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Proteomic Alterations in Human Dermal Fibroblasts under Photo-Induced Pollution Caused by Excessive Solar Irradiations such as Infra-Red, Blue Light, UVA and UVB

Balu Muthaiyah*, Suresh Kumar Ramadoss, Yellapantula Sree Gouri, Balaji Bandyopadhyay*

ITC Life Sciences and Technology Center (ITC LSTC), Bangalore, India
Email: *balu.muthaiyah@itc.in, *balaji.ban@itc.in

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Abstract

Background: The skin serves as the first line of defense for the human body. Direct sunlight contains damaging radiations that can speed up the ageing process of the skin, resulting in wrinkles, leathery skin, dark patches, and solar elastosis. **Objectives:** To evaluate the effect of multiple solar irradiation related factors at the protein level in human dermal fibroblast (HDF). The overall effect of individual solar irradiations such as Infrared A (IRA), blue light (BL), UVA, and UVB on HDF cells and the extent of molecular level aberrations to be assessed and compared against each. **Methods:** Label-free quantitative proteomics (MS/MS) approach has been adopted in this study to observe the protein level changes induced in the HDF cells through various exposures of full light sources. Following that, downstream *insilico* analysis has been carried out. **Results:** In this study, it is demonstrated all the four different solar irradiations significantly contribute to the molecular degeneration of skin cells through various mechanisms. This study confirms that BL down-regulates DNA repair proteins and the skin cells-HDF stimulate the histone proteins as a response mechanism to maintain the chromosomal integrity. **Conclusions:** The proteomics experiment carried out in the current study intends to support the future sun care products based on full light protection technology that can be custom designed to provide complete protection from the solar radiation. Similar technology could enhance the further investigations for deeper understanding of induction, mode of action, and prevention of skin damage from extensive solar irradiation.

Keywords

Full Light Technology, Solar Irradiation, Cellular Damage, DNA Repair & Damage, HDF Cells

1. Introduction

The sun is a powerful light source of emitting radiation of different wavelengths from the gamma-rays to long radio-waves. The sun's light source reaches the skin and contains 6% ultraviolet radiation (UVR), 40% visible light, and 54% infrared radiation (IR) [1]. UVR is known to cause sunburn and is linked to cancer. UVR is recognized to cause DNA damage and oxidative stress in the skin, resulting in not just sunburn, but also long-term structural and functional changes exhibited by photo ageing. Cutaneous sun exposure is likely the most well-studied and well-documented example of environmental factors that hasten the ageing process in one or more organ systems [2].

1.1. Solar Radiation

Sun is the natural and the most powerful light source for emanating radiation of diverse wavelengths from the gamma-rays to long radio-waves. Sunlight consists of a range of rays: visible light, ultraviolet, and infrared light. These rays disturb skin in various detrimental ways.

1.2. Ultraviolet (UV)

There are two basic categories of UV rays that influence the earth's surface UVB and UVA. UVB rays are accountable for generating sunburn. There are approximately 500 times extra UVA rays in sunlight than UVB rays. The UVA rays enter the deeper covers of skin dermis, and stimulate the making of free radicals, which originate oxidative stress that can prime to indirect DNA destruction where the free radicals transform cellular DNA over time [3].

1.3. Blue Light (BL)

The visible light is only a minute portion of the total sun irradiation range but is most significant to human beings [4] [5]. In the case of High-energy visible (HEV) blue light, the primary concern is, the potential cause of photochemical damage in skin tissues, leading to establishment of excited molecular conditions that can bring biochemical alterations within them [6].

1.4. IRA Radiation

Infrared rays account to approximately 30% of the total solar energy reaching the earth's surface. Conventionally, photo dermatology focused largely on physiological, pathophysiological, and beneficial effects of UVB and UVA-radiation whereas wavelengths in the IRA range have long been unnoticed. Skin damage triggered by IRA energy mostly creates the tension of extracellular matrix homeostasis by modifying dermal connective tissue, which clinically manifests as wrinkle formation [7].

New approaches to support peptide measurements have become feasible through the developments in proteomic methodologies and LC-MS/MS-MS analysis. This has helped in uncovering several key biological queries about the

behavioral aspects of numerous proteins. To understand the proteome level changes in HDF cells and the molecular degeneration on diverse solar irradiations such as IRA, BL, UVA and UVB, we have carried out a comprehensive in-house proteomic study.

2. Materials and Methods

2.1. Human Cells

HDF cells were procured from ATCC, USA and cultured in Dulbecco's modified Eagle's medium (DMEM), high glucose (HG) medium, with 10% fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) at 37˚C in a humidified CO_2 (5%) chamber from Thermo Scientific Forma Steri-Cycle i160 CO_2 Incubators-Thermo Fisher Scientific Waltham, MA USA 02451.

2.2. Proteomics Analysis by Nano-HPLC & Mass Spectrometry

For investigating the effect of different solar radiations on the HDF cells, the quantitative proteomics method was carried out as described in this section. HDF cells were seeded at 5×10^4 cells/ml in a 60 mm petri plate and grown in DMEM HG medium and incubated for 24 h at 37˚C. The cells were further exposed individually to UVA at 10 J/cm^2 for 5 minutes from 20 cm distance [8] [9] [10] [11], UVB at 200 mJ/cm^2 for 10 minutes from 20 cm distance [12] [13], BL at 50 mJ/cm^2 for 30 minutes from 40 cm distance [14] [15] [16] and Infrared at 750 J/cm^2 for 1 hour from 20 cm distance [17] [18]. Control cells without any irradiation also maintained separately. These doses were chosen based on the experiments standardized in our lab and based on literature references and ED 50 Value calculations obtained from the MTT assay experiment (data not shown). Time and distance were optimized using cell viability experiments. After the respective incubation period, the cells were subjected for the proteomics analysis that 30 µg of total protein of each sample was subjected to in-solution digestion method [19] [20] [21]. The peptide mixtures were separated using a reverse-phase column nano-high performance liquid chromatography (nanoHPLC) using ThermoFisher Scientific's pico-frit column (0.075 mm × 150 mm, C18). In-solution protein digestion method has been adapted in sample preparation for protein digestion. 100 µg of cell protein extract was re-suspended in 47 µl of 6 M urea, 50 mM ammonium bicarbonate solution. 1 µl of 250 mM TCEP (tris (2 carboxyethyl) phosphine) added in solution and the sample incubated at 37˚C for 30 minutes. Further, 2 µl of 625 mM iodoacetamide was added and the sample was incubated in dark at room temperature (25˚C ± 2˚C) for 45 minutes. Finally, in-solution digestion was carried out by the addition of 450 µl trypsin in 50 mM ammonium bicarbonate with a protein digest ratio of 50:1. The purification of digested peptides was carried out with OASIS 1 ml Cartridge system from Waters Scientific (WAT094225). The purified/enriched protein samples were dried and re-sus-

pended in 0.1% formic acid with 5% acetonitrile in MS water. The peptides were loaded onto an EASY-nano LC system (Proxeon). Peptide mixtures were separated on a C18 reverse-phase column (pico-frit capillary column, 75 μm × 10 cm, New Objective) using a linear gradient of solvent A (0.1% formic acid in 5% acetonitrile) and Solvent B (0.1% formic acid in 95% acetonitrile) at a flow rate 250 nL/min directly into an LTQ-Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA, USA) with nano-ESI source with spray voltage was set at 1.6 KV. Stable spray condition maintained before to sample loading. The instrument operated in data-dependent mode with dynamic exclusion enabled. The MS/MS spectra on the top 20 most abundant peptide ions in full MS scan obtained. The normalized collision induced dissociation was set at 35% for MS/MS.

2.3. Bioinformatics Data Analysis

Data analysis pipeline was set to carry out the analysis of data produced by SEQUEST algorithm incorporated in proteome discoverer software version 1.4 which identifies each tandem mass spectrum individually. Database searching of all raw files performed in the proteome discoverer software against the human protein database of Uniprot developed by The UniProt Consortium [22]. The search performed choosing trypsin as a specific enzyme aligning to the experiment with a maximum of one missed cleavage allowed. Peptide (parent ion) tolerance of 2.5 Da and fragment ion tolerance of 1 Da were allowed, Oxidation (M) as variable modifications, and fixed modifications of carbamidomethylation (C) were used. An in-house Python-based tool, Pro$_t$Lyzer was used for identifying the differentially expressed proteins across and among the samples. The normalization of protein expression in Pro$_t$Lyzer followed as per the standard method with log-odds ratio (base 2) statistics with a mean of individual protein expression in the sample. Quantitative proteomic data analysis performed after global data normalization. From the normalized protein abundance, relative fold change calculation with respect to its control sample been estimated. The downstream pathway from the differentially expressed protein data set carried out using Reactome [23] with all parameters present with human species data set with its *interactome* feature enabled in version 70 release. The protein class for the quantified proteins in this proteomics experiment is assigned from the data curation carried out through Reactome.

3. Results

3.1. Effect of Different Solar Irradiations on HDF Cells with Respect to Skin Aging-Related Proteins

All aging associated proteins such as Collagen alpha-1(1), Collagen alpha-3(VI) chain, Propyl 4-hydroxylase subunit alpha 2, Isoform 3 of Basigin and Isoform 2 of Annexin A4 significantly down regulated upon exposure of UVA, UVB, IRA, and BL (**Figure 1(a)**).

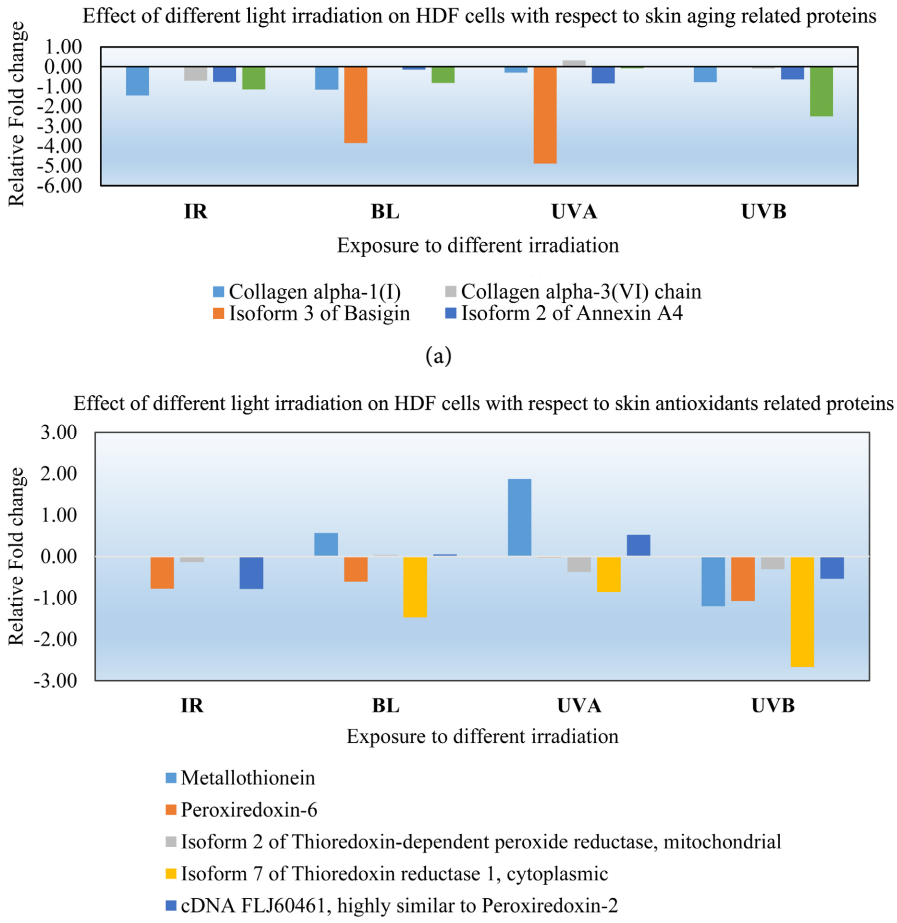


Figure 1. Protein expression fold changes of skin aging-related proteins and skin anti-oxidants related proteins upon exposure to different light irradiation in HDF cells. (a) Represents the fold changes of relevant proteins with respect to the skin aging; (b) Fold change analysis of different skin antioxidant system-related protein expression with re-spect to different light irradiation in HDF cells.

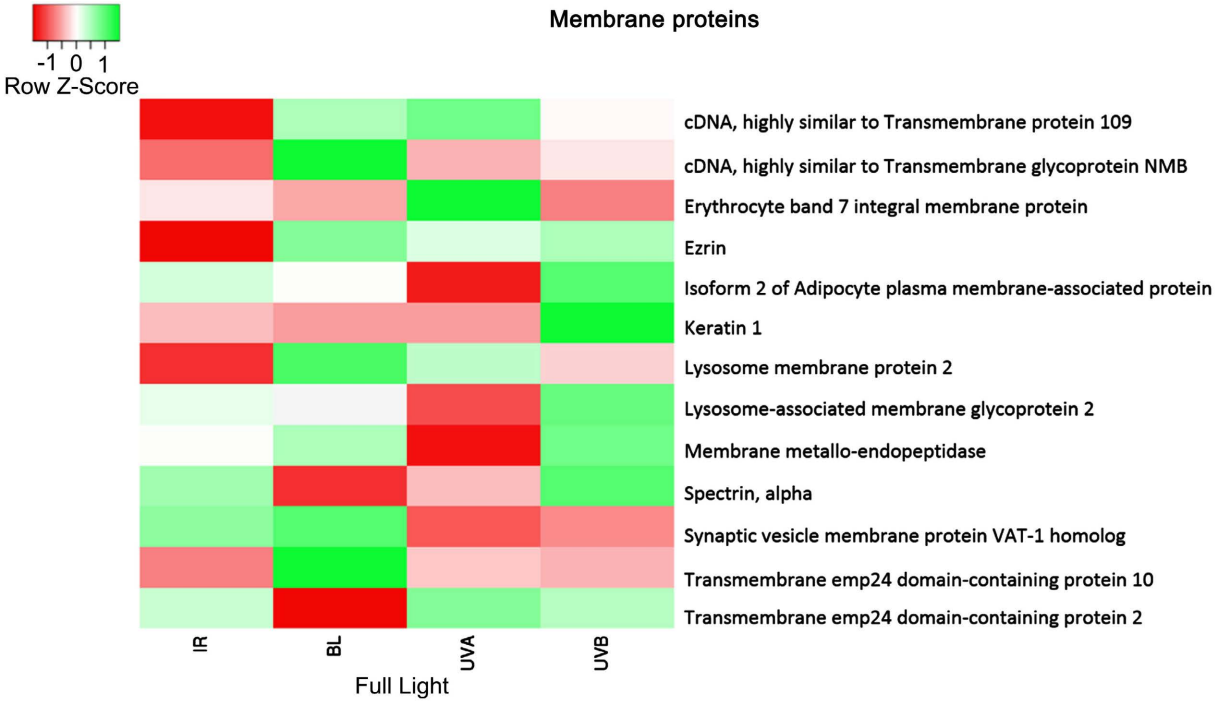
3.2. Effect of Different Solar Irradiations on HDF Cells with Respect to Skin Antioxidants Related Proteins

In comparison to all other solar irradiation, UVB rays significantly alter the proteome profile of skin, especially antioxidant related proteins such as Metal-lothionein, Peroxiredoxin-6, Thioredoxin-dependent peroxide reductase, and Thioredoxin reductase 1 and Peroxiredoxin-2 were significantly down regulated upon exposure of UVB rays which was analyzed by the fold changes of relevant proteins with respect to the anti-oxidants (**Figure 1(b)**). IRA observed to have a moderate down-regulation of the antioxidant proteins.

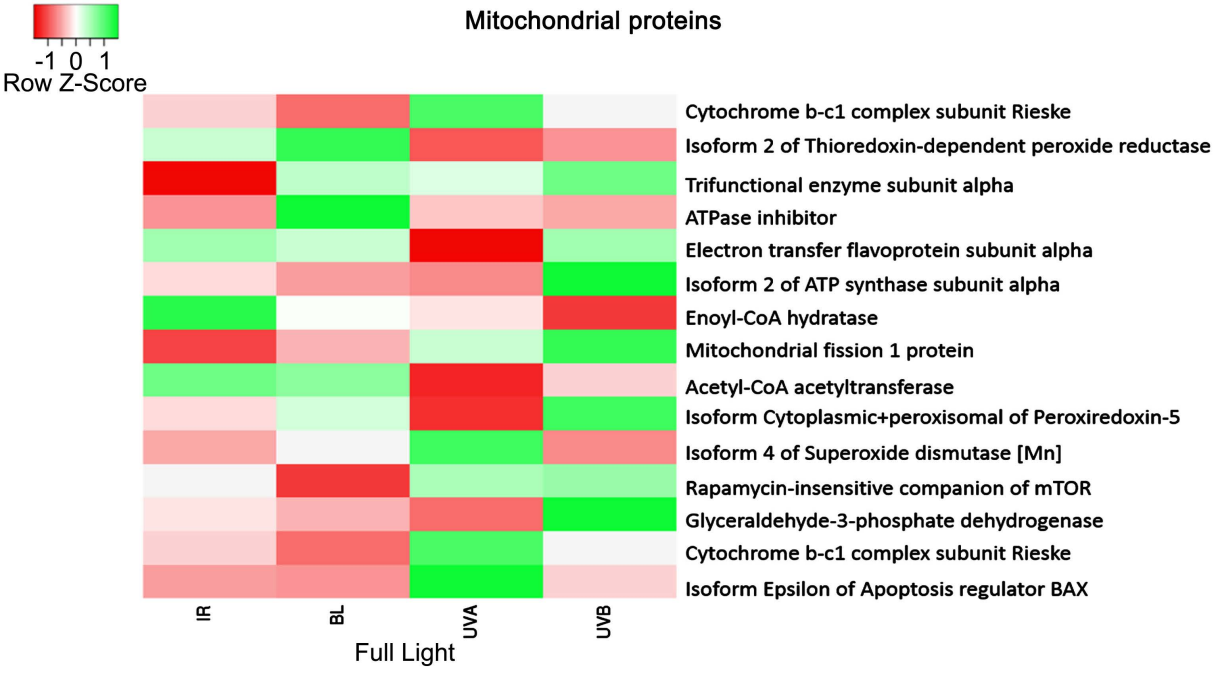
3.3. Effect of Different Light Irradiations on HDF Cells with Respect to Membrane Proteins

While compared to the impact of all solar irradiations, Infrared rays and UVA rays significantly altered the proteome of skin membrane proteins to different

extents, especially the ribosomal related proteins such as Ezrin, Lysosome membrane protein, and transmembrane protein (Figure 2(a)).



(a)



(b)

Figure 2. Heat map analysis of membrane proteins and mitochondrial related proteins with respect to different solar irradiation in HDF cells. (a) Heat map analysis of skin membrane proteins with respect to different light irradiations. Complete proteomics study demonstrated that IRA, Blue light, UVA, and UVB rays are having the impact on cellular membrane proteins present in the fibroblast cells; (b) Heat map analysis of skin mitochondrial proteins.

3.4. Effect of Different Light Irradiations on HDF Cells with Respect to Mitochondrial Related Proteins

The results demonstrated that IRA, Blue light, UVA, and UVB are having the impact on mitochondrial related proteins present in the fibroblast cells. In comparison with each other solar irradiation, Infrared rays and UVA rays significantly alter the proteome of skin, especially the mitochondrial related proteins such as Cytochrome b-c1 complex subunit, Trifunctional enzyme subunit alpha, ATPase inhibitor and Electron transfer flavoproteins (**Figure 2(b)**).

3.5. Effect of Different Light Irradiations on HDF Cells with Respect to Ribosomal Proteins

The results of complete proteomics study demonstrate that IRA, BL, UVA and UVB are having an impact on ribosomal related proteins present in the fibroblast cells. The heat map analysis (**Figure 3(a)**) represents that the IRA and UVA rays significantly alter the proteome of skin, especially the ribosomal related proteins such as 40S ribosomal protein, Heterogeneous nuclear ribonucleoprotein K, 60S acidic ribosomal protein P2, 60S ribosomal protein L7 were significantly down regulated upon exposure of IRA rays.

3.6. Effect of Different Light Irradiations on HDF Cells with Respect to Histone Proteins

The heat map analysis (**Figure 3(b)**) represented that the UVA and UVB rays significantly alter the proteome of skin; especially the histone related proteins such as Histone H4, Histone H3.1, Histone H2B, Histone H2A.J was significantly down regulated upon exposure of UVB rays. Histone H1.2 & Histone H1.5 were significantly down regulated upon exposure of UVA rays in contrast to BL & IRA irradiations which have up regulated the histone proteins.

3.7. Effect of Different Light Irradiations on HDF Cells with Respect to Heat Shock Proteins

The heat map analysis (**Figure 4(a)**) represents that the Infrared and UVB rays significantly alter the proteome of the skin, especially the heat shock related proteins such as HSP-71, HSP-70, and HSP beta1.

3.8. Effect of Different Light Irradiations on HDF Cells with Respect to DNA Repair Proteins

The heat map analysis represents that the IRA and BL significantly down-regulate the proteome of skin, especially the heterogeneous nuclear ribonucleoproteins C1/C2 and K, RuvB-like 1, X-ray repair cross-complementing protein 5 (**Figure 4(b)**).

4. Discussion

The current study uses label-free quantitative proteomics and nano-HPLC methodologies to compare the individual impacts of solar light irradiations such as

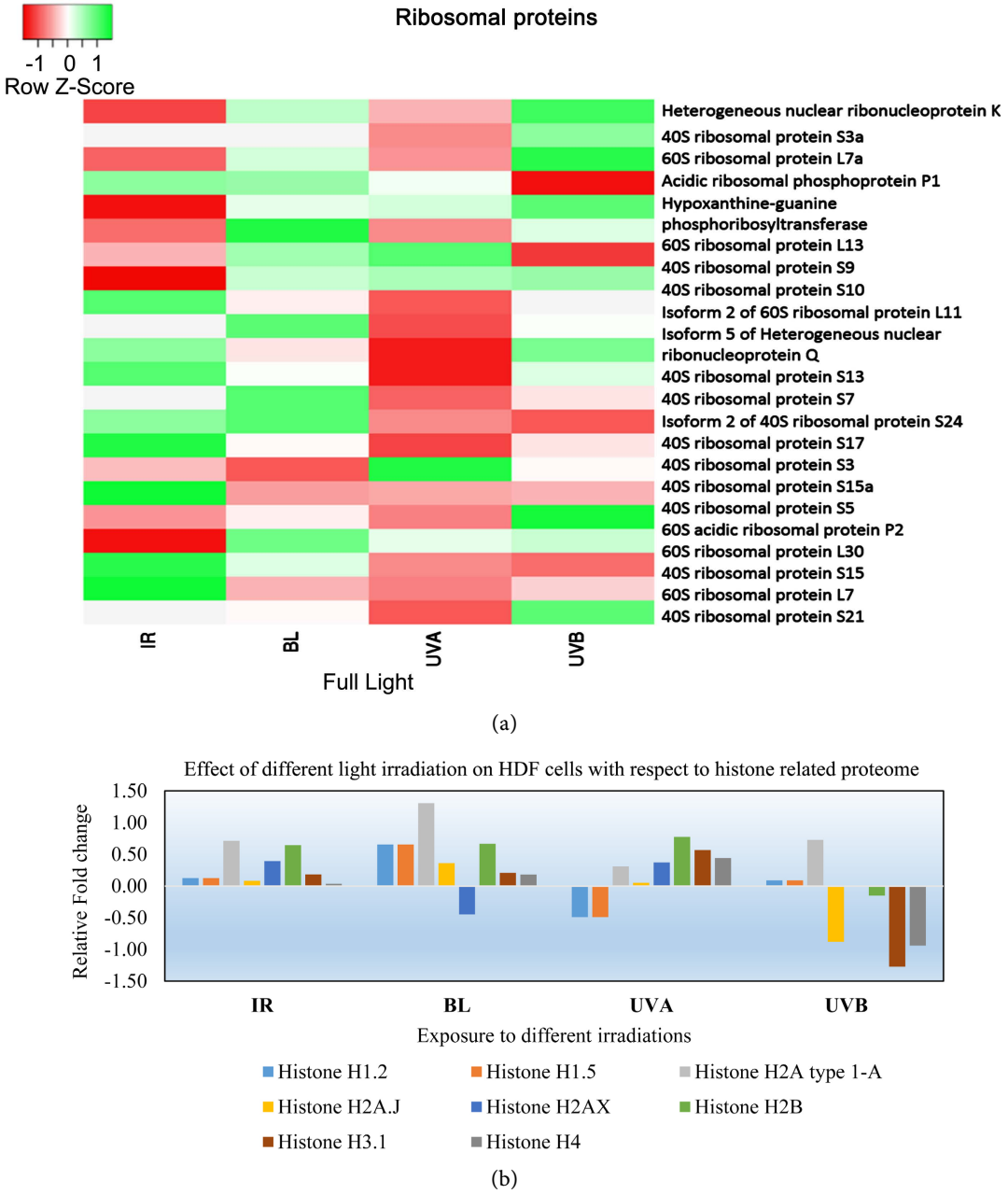
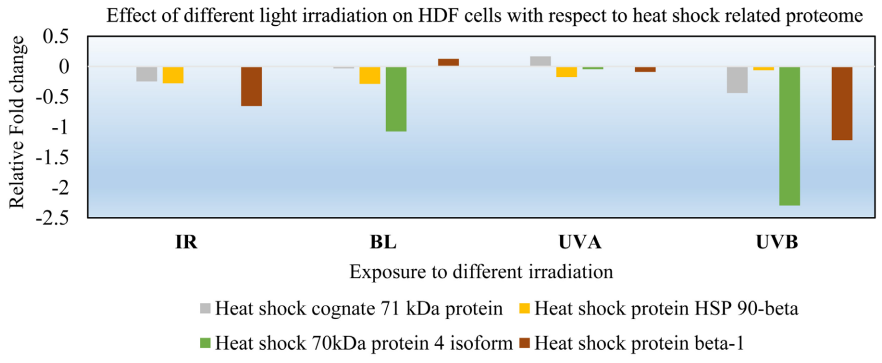
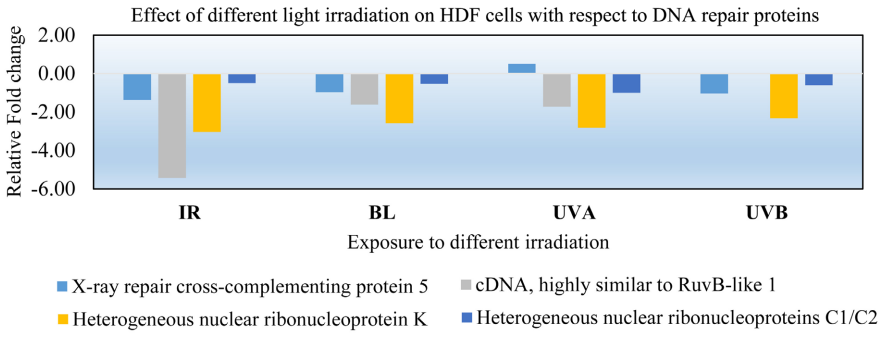


Figure 3. Heat map analysis of ribosome related protein expression and fold change analysis of histone related proteins with respect to different light irradiation in HDF cells. (a) Heat map analysis of ribosomal protein expression with respect to different light irradiation; (b) Fold changes analysis of histone protein expression with respect to different light irradiation.

IRA, BL, UVA, and UVB on primary HDF cells. In contrast to other irradiations, we found that IRA irradiated cells had much lower levels of COL1A1 protein. The COL1A1 protein gives instructions for producing type I collagen, a big molecule. UV rays, in particular UVA rays, penetrate deep into the epidermal and dermal layers of the skin, causing oxidative stress and the stimulation of matrix metalloproteinase (MMPs), which causes the breakdown of collagen and elastin fibres [24] [25].



(a)



(b)

Figure 4. Fold changes analysis of heat shock related proteins and DNA repair proteins and with respect to different light irradiation in HDF cells. (a) Fold change analysis of heat shock protein expression with respect to different light irradiation; (b) Fold change analysis of DNA repair protein expression with respect to different light irradiation.

The current work also confirms the significance of a major intrinsic ageing regulating protein, Basigin isoform, which is considerably down-regulated after UVA exposure. Surprisingly, UVB rays significantly reduce the activity of the prolyl 4-hydroxylase subunit alpha 1, a crucial enzyme in collagen synthesis that is composed of two equivalent alpha subunits and dual beta subunits. As a result, the data suggests that all forms of solar irradiation have a negative impact on collagen metabolism, which is consistent with earlier findings [26].

When exposed to sun irradiation, human skin suffers serious repercussions, including a significant increase in the generation of reactive oxygen species (ROS) [27]. The antioxidant proteins peroxiredoxin and thioredoxin were shown to be significantly altered by UVA ray exposure in our current investigation. Due to the difference in the penetration abilities of UVA and UVB irradiations, the adverse effects on the skin antioxidant system were moderately influenced in UVB induced cells compared to UVA induced cells.

UVB irradiation caused a significant downregulation of metallothionein (MT). MT is a cysteine-rich heavy metal-binding protein that participates in a variety of defensive stress responses and protects cells against oxidants and electrophiles that react rapidly with sulfhydryl groups [28]. UV light inhibits the transcriptional activation of MT-2 in HDF cells, according to Kobayashi *et al.* [29].

The antioxidant pathways are regulated by UVB via modifying Peroxiredoxin 6, a unique member of the peroxiredoxin family [30]. This protein has two active sites, making it a bi-functional enzyme. In comparison to other irradiations investigated, our findings imply that UVB plays a considerable role in affecting skin antioxidant proteins (IRA, BL, UVA). When exposed to infrared rays, Ezrin protein expression is shown to be significantly reduced. This protein serves as a linker between the plasma membrane and the actin cytoskeleton. It also confirms that sun irradiation causes membrane proteins to deregulate, resulting in significant structural and conformational alterations in epidermal proteins [31].

In eukaryotic cells, a cytoskeletal protein called Spectrin lines up the intracellular side of the plasma membrane. In the current work, the Spectrin protein was found to be down-regulated in HDF cells. This shows that different light exposures affect cell membranes by modifying membrane proteins. Membrane proteins are important for maintaining cellular structural homeostasis, which is shown to change when skin cells are exposed to UVA [32].

Mitochondria, is thought to be the primary site of cellular energetics, has been the focus of recent research. We discovered that solar irradiation has an influence on mitochondrial structural and functional proteins as well as organelles in this study [33] [34]. Due to higher penetration capacity of UVA and IRA, proteins implicated in mitochondrial energy metabolism are significantly down-regulated by solar irradiation in this study. As a result, one of the probable targets for blue light-induced oxidative stress in skin cells appears to be the mitochondrial respiratory chain.

Disturbance of ribosomal biogenesis may take place either by augmented or diminished expression of diverse ribosomal apparatuses that promote cell cycle arrest, senescence or apoptosis [35]. Ribosomal biogenesis is one of the potential factors related to the development of skin aging process. Despite the known links between modulators of aging and their roles in translation elongation, it is evident from the current study that the 40S and 60S ribosomal proteins deregulated upon exposure to UVA and infrared rays. Among numerous molecular mechanisms underlying skin aging, alterations of the translation machinery affect the rate and selectivity of protein biosynthesis [36].

The current study also suggests that UVB radiation causes the deregulation of Histone proteins like Histone H4. Histone proteins are essential components in the structure and function of chromatin. Numerous studies published in the last decade, have found that changes in various histones have a major impact on the intrinsic and extrinsic ageing processes [37].

Heat shock proteins are homologous chaperon proteins that are generated in response to environmental stimuli and assist cells recovering from damage [38]. The current study demonstrates that UVB and IRA suppress the heat shock proteins 70 and 71, which could contribute to further skin cellular damage. Both UVA and UVB rays are absorbed by skin cell DNA. Skin undergoes recurrent cycles of damage and repair as a result of UV exposure, and it's ability to self-repair is greatly diminished [39] [40]. UVA irradiation causes damage to HDF

cells by inhibiting a variety of processes, including anti-aging, mitochondrial, membrane proteins, ribosomal proteins, and DNA repair mechanisms. In addition, we discovered that BL and IRA have a considerable impact on DNA repair pathways in dermal cells.

Proteomics appears to be a valuable investigative tool in researching the skin's reaction to UVA, UVB, BL, and IRA irradiation. Based on a thorough understanding of the proteins involved in the skin's response to sun irradiation, the findings from this study could aid in the development of future cosmetic products and/or therapeutic interventions. With the use of proteomics, our study gives preliminary insights and important answers to explain the effects of sun irradiation on human dermal fibroblast cells. More sophisticated methods could further take it to a deeper understanding on the individual impact on the protein metabolism.

5. Summary

The individual effects of solar light irradiations such as IRA, BL, UVA, and UVB irradiations on primary HDF cells by quantitative proteomics using MS-MS and nano-HPLC methods has been summarized in this study (**Figure 5**). It also

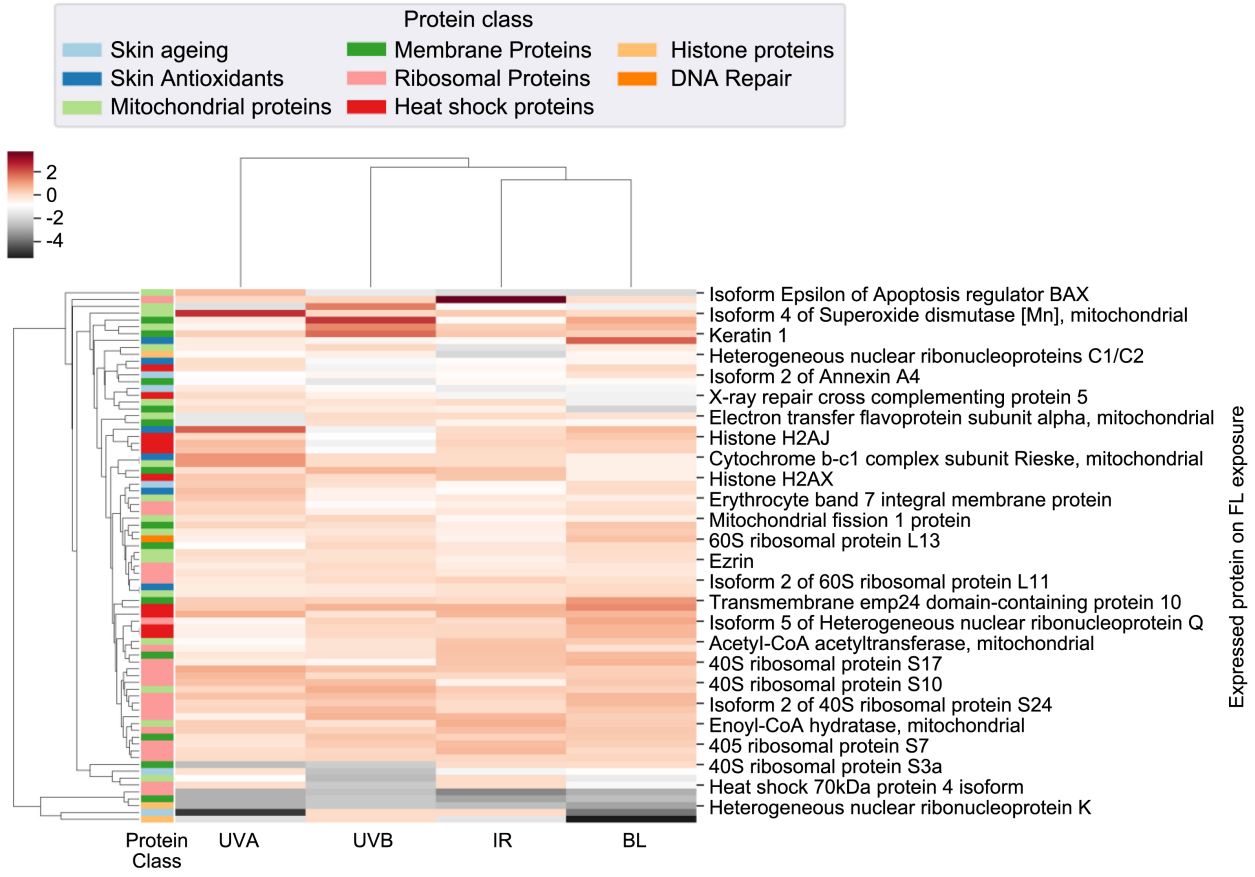


Figure 5. Overall summary of protein expression of proteins involved in skin anti-ageing on different light exposure on HDF cells. Cluster Heat map showing the overall summary of skin proteins expression fold change compared to control (Protein class defined from Reactome database).

shows that upon irradiations in HDF cells, there are detrimental effects observed mainly in collagen metabolism, antioxidant systems, and mitochondrial proteins.

Most of the molecular pathways were significantly down-regulated in IRA exposed cells compared to other solar radiation. UVA has a significant role in skin aging, Anti-oxidants, mitochondrial metabolism, membrane, and ribosomal proteins. UVB exposure has significant role in skin aging, antioxidants proteins, Heat shock proteins, Histone proteins, and DNA repair proteins. Blue light has significant role on antioxidant proteins, Histone proteins, and DNA repair proteins. Interestingly, it is observed from the cluster heatmap that, IR and BL are having more or less similar modulating effect in the skin protein expression. Ribosomal proteins are evenly expressed across upon all four light exposure as observed from the cluster heatmap. The complete data analyzed from the proteomics experiment is given as a supplementary file. Our current study proves that; all the four different light irradiations significantly contribute to the molecular degeneration of skin cells by various mechanisms.

6. Conclusions

Our research indicates that IRA is the most detrimental as it causes the increase in the production of free radicals, which further damages the collagen metabolism by stimulating the MMP1 expression and degrade the collagen. This study also confirms that Blue light down regulates the DNA repair proteins and also stimulates the Histone proteins as response mechanism to the skin chromosomal integrity. The above study demonstrated that there is a need for full light protection in order to cover all deleterious effects caused by solar irradiations. Hence, the future sun care products may need to change their strategies to include different ingredient combinations to protect most of the IRA, UVA, UVB and BL induced skin damages.

Developing novel herbal-based skin care ingredients to improve mitochondrial functions and to address the skin care problems due to solar irradiations could be the new area of science for the cosmetic industry. More than ever before, technology and skin care have collided, paving the opportunity for new skin care research and product creation.

7. Key Highlights of the Study

- Using proteomics to assess the cellular damage caused by various solar radiation, the current study compares the effects of UVA and UVB, IR/BL filtered solar-simulated light on human primary dermal skin fibroblasts.
- The current study has a comprehensive understanding of the proteomics signature of cellular biomarkers in relation to the various effects of solar irradiation on skin cells. It is also able to explain which solar irradiation is most likely to cause cellular damage.
- Unlike other body parts, the skin ages both naturally and as a result of exter-

nal environmental factors like blue light, ultraviolet (UV), infrared (IR), and other wavelengths of light that can accelerate extrinsic aging.

- The effects of non-UV solar radiation, which has a significant impact on photoaging and must be considered when developing a skin-protective routine, are also explained in this study.

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Authors Contributions

Balu Muthaiyah contributed to the conception, design, interpretation of the results and manuscript drafting. Yellapantula Sree Gouri has contributed in the sample preparation and manuscript drafting. Suresh Kumar Ramadoss has involved in the Bioinformatics data analysis and drafting the manuscript. Balaji Bandhyopadhyay has guided and led the overall project. All authors read and approved the final manuscript.

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Availability of Data and Material

Not applicable. The Proteomics raw data resulted in this project and its relevant analysis files are stored in the in-house repository, which is not accessible to public as per the institutional policy.

Conflicts of Interest

The authors declare no competing financial interest and declare no conflict of interest.

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Abbreviations

UVA—Ultraviolet A; UVB—Ultraviolet B; IR—Infrared Radiation; BL—Bluelight; HDF cells—Human Dermal Fibroblast cells; nanoHPLC—nano-High Performance Liquid Chromatography; MS—Masspectrometry.