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AP collagen peptides improve hair growth and quality by promoting human hair cell proliferation and keratin synthesis

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Abstract: Hair is an essential protective and health-maintaining covering for the body, especially the scalp. It is also important esthetically in modern society, where hair loss, whether caused by the severe scalp condition androgenic alopecia or other internal or external factors, has societal and psychological impacts. Preventing the apoptosis of human hair follicle cells, including hair follicle dermal papilla cells (HFDPCs) and human hair follicle keratinocytes (HHFKs), is crucial for hair production, alongside hair strengthening, which is essential in combatting hair loss. Given the structural importance of collagen peptides in the skin matrix and tissues surrounding hair follicles, we studied the function of AP collagen peptides (APCP) as a hair growth and quality improvement agent. APCP was found to stimulate the proliferation of hair follicle cells and counteract H2O2-induced apoptosis. It also significantly suppressed the expression of dickkopf-1 (DKK1) and bone morphogenetic protein 6 (BMP6), which induce hair cell apoptosis in the presence or absence of UVA/B irradiation. Moreover, APCP induced expression of the antioxidant enzymes, catalase and superoxide dismutase 1 (SOD1), while enhancing hair keratin expression in HHFKs. Mechanistically, APCP facilitated hair growth by elevating GAS6 expression and activating PKA and AKT/ERK signaling pathways. Notably, APCP increased levels of phosphor-β-catenin (Ser552/Ser675) as well as total β-catenin, as demonstrated by Western blotting and immunocytochemistry. Lastly, we showed that APCP increased expression of the hair cuticle type I keratins, keratin-32 and 42, in HHFKs. Overall, these findings propose APCP as a promising candidate for safeguarding hair follicle cells and mitigating hair loss by improving hair growth and quality.

Keywords: AP Collagen peptide; dermal papilla cells; hair follicle keratinocytes; hair growth; hair keratins

INTRODUCTION

Hair loss, as exemplified by androgenic alopecia, remains an unsolved issue in modern society. It affects both genders and lacks efficacious treatment options, highlighting uncertainties surrounding its root cause [1]. The hair growth cycle can be divided into three phases: anagen, catagen, and telogen [2]. The typical cycle for maintaining healthy hair in humans is 2-7 years of anagen, 2-4 weeks of catagen, and 3 months of telogen [3]. Disruption of the hair growth cycle caused by a hormonal imbalance, nutrient deficiency, stress, excessive ultraviolet (UV) irradiation, or thermal aging, among other factors, leads to the manifestation of symptoms of hair disorders, such as hair loss [4-9].

Cells involved in the hair-production process play a central role in preventing hair loss. Among the main cell types involved in hair production are human follicle dermal papilla cells (HFDPCs). These cells, located in the hair bulge of the dermis, are essential for inducing and maintaining hair growth [10]. Expression of hair growth-promoting factors in HFDPCs induces the transition from the telogen to the anagen phase [11]. Representative hair growth-promoting factors include vascular endothelial growth factor (VEGF),

epidermal growth factor (EGF), insulin-like growth factor (IGF-1), and hepatocyte growth factor (HGF) [12-16]. Conversely, there are factors that inhibit hair growth, such as dickkopf-1 (DKK1), transforming growth factor beta-1 (TGF-β1), and bone morphogenetic protein (BMP). These factors act early in the process, disrupting cell proliferation and hair growth cycles, and are ultimately detrimental to hair health [17-21].

Wnt/β-catenin signaling is a major pathway involved in hair cell proliferation [22-24]. Other mechanisms important in hair cell proliferation include the protein kinase A (PKA) and AKT/ERK (extracellular signal-regulated kinase) signaling pathways [25]. Activation of the latter signaling is closely linked to the phosphorylation of β-catenin, which has numerous potential phosphorylation sites. The phosphorylation of β-catenin by different kinases at specific sites can either enhance downstream signal transduction activity or trigger β-catenin degradation, depending on the location. [26]. β-catenin phosphorylated at Ser675 by PKA is stabilized and can induce transcriptional activity in the nucleus [27-28]. Furthermore, AKT-mediated phosphorylation of β-catenin at Ser552 promotes its dissociation from cell junctions, facilitating its translocation to the nucleus, where it directly stimulates TCF/LEF-1 transcriptional activity. Thus, it induces increased expression of genes related to hair growth, such as c-myc and cyclin D1 [29-30].

In addition to the primary process of promoting hair growth by stimulating the proliferation of dermal papilla cells and subsequent hair production, the quality and overall health of the hair itself are significant factors to consider. Hair consists of three structures: the cuticle (10-15%), cortex (85-90%), and medulla (0-5%) [31]. The cortex, which is the intermediate layer, is composed of keratin-rich cortical cells and intercellular binding substances, which determine the elasticity and color of the hair [32]. The cuticle or outer layer provides resilience and strength to the hair, contributing significantly to its shine and texture. Keratin is a major component of hair, accounting for up to 95% of the hair structure. The human genome has 28 type I and 26 type II keratin genes. Among hair keratin genes, 11 (KRT31-KRT40) encode type I keratin, and 6 (KRT81-KRT86) encode type II keratin [33]. Thus, there are 17 different types of hair keratin whose induction could be key to hair health.

Collagen peptide, a structural protein found in all connective tissues, including the skin matrix and the tissues surrounding follicles, can be absorbed into the bloodstream and act throughout the body [34]. AP collagen peptide (APCP), a collagen tripeptide-rich hydrolysate extracted from the scales of golden threadfin bream (*Nemipterus virgatus*), contains 3% glycine-proline-hydroxyproline. Previous studies have shown that APCP prevents cellular aging and counters the decline in collagen type I in skin fibroblasts, typically induced by the stress hormone cortisol [35-36]. When taken orally, it is also known to improve skin barrier function by increasing skin moisture and natural moisturizing factors, such as ceramides, in the stratum corneum [37]. In this study, we investigated the protective potential of APCP on hair cells. To this end, we tested the ability of APCP to promote cell proliferation and inhibit apoptosis and analyzed the expression of hair growth-related factors, antioxidant enzymes, and keratin to identify possible strategies for improving hair growth and quality.

MATERIALS AND METHODS

Materials

The AP collagen peptides were provided by AMOREPACIFIC R&I Center (Yongin, Korea). H_2O_2 was purchased from Sigma Aldrich (St. Louis, MO, USA). All PCR primers were purchased from Bio-Rad (Hercules, CA, USA). Antibodies against β-actin and β-catenin were purchased from Abcam (Cambridge, UK), and those against AKT, phospho-AKT, ERK, phospho-ERK, phospho-PKA substrate, and phospho-β-catenin (Ser552/Ser675) were purchased from Cell Signaling Technology (Danvers, MA, USA). The antibody against human type I+II hair keratins was obtained from Progen (Seoul, Korea).

Hair follicle cell culture

HFDPCs, cell growth medium, and supplement were purchased from PromoCell (Heidelberg, Germany). HHFKs, culture medium, and supplement were obtained from ScienCell (Carlsbad, CA, USA). HFDPCs and HHFKs were cultured in a medium containing a supplement and 1% penicillin/streptomycin solution.

Cells were incubated at 37°C in a humidified 5% CO2 incubator. HFDPCs and HHFKs in passages 3-5 were used for experiments.

Cell viability, proliferation, and apoptosis inhibitory assay

Cell viability was assessed using a Quanti-MAX WST-8 cell viability assay kit (Biomax, Seoul, Korea). Absorbance was measured at 450 nm using a microplate reader (BioTek, Winooski, VT, USA). For cell proliferation assays, cells were incubated in a medium containing only 20% of the supplement; a group of cells grown in a culture medium containing the entire supplement was used as a positive control. For apoptosis inhibitory assays, H_2O_2 was used as a stimulator, and niacinamide was used as a positive control. For UV stimulation, cells were irradiated with UVB (20 mJ/cm^2) or UVA (2 J/cm^2) using a Biosun Ultraviolet system (Vilber, Eberhardzell, Germany).

Co-culture and wound healing assay

In cell migration assays, HFDPCs (10×10^4 cells/well) and HHFKs (20×10^4 cells/well) were co-cultured by seeding them together in a 6-well plate. Media containing 40% of the supplement suitable for each cell type were mixed at a 1:1 ratio. After 24 h, a "wound" was introduced by scratching a line in cell monolayers, and then cells were treated with APCP. Recovery, reflecting the migration of cells into the wounded area, was observed initially and then at 12 and 24 h using microscopy.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

HFDPCs and HHFKs were treated with APCP for 24 h in medium. After washing with cold phosphate-buffered saline (PBS; Biosesang, Seongnam, Korea), cells were lysed with TRIzol (Thermo Fisher Scientific, Waltham, MA, USA), after which RNA was extracted with chloroform, precipitated with isopropanol, washed with 70% ethanol, and quantified RNA using a spectrophotometer. cDNA was then synthesized from quantified RNA and used for qRT-PCR, which was performed using SYBR Green reagents (Bio-Rad).

Western blotting

HFDPCs and HHFKs were treated with APCP for 24 or 96 h in medium. After washing with cold PBS, cells were lysed with RIPA lysis buffer (Cell Signaling Technology) containing protease and phosphatase inhibitors. Lysates containing an equivalent amount of protein, measured using the Pi BCA Protein Assay kit (Sigma Aldrich), were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and then transferred to a nitrocellulose membrane (Bio-Rad). Membranes were blocked with Tris-buffered saline (TBS) containing 5% nonfat skimmed milk powder. After incubating with the primary antibody overnight, membranes were incubated with the secondary antibody for 1 h. Immunoreactive bands were visualized using Clarity Western ECL substrate (Bio-Rad).

Immunocytochemistry (ICC)

HFDPCs (1.3×10^4 cells/well) plated in 8-well chamber slides were treated with APCP for 24 h. After that, cells were fixed with 4% paraformaldehyde in PBS for 30 min and permeabilized with 0.1% Triton X-100 by incubating in PBS for 15 min. Cells were immunostained with anti-β-catenin primary antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies and counterstained with DAPI (4',6-diamidino-2-phenylindole), after which signals were visualized using a fluorescence microscope.

Statistical analysis

Data are expressed as means ± standard deviation (SD) of three independent experiments. The statistical significance of data was assessed using the Student's t-tests. $P < 0.05$ was considered significant; individual P values are indicated in figure legends.

RESULTS

Cell proliferation and migration, and anti-apoptotic effects of APCP on human hair cells

To determine the effects of APCP on cell viability, HFDPCs and HHFKs were treated with APCP for 24 h. Fig. 1A shows that APCP was not cytotoxic for either

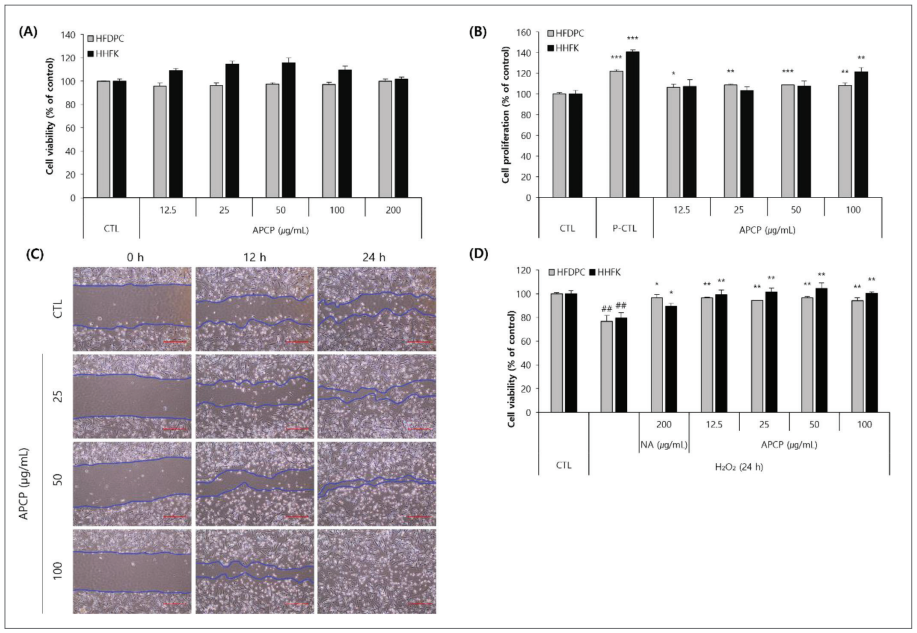

Fig. 1. Proliferation-promoting and apoptosis-inhibiting effects of APCP on human hair cells. **A** – Effects of APCP on HFDPC and HHFK cell viability after 24-h treatment. **B** – Proliferation-promoting effect of APCP on HFDPCs and HHFKs after 72-h treatment. A medium containing the entire supplement was used as a positive control; other conditions used a medium containing 20% of the supplement. Cell viability and proliferation rates were evaluated using WST-8 assays. Data are presented as means±SD (*P<0.05, **P<0.01, ***P < 0.001 vs. control [untreated] cells). **C** – Cell migration-promoting effect of APCP in HFDPC–HHFK co-cultures. For co-culture, a medium containing 40% of the supplement suitable for each cell type was mixed at a 1:1 ratio. Scale bars, 500 μm. **D** – Apoptosis-inhibiting effects of APCP on HFDPCs and HHFKs. H_2O_2, a representative apoptosis-induced ROS, was used at concentrations of 250 μM and 490 μM on HFDPCs and HHFKs, respectively. NA-niacinamide, a form of vitamin B, was used as a positive control. Data are presented as means±SD (##P<0.01 vs. control [untreated] cells; *P<0.05, **P<0.01 vs. H_2O_2 alone).

HFDPCs or HHFKs up to a concentration of 200 μg/mL. To provide a margin of safety, APCP concentrations up to 100 μg/mL were used in subsequent experiments. Since hair cell proliferation is a reliable indicator of hair growth, we investigated the effect of APCP on the proliferation rate of HFDPCs and HHFKs. Treatment of hair cells with various concentrations of APCP for 72 h induced proliferation of HFDPCs and HHFKs (Fig. 1B). To assess interactions of hair cells and APCP, the co-culture migration assay was performed and results showed that treatment with APCP markedly increased the migration of HFDPCs and HHFKs in co-cultures (Fig. 1C). To verify the apoptosis-inhibitory effect of APCP, we treated cells with H_2O_2, a representative reactive oxygen species (ROS) and alopecia stimulator. H_2O_2 reduced hair cell viability by over 20%, indicating H_2O_2-induced apoptosis and cell dysfunction. Notably, APCP treatment mitigated this effect, acting in a concentration-dependent manner to restore the viability of hair cells to a level similar to that of controls (Fig. 1D). Niacinamide (NA, 200 μg/mL), a form of vitamin B used as a positive control, also inhibited the apoptosis of HFDPCs and HHFKs.

Effects of APCP on the expression of hair growth-related factors in human hair cells

To determine whether APCP inhibits apoptosis-related factors, we analyzed the gene expression of DKK1 and BMP6 using real-time quantitative RT-PCR. APCP reduced the expression of DKK1 mRNA levels in HFDPCs and HHFKs in a concentration-dependent manner (Fig. 2A). Interestingly, APCP had no significant effect on BMP6 mRNA expression in HFDPCs but significantly decreased BMP6 mRNA levels in HHFKs (Fig. 2B). In additional experiments designed to determine whether APCP is beneficial to hair health in the context of environmental stress, we tested its effect on hair cells exposed to UVA and UVB irradiation, which causes alopecia. UVA (2 J/cm^2) and UVB (20 mJ/cm^2) irradiation increased the level of DKK1 mRNA in both HFDPCs and HHFKs (Fig. 2C and 2D). APCP treatment caused a concentration-dependent decrease in both cell types of UVA- and UVB-induced DKK1. It also significantly increased the expression of mRNAs encoding the hair growth-promoting factors, EGF and VEGF, in both HFDPCs and HHFKs (Supplementary Fig. 1A and B).

Antioxidant effect of APCP on human hair cells

To investigate the antioxidant effect of APCP *in vitro*, we examined the expression of the antioxidant enzymes catalase (CAT) and superoxide dismutase 1 (SOD1) in hair cells. To this end, we cultured HFDPCs and HHFKs in a medium containing 25, 50, or 100 μg/mL

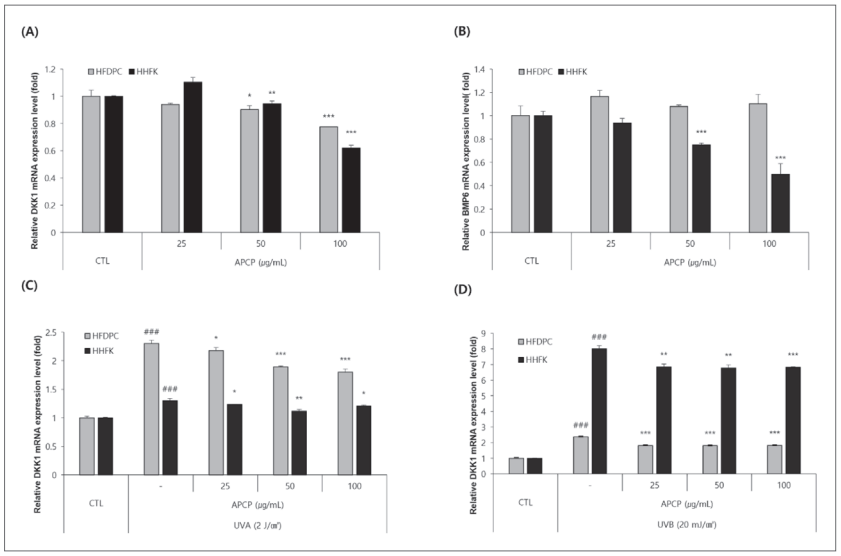

Fig. 2. Effects of APCP on the expression of genes encoding hair growth-inhibitory factors in human hair cells. **A**, **B** – HFDPCs and HHFKs treated with APCP for 24 h. **C**, **D** – HFDPCs and HHFKs were stimulated with UVA (2 J/cm^2) or UVB (20 mJ/cm^2) and incubated with APCP at the indicated concentration for 24 h. mRNA expression of hair growth-inhibitory factors was determined by qRT-PCR. Results are normalized to the expression of GAPDH. Data are expressed as means ± SD (A, B: *P<0.05, **P<0.01, ***P<0.001 vs. control [untreated] cells. C, D: ###P<0.001 vs. control [untreated] cells; *P<0.05, **P<0.01, ***P<0.001 vs. UVA or UVB alone).

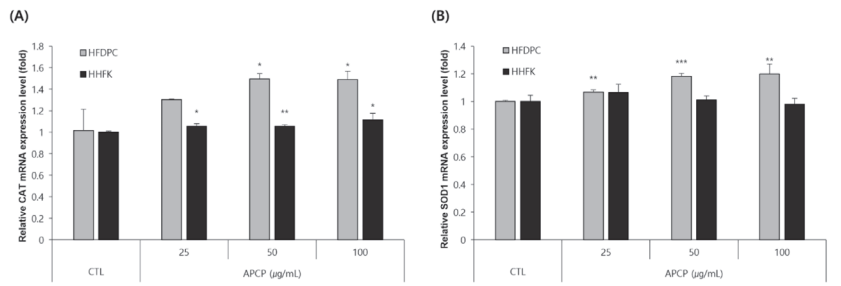

Fig. 3. Antioxidant effects of APCP on human hair cells. **A**, **B** – HFDPCs and HHFKs were treated with APCP for 24 h, and mRNA expression of antioxidant enzymes (CAT and SOD1) was determined by qRT-PCR. Results were normalized to the expression of β-actin. Data are expressed as means±SD (*P<0.05, **P<0.01, ***P<0.001 vs. control [untreated] cells).

of APCP for 24 h and measured mRNA levels of CAT and SOD1. In HFDPCs, both CAT and SOD1 mRNA expression levels were markedly increased by APCP (Fig. 3A and 3B). In HHFKs, APCP led to a modest but significant increase in CAT gene expression, but it had no significant effect on SOD1 expression. DPPH scavenging assays revealed that APCP lacked ROS-scavenging activity (Supplementary Fig. S2).

Molecular mechanism underlying APCP-induced HFDPC proliferation

Next, we investigated the molecular mechanism responsible for the APCP-induced proliferation of HFDPCs. As seen in Figure 4A, APCP induced mRNA expression of GAS6, a marker of cell proliferation. To further elucidate the mechanism underlying hair cell proliferation induced by APCP, we examined the expression of related signaling molecules by Western blot analysis. Given the critical role of activating the β-catenin signaling pathway for hair cell growth, we evaluated the β-catenin protein levels and phosphorylation status. Treatment with 25, 50 or 100 μg/mL of APCP significantly increased β-catenin phosphorylation (Ser552/Ser675) and increased total β-catenin protein levels (Fig. 4B). Immunofluorescence staining confirmed that APCP increased the expression of β-catenin and further revealed that APCP induced the migration of stabilized β-catenin from the cytoplasm to the nucleus, as evidenced by the overlap between green (β-catenin) and blue (nucleus) staining (Fig. 4C). AKT/ERK signaling stabilizes β-catenin by phosphorylating it at Ser552, facilitating its translocation to the nucleus. Moreover, β-catenin undergoes phosphorylation at Ser675 by PKA. Accordingly, we assessed AKT, ERK, and PKA activation in APCP-treated HFDPCs by determining their phosphorylation status using Western blotting. Treatment with APCP for 24 h caused a concentration-dependent increase in the phosphorylation of AKT and ERK in HFDPCs (Fig. 4D). Next, we confirmed the phosphorylation of a PKA substrate to assess PKA activation. As shown in Figure 4E, APCP induced a concentration-dependent

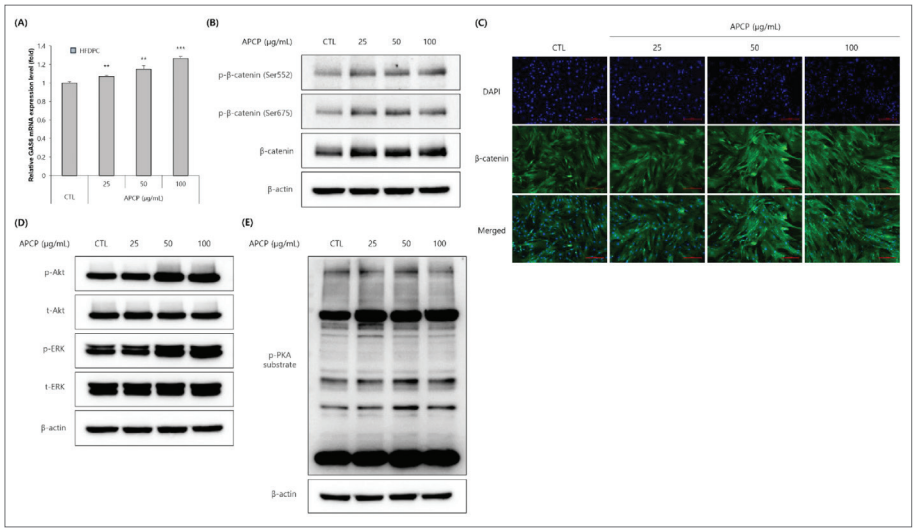

Fig. 4. Mechanisms underlying APCP-induced HFDPC cell proliferation. **A** – HFDPCs were incubated with APCP for 24 h, and the production of the cell proliferation marker, GAS6, was examined by qRT-PCR. mRNA expression was normalized to that of β-actin. Data are presented as means ± SD (**P < 0.01, ***P < 0.001 vs. control [untreated] cells). **B-E** – HFDPCs were treated with APCP for 24 h, and then phospho-ERK, phospho-AKT, phospho-PKA substrate, and phospho-β-catenin (Ser522/Ser675) protein levels were determined by Western blot analysis. β-actin was used as a loading control. **C** – Immunocytochemical (ICC) analysis showing merged images of β-catenin (green) staining and DAPI (nucleus, blue) signals. Scale bars, 200 μm.

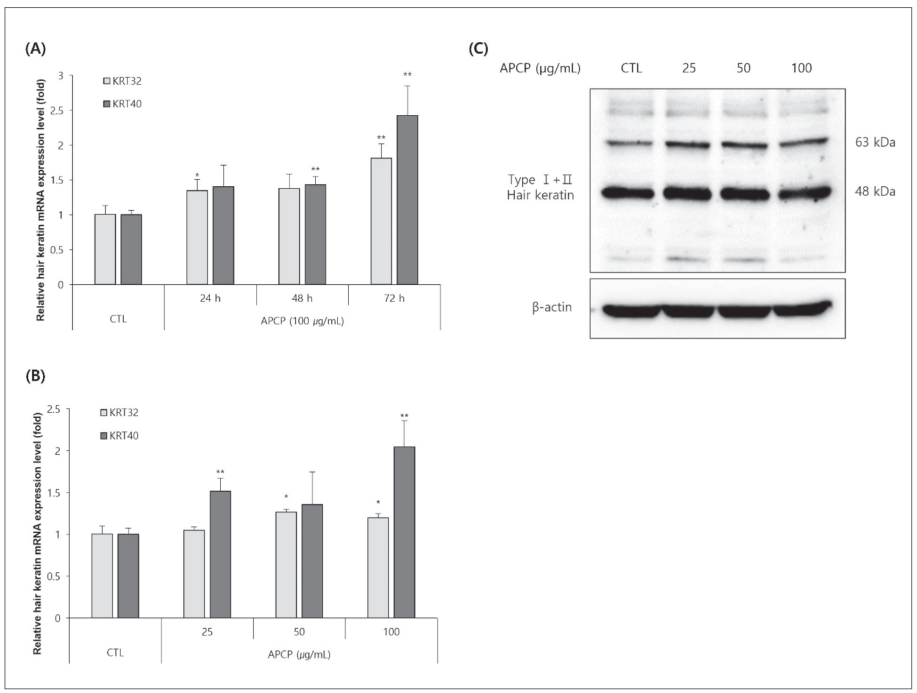

Fig. 5. Effect of APCP on hair keratin expression in HHFKs. **A** – HHFKs were incubated with 100 μg/mL of APCP for 24, 48 or 72 h. **B** – HHFKs were incubated with the indicated concentrations (25 to 100 μg/mL) APCP for 72 h. KRT32 and KRT40 mRNA expression were examined by qRT-PCR and normalized to that of GAPDH. Data are presented as means±SD (*P<0.05, **P<0.01 vs. control [untreated] cells). **C** – Induction of type I + II hair keratin expression in HHFKs by treatment with APCP for 96 h, determined by Western blot analysis. β-actin was used as a loading control.

increase in phospho-PKA substrate in HFDPCs.

APCP induction of hair keratin gene expression in HHFKs

Lastly, we explored the effect of APCP on the expression of hair keratin genes. Applying APCP to HHFKs induced a significant time- and concentration-dependent increase in mRNA for the type I hair keratins, KRT32 and KRT40 (Fig. 5A and 5B). Western blot analysis confirmed these results at the protein level, showing that treatment with APCP for 96 h increased the synthesis of both KRT32 (48 kDa) and KRT40 (50 kDa). As shown in Figure 5C, we further found that APCP promoted the expression of a 63 kDa protein consistent with type II hair keratins (KRT81-KRT86). However, it was impossible to distinguish which specific keratins were induced.

DISCUSSION

In this study, we present the initial evidence of the hair growth-promoting and quality-improving effects of APCP, using HFDPCs and HHFKs as *in vitro* models. HFDPCs represent a significant hair cell type crucial for regulating the hair growth cycle and hair follicle development [38], while HHFKs are follicular keratinocytes responsible for producing hair keratin, a primary constituent of hair. Hair growth-promoting factors secreted from HFDPCs induce cell proliferation and differentiation and mediate the growth of HHFKs through intercellular interactions during the hair biogenesis process

[39]. We verified the effectiveness of APCP in stimulating cell proliferation in HFDPCs and HHFKs. We demonstrated its capacity to facilitate cell migration under HFDPC/HHFK co-culture conditions, thus establishing the ability of APCP to promote the proliferation of hair cells.

Hair growth-inhibitory factors such as BMP6, secreted by HFDPCs, suppress the transition from the telogen to anagen phase of the hair growth cycle by blocking the Wnt signaling pathway, which stimulates hair growth [40]. In addition, many dermatologic disorders, including atopic dermatitis, vitiligo, and alopecia areata, are accompanied by increased ROS [41]. In this study, we showed that APCP inhibited H_2O_2-induced apoptosis in HFDPCs and HHFKs, an effect that we infer is caused, at least in part, by the increased expression of antioxidant enzymes in these hair cells (Fig. 3). We further investigated the effect of UV irradiation on the expression of hair growth-inhibitory factors. UV irradiation is known to induce DNA damage and the generation of reactive oxygen species (ROS), consequently disrupting the growth cycle of hair follicles and compromising their cellular function. [42]. At the mRNA level, UVA/UVB stimulation markedly increased the expression of DKK1 in HFDPCs and HHFKs; this effect was significantly inhibited by APCP, which also suppressed DKK1 expression in the control, unirradiated cells (Fig. 2). These results suggest the possibility that APCP would also suppress hair loss caused by natural external conditions. However, changes in the expression of genes encoding hair growth factors and hair growth-inhibitory factors induced by APCP treatment in hair cells exposed to UV or hair loss inducers have not yet been identified, so further research on the subject is needed.

Our investigation of the mechanism underlying APCP-induced HFDPC proliferation revealed that APCP also increased the mRNA expression of GAS6, which induces cell proliferation through its mitosis-promoting effects [43-44]. The Wnt/β-catenin signaling pathway is well known to play an essential role in the proliferation and follicular growth of HFDPCs. Depending on the site of phosphorylation, β-catenin can follow two pathways: it may undergo degradation or translocate to the nucleus, where it facilitates transcription. The β-catenin-destroying complex that phosphorylates β-catenin consists of glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), axin, and casein kinase 1 (CK-1) [45-46]. Ubiquitinated β-catenin resulting from the action of this complex subsequently undergoes proteosome-mediated degradation. In contrast, phosphorylation of β-catenin by PKA and AKT/ERK signaling pathways promotes β-catenin migration to the nucleus. Specifically, PKA activation leads to the phosphorylation of β-catenin at Ser675, thereby stabilizing it, whereas AKT/ERK signaling induces phosphorylation of β-catenin at Ser552, thereby promoting gene expression through the transcriptional activity of β-catenin. Phospho-AKT also inhibits the activation of phospho-GSK3β [47], suppressing β-catenin ubiquitination. We verified that APCP promotes the phosphorylation of the PKA substrates, AKT and ERK, and increases the expression of phospho-β-catenin (Ser552/Ser675) and total β-catenin protein. Immunocytochemical analysis further revealed that this APCP-induced increase in β-catenin was detectable by fluorescence microscopy in HFDPCs. In addition, we confirmed that APCP activates the AKT/ERK signaling pathway in HHFKs (Supplementary Fig. 3). Taken together, these findings suggest that APCP stimulated the proliferation and growth of hair cells through a PKA/AKT/ERK-mediated β-catenin signaling pathway.

Improved hair growth is also closely related to keratin production. Since keratin is the main component of hair, increasing keratin synthesis during hair formation has the potential to improve hair quality [48-49]. In HHFKs, we verified the hair keratin expression-promoting effects of APCP at both mRNA and protein levels. Previous studies have shown that the type I hair keratins, KRT32 and KRT40, contribute significantly to the synthesis of keratin in the cuticle, among hair structures [50]. In this study, we found that APCP induced a time- and concentration-dependent increase in KRT32 and KRT40 mRNA levels. It also promoted the expression of both type I hair keratins (KRT31-KRT40) and type II hair keratins (KRT81-KRT86) at the protein level (Fig. 5C). Based on the data presented, we deduce that APCP may regulate hair density and strengthen hair by increasing keratin expression, resulting in healthier hair.

In conclusion, this study represents the first evidence that APCP can stimulate proliferation, inhibit apoptosis, and increase the expression of antioxidant

enzymes and keratin in hair cells. Therefore, we propose that APCP may serve as a promising bioactive supplement capable of enhancing both hair growth and hair quality.

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Author contributions: D Shim and IH Bae contributed equally to this work. Conceptualization, CS Lee and J Roh; methodology, D Shim, IH Bae, JO Chung, HW Mok and W Kim; data curation, D Shim and IH Bae; writing, original draft preparation, D Shim and IH Bae; writing, review and editing, CS Lee and J Roh; supervision, CS Lee and J Roh. All authors have read and agreed to the published version of the manuscript.

Conflict of interest disclosure: The authors report no conflicts of interest related to this work.

Data availability: Data underlying the reported findings have been provided as a raw dataset, which is available here: https://www.serbiosoc.org.rs/NewUploads/Uploads/Shim%20et%20al_Raw%20Dataset.xlsx

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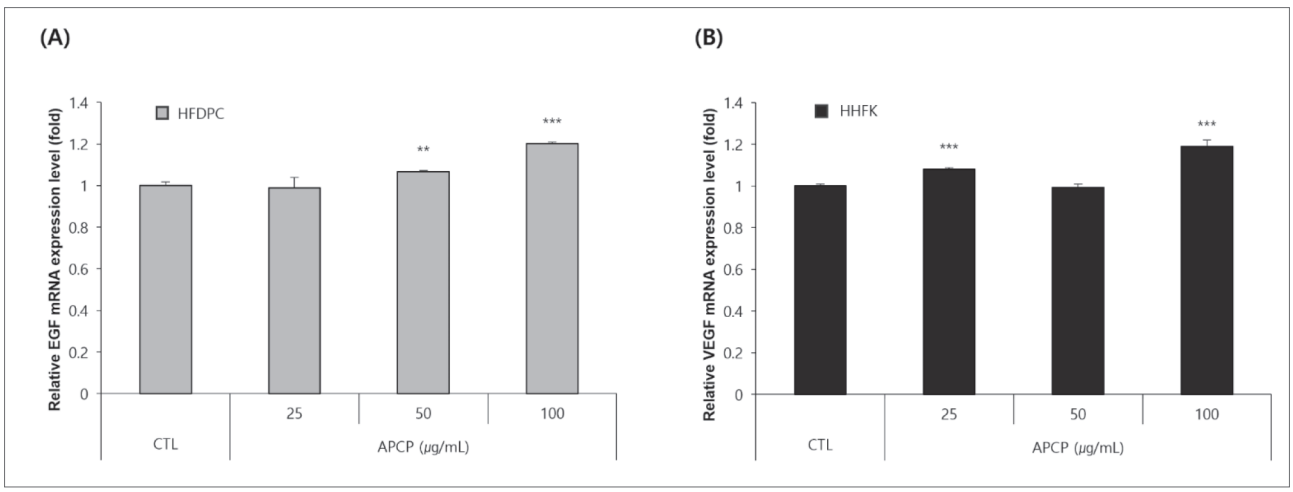
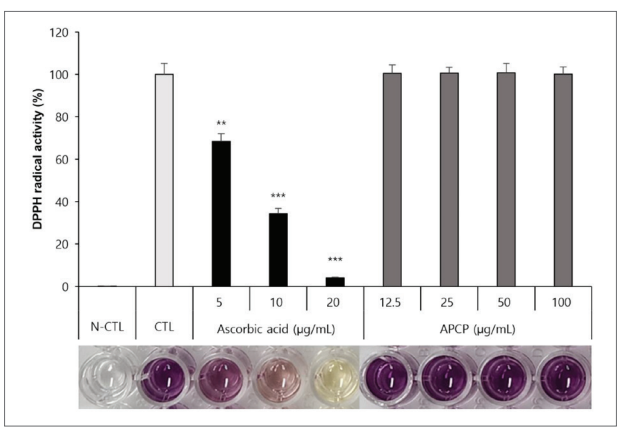
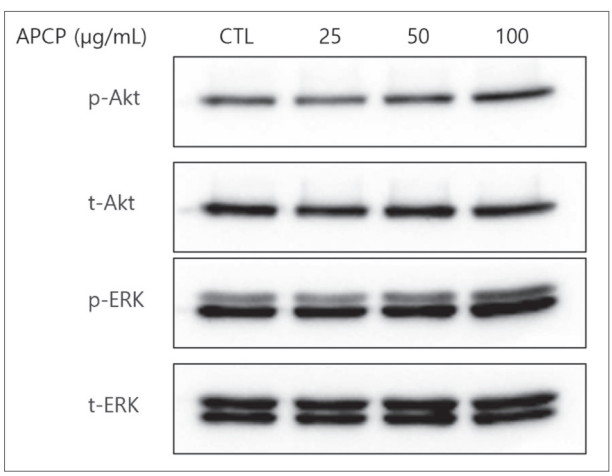
SUPPLEMENTARY MATERIAL



Supplementary Fig. S1. Effect of APCP on the expression of genes encoding hair growth-promoting factors in human hair cells. (A, B) HFDPCs and HHFKs were treated with APCP for 24 h, and the expression of mRNA for hair growth-promoting factors was determined by qRT-PCR. mRNA expression was normalized to that of GAPDH. Data are presented as means ± SD (*P < 0.05, **P < 0.01, ***P < 0.001 vs. control [untreated] cells).



Supplementary Fig. S2. Effect of APCP on DPPH radical activity, determined by measuring absorbance at 520 nm. Ascorbic acid was used as a positive control. Data are presented as means±SD (**P <0.01, ***P<0.001 vs. controls).



Supplementary Fig. S3. Mechanism underlying APCP-induced HHFK cell proliferation. HHFKs were treated with APCP for 24 h, and phospho-ERK and phospho-AKT protein levels were determined by Western blot analysis. Quantified proteins in HHFK cell extract were resolved by SDS-PAGE on 10% gels and detected using specific antibodies.