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Paeonia Suffruticosa Callus Extract for Anti-Photoaging and Skin Whitening: Cellular and Transcriptomic Insights

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Abstract

Tree peony (*Paeonia Suffruticosa*) is a traditional Chinese medicinal herb with significant therapeutic value. Although its effects on the skin have been preliminarily studied, comprehensive and systematic studies remain limited. In this study, we utilized plant callus culture techniques and transcriptome sequencing to investigate the anti-photoaging effects of peony callus extracts (PCE) on primary human dermal fibroblasts (HDFs). Additionally, the potential skin-whitening activity of PCE was assessed through melanin content quantification and RT-qPCR analysis of melanogenesis-related genes in B16F10 cells. Our results demonstrate that PCE significantly upregulated the expression of several extracellular matrix (ECM)-related genes, autophagy-related genes, and NAD^+ metabolism-related genes, suggesting its potential in counteracting photoaging processes. Furthermore, PCE notably reduced melanin content and inhibited the expression of key melanogenesis-related genes, providing evidence for its role in regulating skin pigmentation. Through a systematic analysis, this study identifies numerous novel molecular targets and mechanisms of action underlying the effects of PCE. These findings highlight the promising potential of PCE for future applications in cosmetic formulations aimed at addressing photoaging and pigmentation-related concerns.

Keywords: Peony callus extracts; anti-photoaging; skin-whitening

1. Introduction

Tree peony, a deciduous shrub of the *Paeoniaceae* family, includes the historically renowned cultivar 'Yucui Hehua', known as the "King of Peonies", which originated during the Ming Dynasty (circa 1610). Peony extract is rich in various phytochemicals, including paeoniflorin [1], pentagalloyl glucose [2], and paeonol [3], which provide significant medicinal value. In recent years, increasing interest in the application of plant extracts in cosmetics has highlighted the potential of peony extract for cosmeceutical applications.

Ultraviolet (UV) radiation is one of the most common external factors contributing to premature skin aging [4]. Skin areas such as the face, hands, and arms are particularly susceptible to photoaging due to frequent UV exposure, which manifests as dryness, uneven pigmentation, freckles, hyperpigmentation, wrinkles, and loss of elasticity [5]. Thus, intervention measures against photoaging, such as the use of cosmeceuticals with anti-photoaging properties, are essential for preventing and alleviating skin aging. According to our previous research, PCE exhibits anti-photoaging and anti-inflammatory effects [6]. In-depth research on its molecular mechanism will help reveal the unique role of peony in the prevention and treatment of photoaging. It will also provide a scientific basis for the development of new anti-photoaging products.

In addition to its known anti-photoaging and antioxidant properties, peony also shows potential whitening effects. Lu et al. [7] demonstrated that oxidized paeoniflorin inhibited melanin synthesis. While previous studies mainly focused on tyrosinase inhibition, melanin synthesis is a complex process regulated by multiple genes and pathways. UV radiation, α-MSH, and cAMP inducers can all promote melanogenesis [8]. *MITF* plays a central role by regulating genes such as *TYR* involved in melanin production and melanosome formation [9]. Therefore, further investigation into the molecular mechanisms of peony's whitening effect is essential to support its cosmetic applications.

Therefore, this study aimed to investigate the potential skin-protective effects of PCE. RNA-sequencing (RNA-seq) was employed to analyze gene expression changes in UVA-induced senescent dermal fibroblasts, with the goal of exploring the involvement of photoaging-related pathways. To evaluate its whitening activity, B16F10 cells were treated with PCE, and RT-qPCR was used to examine the underlying molecular mechanisms.

2. Materials and Methods

2.1. Preparation of PCE

PCE from Yucui Hehua peony was prepared according to previously published protocols [6].

2.2. Cell culture and viability measurement

HDFs and B16F10 cells were obtained from Guangdong BioCell Biotechnology Co., Ltd (Guangdong, China) and the Chinese Academy of Sciences Cell Bank (Beijing, China), respectively. HDFs were cultured in DMEM, and B16F10 cells in RPMI-1640, both under standard conditions (37°C, 5% CO_2). For viability assessment, cells were seeded into 96-well plates and treated with gradient concentrations of PCE for 24 h. After incubation with CCK-8 reagent for 2 h, cell viability was determined by measuring absorbance at 450 nm.

2.3. PCE treatment of photodamaged HDFs and RNA-seq

After 24 hours of treatment with either PCE or DMEM medium (solvent control and UVA groups), HDFs were irradiated with UVA （30 J/cm²）, except for the solvent control group. Cells were then collected, and total RNA was extracted for RNA-seq analysis to evaluate the effects of PCE on photoaging. High-throughput sequencing was conducted on an Illumina platform by

Novogene Co., Ltd. (Beijing, China). Differential expression analysis was conducted between the model and PCE-treated groups. Genes with an adjusted p-value < 0.05 were considered differentially expressed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed to identify significantly enriched biological processes and pathways.

2.4. Melanin content determination and RT-qPCR analysis of melanogenesis-related genes

Cells were treated with PCE or arbutin in the presence of IBMX, and melanin content was measured spectrophotometrically. RT-qPCR was performed to evaluate the expression levels of key genes involved in melanin production, including *Mitf*, *Tyr*, and *Tyrp1*. Gene expression levels were analyzed using the $2^{-\triangle\triangle CT}$ method. Primer sequences are listed in **Table 1**.

Table 1. Primers used in RT-qPCR.

Gene	Forward	Reverse
Gapdh	TGGCCTTCCGTGTTCCTAC	GAGTTGCTGTTGAAGTCGCA
Mitf	CAAATGGCAAATACGTTACCCG	CAATGCTCTTGCTTCAGACTCT
Tyr	CACCTGAGGGACCACTATTACG	GGCAGTTCTATCCATTGATCCAG
Tyrp1	ATGAAATCTTACAACGTCCTCCC	GCACACTCTCGTGGAAACTGA

3. Results

3.1. Effects of PCE on cell viability

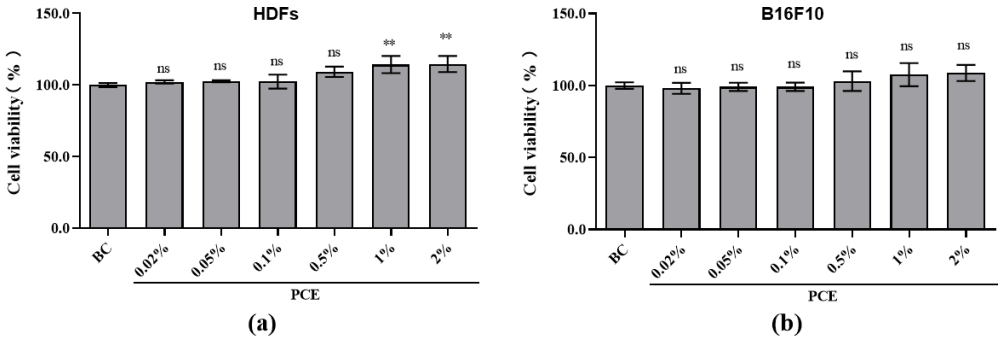

Figure 1. Effect of PCE on cell viability in HDFs and B16F10. (a) Effects of PCE at different concentrations on the cell viability in HDFs. (b) Effects of PCE at different concentrations on the cell viability in B16F10. Statistical analysis was performed using one-way ANOVA. ns, $P > 0.05$, $^{**}P < 0.01$; n=3.

To measure cellular tolerance to PCE, the effect of PCE on the cell viability of HDFs and B16F10 were determined by WST-8. Cell viability remained above 100% in HDFs (Figure 1a) and above 98% in B16F10 cells (Figure 1b) across all tested concentrations, indicating that the tested concentrations were non-toxic to both cell types.

3.2. Evaluation of anti-photoaging effect of PCE

RNA-seq technology was employed to investigate the anti-photoaging mechanisms of PCE in cultured HDFs. Transcriptomic profiles of PCE-treated cells were compared with those of the

UVA-treated group (30 J/cm²). A total of 4,025 differentially expressed genes (DEGs) were identified following PCE treatment (|log2FC| ≥ 0, p-value ≤ 0.05), including 2,033 upregulated and 1,992 downregulated genes **(Figure 2a**). Gene Ontology (GO) enrichment analysis revealed that the DEGs were mainly involved in cell-substrate adhesion, cell-substrate junctions, cell adhesion molecule binding, and regulation of the mitotic cell cycle (**Figure 2b & 2c**).

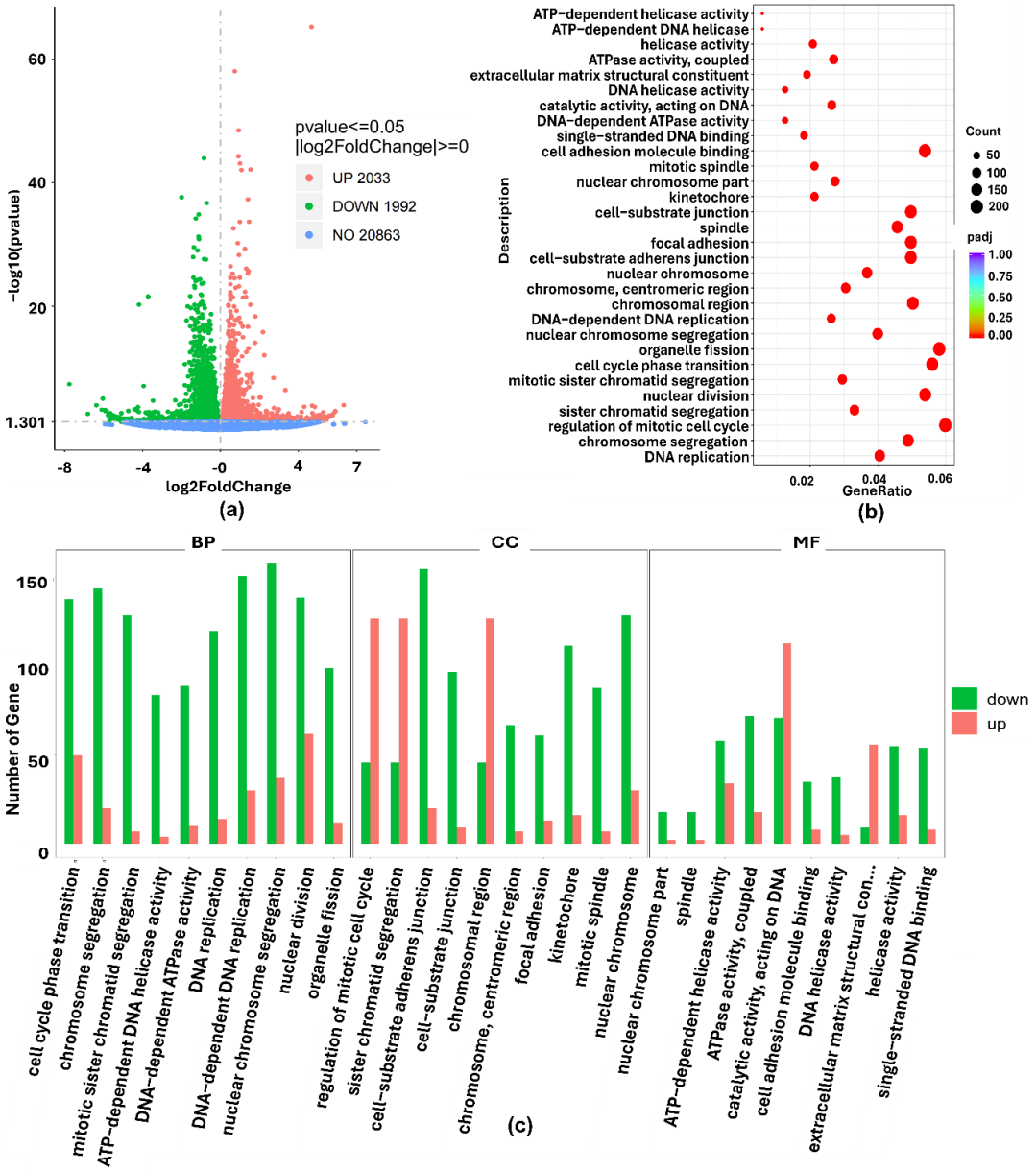

Figure 2. Identification of DEGs and GO enrichment analysis. Data are presented as mean ± SD (n = 3). (a) Volcano plot of DEGs: upregulated genes are shown in red and downregulated genes in green (p-value < 0.05, |log2FC| ≥ 0). (b) Top 10 enriched GO terms in BP (Biological Process), CC (Cellular Component), and MF (Molecular Function) categories, ranked by adjusted p-value. GeneRatio represents the proportion of enriched DEGs to total DEGs. Dot size and color indicate the number of enriched DEGs and statistical significance, respectively.

(c) Significantly enriched CC and MF terms related to cell–substrate junction and cell adhesion molecule binding, each enriched by over 100 DEGs.

KEGG pathway analysis revealed significant enrichment in ECM–receptor interaction, phagosome formation, and energy metabolism pathways. Notably, several ECM-related genes (e.g., *COL1A1*, *COL6A1*, *LAMA2*, *LAMB1*), along with autophagy- and metabolism-associated genes such as *ATG12*, *PCK2*, *SIRT2*, and *NADSYN1*, showed increased expression (**Table 2**). These findings suggest that PCE may promote skin repair by modulating ECM remodeling, autophagy, and energy metabolism pathways.

Table 2. Functional characterization of differentially expressed genes in the UVA-induced photoaging model.

Gene_ ID	Gene Name	Gene Description	Log2FC (1%PCE+UVA/ UVA)	p-value (1%PCE+UVA/ UVA)
2309	*FOXO3*	forkhead box O3	0.35	0.00
1277	*COL1A1*	collagen type I alpha 1 chain	0.38	0.00
1291	*COL6A1*	collagen type VI alpha 1 chain	0.42	0.00
1292	*COL6A2*	collagen type VI alpha 2 chain	0.26	0.00
1293	*COL6A3*	collagen type VI alpha 3 chain	0.34	0.00
3908	*LAMA2*	laminin subunit alpha 2	0.86	0.00
3912	*LAMB1*	laminin subunit beta 1	0.12	0.01
9140	*ATG12*	autophagy related 12	0.40	0.00
5106	*PCK2*	phosphoenolpyruvate carbox-ykinase 2	2.52	0.04
55191	*NADSYN1*	NAD synthetase 1	0.42	0.00
22933	*SIRT2*	NAD-Dependent Protein Defatty-Acylase Sirtuin-2	1.34	0.02

3.3. Measurement of Melanin Content

The whitening effect of PCE on B16F10 cells was evaluated using a melanin synthesis inhibition assay. Cells were treated with PCE (0.05%–2%) in the presence of IBMX (100 μM), and compared to the IBMX-only model group. As shown in **Figure 3a**, melanin production was significantly reduced in a dose-dependent manner following PCE treatment. Quantitative analysis revealed that PCE at 0.05%, 0.1%, 0.5%, 1%, and 2% inhibited melanin synthesis by approximately 28.43%, 43.33%, 53.29%, 77.68%, and 79.67%, respectively ($P < 0.001$; **Figure 3b**). Arbutin (100 ppm), used as a positive control, showed an inhibition rate of 59.04%. These results indicate that PCE at concentrations of 0.05%–2% exhibits significant anti-melanogenic activity in B16F10 cells, with 1% and 2% showing stronger inhibitory effects than arbutin, suggesting its potential as a skin-whitening agent.

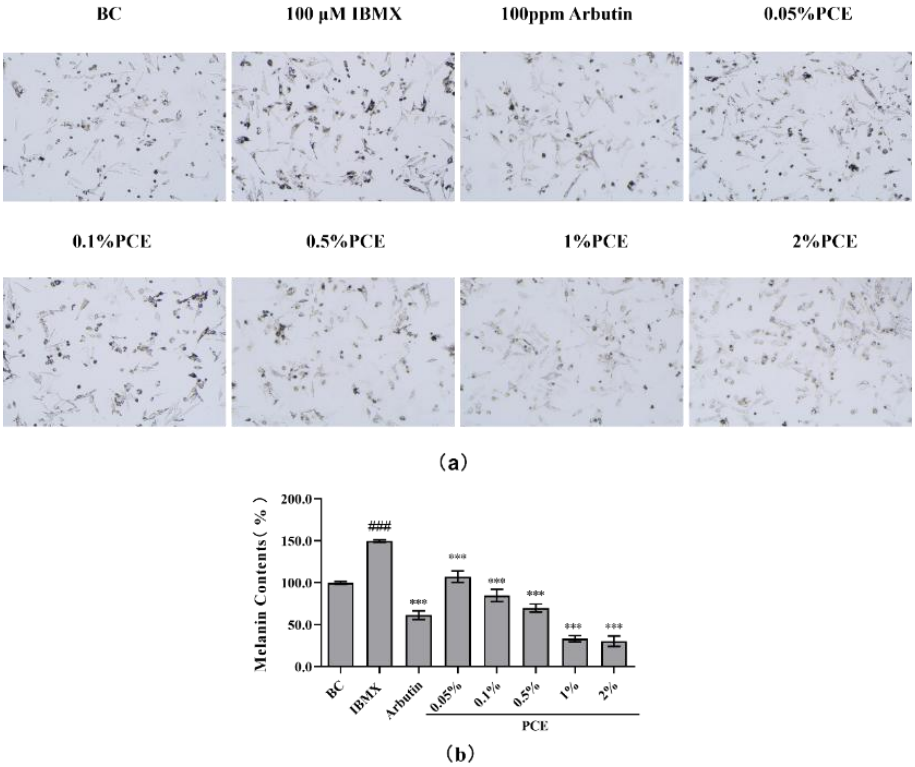

Figure 3. Effect of PCE on IBMX-induced melanin production. (a) Microscopic image of melanin distribution in B16F10 cells. (b) Quantitative analysis of melanin content. One-way ANOVA was used for statistical analysis. ***P < 0.001. Student's t-test was used for comparison between the control and model groups (IBMX-treated). $^{###}P$ < 0.001; n = 3.

3.4. Expression Analysis of Melanogenesis-Related Genes by RT-qPCR

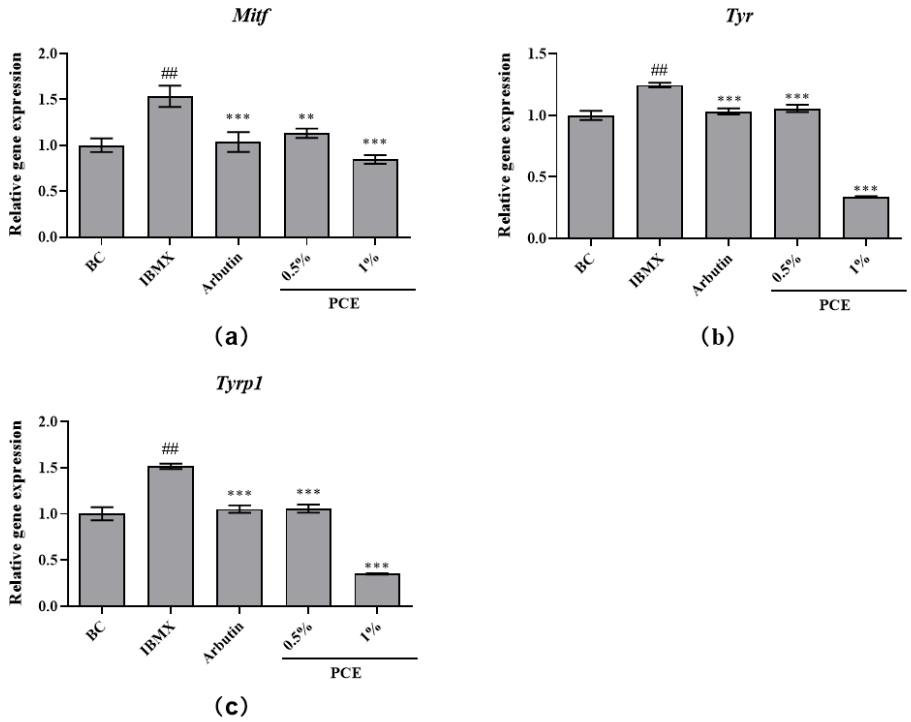

Figure 4. Effect of PCE on IBMX-induced expression of melanogenesis-related genes. (a–c)

Relative mRNA expression levels of *Mitf*, *Tyr*, and *Tyrp1* in B16F10 cells. One-way ANOVA was used for statistical analysis. $^{**}P < 0.01$; $^{***}P < 0.001$. Student's t-test was applied for comparison between the control and model groups. $^{\#\#}P < 0.01$; n = 3.

To investigate the effects of PCE on melanogenesis at the molecular level, we measured the expression levels of *Mitf*, *Tyr*, and *Tyrp1* genes in B16F10 cells using RT-qPCR. Cells were treated with PCE (0.5% and 1%) in the presence of IBMX (100 μM) for 48 h, and arbutin (100 ppm) served as a positive control. As shown in **Figure 4**, treatment with PCE significantly downregulated the expression of *Mitf*, *Tyr*, and *Tyrp1* in a dose-dependent manner ($p < 0.001$). Arbutin similarly reduced the expression of these genes. These results indicate that PCE may exert its whitening effects by inhibiting the expression of key genes involved in melanin biosynthesis.

4. Discussion

This study systematically evaluated the photoprotective and skin-whitening effects of PCE. Our results demonstrated that PCE significantly reduced UVA-induced cellular damage and inhibited melanin synthesis *in vitro*. These findings are consistent with previous studies highlighting UVB damage-repair properties [6] and the whitening [8] of *Paeonia*. Our study further confirms the photoprotective effects of PCE. It also demonstrates that PCE reduces melanin production by suppressing the expression of MITF and tyrosinase-related genes, thereby exerting skin-whitening effects. These data support the hypothesis that PCE can improve both photodamage and pigmentation through multiple molecular pathways.

RNA-sequencing enabled comprehensive profiling of gene expression changes. In UVA-stimulated human dermal fibroblasts (HDFs) following PCE treatment, differentially expressed genes (DEGs) (**Table 2**) were identified and found to be enriched in extracellular matrix (ECM)-related processes, autophagy, and NAD^+ metabolism. Notably, ECM-related genes such as *COL1A1*, *LAMA2*, and *LAMB1* were significantly upregulated. *COL1A1* encodes the collagen type I alpha 1 chain, a major structural component of the dermal extracellular matrix that plays a critical role in maintaining skin strength, elasticity, and structural integrity [10]. *LAMA2* and *LAMB1* encode laminin subunits that are critical for basement membrane assembly and tissue organization. Given their roles in basement membrane assembly, upregulation of *LAMA2* may contribute to enhanced dermal strength and stability, thereby supporting the structural integrity of the skin [11]. Meanwhile, *LAMB1* plays an important role in regulating cell adhesion and motility; its increased expression may influence fundamental cellular functions, especially in the context of wound healing and tissue regeneration [12]. Their upregulation suggests that PCE may enhance dermal structural integrity, thereby improving skin resilience against UV-induced damage. Autophagy, a key cellular homeostasis mechanism, was also promoted following PCE treatment, as evidenced by increased expression of *ATG12* and *PCK2*. *ATG12* is a key gene involved in the autophagic pathway, and its upregulation may facilitate autophagy, thereby promoting the clearance of UV-induced damaged proteins and organelles to protect cells [13]. PCK2 has been shown to activate the PI3K-Akt and AMPK-autophagy pathways, enhancing cell survival and promoting recovery following UV-induced oxidative stress [14]. The

SIRT2 gene encodes a member of the sirtuin family of proteins, which are evolutionarily conserved NAD^+-dependent enzymes with deacetylase and mono-ADP-ribosyltransferase activities. Studies have shown that sirtuins can regulate lifespan in lower organisms and are involved in the pathogenesis of age-related diseases in mammals [15]. *NADSYN1* (NAD synthetase 1) is a protein-coding gene that catalyzes the final step in the *de novo* biosynthesis of nicotinamide adenine dinucleotide (NAD^+), a vital coenzyme in redox metabolism, a precursor of several signaling molecules, and a substrate for protein post-translational modifications [16]. The enzymatic activity of sirtuins strictly depends on the intracellular NAD^+ level. Therefore, increased *NADSYN1* expression may enhance NAD^+ biosynthesis, which may indirectly affect the activity of *SIRT2*. *SIRT2* has been shown to activate the autophagy pathway and alleviate oxidative stress. According to the research of Zhang et al., [17] NAD^+ can directly enhance the antioxidant capacity of cells by activating SIRT2, ERK and Nrf2 pathways. Collectively, these results indicate that PCE activates multiple protective mechanisms to mitigate photodamage in skin cells.

Melanogenesis is a complex biological process regulated by multiple enzymes and transcription factors [18], among which MITF plays a central role. As a key regulator of melanogenesis, MITF binds to promoter regions of melanogenic genes to activate their transcription [19]. TYR (tyrosinase) is one of its main targets. TYR catalyzes the conversion of L-tyrosine to L-DOPA, which is the first step in melanin production [20]. MITF also regulates TYRP1 (tyrosinase-related protein 1), which is involved in melanin maturation and stability [21]. Thus, MITF, TYR, and TYRP1 play essential roles in controlling melanin synthesis. In this study, to evaluate the whitening potential of PCE, B16F10 cells were stimulated with IBMX. Melanin content and the expression of melanogenic genes (*Mitf, Tyr, Tyrp1*) were measured (**Figures 3 and 4**). PCE significantly suppressed IBMX-induced melanin synthesis and downregulated the expression of these melanogenic genes in a dose-dependent manner, suggesting that it inhibits melanogenesis via suppression of the MITF-TYR axis. Notably, compared with common whitening agents such as kojic acid, peony extract showed good inhibitory effects even at low concentrations, indicating its superior safety and biocompatibility. In recent years, the use of natural plant extracts in cosmetics has attracted significant attention, especially those with antioxidant, whitening and anti-aging properties [22]. This study demonstrated the potential of peony extract in photodamage protection and skin whitening, providing new scientific evidence for the applications of active ingredients from natural plants. It also provides theoretical support for the cosmetic industry to develop effective and safe whitening and sunscreen skin care products.

5. Conclusion

This study systematically investigated the functions of PCE in anti-photoaging and skin pigmentation through cellular experiments and transcriptomic sequencing. Numerous novel targets and mechanisms of action were identified, demonstrating the promising potential of PCE for further exploration in cosmetic applications.

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