, ESPL1, CCNB1, CCNE1, KIF23 and DLGAP5 had diagnostic value which distinguishes SC patients apart from the rest of the population; having the ability to discriminate between people with skin cancer and healthy individuals as a diagnostic marker. Cyclin-dependent kinases 4 (CDK4) trigger a G1 arrest by playing important roles in the control of the cell cycle [63] and are overexpressed in human skin cancers [59]. During the first gap phase (G1 to S) of the cell cycle, CDK4 inhibitors keep everything under control [18]; in addition, over 90 percent of people with melanoma had genetic abnormalities in the CDK4 pathway [58]. Cyclin-dependent kinase inhibitor 1 (CDKN1A) was downregulated in melanomas, suggesting a possible involvement in carcinogenesis [70]. Raising the polo-like kinase 1 (PLK1) expression in human melanoma like BCC and SCC is considerably overexpressed. There was also a considerable G2/M cell cycle arrest, mitotic catastrophe and induce apoptosis in melanoma cells when PLK1 was found to be anti-proliferative [55]. The expression of G1/S-specific cyclin-D1 (CCND1) mRNA genes and proteins is associated with the development of SC [14], which may be altered by mutations in BRAF and NRAS [66]. G1/S and S/G2

transitions are controlled by cyclin-dependent kinase 2 (CDK2) which helps melanomas cells aggressive by regulating CDK2 expression [44][57]. Aurora kinase A (AURKA) is a key protein in controlling the G2 to M transition in mitosis and may serve as an oncogene [8]. Cell cycle regulation during the G1/S and G2/M transition in certain somatic cells is thought to be mediated by cyclin-A1 (CCNA1) protein [22]. There is evidence to suggest that separase autocleavage or the cleaved fragments play an essential role in cell cycle progression when extra spindle pole bodies like 1 (ESPL1) protein is present [72]. Following quantitative real-time reverse transcriptase-PCR analysis of the major target genes PTCH1, GLI1, GLI2 and CCNB1; in both normal and malignant skin, the average E3 ubiquitin ligase CCNB1p1 (CCNB1) transcript level was the same between the two groups [29]. G1/S-specific cyclin-E1 (CCNE1) increase may be a significant biomarker for patients with acral melanoma to predict treatment success [68]. Microtubule-dependent and Rho-mediated signalling are necessary for developing the myosin contractile ring during cell cycle cytokinesis and kinesin-like protein (KIF23) fulfils this purpose [28]. A potential cell cycle regulator, discs large-associated protein 5 (DLGAP5) may have a role in developing cancer cells including their carcinogenesis [11]. AURKA, CDK2, CDK4, CCNB1, ESPL1 and PLK1 showed a significant survival rate in the group with greater levels of expression, while AURKA, PLK1, CDK2, and DLGAP5 were found in higher expression levels in tumor tissue. Additionally, CDK4 has immune-modulatory properties and inhibiting CDK4 via the reduction of NFAT family members can significantly increase T cell activation [11]. Anti-melanoma immune checkpoint drugs can slow tumor development by decreasing CDK4/6 activity, slowing the resistance pathway and inducing senescence [25]. Melanoma patients who have resisted anti-PD-1 treatment had evident CDK4 pathway abnormalities including CDKN2A loss, CDK4 and CCND1 gain which were confirmed through the whole genome and RNA sequencing profiling studies [71]. X. Wu et al. studies established a rationale for treating acral melanoma using CDK inhibitors [68]. Melanoma preclinical trials using CDK4/6 inhibition along with genetic analysis for the pathway give a foundation for treating various diseases with targeted agents. An in-vitro investigation done by T. Schmit et al. found that PLK1 is overexpressed in both protein and mRNA levels in SCC (A253 and A431) relative to normal keratinocytes (NHEK) [55]. As a therapeutic target for cSCC and PLK1 has the advantage of already having numerous small molecule inhibitors in clinical trials [56, 10]. These findings add together to show that cSCC treatment focusing on PLK1 holds a lot of potentials. Aurora kinase and FOXM1 proteins play a crucial role in melanoma formation implying that AURKA expression affects disease prognosis [48]. As AURKA is highly expressed in melanomas, inhibiting it may have the effect of slowing tumor growth indicates the development of new therapies of SC treatment [4]. Table 3 showed the detailed information of identified 7 TFs that regulate transcriptional activity during tumor cell proliferation. Only GATA2 was shown to be unrelated to the development of skin cancer in our investigation, as verified by literature analysis. We also found several miRNAs molecules; among these mir-7-5p has anti-tumor effects via preventing melanoma cell invasion and migration [16]; and mir-34a-5p suppresses cell proliferation in melanoma cell [33]. In treating head and neck squamous cell carcinoma, valproic acid (VPA) has shown effectiveness was confirmed by R. B. Erlich et al. studies [13]; VPA sensitisation effects can be used to target drug resistance in melanoma cells [27]. Preconditioning skin cancers with calcitriol (the active form of Vitamin D) before ALA-PDT dramatically enhances treatment outcomes, according to K. Rollakanti, S. Anand, and E. V Maytin studies [51]. G. Park et al. demonstrated that a new dietary component coumestrol may help prevent and treat UVB-induced skin cancer [43]. Cyclosporine A lessens skin cancer cell death in transplant patients by inhibiting MPTP in a model of the disease's occurrence in keratinocytes [39]. The pathway analysis recommended that the regulation of cyclin-dependent protein serine/threonine kinase activity, cytoskeleton pathway, receptor-ligand activity and the cell cycle regulator were the most significant pathways for inhibiting the expression of associated genes. Selection of hub genes from PPI, survival and expression level analysis of hub genes, GRN studies and protein-chemical interaction analysis indicated that AURKA, PLK1 and CDK4 could be served as the most potential biomarker in skin cancer therapy. The GATA2 transcription factors may also serve as novel biomarkers in the treatment of skin cancer. Several miRNAs identified in our study, including mir-7-5p, and mir 34a-5p are all candidates for therapeutic intervention in melanomas because of their roles in tumor growth and progression. Valproic acid, coumestrol, calcitriol and Cyclosporine A were indicated the potential therapeutic agent in a melanoma cell. These findings expand our understanding of UV-induced skin tumorigenesis and tumor progression and suggest targeting AURKA, CDK4, and PLK1 genes can help prevent or treat UV-associated skin cancer.

5. Conclusions

The emergence of omics methods has given bioinformatics a substantial boost in recent decades. Several gene expression changes are linked with human cancer, and the RNA sequence is one of the most commonly utilized omics techniques for their detection. When it comes to diagnosing cancer, the most frequently utilized omics technique is using RNA sequences to uncover the myriad of gene expression alterations linked with various types of human malignancies. RNA sequence data was used in this work to examine the transcriptome of a collection of UV-irradiated human keratinocyte lesions using a constrained pipeline method; we were able to separate 21 different case states from the merging of three sub-datasets of SC by using 32 common DEGs. Three differential genes (AURKA, CDK4 and PLK1) were identified through gene ontology and molecular pathway, protein-protein interaction, survival and expression analysis, gene-regulatory network and protein-chemical interactions. Based on findings and explanations in this study, the AURKA, CDK4 and PLK1 genes have been identified as viable biomarkers for skin cancer in mostly metabolic and cell cycle regulatory pathways. According to our findings, these three genes should serve as possible biomarkers for the early identification of UV-mediated SC. The AURKA, CDK4 and PLK1 proteins expression need to be validated as clinical biomarkers through further investigational and experimental approaches to understand the pathological process better and anticipate UV-irradiated SC diagnosis.

Table 3: Summary of transcriptional and post-transcriptional regulatory biomolecules of DEGs overlapped among three groups of UV-mediated skin cancer that includes (a) Regulatory Transcription Factors and (b) Regulatory microRNAs

(a) Regulatory Transcription Factors			
TFs name	Description	Function	Type
FOXC1	Forkhead Box C1	NA-binding transcription factor activity and transcription factor binding	Stress-responsive
GATA2	GATA binding protein 2	DNA-binding transcription factor activity and chromatin binding	Oxidative stress-responsive
MYC	Myc Proto-Oncogene	Regulating numerous biological activities that contribute to tumorigenesis	Cell cycle regulator
TFAP2A	Transcription Factor AP-2 Alpha	DNA-binding transcription factor activity and sequence-specific DNA binding	DNA regulator
MAX	Myc-associated factor X	Regulating numerous biological activities that contribute to tumorigenesis	Cell cycle regulator
FOXL1	Forkhead Box L1	NA-binding transcription factor activity and DNA-binding transcription factor activity	Stress-responsive
PPARG	Peroxisome Proliferator Activated Receptor Gamma	DNA-binding transcription factor activity and chromatin binding	DNA regulator
(b)Regulatory microRNAs			
miRNA Name	Description	Function	
hsa - mir-1-3p	MicroRNA 1	Suppresses the proliferation and invasion of cancer cells	
hsa - mir-7-5p	MicroRNA 7	Inhibits melanoma cell migration and invasion	
hsa - mir-34a-5p	MicroRNA 34a	Suppresses cell proliferation in melanoma cells	
hsa - mir-124-3p	MicroRNA 124	Leads to malignant progression of cancer, including cell apoptosis, migration and invasion	

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Ethics statement

The authors have no relevant financial or non-financial interests to disclose.

Consent to participate and for publication

Informed consent was obtained from all individual participants included in the study.

Authors' contributions

Md. Arju Hossain: Conceptualization, Methodology, Software, Data curation & Writing. **Asif Ahsan:** Software, Validation, Writing - Original draft preparation & editing. **Md. Imran Hasan:** Methodology, Software, Data curation, Validation & Writing. **Md. Arif khan:** Data curation & Writing. **Md Sipon Miah:** Data curation, Validation, reviewing & editing. **Md Habibur Rahman:** Review & editing.

Availability of data and material

The data that supports the findings of this study are openly available in NCBI.

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