Clinical, Cosmetic and Investigational Dermatology

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Tape-Strip Proteomic Analysis of Female Melasma Skin Lesions in a Chinese Population

Yi Yang[1,*], Zheng Zhao[2,*], Lulu Lu[2], Na Gao[2], Jiangang Hu[2], Gangwen Han[2], Xiaolei Ma[2]

[1]Department of Aesthetic and Plastic Surgery, Peking University International Hospital, Beijing, People's Republic of China; [2]Department of Dermatology, Peking University International Hospital, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xiaolei Ma, Department of Dermatology, Peking University International Hospital, No. 1 Life Park Road, Life Science Park of Zhongguancun, Changping District, Beijing, People's Republic of China, Email superma.xiaolei@163.com

Purpose: Melasma is a common, chronic, and recurring disorder of hyperpigmentation arising from hyperfunctional melanocytes that deposit excessive amounts of melanin in the epidermis and dermis. The pathophysiology of melasma remains unclear and the treatment is challenging. Proteomic Analysis may contribute to understand the pathogenesis of melasma.

Patients and Methods: In this study, transepidermal water loss(TWEL) was evaluated to assess skin barrier function. Melasma area and severity index score was used to measure the severity of melasma. Data independent acquisition mass spectrometry was used to perform a comparative analysis of protein expression in female skin samples(cheeks) from 8 healthy controls and 8 melasma subjects. The hospital anxiety and depression scale were used to assess the anxiety and depression levels of the melasma patients.

Results: The results showed that the melasma patients had higher TEWL values than the controls (12.95 ± 2.44 versus 6.86 ± 2.19, $p < 0.01$). Quantitative proteomic analysis identified a total of 230 differentially expressed proteins, including 193 upregulated and 37 downregulated. Enrichment analysis of these proteins based on GO, KEGG databases and protein-protein interaction analysis revealed that functional cluster associated with skin barrier (which included ALB, ANXA5, HSPB1, IQGAP1, S100A7), immunity and inflammation (which included YWHAZ, YWHAE, HSPA5, CSNK2B), melanogenesis (which included ALDH1A1, YWHAH, NDRG2, PMEL, APOE), psychoneurosis (which included YWHAE,YWHAH, PFN1, C3) and hormone (which included ARPC2, HSC70 and HSP70).

Conclusion: Our non-invasive proteomics analysis of human epidermal proteins may guide future research on female melasma and help in the development of treatments for melasma.

Keywords: proteomic, female melasma, Chinese population, tape-strip

Introduction

Melasma is a prevalent, chronic, and recurrent dermatological condition characterized by hyperfunctional melanocytes, leading to excessive melanin deposition in the epidermis and dermis.[1] It is particularly common among females of reproductive age and in areas of high sun exposure, notably on the face.[2] The condition is challenging to treat and prone to relapse, with significant psychosocial impact, including feelings of shame and reduced self-confidence.[3] While the precise pathophysiology of melasma remains unclear, potential contributing factors include genetic influences, sun exposure, hormones sensitivity, pregnancy, and medication use.[4] Further research into melasma is urgently needed.

Data-independent acquisition (DIA) mass spectrometry is an emerging technology that holds significant potential for deep and accurate proteomics with enhanced reproducibility.[5] The integration of proteomics into disease research efforts has broadened our understanding of the disease molecular profile through the discovery of new biomarkers. Furthermore, proteomics supports the development of targeted treatments, ultimately advancing personalized medicine. Although several proteomic studies on melasma have been reported in recent years, few studies have examined the proteomic analysis of melasma in the Chinese population using a non-invasive tape stripping technique.[6,7] In this study, we employed proteomic analysis using an Astral Mass Analyzer for Quantitative proteomics via data-Independent

Received: 2 May 2025
Accepted: 26 August 2025
Published: 12 September 2025

acquisition technology. The objective was to assess the potential of skin proteomics as a non-invasive approach for identifying novel protein biomarkers associated with melasma and to explore their role in the disease pathogenesis by investigating known signaling pathways.

Materials and Methods

Study Participants

The study protocols were received approval from the ethics committee of Peking University International Hospital (The approval number from the ethics committee is 2021-KY-0024-01). And all methods were performed in accordance with the relevant guidelines and regulations. Informed consent to participate in the study was obtained from each patient and healthy person before enrolling in the study. Informed consent was obtained from all participants prior to enrollment. The sample comprised eight individuals with melasma (mean age: 38.87 ± 3.31 years) and eight healthy controls (mean age: 37.75 ± 3.11 years), all residing in the Beijing area. The diagnosis of melasma was based on characteristic clinical manifestations and confirmed through a Wood's lamp examination. Comprehensive examinations and assessments were conducted to exclude post-inflammatory pigmentation, freckles, nevus fuscoceruleus zygomaticus, solar lentigines, melanosis, and other dermatological conditions contributing to skin pigmentation. Participants were required to abstain from treatments or medications that might interfere with the study for at least two months prior to enrolment. Exclusion criteria included chemical peels, oral tranexamic acid and polypodium leucotoma, topical hydroquinone, laser therapy and light devices.

Protein Sampling

Stratum corneum samples from the malar area were collected using 3M medical tape. Before sampling, the area was gently wiped with a 5% saline swab. The tape was applied to the skin with uniform pressure for three minutes, and three consecutive collections were performed at the same site. To ensure consistency and minimize variability, the same technician conducted all procedures. All samples were immediately stored at −80°C until further analysis.

The Melasma Area and Severity Index (MASI) is a scoring system designed to evaluate the severity of melasma based on three subjective factors: the area of involvement(A), darkness (D), and homogeneity (H).[8] These factors are assessed in four facial regions: the forehead (f), right malar region (rm), left malar region (lm), and chin (c), each of which is weighted at 30%, 30%, 30%, and 10% of the total face, respectively. The extent of involvement in each region is scored on a scale from 0 to 6, with 0 indicates no involvement, 1 represents less than 10–29%, and so on, up to 6 representing 90–100% involvement. Darkness and homogeneity are rated on a scale of 0 to 4, where 0 indicating absence,1 is slight, 2 is mild, 3 is marked and 4 is maximum. The MASI score is calculated by summing the products of the area, darkness, homogeneity scores for each region, yielding a total score ranging from 0 to 48.

TEWL Measurement for Skin Barrier Assessment

TEWL was measured at melasma site using a portable VapoMeter (TM300, CK, Cologne, Germany) in patients and the same area in matched controls. Measurements were taken every 6 to 8 seconds per reading. For each participant, three consecutive readings were collected at the same site and averaged.

Hospital Anxiety and Depression Scale (HADS)

The HADS is a psychometric instrument used to assess the severity of anxiety and depression.[9] It consists of 14 items, evenly divided between the anxiety and the depression subscales. Six items are reverse-scored, with five in the depression subscale and one in the anxiety subscale. Scores on each subscale are categorized as follows:

- 0–7: No symptoms
- 8–10: Possible anxiety or depression
- 11–21: Definite presence of the condition

Skin Proteomic Analysis

The samples were treated with 50 nM TEAB (tetraethyl ammonium bromide) in 200μL, and trypsin (in 50 mM ammonium bicarbonate) was added at an enzyme-to-protein ratio of 1:50. The samples were digested at 37°C for 16 hours. The resulting peptides were desalted, dried and re-solubilized by adding 0.1% formic acid (FA) solution.

Data were acquired using a Thermo Scientific™ Vanquish™ Neo UHPLC system coupled with an Orbitrap Astral mass spectrometer, equipped with a Thermo Scientific™ Easy-spray™ source. The data were acquired in data-independent acquisition mode with a normalized collision energy of 25% and a default charge state of 2. For quantitative analysis, a 110 mm Thermo Scientific™ μPac™ Neo HPLC column was used, with a 7-minute gradient from 4% to 99% B at a flow rate of 2.5 μL/min. MS1 spectra were acquired in the Orbitrap at a resolving power of 240,000 every 0.6 s, and MS/MS spectra were recorded in the Astral analyzer with varying injection times as specified for each experiment. Raw data were analyzed using DIA-NN v1.8.1 in library-free mode and searched against the same Uniprot Human database (uniprot-UP000005640_9606.fasta).

Statistical Analysis

Categorical data were presented as counts (percentages) and analyzed using chi-square tests. Continuous variables with a normal distribution were expressed as mean ± standard deviation and analyzed using *t*-tests. Continuous variables that did not follow a normal distribution were presented as median (interquartile range) and analyzed using the Mann–Whitney *U*-test. A p-value of less than 0.05 was considered statistically significant.

The fold-change (FC) for each group pair was calculated using the mean values of the groups. A two-sided unpaired Welch's *t*-test was employed to determine statistically significant differences between the groups. Differentially expressed proteins (DEPs) were selected based on the following thresholds: A fold change (FC) of ≥1.5 or ≤0.67, with a p-value of <0.05 considered statistically significant. To improve the reliability of the results, the p-values were adjusted for false discovery rate (FDR) using the Benjamini and Hochberg method.

Bioinformatics Analysis

Protein annotation, including cellular component, molecular function, and biological process, was obtained from the Gene Ontology database (http://www.geneontology.org/). A biological signaling pathway analysis was conducted using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html/). The selected proteomic genes underwent enrichment analysis using DAVID version 6.8 (The Database for Annotation, Visualization and Integrated Discovery; https://david.ncifcrf.gov/)[10,11] with Mus musculus as the reference species. The Gene Ontology (GO) enrichment analysis was conducted using the Gene Ontology tool, while KEGG pathways were analysed using the KEGG_PATHWAY tool. A p-value of less than 0.05 was considered statistically significant.

Results

Characteristics of the Participants

The section describes the characteristics of the participants. Table 1 summarizes the clinical characteristics of 16 participants. The patients were classified into Fitzpatrick phototypes III (5 patients; 62.5%) and IV (3 patients, 37.5%). They exhibited bilateral light-to-dark brown asymptomatic macules with irregular borders. The malar type was observed in four cases, with lesions located over the nose and malar cheeks. The centrofacial type was observed in four cases, with lesions located on the cheeks, nose, forehead, and upper lip. The mean age of patients with melasma was 38.87 ± 3.31 years, and the mean duration of the disease was 3.12 years. A total of 87.5% of patients (7/8) reported a direct relationship between intense sun exposure, mental stress, and poor sleep with the recurrent exacerbation of the rash. The presence of melasma had a notable impact on the psychosocial well-being of patients, as evidenced by the average HADS score of 9.125 ± 1.125. A majority (75%) of patients (6/8) reported feelings of shame and reduced self-confidence.

Table 1 Characteristics of the Participants

Number	Age	Course	Type	TEWL	HADS Score	MASI Score
P1	36	2	Malar type	10.4	8	15.82
P2	42	3	Malar type	11.38	9	13.24
P3	38	3	Centrofacial type	14.62	10	19.86
P4	40	4	Centrofacial type	10.29	10	17.54
P5	38	3	Malar type	13.02	11	12.48
P6	45	6	Centrofacial type	12.28	9	14.26
P7	37	2	Centrofacial type	17.56	8	13.88
P8	35	2	Malar type	14.03	8	10.38
C1	35	NA	NA	7.41	4	NA
C2	33	NA	NA	4.83	3	NA
C3	42	NA	NA	5.11	5	NA
C4	37	NA	NA	5.35	1	NA
C5	36	NA	NA	4.71	0	NA
C6	40	NA	NA	7.93	3	NA
C7	41	NA	NA	10.46	4	NA
C8	38	NA	NA	9.12	2	NA

Abbreviations: TEWL, Transepidermal Water Loss; HADS, Hospital Anxiety and Depression Scale; MASI, The Melasma Area and Severity Index; P, patients; C, controls; NA, not available.

Skin Barrier Function

The function of the human skin barrier is commonly assessed by measuring TEWL. Elevated TEWL levels typically indicate the dysfunction of skin barrier. In this study, melasma patients exhibited significantly higher TEWL values compared to controls (12.95 ± 2.44 versus 6.86 ± 2.19, $p < 0.01$), suggesting an association between melasma and skin barrier dysfunction.

Quantitative Protein Detection

A spectral library of human skin proteins was established and analyzed using data-independent acquisition (DIA) mass spectrometry. The library was obtained from 8 melasma patients and 8 healthy controls. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org) via the iProX partner repository[4,12] with the dataset identifier PXD057929.

A total of 15882 peptides and 2227 proteins were identified. A fold change of$\geq$1.5 or $\leq$0.67and $p<0.05$ were used as the screening criteria for differentially expressed proteins(DEPs). A total of 230 proteins were found to be differentially expressed in melasma patients when compared to healthy controls. Of these, 193 proteins were upregulated, while 37 were downregulated. A cluster analysis was performed to illustrate the expression differences between the two groups (Figure 1). Principal component analysis (PCA) and volcano plots, as supervised multivariate data analysis methods, demonstrated that the differential proteins segregated into two distinct clusters, allowing differentiation between the patient group and the healthy controls (Figures 2 and 3).

Gene Ontology (GO) Enrichment Analysis

Gene Ontology (GO) annotation analysis was conducted to evaluate the functional properties of all identified proteins, using the UniProt database and the R 3.6 software package (clusterProfiler). The results of this analysis are presented in Figure 4. The most enriched biological processes in the upregulated proteins included biological regulation, cellular processes, detoxification, immune response, homeostatic processes, metabolic processes, pigmentation, response to stimuli, and developmental processes. The most enriched cellular components were identified as cellular anatomical entities and protein-containing complexes. The most enriched molecular functions included binding, antioxidant activity, ATP-dependent activity, catalytic activity, molecular adaptor activity, molecular function regulator activity, structural molecule activity, transcription regulator activity, translation regulator activity, and transporter activity.

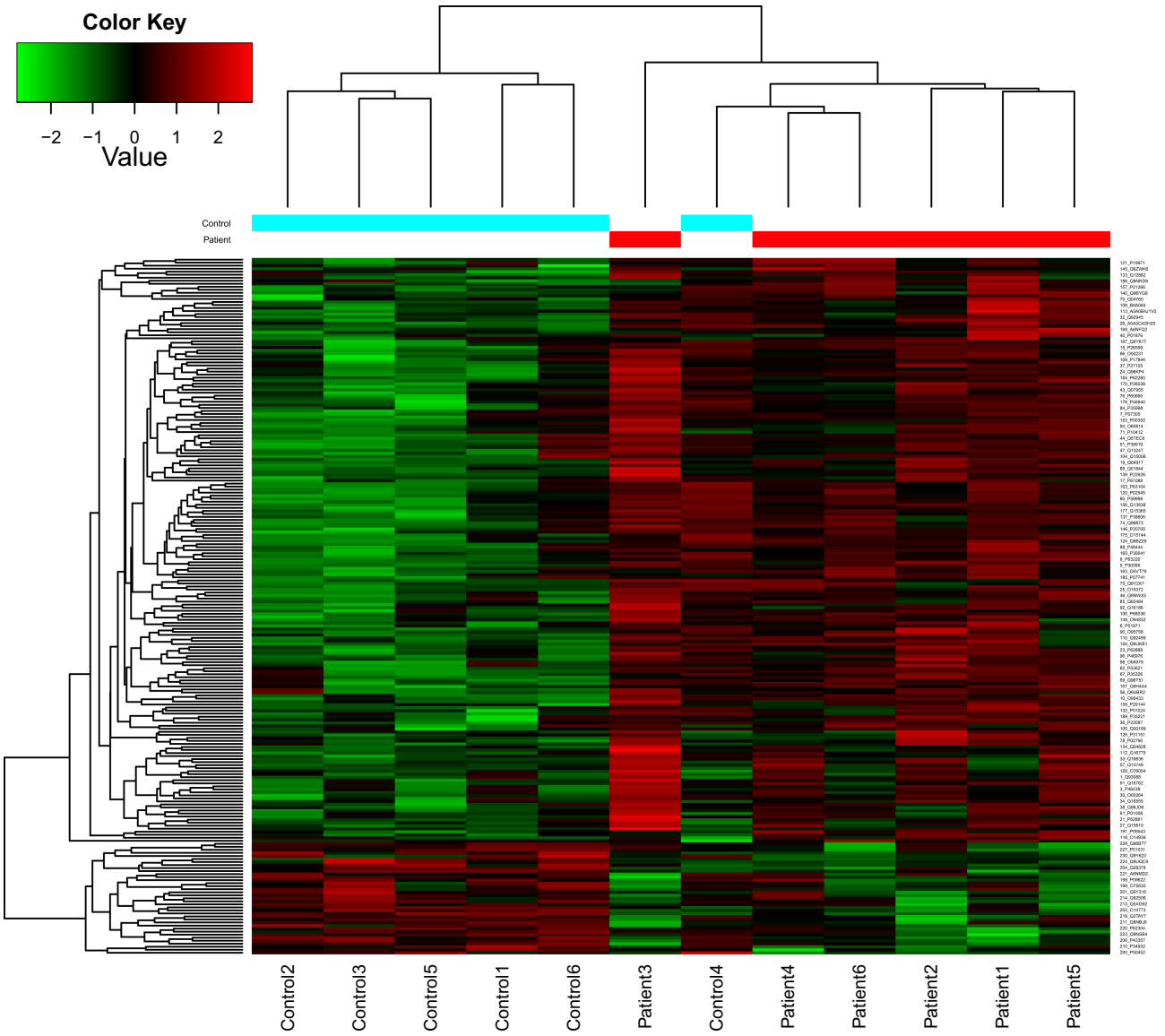

Figure 1 Cluster analysis chart. Heat map showing the individual proteins that were significantly different in the melasma samples compared to the control samples after adjustment by false discovery rate.

A gene ontology (GO) enrichment analysis was performed on the identified proteins to determine the biological functions significantly associated with the differentially expressed proteins. It was observed that, in general, both up- and down-regulated differentially expressed proteins (DEPs) are involved in similar structural or functional processes (Figure 5). However, specific enrichments were noted for upregulated proteins in certain GO terms within the biological process and molecular functions categories, including pigmentation, growth, rhythmic process, molecular carrier activity, molecular transducer activity, and translation regulation.

KEGG Pathway Analysis

The KEGG pathway analysis for the identified differential proteins (DEPs) was conducted to identify the most important biochemical metabolic and signal transduction pathways. The results showed that upregulated proteins were annotated to several significant pathways, including metabolic pathways, gluconeogenesis, PI3K-Akt signaling pathway, Neutrophil extracellular trap formation, MAPK signaling pathway, glutathione metabolism, calcium signaling pathway, NF-kappa B signaling pathway, HIF-1 signaling pathway, sphingolipid signaling pathway, and VEGF signaling pathway.

https://doi.org/10.2147/CCID.S538073

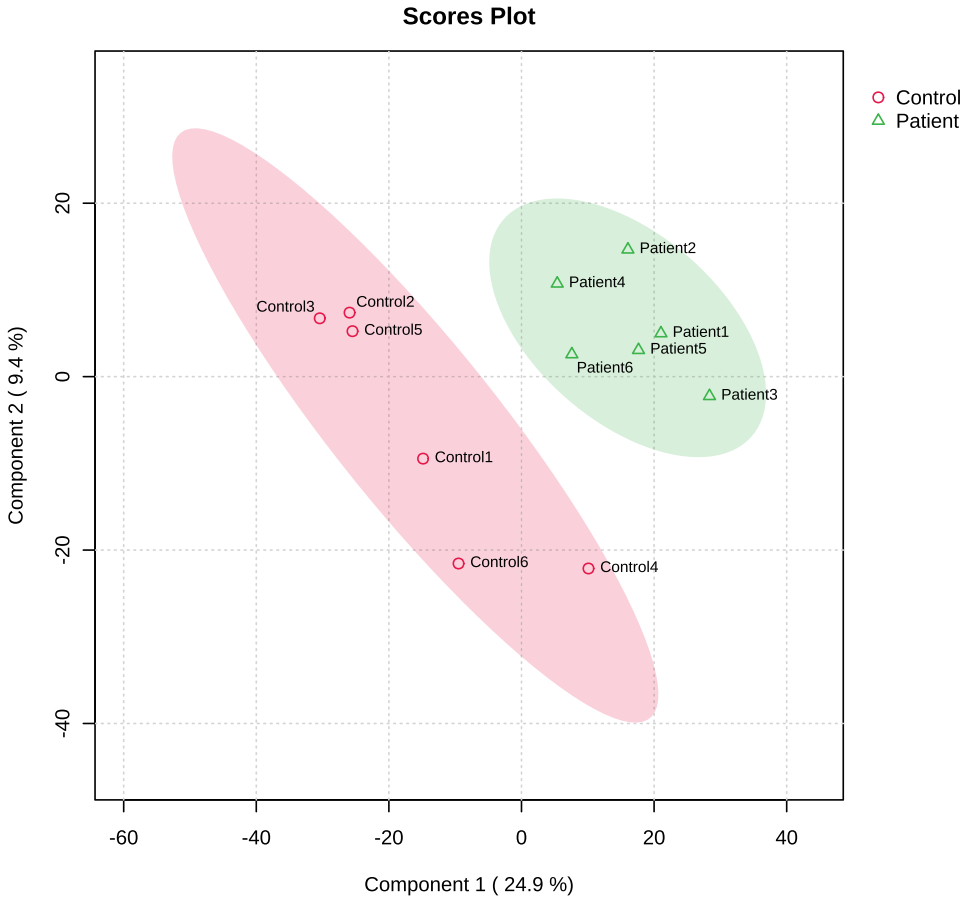

Figure 2 PCA Plot. PCA plot showing the overall distinction between the control and melasma groups.

Additionally, the natural killer cell-mediated cytotoxicity pathway was identified. Other significant pathways included the B cell receptor signaling pathway, pentose phosphate pathway, Wnt signaling pathway, complement and coagulation cascades, antigen processing and presentation, IL-17 signaling pathway, the GnRH signaling pathway, oxytocin signaling pathway, fatty acid degradation pathway, estrogen signaling pathway, tyrosine metabolism pathway, tryptophan metabolism pathway, nitrogen metabolism pathway, biosynthesis of unsaturated fatty acids pathway, and melanogenesis pathways. The most significantly enriched pathways were glycolysis/gluconeogenesis, the PI3K-AKT signaling pathway, and neutrophil extracellular trap formation (Figure 6).

The downregulated proteins were annotated to the following pathways: metabolic pathways, fatty acid metabolism and neuroactive ligand-receptor interaction pathways. The most significantly enriched ones were metabolic pathways and oxidative phosphorylation (Figure 7).

Subcellular Location and Protein-Protein Interaction Network Analyses

The subcellular location of the identified differentially expressed proteins (DEPs) indicated that the cytoplasm, nucleus, mitochondrion, and extracellular structures were the most represented compartments (Figure 8). Subsequently, the proteins were analyzed using STRING 12.0 for protein-protein interactions (Figure 9). PPI (protein-protein interaction) analysis identified five main clusters, corresponding to five broad functions:

- Immunity and inflammation: YWHAZ, YWHAE, HSPA5, CSNK2B
- Melanogenesis: ALDH1A1, YWHAH, NDRG2, PMEL, APOE
- Psychoneurosis: YWHAE, YWHAH, PFN1, C3

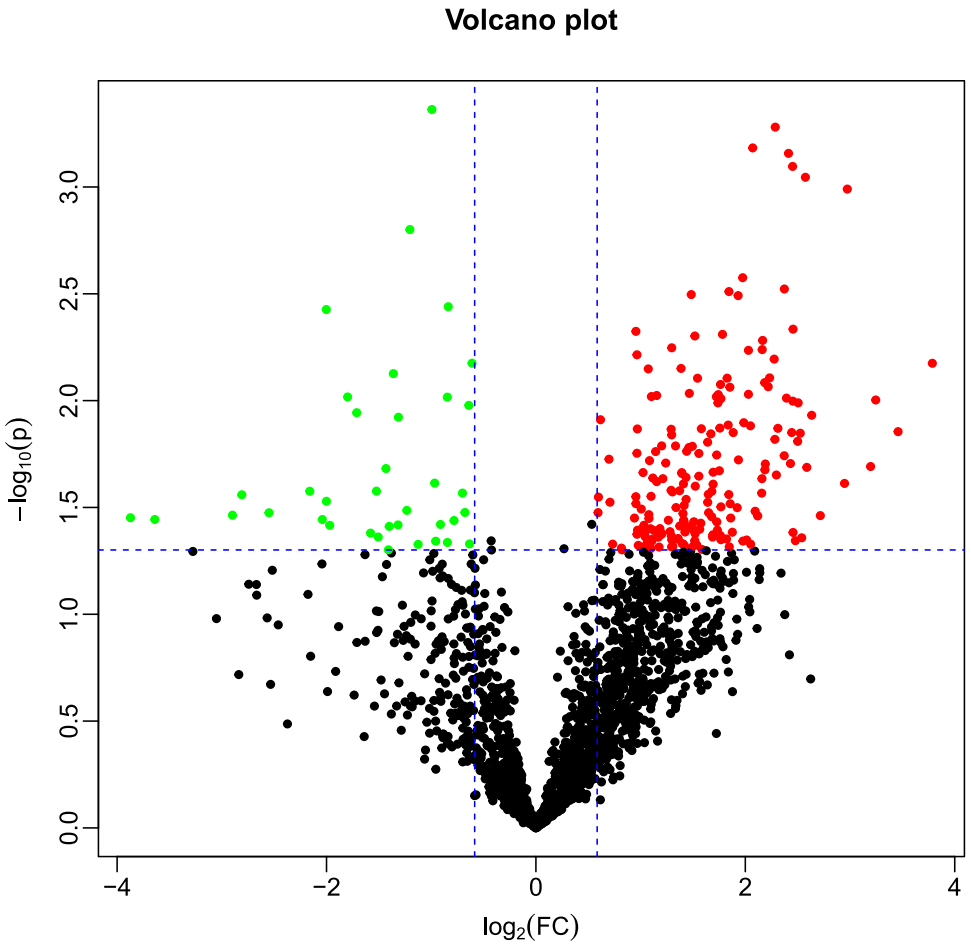

Figure 3 Volcano plots. Volcano plots summarizing findings from proteomics analysis of human melasma and control.

Additionally, the identified DEPs were involved in various biological processes, including hormone metabolism, associated with ARPC2, HSC70, and HSP70.

Discussion

Melasma is a common, acquired cutaneous pigmentary disorder that is more prevalent in women than men. The overall prevalence of melasma in the general population is approximately 1%, although it can range from 9% to 40% in high-risk populations.[2,13] The etiology of melasma remains unclear, despite several proposed contributing factors, including genetic predisposition, sun exposure, hormonal sensitivity, pregnancy, and medication use. The therapeutic management of melasma presents a significant challenge due to its high recurrence rate, and the optimal treatment remains to be established.[13,14]

Recently, there has been a growing body of research utilizing proteomics in skin diseases such as atopic dermatitis, psoriasis, and melanoma. This has led to the discovery of novel biomarkers, contributing to a deeper understanding of the molecular profiles of these diseases.[15–20]Xu et al[21] has reported to utilizes Tape Strip to investigate lipid profiles in melasma, however, the proteomic characterization of the stratum corneum of patients with melasma using a tape stripping technique in the Chinese population has not been reported to date. To gain further insight, we conducted proteomic analyses to identify a panel of promising biomarkers for melasma.

The results of our study indicated that, following GO enrichment and cluster analysis, and KEGG-based pathway analysis, the upregulated proteins were enriched in several key biological processes, including immunity and inflammation, the skin barrier, melanogenesis, psychoneurosis and hormone. These findings suggest that these five aspects are involved in the pathogenesis of melasma.

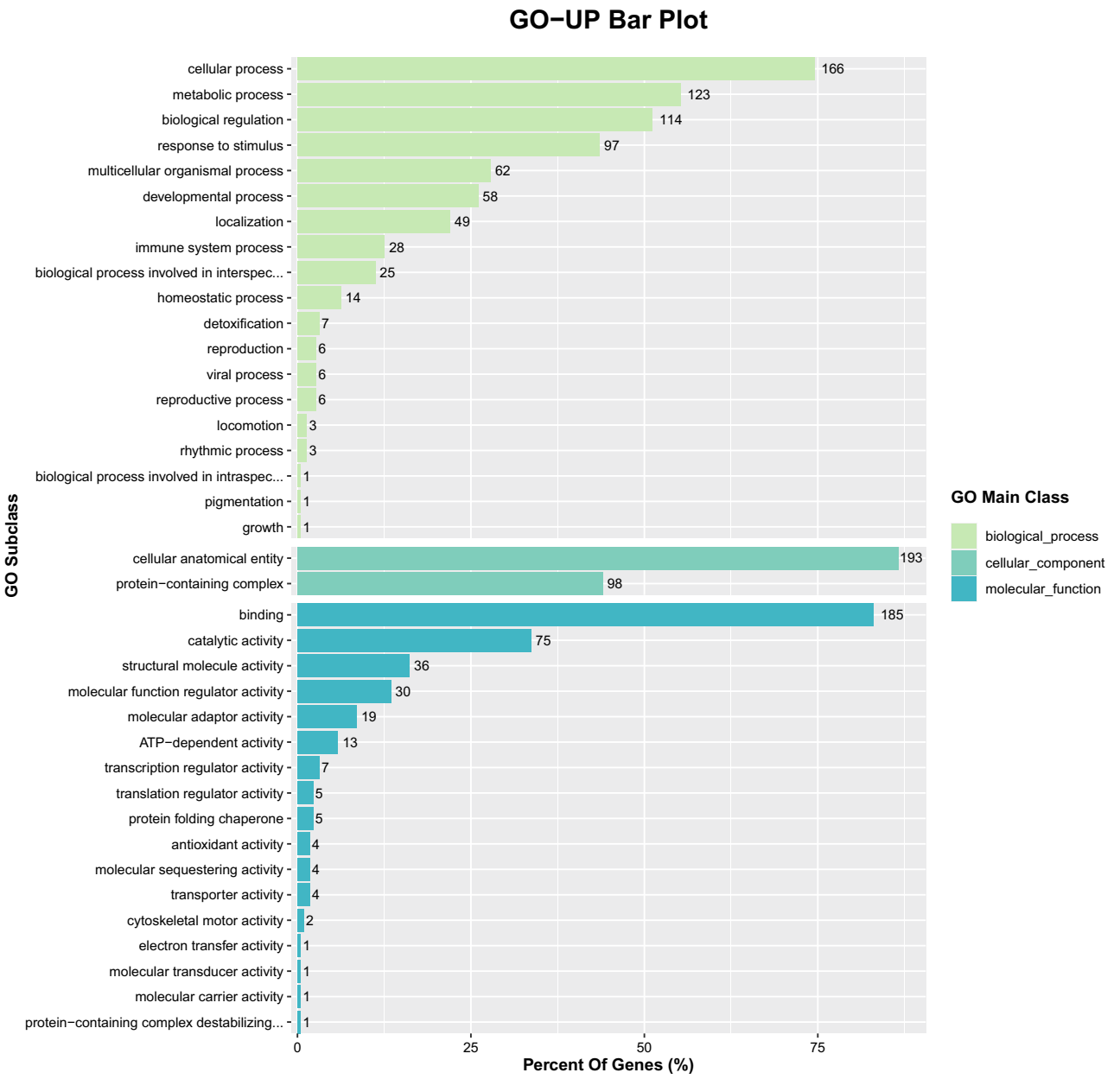

Figure 4 Gene ontology (GO) classification of the up differentially expressed proteins between the melasma and control groups. The axes indicate the number and per-cent of proteins in each GO term.

Chronic Inflammation and Its Role in Melasma Pathogenesis

There is mounting evidence that chronic inflammation plays a role in the pathogenesis of melasma, which may partly explain the high frequency of recurrences. Several studies have demonstrated the presence of CD4+ T cells, CD68 + macrophages, and mast cells in the infiltrates of melasma patients.[22,23] Our data revealed that multiple differentially expressed proteins DEPs upregulated in melasma are related to immunity and inflammation, including YWHAZ (14-3-3ζδ), YWHAE (14-3-3ε), HSPA5 (endoplasmic reticulum chaperone Bip), and CSNK2B (casein kinase II). These proteins were upregulated in melasma and are associated with immunity and inflammation. The 14-3-3 proteins constitute a family of conserved regulatory molecules expressed in eukaryotic cells. They play pivotal roles in various essential regulatory processes, including immunity.[24,25]

B cells exert a tonic inhibitory effect on T cell trafficking by secreting a peptide called PEPITEM, which is proteolytically derived from the YWHAZ 14-3-3 zeta delta 14-3-3.ζδ) protein. In animal models of peritonitis, hepatic

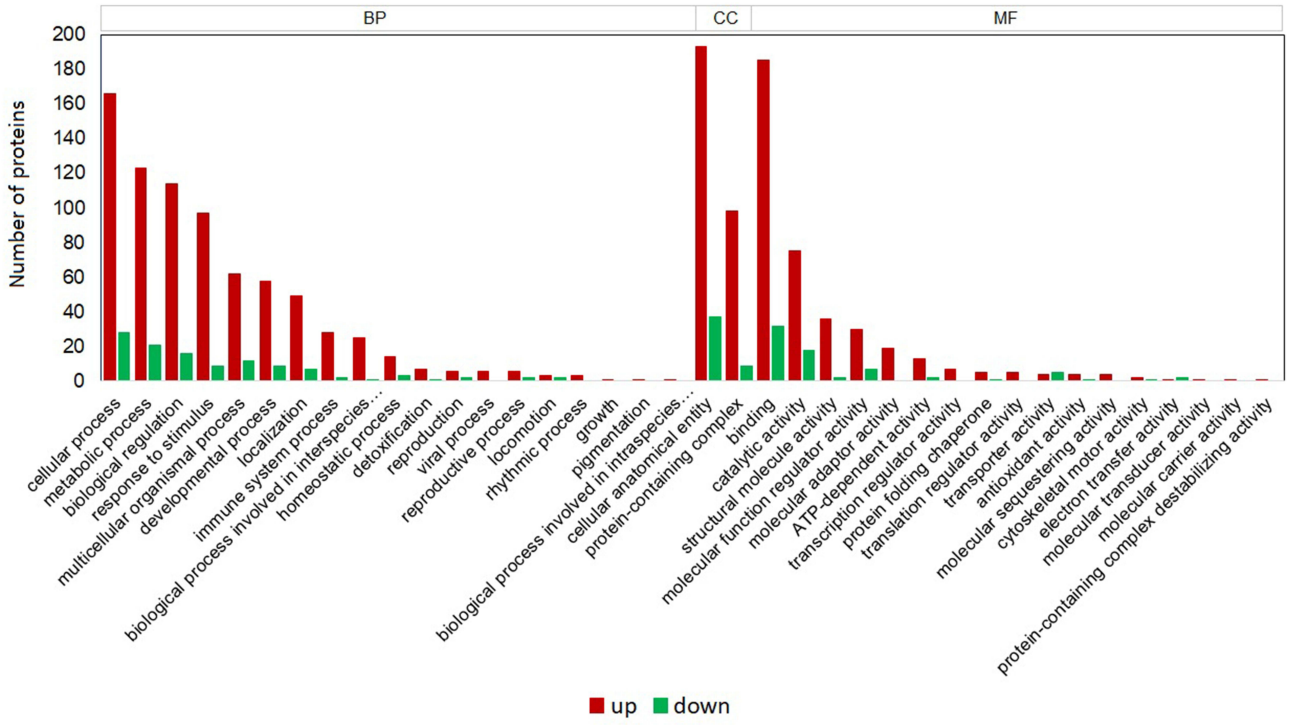

Figure 5 Gene ontology (GO) classification of all the differentially expressed proteins between the melasma and control groups. The axes indicate the number and per-cent of proteins in each GO term.

ischemia-reperfusion injury, Salmonella infection, uveitis and Sjögren's syndrome, PEPITEM has been shown to reduce T cell recruitment into inflamed tissues.[26] YWHAE 14-3-3ε is indispensable for TNFR2 signaling-mediated regulation of macrophage polarization and switching.[27] Several studies have identified YWHAE 14-3-3ε as key component of the TNFR2 receptor complex, particularly in its role in macrophage polarization, with potential therapeutic implications for inflammatory and autoimmune diseases through the activation of the TNFR2/14-3-3ε anti-inflammatory pathway.[28]

HSPA5, the endoplasmic reticulum chaperone Bip plays a pivotal role in protein folding and quality control within the endoplasmic reticulum lumen. As a regulator of ER function, BiP is associated with several pathological conditions, including cancer, cardiovascular disease, neurodegenerative disease, and immunological diseases.[29] It is considered as a target for immunogenic cell death inducers.[30] Inducible heat shock protein (HSP)70 is a molecular chaperone that facilitates correct protein folding. Following stress conditions, HSP70 is markedly upregulated, mediating cytoprotective functions. Furthermore, HSP70 can trigger innate and adaptive immune responses, facilitating antigen recognition by the immune system and acting as a cytokine.[31]

CSNK2B, also known as Casein kinase II, is a highly conserved serine/threonine or tyrosine kinase enzyme expressed ubiquitously. It regulates diverse signaling pathways responsible for various cellular processes and interacts with over 500 known substrates. CK2's plays a regulatory role in key signaling events, including NF-κB, JAK/STAT, HIF-1α, and COX-2 pathways. The ERK/MAPK, Notch, AKT, Ikaros and Wnt signaling pathways have been showed to influence immune cells development and function.[32,33]

Defective skin barrier function in melasma plays a critical role in the pathogenesis of melasma.[34] This study demonstrates notable differences in TEWL, a marker of barrier function, between lesions and healthy skin following repeated tape stripping. GO enrichment and KEGG-based pathway analyses indicated that upregulated proteins were enriched in disorders of skin lipid metabolism, including the sphingolipid signaling pathway, fatty acid degradation, and biosynthesis of unsaturated fatty acids pathways. These findings align with those of D. J. Lee,[34] who suggested that altered skin lipid metabolism contributes to defective skin barrier function in melasma based on microarray analysis.

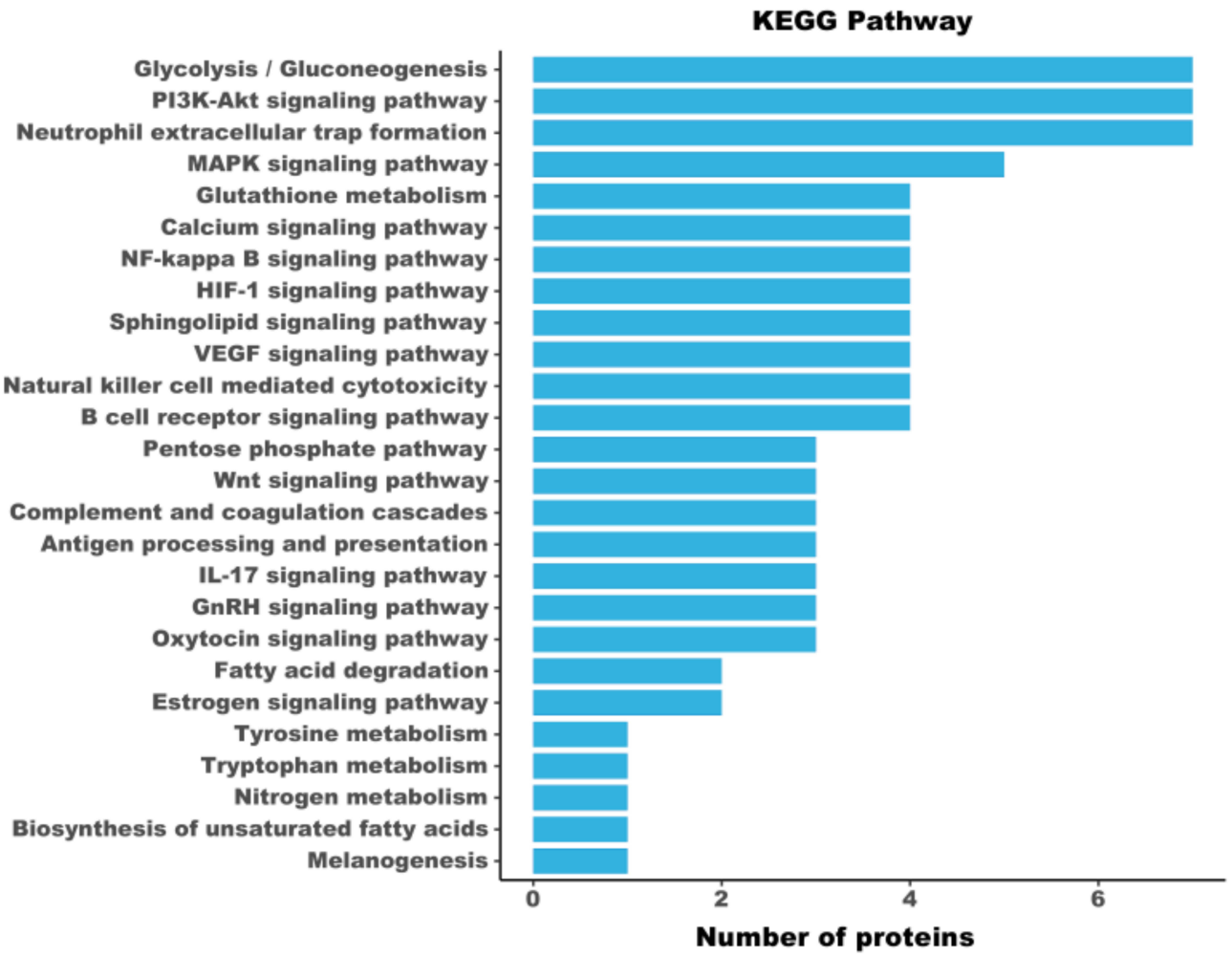

Figure 6 KEGG analysis of the proteins showing up differential expression. Significant enrichment analysis of the proteins showing up differential expression between the melasma and control groups using the KEGG and Reactome databases.

In the present study, the expression of several proteins, including ALB (albumin), ANXA5 (Annexin A5), HSPB1 (heat shock proteinsB1), IQGAP1 (Ras GTPase-activating-like protein), S100A7 (Protein S100-A7) was found to be elevated in patients with melasma. Albumin has been demonstrated to serve as a potential marker for pruritus in older adults with disrupted skin barrier function and localized skin inflammation.[35] Annexin A5, which accumulates at the nuclear envelope, is a biomarker of cellular ageing and is observed in aged human skin.[36] It plays a significant role in regulating macrophage inflammation and the promotion of epithelialization.[37] Ras GTPase-activating-like protein IQGAP1 acts as an effector molecule for Cdc42 and Rac1, members of the Rho small GTPase-activating-like family of the Rho small GTPase family play a pivotal role in regulating E-cadherin-mediated cell adhesion.[38] S100A7, also known as psoriasin, was initially identified three decades ago as a protein that is highly expressed in psoriatic keratinocytes.[39] Recent studies indicate that psoriasin functions as an antimicrobial and immunomodulatory protein and serves as a critical effector molecule of the cutaneous barrier. The protein comprises five α-helices, each of which contains a single calcium-binding EF-hand.[40]

Psoriasin expression can be induced by various factors, including bacteria stimulation and proinflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, IL-17 and - IL 22.[39] Elevated psoriasin expression has also been observed in several dermatological conditions, including psoriasis, atopic dermatitis, lichen sclerosus, actinic keratoses, acne inversa, and Darier disease.[41–45]These finding suggest that the identified proteins may be associated with skin barrier function, potentially contributing to a dry skin phenotype.

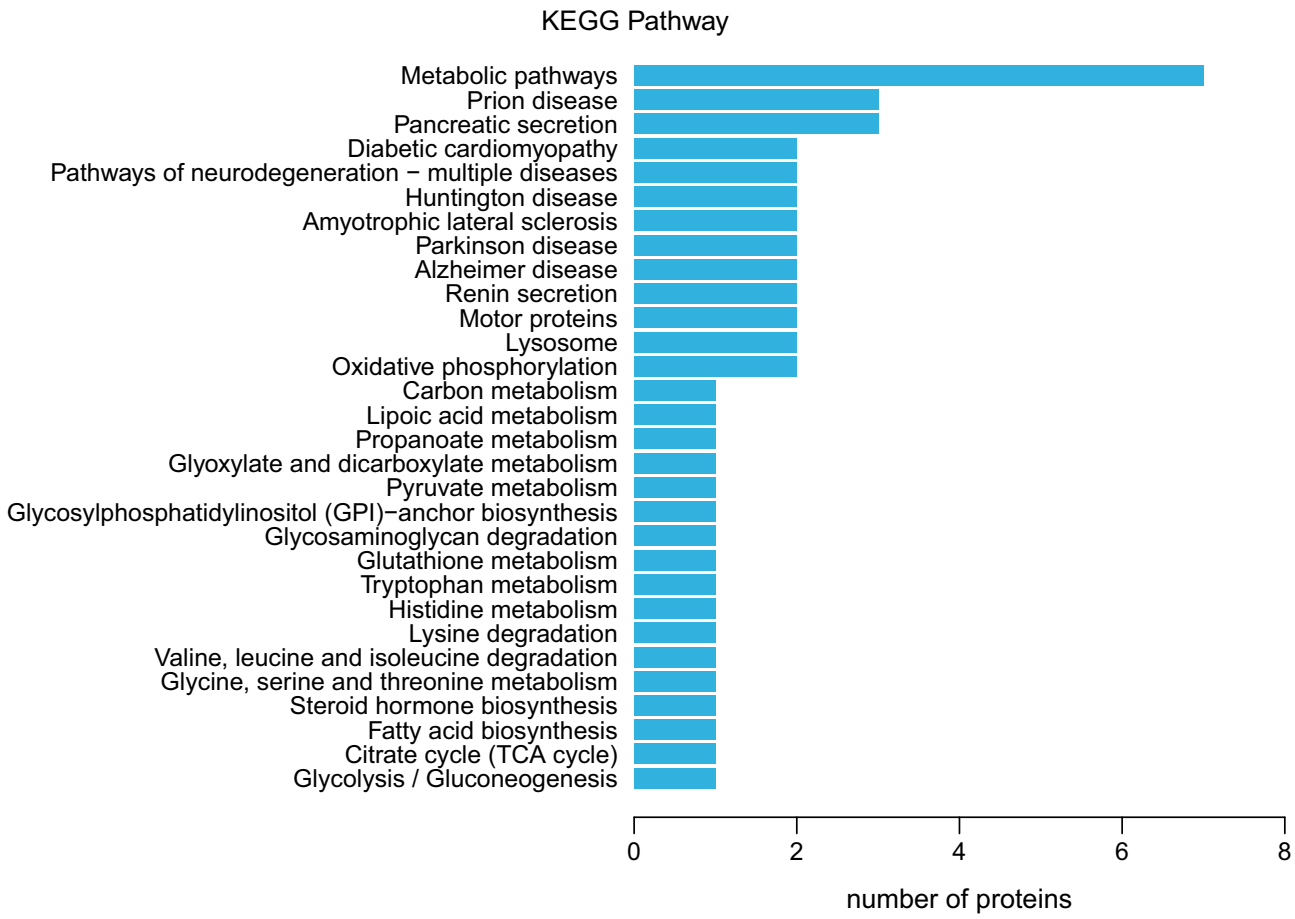

Figure 7 KEGG analysis of the proteins showing down differential expression. Significant enrichment analysis of the proteins showing down differential expression between the melasma and control groups using the KEGG and Reactome databases.

Melanin Production and Melanogenesis Pathways

Melasma is defined as hyperpigmentation of the skin. Melanin is produced in melanocytes through the process of melanogenesis, a series of biochemical and enzymatic reactions catalyzed by tyrosinase and other related proteins.[46] Our study demonstrated that the upregulated proteins were enriched in pigmentation-related pathways, including the PI3K-Akt signaling pathway, MAPK signaling pathway, glutathione metabolism, Wnt signaling pathway, and tyrosine metabolism, as identified by GO enrichment and KEGG-based cluster analysis. These pathways are all associated with melanogenesis.

The key upregulated proteins identified in this study included ALDH1A1 (aldehyde dehydrogenase 1A1), YWHAH (14-3-3 protein epsilon), NDRG2 (protein NDRG2), PMEL (promyelocytic leukemia protein), and APOE (apolipoprotein E). Aldehyde dehydrogenase 1A1 (ALDH1A1), an enzyme that catalyzes the conversion of lipid aldehydes to lipid carboxylic acids, plays a multitude of roles in melanogenesis.[46] Paterson et al[47] demonstrated that the ALDH1A1 substrate 9-cis retinal and its product 9-cis retinoic acid potently induced the accumulation of MITF mRNA, tyrosinase mRNA and melanin, suggesting that ALDH1A1 regulates melanogenesis through this conversion. Inhibitors of ALDH1A1, such as cyanamide or Angeli's salt, have been shown to effectively suppress pigmentation in human skin cells.

The 14-3-3 protein epsilon (YWHAH) act as an adapter protein regulating various signaling pathways, both general and specialized. Yin et al[48] demonstrated that YWHAE plays a role in the pathogenesis of atopic dermatitis (AD) and tyrosinase-mediated pigmentation.

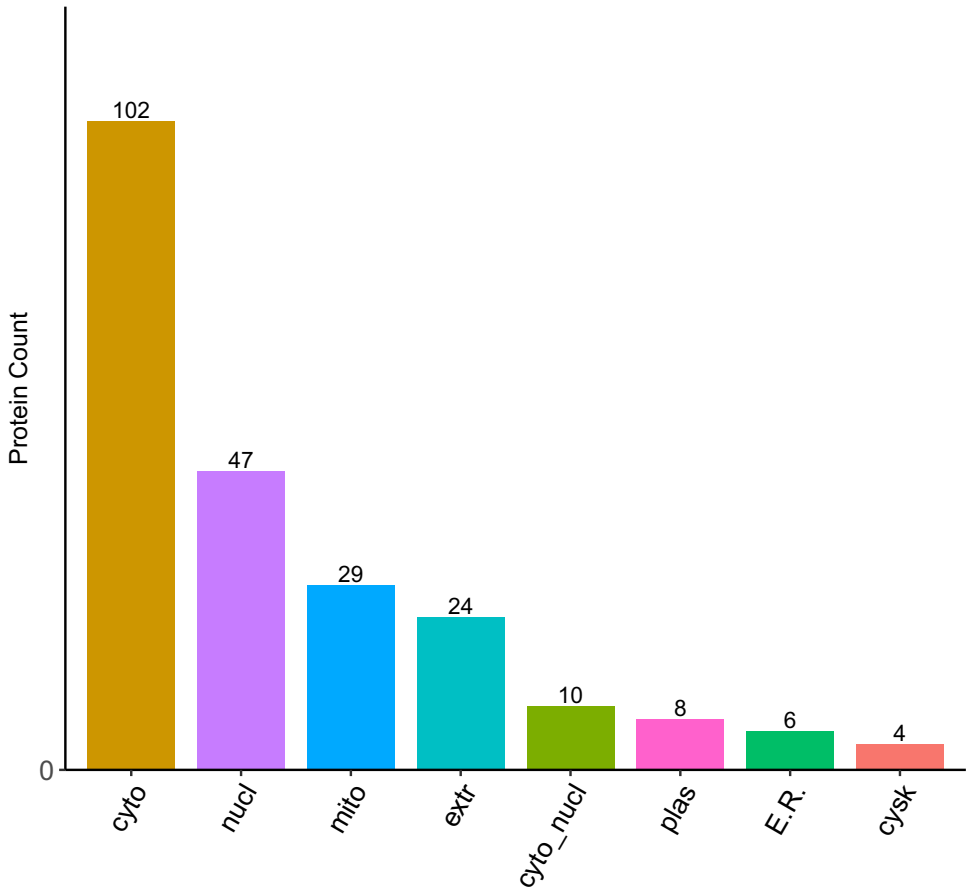

Figure 8 The subcellular location of the identified differentially expressed proteins. The subcellular location of the identified differentially expressed proteins (DEPs) indicated that the cytoplasm, nucleus, mitochondrion, and extracellular structures were the most represented compartments.

NDRG2 (*N*-myc downstream-regulated gene 2) a member of the NDRG family, regulates the Wnt signaling pathway. NDRG2 impedes cAMP- and β-catenin-mediated activation of the MITF promoter, thereby suppressing melanogenesis via downstream MITF target genes, including tyrosinase, TYRP1, and DCT.[43]

PMEL, a melanosomal protein, forms physiological amyloids critical for melanosome morphogenesis and pigmentation. In stages III and IV melanosomes, the amyloid matrix provides a platform for eumelanin precursors accumulation, facilitating pigment formation.[49]

Apolipoprotein E (APOE) regulate PMEL, pigment cell-specific premelanosomal protein. The biogenesis and maturation of melanosomes begin with an unpigmented stage, which leads to the formation of luminal fibrils derived from (PMEL). Aberrant expression of APOE may result in hyperpigmentation.[50]

Our study indicates that melasma patients exhibit statistically significantly higher levels of anxiety and depression compared to healthy controls. Furthermore, the expression of specific proteins associated with depression and anxiety, including YWHAE (14-3-3 protein epsilon, YWHAH (14-3-3 protein eta), PFN1 (Profilin-1), C3, and C3a, was elevated in individuals with melasma. The seven 14-3-3 isoforms constitute approximately 1% of total soluble brain proteins, and they play pivotal roles in nervous system development and cortical development. These proteins have recently been identified as pivotal regulators of various neurodevelopmental processes.[51] Among these, YWHAE (14-3-3 protein epsilon) has been linked to suicide victims,[52] while YWHAH (14-3-3 protein eta) has been identified as a candidate gene for schizophrenia.[53] PFN1 (Profilin-1) was detected in the anterior cingulate cortex of individuals diagnosed with major depressive disorder, where it functions as a suppressor of both tissue remodeling and immune response suppression.[54]

Dysfunction of the complement system has also been implicated in the pathophysiology of major depressive disorder. Experimental evidence suggests that complement C3 signaling contributes to the etiology of depression. Luo et al[55] proposed

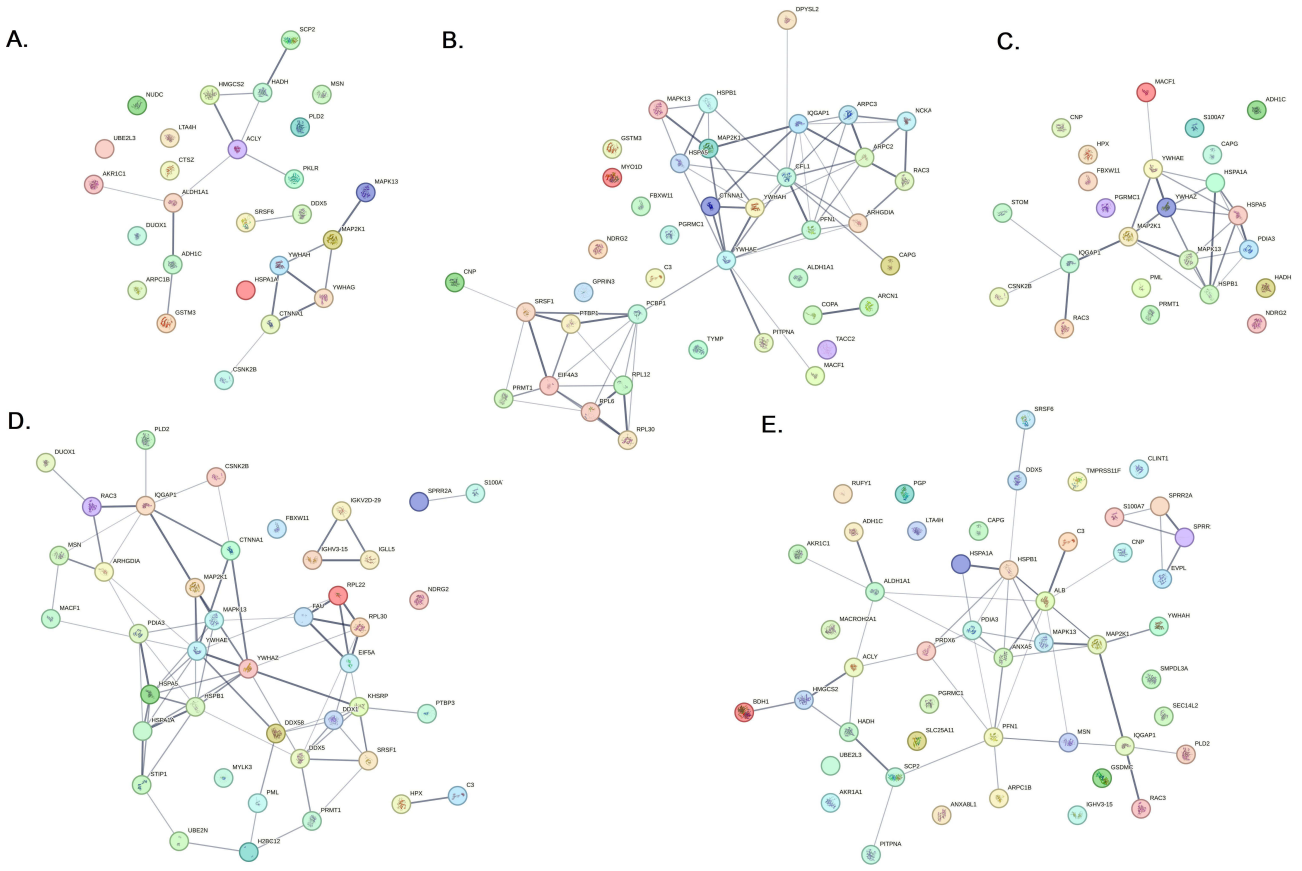

Figure 9 PPI network of differentially expressed proteins using STRING software. (**A**) Hormone -related PPI network (**B**) Psychoneurosis-related PPI network. (**C**) Melanogenesis-related PPI network (**D**) Immunity and inflammation -related PPI network (**E**) Skin barrier function-related PPI network.

that C3 and C3a are involved in the pathophysiology of major depressive disorder via immune activation mechanism. Additionally, Hao et al[56] demonstrated that gut dysbiosis induces depression-like behaviors through complement C3-mediated abnormal synapse pruning in microglia. These proteins have been shown to influence the nervous system, which is hypothesized to modulate the microenvironment surrounding melanocytes and affect melanogenesis.

Melasma is most prevalent in females of reproductive age, and lesions frequently occur following the use of oral contraceptives, pregnancy, menopause, or hormone replacement therapy. This has led to the assumption that hormones play a significant role in the etiology of melasma. Studies have demonstrated that estrogens stimulate melanogenesis by increasing the expression of alpha-MSH and inducing the synthesis of tyrosinase and tyrosinase-related proteins 1 and 2.[57] KEGG- based cluster analysis from this study revealed that the estrogen signaling pathway, GnRH signaling pathway, and GnRH secretion are associated with melasma. Several differentially expressed proteins (DEPs) related to the hormones exhibited higher levels in the melasma. Actin remodeling is a fundamental process for cellular signaling, motility and survival in all cells. ARPC2 (Actin Related Protein 2/3 Complex) is an essential actin nucleation and branching protein. One of its subunits, Arpc1b, has been demonstrated to exhibit estrogen responsiveness via estrogen receptor beta.[58] Additionally, 70 kD heat-shock proteins, HSC70 and HSP70, are proposed to regulate steroid hormone-dependent transcription and apoptosis by mediating the diverse functions of BAG-1, a multifunctional protein that associates with steroid hormone receptors and the anti-apoptotic BCL-2 protein.[59]

KEGG-based analysis revealed elevated VEGF signaling, suggesting enhanced vascularization in the pathology of melasma. This finding aligns with the hypothesis proposed by Kim, who reported a correlation between the number of blood vessels and the extent of hyperpigmentation.[4] VEGF has also been shown to stimulate the release of arachidonic acid, which potentially influences melanogenesis.[60] Rodríguez-Arámbula A's[22] observation of increased COX-2 in

https://doi.org/10.2147/CCID.S538073

melasma lesions supports this connection. Additionally, melanocytes have been observed to produce vascular endothelial growth factor (VEGF).[61] Our study demonstrates that both C3 and NDRG2 are involved in regulating VEGF production.

Conclusion

This study represents the investigation into proteomic alteration in tape-strips obtained from melasma skin lesions in Chinese populations. Differential protein analysis identified five main categories of proteins involved in the pathogenesis of melasma: immunity and inflammation, the skin barrier, melanogenesis, psychoneurosis, and hormones. Among the differentially expressed proteins DEPs, the heat shock protein family and 14-3-3 protein family were implicated in multiple aspects of melasma pathology. These proteins are anticipated to serve as potential biomarkers for melasma. Our dataset provides a valuable resource for the scientific community, especially given the limited clinical proteomic data available for melasma. However, this study is not without its limitations. The sample size was limited, necessitating larger cohort studies of melasma to validate these findings and conclusions. Besides, melasma is potentially an epidermal-dermal condition, we employed 3M medical tape for sampling, resulting in samples only from the epidermis and consequently lacking dermal information further validation of the DEPs in larger cohorts, including healthy controls and dermal information are necessary to gain deeper insight into the pathogenesis of melasma.

Abbreviations

DEPs, Differentially expressed proteins; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; VEGF, Vascular Endothelial Growth Factor; HADS, Hospital Anxiety and Depression Scale; TEWL, Transepidermal Water Loss.

Data Sharing Statement

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org) via the iProX partner repository[4,12] with the dataset identifier PXD057929. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Ethics Approval and Informed Consent

The study protocols were received approval from the ethics committee of Peking University International Hospital (number:2021-KY-0024-01). And all methods were performed in accordance with the relevant guidelines and regulations. Informed consent to participate in the study was obtained from each patient and healthy person before enrolling in the study. Informed consent was obtained from all participants prior to enrollment. The research has been carried out complies with the Declaration of Helsinki, and that all subjects provided written informed consent.

Consent for Publication

All the authors and participants confirm that the details of any recordings can be published.

Acknowledgments

The authors thank Hongchen Jin for proofreading the manuscript.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

The work was supported by research grant from National Natural Science Foundation of China, Grant/Award Number:82404136 to Xiaolei Ma.

Disclosure

The authors declare no competing interests in this work.

References

1. Kwon S-H, Na J-I, Choi J-Y, Park K-C. Melasma: updates and perspectives. *Exp Dermatol*. 2019;28(6):704–708. doi:10.1111/exd.13844
2. Neagu N, Conforti C, Agozzino M, et al. Melasma treatment: a systematic review. *J Dermatol Treatment*. 2022;33(4):1816–1837. doi:10.1080/09546634.2021.1914313
3. Mahajan VK, Patil A, Blicharz L, et al. Medical therapies for melasma. *J Cosmetic Dermatol*. 2022;21(9):3707–3728. doi:10.1111/jocd.15242
4. Alauzet C, Marchandin H, Lozniewski A. New insights into prevotella diversity and medical microbiology. *Future Microbiol*. 2010;5(11):1695–1718. doi:10.2217/fmb.10.126
5. Zhang F, Ge W, Ruan G, Cai X, Guo T. Data-independent acquisition mass spectrometry-based proteomics and software tools: a glimpse in 2020. *Proteomics*. 2020;20(17–18):e1900276. doi:10.1002/pmic.201900276
6. Zhang X, Chen Y, Yang H, et al. Plasma metabolomics indicates potential biomarkers and abnormal metabolic pathways in female melasma patients. *Annals Dermatol*. 2024;36(5):300–309. doi:10.5021/ad.23.141
7. Schaefer LV, Pontes LG, Cavassan NRV, Santos LDD, Miot HA. Proteomic study of facial melasma. *Anais brasileiros de dermatologia*. 2022;97(6):808–814. doi:10.1016/j.abd.2021.06.010
8. Erduran F, Hayran Y, Emre S, Eren F, Iyidal AY, Ö E. Oxidative stress in patients with melasma: an evaluation of the correlation of the thiol/disulfide homeostasis parameters and modified MASI score. *Cutis*. 2024;113(6):264–268. doi:10.12788/cutis.1036
9. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the hospital anxiety and depression scale. An updated literature review. *J Psychosomatic Res*. 2002;52(2):69–77. doi:10.1016/s0022-3999(01)00296-3
10. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protocols*. 2009;4(1):44–57. doi:10.1038/nprot.2008.211
11. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37(1):1–13. doi:10.1093/nar/gkn923
12. Chen T, Ma J, Liu Y, et al. iProX in 2021: connecting proteomics data sharing with big data. *Nucleic Acids Res*. 2022;50(D1):D1522–d1527. doi:10.1093/nar/gkab1081
13. Lai D, Zhou S, Cheng S, Liu H, Cui Y. Laser therapy in the treatment of melasma: a systematic review and meta-analysis. *Lasers Med Sci*. 2022;37(4):2099–2110. doi:10.1007/s10103-022-03514-2
14. Desai S, Chan L, Handog E, et al. Optimizing melasma management with topical tranexamic acid: an expert consensus. *Journal of Drugs in Dermatology: JDD*. 2023;22(4):386–392. doi:10.36849/jdd.7104
15. Pavel AB, Zhou L, Diaz A, et al. The proteomic skin profile of moderate-to-severe atopic dermatitis patients shows an inflammatory signature. *J Am Academy Dermatol*. 2020;82(3):690–699. doi:10.1016/j.jaad.2019.10.039
16. Chularojanamontri L, Charoenpipatsin N, Silpa-Archa N, Wongpraparut C, Thongboonkerd V. Proteomics in psoriasis. *Int J Mol Sci*. 2019;20(5). doi:10.3390/ijms20051141
17. He H, Olesen CM, Pavel AB, et al. Tape-strip proteomic profiling of atopic dermatitis on dupilumab identifies minimally invasive biomarkers. *Front Immunol*. 2020;11:1768. doi:10.3389/fimmu.2020.01768
18. Al-Janabi A, Martin P, Khan AR, et al. Integrated proteomics and genomics analysis of paradoxical eczema in psoriasis patients treated with biologics. *J Allergy Clin Immunol*. 2023;152(5):1237–1246. doi:10.1016/j.jaci.2023.07.011
19. Goleva E, Calatroni A, LeBeau P, et al. Skin tape proteomics identifies pathways associated with transepidermal water loss and allergen polysensitization in atopic dermatitis. *J Allergy Clin Immunol*. 2020;146(6):1367–1378. doi:10.1016/j.jaci.2020.04.022
20. Harel M, Ortenberg R, Varanasi SK, et al. Proteomics of melanoma response to immunotherapy reveals mitochondrial dependence. *Cell*. 2019;179(1):236–250.e18. doi:10.1016/j.cell.2019.08.012
21. Xu J, Lu H, Luo H, et al. Tape stripping and lipidomics reveal skin surface lipid abnormity in female melasma. *Pigment Cell Melanoma Res*. 2021;34(6):1105–1111. doi:10.1111/pcmr.12984
22. Rodríguez-Arámbula A, Torres-álvarez B, Cortés-García D, Fuentes-Ahumada C, Castanedo-Cázares JP. CD4, IL-17, and COX-2 Are associated with subclinical inflammation in Malar Melasma. *Am J Dermatopathol*. 2015;37(10):761–766. doi:10.1097/dad.0000000000000378
23. Espósito ACC, Cassiano DP, da Silva CN, et al. Update on melasma-part i: pathogenesis. *Dermatol Therapy*. 2022;12(9):1967–1988. doi:10.1007/s13555-022-00779-x
24. Fu H, Subramanian RR, Masters SC. 14-3-3 proteins: structure, function, and regulation. *Annual Rev Pharmacol Toxicol*. 2000;40:617–647. doi:10.1146/annurev.pharmtox.40.1.617
25. Fan X, Cui L, Zeng Y, Song W, Gaur U, Yang M. 14-3-3 proteins are on the crossroads of cancer, aging, and age-related neurodegenerative disease. *Int J Mol Sci*. 2019;20(14). doi:10.3390/ijms20143518
26. Chimen M, McGettrick HM, Apta B, et al. Homeostatic regulation of T cell trafficking by a B cell-derived peptide is impaired in autoimmune and chronic inflammatory disease. *Nat Med*. 2015;21(5):467–475. doi:10.1038/nm.3842
27. Millerand M, Sudre L, Nefla M, et al. Activation of innate immunity by 14-3-3 ε, a new potential alarmin in osteoarthritis. *Osteoarthritis Cartilage*. 2020;28(5):646–657. doi:10.1016/j.joca.2020.03.002
28. Fu W, Hettinghouse A, Chen Y, et al. 14-3-3 epsilon is an intracellular component of TNFR2 receptor complex and its activation protects against osteoarthritis. *Annals Rheumatic Dis*. 2021;80(12):1615–1627. doi:10.1136/annrheumdis-2021-220000
29. Wang J, Lee J, Liem D, Ping P. HSPA5 gene encoding Hsp70 chaperone BiP in the endoplasmic reticulum. *Gene*. 2017;618:14–23. doi:10.1016/j.gene.2017.03.005
30. Kepp O, Bezu L, Kroemer G. The endoplasmic reticulum chaperone BiP: a target for immunogenic cell death inducers? *Oncoimmunology*. 2022;11(1):2092328. doi:10.1080/2162402x.2022.2092328
31. Mansilla MJ, Comabella M, Río J, et al. Up-regulation of inducible heat shock protein-70 expression in multiple sclerosis patients. *Autoimmunity*. 2014;47(2):127–133. doi:10.3109/08916934.2013.866104

32. Rui L, Kang P, Shao J, et al. The chloroplast-localized casein kinase II α subunit, CPCK2, negatively regulates plant innate immunity through promoting S-nitrosylation of SABP3. *Plant J Cell Mol Biol*. 2024;120(2):552–568. doi:10.1111/tpj.17000
33. Husain K, Williamson TT, Nelson N, Ghansah T. Protein kinase 2 (CK2): a potential regulator of immune cell development and function in cancer. *Immunol Med*. 2021;44(3):159–174. doi:10.1080/25785826.2020.1843267
34. Lee DJ, Lee J, Ha J, Park KC, Ortonne JP, Kang HY. Defective barrier function in melasma skin. *J Eur Academy Dermatol Venereol*. 2012;26 (12):1533–1537. doi:10.1111/j.1468-3083.2011.04337.x
35. Sari DW, Minematsu T, Yoshida M, et al. Validity of skin blot examination for albumin and nerve growth factor β to detect itching of the skin in Indonesian older adults. *J Tissue Viability*. 2021;30(1):42–50. doi:10.1016/j.jtv.2020.10.001
36. Klement K, Melle C, Murzik U, Diekmann S, Norgauer J, Hemmerich P. Accumulation of annexin A5 at the nuclear envelope is a biomarker of cellular aging. *Mech Ageing Develop*. 2012;133(7):508–522. doi:10.1016/j.mad.2012.06.003
37. Kang B, Jia Z, Dong Y, Li W, Zhang W. Recombinant human annexin A5 accelerates diabetic wounds healing by regulating skin inflammation. *Regenerative Therap*. 2024;27:342–353. doi:10.1016/j.reth.2024.03.013
38. Presslauer S, Hinterhuber G, Cauza K, et al. RasGAP-like protein IQGAP1 is expressed by human keratinocytes and recognized by autoantibodies in association with bullous skin disease. *J Investig Dermatol*. 2003;120(3):365–371. doi:10.1046/j.1523-1747.2003.12070.x
39. Gläser R, Köten B, Wittersheim M, Harder J. Psoriasin: key molecule of the cutaneous barrier? *J der Deutschen Dermatologischen Gesellschaft*. 2011;9(11):897–902. doi:10.1111/j.1610-0387.2011.07683.x
40. Watson PH, Leygue ER, Murphy LC. Psoriasin (S100A7). *Int J Biochem Cell Biol*. 1998;30(5):567–571. doi:10.1016/s1357-2725(97)00066-6
41. Batycka-Baran A, Baran W, Nowicka-Suszko D, et al. Serum concentration and skin expression of S100A7 (Psoriasin) in patients suffering from hidradenitis suppurativa. *Dermatology*. 2021;237(5):733–739. doi:10.1159/000510689
42. Hermes BM, Rademacher F, Chung C, et al. Skin microbiota analysis in patients with anorexia nervosa and healthy-weight controls reveals microbial indicators of healthy weight and associations with the antimicrobial peptide psoriasin. *Sci Rep*. 2022;12(1):15515. doi:10.1038/s41598-022-19676-6
43. Liang H, Li J, Zhang K. Pathogenic role of S100 proteins in psoriasis. *Front Immunol*. 2023;14:1191645. doi:10.3389/fimmu.2023.1191645
44. Gläser R, Meyer-Hoffert U, Harder J, et al. The antimicrobial protein psoriasin (S100A7) is upregulated in atopic dermatitis and after experimental skin barrier disruption. *J Investigative dermatol*. 2009;129(3):641–649. doi:10.1038/jid.2008.268
45. Stolte KN, Danker K, Witt M, Ebhardt H, Dommisch H. Upregulation of psoriasin/S100A7 correlates with clinical severity in patients with oral lichen planus. *Clin Oral Investig*. 2024;28(6):318. doi:10.1007/s00784-024-05717-z
46. D'Mello SA, Finlay GJ, Baguley BC, Askarian-Amiri ME. Signaling pathways in melanogenesis. *Int J Mol Sci*. 2016;17(7). doi:10.3390/ijms17071144
47. Paterson EK, Ho H, Kapadia R, Ganesan AK. 9-cis retinoic acid is the ALDH1A1 product that stimulates melanogenesis. *Exp Dermatol*. 2013;22 (3):202–209. doi:10.1111/exd.12099
48. Yin SJ, Lee JR, Kwak H, et al. Functional study of 14-3-3 protein epsilon (YWHAE) in keratinocytes: microarray integrating bioinformatics approaches. *J Biomol Structure Dynamics*. 2020;38(9):2633–2649. doi:10.1080/07391102.2019.1637282
49. Bissig C, Rochin L, van Niel G. PMEL amyloid fibril formation: the bright steps of pigmentation. *Int J Mol Sci*. 2016;17(9). doi:10.3390/ijms17091438
50. Toops KA, Tan LX, Lakkaraju A. Apolipoprotein E Isoforms and AMD. *Adv Exp Med Biol*. 2016;854:3–9. doi:10.1007/978-3-319-17121-0_1
51. Cornell B, Toyo-Oka K. 14-3-3 proteins in brain development: neurogenesis, neuronal migration and neuromorphogenesis. *Front Mol Neurosci*. 2017;10:318. doi:10.3389/fnmol.2017.00318
52. Yanagi M, Shirakawa O, Kitamura N, et al. Association of 14-3-3 epsilon gene haplotype with completed suicide in Japanese. *J Human Genetics*. 2005;50(4):210–216. doi:10.1007/s10038-005-0241-0
53. Toyooka K, Muratake T, Tanaka T, et al. 14-3-3 protein eta chain gene (YWHAH) polymorphism and its genetic association with schizophrenia. *Am J Med Genetics*. 1999;88(2):164–167.
54. Qi YJ, Lu YR, Shi LG, et al. Distinct proteomic profiles in prefrontal subareas of elderly major depressive disorder and bipolar disorder patients. *Translational Psychiatry*. 2022;12(1):275. doi:10.1038/s41398-022-02040-7
55. Luo X, Fang Z, Lin L, Xu H, Huang Q, Zhang H. Plasma complement C3 and C3a are increased in major depressive disorder independent of childhood trauma. *BMC Psychiatry*. 2022;22(1):741. doi:10.1186/s12888-022-04410-3
56. Zhang B, Ma S, Rachmin I, et al. Hyperactivation of sympathetic nerves drives depletion of melanocyte stem cells. *Nature*. 2020;577:7792):676–681. doi:10.1038/s41586-020-1935-3
57. Filoni A, Mariano M, Cameli N. Melasma: how hormones can modulate skin pigmentation. *J Cosmetic Dermatol*. 2019;18(2):458–463. doi:10.1111/jocd.12877
58. Liu Y, Jones C, Coward K. An investigation of mechanisms underlying mouse blastocyst hatching: a ribonucleic acid sequencing study. *F&S Sci*. 2022;3(1):35–48. doi:10.1016/j.xfss.2021.12.003
59. Jorge AD, Stati AO, Roig LV, Ponce G, Jorge OA, Ciocca DR. Steroid receptors and heat-shock proteins in patients with primary biliary cirrhosis. *Hepatology*. 1993;18(5):1108–1114.
60. Yang S, Wei S, Pozzi A, Capdevila JH. The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. *Arch Biochem Biophysics*. 2009;489(1–2):82–91. doi:10.1016/j.abb.2009.05.006
61. Zhu JW, Ni YJ, Tong XY, Guo X, Wu XP. Activation of VEGF receptors in response to UVB promotes cell proliferation and melanogenesis of normal human melanocytes. *Exp Cell Res*. 2020;387(2):111798. doi:10.1016/j.yexcr.2019.111798