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Research Article

Ginsenoside Re prevents 3-methyladenine-induced catagen phase acceleration by regulating Wnt/β-catenin signaling in human dermal papilla cells

Gyusang Jeong [a, b], Seung Hyun Shin [a], Su Na Kim [a], Yongjoo Na [a], Byung Cheol Park [b, **], Jeong Hun Cho [a], Won-Seok Park [a], Hyoung-June Kim [a, *]

[a] AMOREPACIFIC Research and Innovation Center, Yongin-si, Republic of Korea
[b] Department of Dermatology, College of Medicine, Dankook University, Cheonan-si, Republic of Korea

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ABSTRACT

Background: The human hair follicle undergoes cyclic phases—anagen, catagen, and telogen—throughout its lifetime. This cyclic transition has been studied as a target for treating hair loss. Recently, correlation between the inhibition of autophagy and acceleration of the catagen phase in human hair follicles was investigated. However, the role of autophagy in human dermal papilla cells (hDPCs), which is involved in the development and growth of hair follicles, is not known. We hypothesized that acceleration of hair catagen phase upon inhibition of autophagy is due to the downregulation of Wnt/β-catenin signaling in hDPCs, and that components of *Panax ginseng* extract can increase the autophagic flux in hDPCs.

Methods: We generated an autophagy-inhibited condition using 3-methyladenine (3-MA), a specific autophagy inhibitor, and investigated the regulation of Wnt/β-catenin signaling using the luciferase reporter assay, qRT-PCR, and western blot analysis. In addition, cells were cotreated with ginsenoside Re and 3-MA and their roles in inhibiting autophagosome formation were investigated.

Results: We found that the unstimulated anagen phase dermal papilla region expressed the autophagy marker, LC3. Transcription of Wnt-related genes and nuclear translocation of β-catenin were reduced after treatment of hDPCs with 3-MA. In addition, treatment with the combination of ginsenoside Re and 3-MA changed the Wnt activity and hair cycle by restoring autophagy.

Conclusions: Our results suggest that autophagy inhibition in hDPCs accelerates the catagen phase by downregulating Wnt/β-catenin signaling. Furthermore, ginsenoside Re, which increased autophagy in hDPCs, could be useful for reducing hair loss caused by abnormal inhibition of autophagy.

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1. Introduction

The human hair follicle is a dynamic organ that changes continuously. The life cycle of a hair follicle can be divided into a growth phase (anagen), a regression phase (catagen), and a rest phase (telogen); these phases are repeated during the lifetime of hair follicles. In the anagen phase, the hair shaft grows continuously; however, during catagen and telogen, the growth of shaft decreases and there are structural changes in preparation for the next hair cycle [1–5]. The possibility of hair loss increases if the transition to catagen is accelerated or that to anagen is inhibited. Hence, these transitions are being studied extensively as targets for treating hair loss [6–10]. The mesenchymal and epithelial parts of the hair follicle interact with and regulate each other during each stage. The dermal papilla, located at the base of the hair follicle, is a special mesenchymal part that plays crucial roles in the development and regeneration of hair follicles. Various signals from the dermal papilla regulate the hair cycle via interaction with the epithelial compartment [11]. Among them, the Wnt/β-catenin signaling is known to be the most important signaling pathway required for maintenance of the anagen phase [12] and its connection with autophagy has been investigated in different types of cells and organs [13–15].

* Corresponding author. 1920, Yonggu-daero, Giheung-gu, Yongin-si, Gyeonggi-do, 170174, Republic of Korea.
** Corresponding author. Department of Dermatology, College of Medicine, Dankook University, Cheonan-si, 31116, Republic of Korea.
E-mail address: leojune@amorepacific.com (H.-J. Kim).

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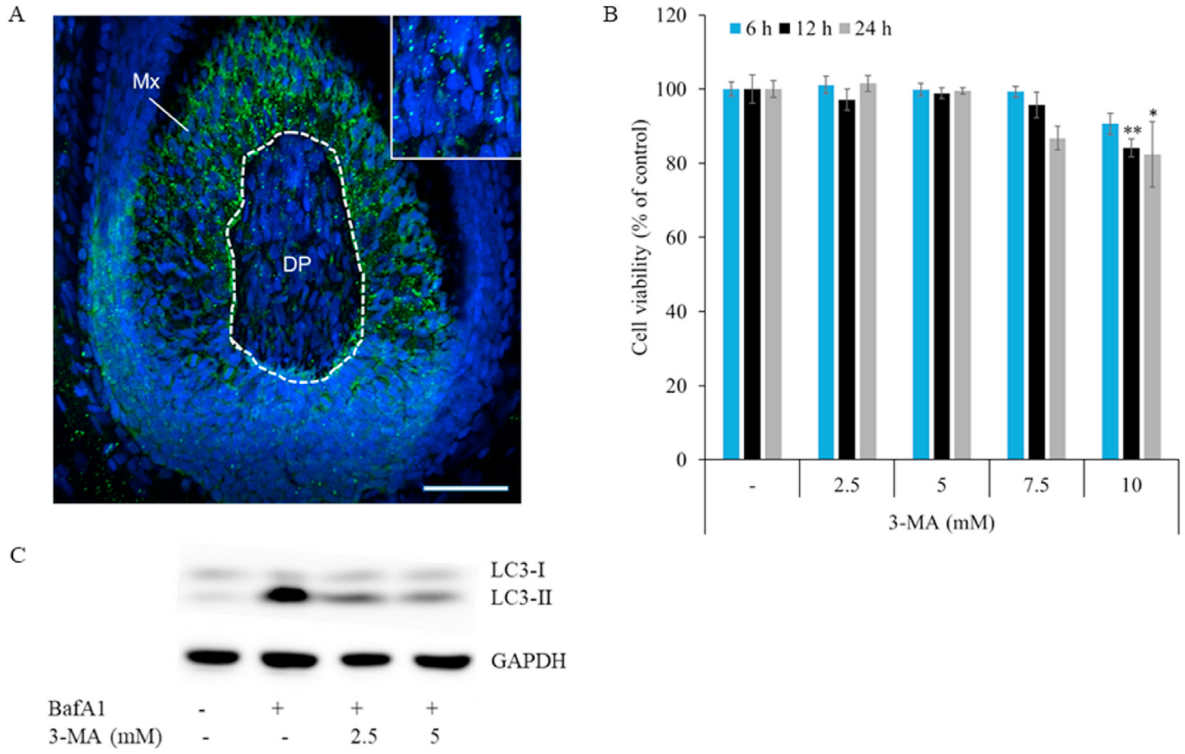

Fig. 1. 3-MA inhibits autophagy in hDPCs. (A) LC3 puncta in the anagen human hair follicle dermal papilla region (Mx: matrix, DP: dermal papilla, Scale bar = 50 μm, Blue: DAPI, Green: LC3B). (B) CCK-8 assay. (C) LC3 conversion in hDPCs assessed using western blot analysis after 6 h of 3-MA treatment. Autophagy was assessed after bafilomycin A1 treatment (*$p < 0.05$, **$p < 0.01$ vs. non-treated).

Autophagy is a stress response system for cellular homeostasis that has been actively researched in the context of aging, metabolic disease, and stress [16–22]. However, studies on the role of autophagy in the development of hair follicle and hair loss are limited. Recent studies indicate that autophagy is more active in the matrix and outer root sheath (ORS) region in the anagen phase, and that inhibition of autophagy accelerates the hair regression phase. Substances that enhance autophagy in the hair follicle can extend the anagen phase [23,24]. However, the mechanisms underlying the regulation of hair cycle and function of autophagy in dermal papilla remain unknown.

In this study, we investigated the role of autophagy in human dermal papilla cells (hDPCs) using 3-methyladenine (3-MA), an autophagy inhibitor. We also evaluated the effect of ginsenoside Re, one of the main components of the *Panax ginseng* extract, on the autophagy-suppressed environment induced by 3-MA.

2. Materials and methods

2.1. Reagents

3-MA (M9281), bafilomycin A1 (BafA1, B1793), and ginsenoside Re (77960) were purchased from Sigma-Aldrich (MO, US). BafA1 and ginsenoside Re were dissolved in dimethyl sulfoxide (Sigma-Aldrich, D8418). 3-MA was dissolved directly in culture medium and used for experiments.

2.2. Cell culture

Primary hDPCs were obtained from the Kyungpook National University (Daegu, Korea) and cultured in low glucose Dulbecco's modified Eagle's medium (DMEM, Clytia, England, SH30021.01) with 10% fetal bovine serum (Welgene, Korea, S001-01) and 1% antibiotic-antimycotic solution (Sigma-Aldrich, A5955). The Wnt reporter cell line (Enzo Lifesciences, NY. US, 61002) was cultured in high glucose DMEM (Clytia, SH30243.01) with 10% fetal bovine serum and 1% antibiotic-antimycotic solution. For quantitative reverse transcription polymerase chain reaction (qRT-PCR) and western blotting, 3×10^5 hDPCs were seeded per well in 60 mm dishes (Falcon, GA, US, 353004) and treated with 3-MA, BafA1, and ginsenoside Re.

2.3. Cell viability assay

hDPCs were seeded in a 96-well plate at a density of 3.5×10^3 cells/well ($n = 3$). The cells were treated with different concentrations of 3-MA for 24 h, following which cell viability was determined using the cell counting kit 8 (CCK8) assay (Dojindo, MD, US, CK04).

2.4. Human hair follicle organ culture

The use of human hair follicles was approved by the Ethics Committee of the Dankook University Hospital (Cheonan, Korea) (approval no. DKUH-2020-11-004-001). Anagen stage hair follicles isolated from the occipital region of the scalp were used for organ culture ($n = 12$). The hair follicles were maintained in William's E medium (Sigma-Aldrich, W1878) supplemented with 5% fetal bovine serum, 1% antibiotics-antimycotic solution, 0.1% amphotericin B (Gibco, MD, US, 15290026), 10 ng/mL hydrocortisone (Sigma-Aldrich, H6909), 10 μg/mL insulin (Sigma-Aldrich, I9276), and 2 mM L-glutamine (Sigma-Aldrich, G7513). The culture medium was changed once every 2 or 3 days, and shaft elongation and hair cycle were analyzed using a stereoscopic microscope (Olympus, Japan, SZX16).

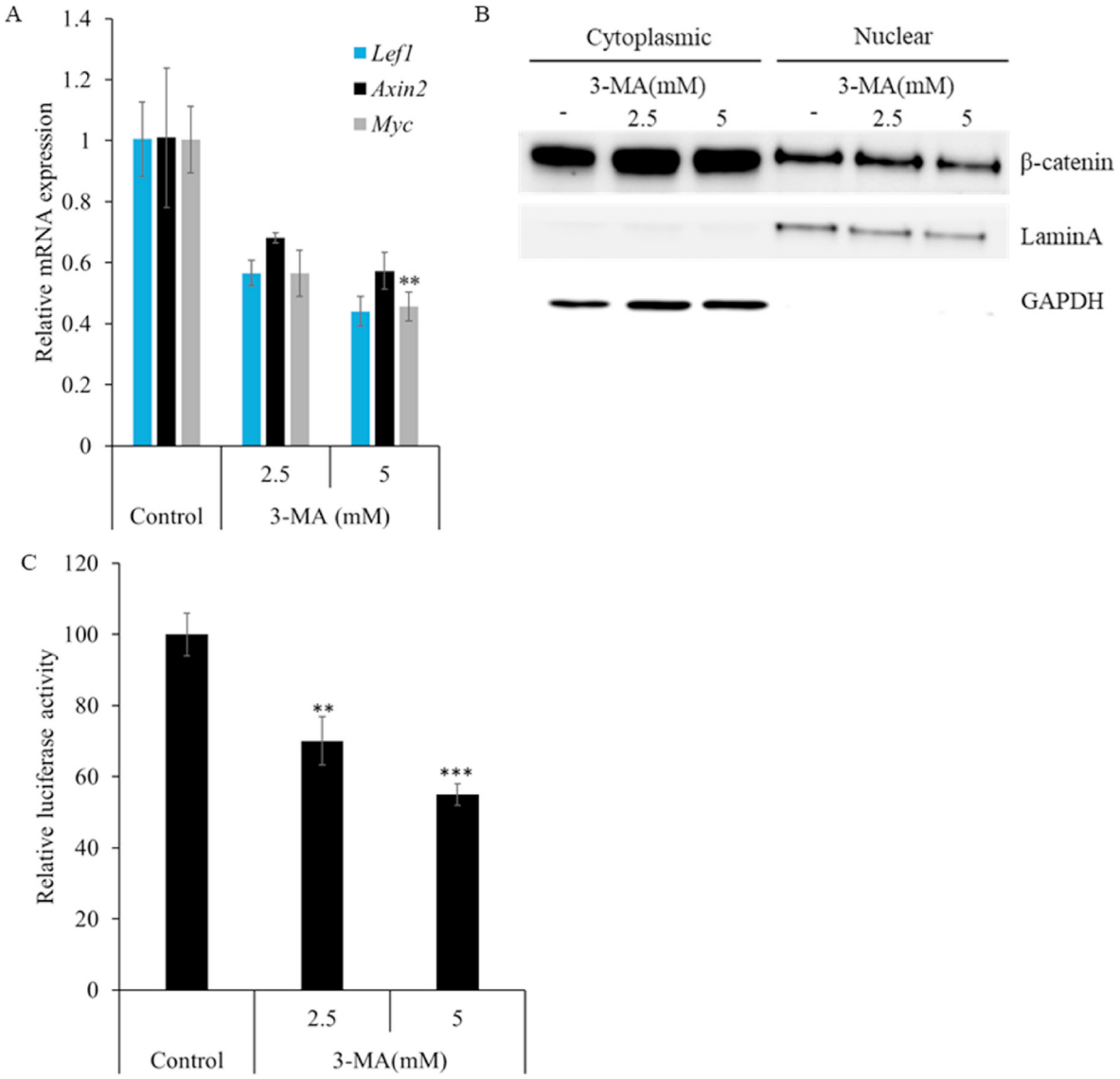

Fig. 2. 3-MA reduced Wnt/β-catenin signaling. (A) qRT-PCR analysis for assessing the transcription of Wnt/β-catenin signaling-related genes. (B) Nuclear translocation of β-catenin assessed using western blot analysis. (C) Luciferase activity using a Wnt reporter cell line (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control).

2.5. qRT-PCR

Total RNA was collected from hDPCs using the RNeasy mini kit (Qiagen, Germany, 74104). One microgram of the extracted RNA was used to synthesize cDNA using a cDNA synthesis kit (Takara, Japan, 6110A). qRT-PCR was performed using a PCR master mix (Applied Biosystems, MA, US, 43-044-37) and TaqMan probes, and the results were analyzed using the ΔΔCq method. The data are presented as mean ± standard error of mean (SEM) of three independent experiments ($n = 3$). The following TaqMan probes were used: *Myc* (Hs00153408_m1), *Lef1* (Hs01547250_m1), *Axin2* (Hs00610344_m1), and *GAPDH* (Hs02786624_g1).

2.6. Western blot analysis

Total protein was extracted using radioimmunoprecipitation assay lysis and extraction buffer (Thermo Fisher, MA, US, 89900), supplemented with a protease inhibitor cocktail (Sigma-Aldrich, P8340) and a phosphatase inhibitor cocktail (Sigma-Aldrich, P0044). The nuclear extraction kit (Sigma-Aldrich, NXTRACT) was used for extracting the nuclear fraction per the manufacturer's instructions. The extracted proteins were quantified and fractionated, and transferred onto a membrane for antigen–antibody reaction. The protein was visualized using the enhanced chemiluminescence (ECL) solution (Thermo Fisher, 32109). The following antibodies were used: anti-LC3B (1:300, Novus Biologicals, CO, US, NB100-2220), anti-Beclin 1 (1:300, Novus Biologicals, NB110-87318), anti-GAPDH (1:1000, Santa Cruz Biotechnology, TX, US, sc-32233), anti-lamin A (1:1000, Santa Cruz Biotechnology, sc-518013), and anti-β-catenin (1:500, Cell Signaling, MA, US, 8480T). Mouse IgG kappa binding protein conjugated to horse radish peroxidase (HRP) (1:5000, Santa Cruz Biotechnology, sc-516102) and mouse anti-rabbit IgG-HRP (1:2000, Santa Cruz Biotechnology, sc-2357) were used as secondary antibodies.

2.7. Luciferase assay

The Wnt reporter cell line (Enzo life sciences, ENZ-61002) was seeded in a collagen type I-coated white 96-well plate (Corning, NY, US, 354650) at a density of 1.5×10^4 cells/well ($n = 3$). After 24 h,

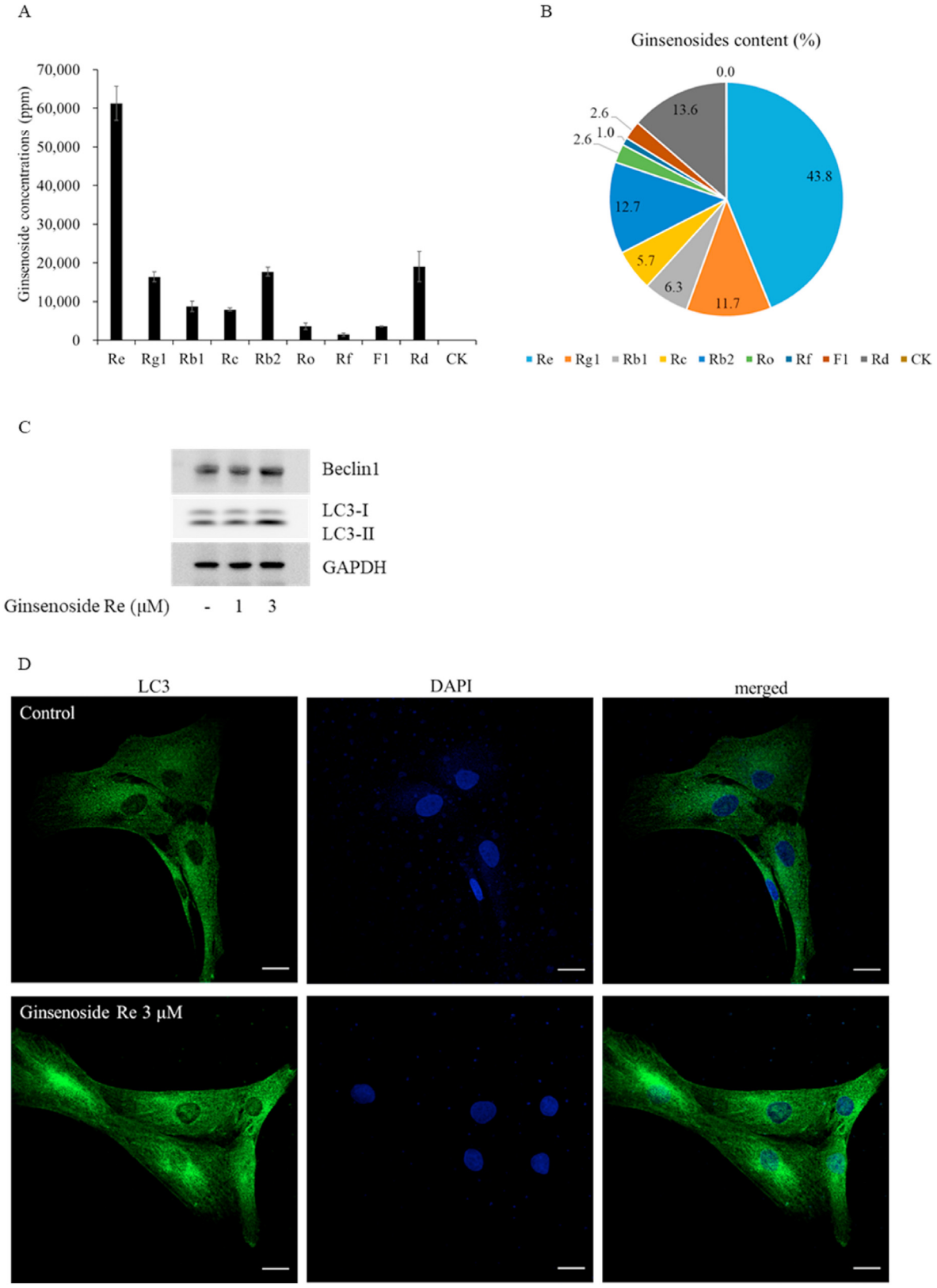

Fig. 3. Induction of autophagy in hDPCs by ginsenoside Re. The content of ginsenoside Re is the highest in the *Panax ginseng* extract. (A, B) hDPCs were treated with ginsenoside Re for 6 h and autophagy was assessed using western blot analysis and (C) immunocytochemistry (D, Scale bar = 20 μm, Blue: DAPI, Green: LC3B).

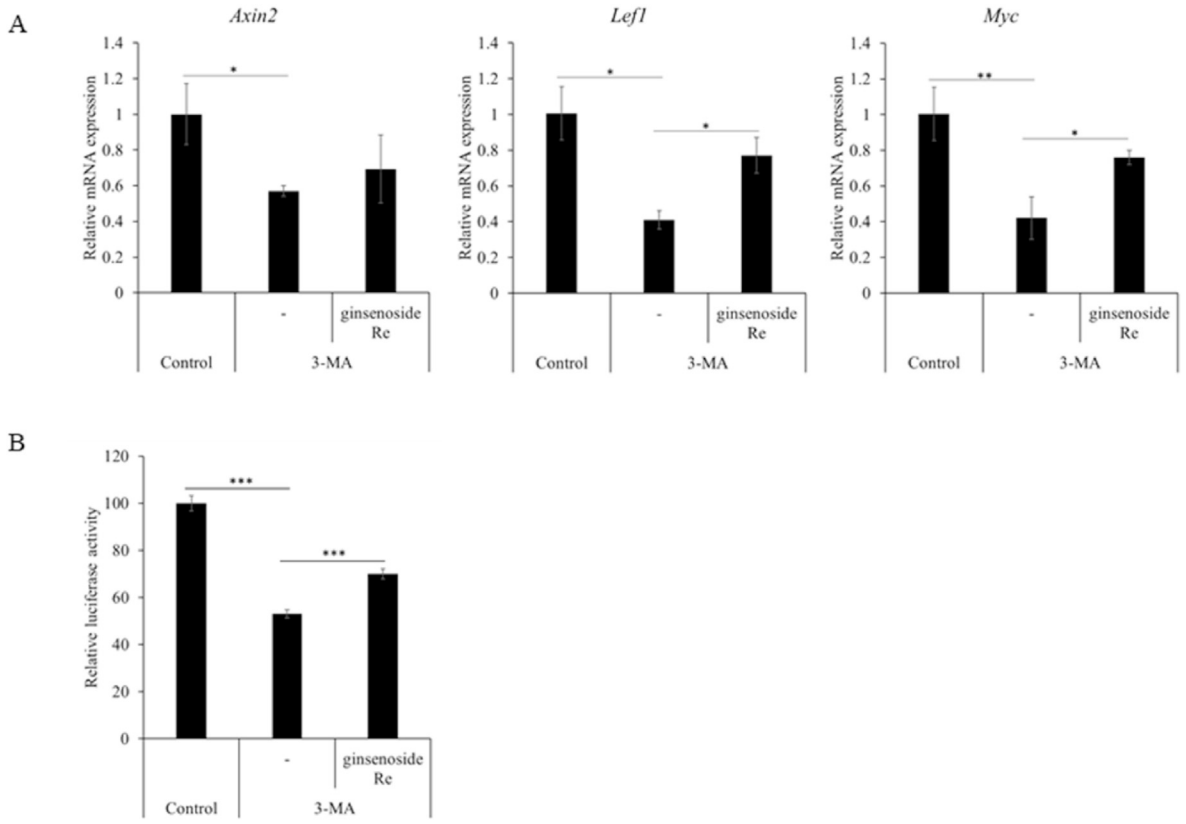

Fig. 4. Effect of ginsenoside Re on the autophagy-inhibited condition generated using 3-MA (5 mM) treatment. (A) Wnt-related gene expression and (B) luciferase activity were analyzed with and without 3 μM ginsenoside Re (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. control).

the cells were treated with 3-MA and ginsenoside Re and incubated for 24 h. The luciferase activity was assessed using the luciferase assay system (Promega, WI, US, E610).

2.8. Immunofluorescence

For immunocytochemistry, hDPCs were seeded in 4-chamber slides (Thermo Fisher, 154526) at a density of 1×10^4 cells/well and treated with ginsenoside Re for 6 h, following which, the cells were fixed with 4% formaldehyde for 15 min and permeabilized with 0.3% Triton X-100 for 10 min. The cells were blocked with a blocking buffer (Abcam, England, ab126587) for 1 h and incubated with anti-LC3B antibody (1:200, Novus Biotechnology, NB100-2220) overnight at 4 °C. After incubation with the primary antibody, the cells were washed and incubated with goat anti-rabbit IgG secondary antibody (1:1000, Thermo Fisher, A-11034). LC3B and DAPI were detected using confocal microscopy.

For immunohistochemistry, scalp tissue containing anagen stage hair follicles was used for LC3 staining. The scalp tissue was cut into 200 μm-thick sections using a vibrator and the sections were cleared using a tissue clearing solution (Binaree, Korea, HROI-101). The tissue sections were permeabilized overnight using 0.1% Triton X-100 and treated for 4 days with anti-LC3B antibody (Novus Biologicals, NB100-2220). The sample was incubated with the secondary antibody (Abcam, ab150081) for 24 h and with DAPI for 1 h. After adding the mounting and storage solution (Binaree, HROI-101), the staining pattern was visualized using confocal microscopy.

2.9. Quantitation of ginsenoside content in the P. ginseng extract

Five grams of the *P. ginseng* extract was dissolved in 50 mL methanol, following which water was added to a final volume of 100 mL. The solution was filtered and used as the sample solution. Ginsenosides for standard solution were purchased from Sigma-Aldrich, dissolved in 50 mL methanol, and filtered. The ginsenoside content in the sample was determined using high performance liquid chromatography-mass spectrometry (TQ-D system, Waters, USA). The mobile phase was composed of 0.1% (v/v) formic acid (A) and acetonitrile (B); the column temperature was set to 40 °C and the flow rate was 0.3 mL/min. The gradient conditions used were as follows: 0 min, 80% A/20% B; 0.1 min 80% A/20% B; 2 min 68% A/32% B; 7 min, 67% A/33% B; 20 min, 48% A/52% B; 23 min, 47% A/53% B; 26 min, 20% A/80% B; 26.9 min, 0% A/100% B; 27 min, 80% A/20% B; 30 min, 80% A/20% B. The experiment was repeated thrice, and the content was expressed as mean ± standard deviation of the ratio among the 10 types of saponins.

2.10. Statistical analysis

All experimental data are presented as mean ± standard error of mean (SEM). The Student's *t*-test was used for analysis of differences between two groups. *P*-values less than 0.05 (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) were considered statistically significant.

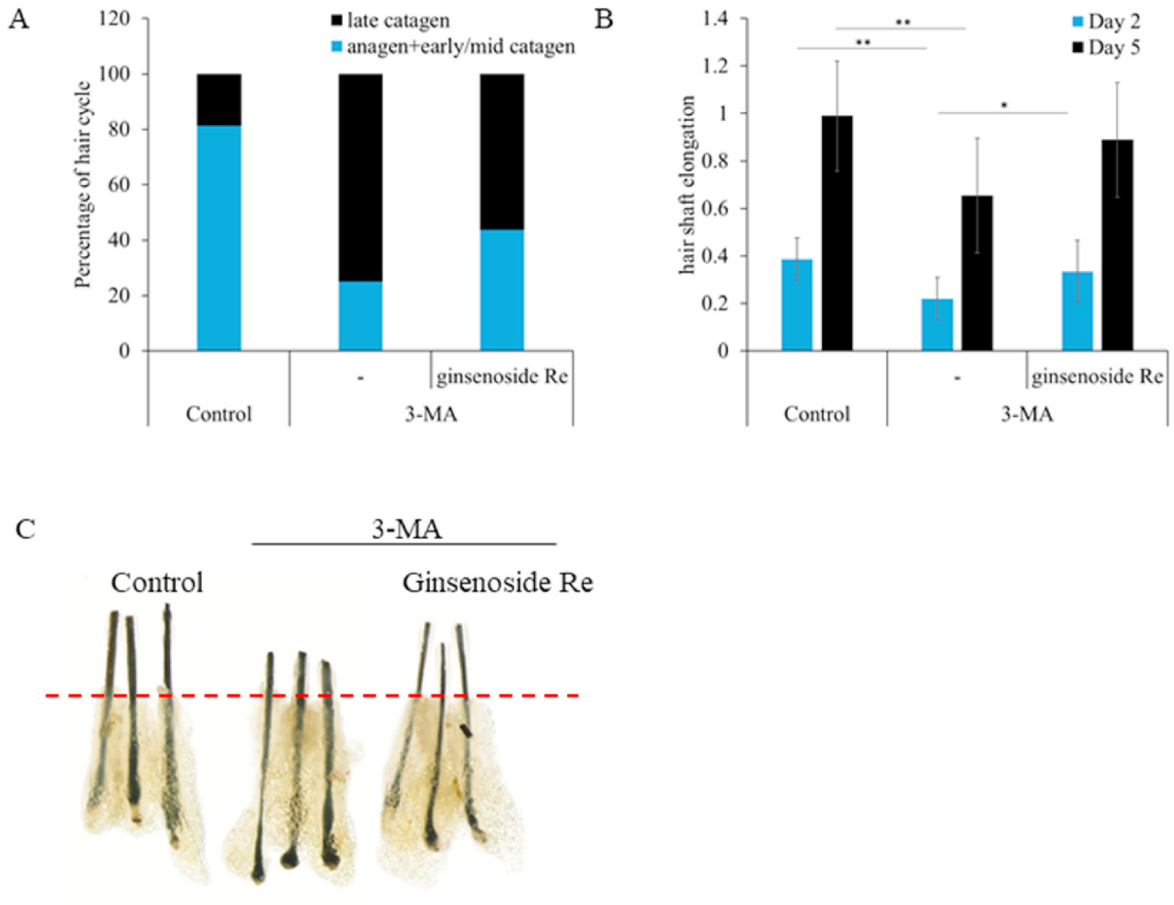

Fig. 5. Ginsenoside Re reverses 3-MA (5mM)-induced acceleration of catagen and inhibition of hair growth in organ culture. The hair follicles treated with and without 3 μM ginsenoside Re. (A) Hair cycle was calculated on day 7 as reported previously [47,48]. (B) Hair elongation was measured on days 2 and 5. (C) Images of hair follicle organ culture on day 5 (*$p < 0.05$, **$p < 0.01$ vs. control).

3. Results

3.1. 3-MA suppresses autophagy in hDPCs

We performed immunofluorescence to identify autophagy in the hair follicle dermal papilla region. The human hair follicle, which is considered the anagen hair stage, was isolated and immediately stained for the autophagy marker, LC3. The expression of LC3 was observed in the hair follicle dermal papilla region, which was lower than that in ORS and matrix cell (Fig. 1A).

To determine the effect of autophagy on the dermal papilla region, hDPCs were treated with 5 mM 3-MA, as the viability of dermal papilla cells is not affected at concentrations lower than this (Fig. 1B). As LC3-I to LC3-II conversion is typically used for identifying autophagy [25], we assessed the levels of LC3-I and II using western blot analysis. To identify an appropriate autophagy inhibitor for hDPCs, we used 3-MA and BafA1. 3-MA is a known inhibitor of the early stages of autophagy, which inhibits a class III phosphatidylinositol-3 kinase, whereas BafA1 is commonly used as an inhibitor of the latter stages of autophagy, which hinders the autophagosome–lysosome fusion by inhibiting vacuolar H^+ ATPase [26]. We observed a decrease in LC3 conversion in hDPCs after treatment with 2.5 and 5 mM 3-MA in the presence of BafA1 (Fig. 1C). These results indicate that 3-MA is an appropriate material for autophagic inhibition in hDPCs.

3.2. 3-MA downregulates Wnt/β-catenin signaling

Wnt/β-catenin signaling plays an important role in the activation of hair follicle stem cells and in the proliferation of hair germ cells essential for the growth and regeneration of hair [27]. In addition, Wnt/β-catenin signaling in human dermal papilla is essential for hair growth and for the maintenance of the anagen phase [12]. Several studies have suggested an association between autophagy and Wnt/β-catenin signaling [13–15]. Moreover, alterations in autophagy during the transition from anagen to catagen have been observed [24]. Therefore, we hypothesized that catagen transition following autophagy inhibition may be due to Wnt/β-catenin regulation in hDPCs. To confirm this hypothesis, we assessed the changes in dermal papilla after 3-MA treatment. Canonical Wnt target genes, including *LEF1*, *AXIN2*, and *MYC*, were downregulated in hDPCs after 3-MA treatment (Fig. 2A), and nuclear translocation of β-catenin was also reduced (Fig. 2B). In addition, luciferase activity of the Wnt reporter cell line reduced significantly by up to 50% after 3-MA treatment (Fig. 2C).

3.3. Ginsenoside Re increases autophagy in hDPCs

We investigated the ability of the *P. ginseng* extract to regulate autophagy in hDPCs (Fig. S1). The *P. ginseng* extract contains various ginsenosides [28,29], among which ginsenoside Re is the most abundant (Fig. 3A and B). Ginseng components can induce

autophagy [30–32]; however, compared with studies on other compounds of *P. ginseng*, those on the effect of ginsenoside Re on autophagy in hDPCs are scarce. Therefore, we investigated whether ginsenoside Re enhances autophagy in hDPCs. We found that LC3-II conversion, Beclin 1 expression (Fig. 3C), and LC3 puncta (LC3-positive fluorescent dots) (Fig. 3D) increased when hDPCs were treated with ginsenoside Re. Based on our results, we conclude that the ginsenoside Re-enriched extract can increase the autophagy of hDPCs.

3.4. Ginsenoside Re recovers 3-MA-induced down-regulation of Wnt/ β-catenin signaling

We previously investigated the changes in 3-MA-induced suppression of autophagy and found that Wnt/β-catenin target genes are downregulated by 3-MA. Therefore, we investigated whether ginsenoside Re could restore the 3-MA-induced inhibition of Wnt/β-catenin target genes, *LEF1*, *AXIN2*, and *MYC*, in hDPCs. *LEF1* is a key regulator of the Wnt/β-catenin pathway and works together with β-catenin to regulate the expression of other Wnt target genes. *AXIN2* and *MYC* are Wnt target genes, and hence, these three factors are sufficient to confirm regulation of Wnt signaling [33–35]. We observed that 3-MA significantly lowered the expression of all the three genes, and that the mRNA expression of *LEF1* and *MYC* was restored by ginsenoside Re after 24 h (Fig. 4A). In addition, we confirmed that 3-MA significantly reduced the activity of the Wnt luciferase reporter cells, which was significantly recovered by ginsenoside Re treatment (Fig. 4B).

3.5. Ginsenoside Re prevents 3-MA induced anagen-to-catagen transition in human hair follicles

We observed that 3-MA accelerated the anagen-to-catagen transition, and that ginsenoside Re was able to overcome the 3-MA-induced suppression of autophagy. Therefore, we investigated whether ginsenoside Re can prevent catagen acceleration or mitigate the consequences associated with the catagen phase after 3-MA treatment. In other words, we expected that ginsenoside Re may recover the catagen acceleration caused by the inhibition of autophagy. Toward this end, we used a human follicle organ model to measure the elongation of hair shaft. As observed for hDPCs, 3-MA treatment changed the growth of human hair follicles by accelerating catagen. Hair growth was also reduced in human hair follicle organ culture. In contrast, ginsenoside Re reversed the 3-MA-induced anagen-to-catagen transition (Fig. 5A) and retarded the growth of3-MA-treated hair follicles (Fig. 5B).

4. Discussion

The Wnt/β-catenin is an important signaling pathway that maintains tissue development and homeostasis. The Wnt protein exists in 19 isoforms in mammals, including humans. These glycoproteins bind to frizzed and LRP5/6 coreceptors and activate the Wnt/β-catenin pathway. β-catenin accumulates in the nucleus and regulates the expression of Wnt-response target genes and induces various intracellular actions [36–38]. The Wnt/β-catenin affects the pathogenesis of most of the skin diseases [39] and also plays an important role in the morphogenesis and development of hair follicles and in the regulation of the hair cycle. Wnt3a regulates the expression of the hair cycle regulatory genes in dermal papilla cells. DHT, an androgenetic alopecia inducer, downregulates Wnt5a and Wnt10b in dermal papilla cells [40–42]. Therefore, various Wnt isoforms affect cellular processes in dermal papilla cells; other, hitherto undiscovered, mechanisms might also exist.

Previous studies on autophagy in hair follicles have focused on the inhibition or induction of this process. Strong autophagy flux is observed in the matrix and ORS region in the anagen phase. Autophagy inducers, such as α-ketoglutarate, α-ketobutyrate, rapamycin, and metformin, are efficient stimulators of hair growth and anagen phase maintenance. Moreover, the anagen-to-catagen transition is accelerated when the autophagy flux is suppressed [23,24]. Therefore, the abnormal reduction in the autophagy flux can be a target for hair loss. However, autophagy in the dermal papilla and the mechanism of the regulation of hair cycle are not well understood and investigated in this study.

We show that the normal autophagy flux in the hair follicle dermal papilla region plays an important role in maintaining the anagen phase. The autophagy marker, LC3, is expressed in the dermal papilla region without any stimulation in the anagen phase of the human hair follicles (Fig. 1A). Inhibition of autophagy by 3-MA accelerated the anagen-to-catagen transition by reducing the Wnt/β-catenin signaling in dermal papilla cells. In addition, one of the major components of the *P. ginseng* extract, ginsenoside Re, reversed the inhibition of hair growth by enhancing the autophagy flux (Figs. 3–5). We previously found that the *P. ginseng* extract possesses anti-hair loss properties. The *P. ginseng* extract and its major components, such as ginsenoside Re, Rg_1 and Rb_1, affect hair growth, and their mechanism of action is similar to that of the Food and Drug Administration-approved minoxidil [43]. The catagen-like changes induced by DKK-1, known for its hair loss ability, were restored by the *P. ginseng* extract [44]. In addition, the results of this study suggest that the *P. ginseng* extract and ginsenoside Re have anti-hair loss effect through an as yet unknown mechanism.

Our study has one limitation. The dermal papilla exists as a 3D sphere in the anagen phase hair follicles in the absence of proliferation [45,46]. However, we used 2D cell culture in this study, which may have influenced the regulation of autophagy. Hence, further studies using dermal papilla spheres are required.

In conclusion, we demonstrate that inhibition of autophagy downregulates the Wnt/β-catenin signaling in hDPCs, which accelerates the catagen phase in the hair follicle. Ginsenoside Re can reverse autophagy inhibition-induced anagen-to-catagen transition. Therefore, ginsenoside Re may be used as a possible therapeutic agent for alleviating hair loss or alopecia.

Author contributions

Conceptualization: GJ, WSP, and YN. Methodology: GJ, SHS and BCP. Investigation: GJ and JHC. Formal analysis: GJ, SHS, SNK and BCP. Resources: BCP. Writing draft: GJ, JHC, and HK. Supervision: HK and BCP. All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2022.11.002.

References

[1] Alonso L, Fuchs E. The hair cycle. J Cell Sci 2006;119:391–3. https://doi.org/10.1242/jcs.02793.

[2] Geyfman M, Plikus MV, Treffeisen E, Andersen B, Paus R. Resting no more: re-defining telogen, the maintenance stage of the hair growth cycle. Biol Rev Camb Philos Soc 2015;90:1179–96. https://doi.org/10.1111/brv.12151.
[3] Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic mini-organ. Curr Biol 2009;19:R132–42. https://doi.org/10.1016/j.cub.2008.12.005.
[4] Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev 2001;81:449–94. https://doi.org/10.1152/physrev.2001.81.1.449.
[5] Paus R, Foitzik K. In search of the "hair cycle clock": a guided tour. Differentiation 2004;72:489–511. https://doi.org/10.1111/j.1432-0436.2004.07209004.x.
[6] Lee NE, Park SD, Hwang H, Choi SH, Lee RM, Nam SM, Choi JH, Rhim H, Cho IH, Kim HC, et al. Effects of a gintonin-enriched fraction on hair growth: an in vitro and in vivo study. J Ginseng Res 2020;44:168–77. https://doi.org/10.1016/j.jgr.2019.05.013.
[7] Keum DI, Pi LQ, Hwang ST, Lee WS. Protective effect of Korean Red Ginseng against chemotherapeutic drug-induced premature catagen development assessed with human hair follicle organ culture model. J Ginseng Res 2016;40:169–75. https://doi.org/10.1016/j.jgr.2015.07.004.
[8] Zhou L, Wang H, Jing J, Yu L, Wu X, Lu Z. Morroniside regulates hair growth and cycle transition via activation of the Wnt/β-catenin signaling pathway. Sci Rep 2018;8:13785. https://doi.org/10.1038/s41598-018-32138-2.
[9] Castela M, Linay F, Roy E, Moguelet P, Xu J, Holzenberger M, Khosrotehrani K, Aractingi S. Igf1r signalling acts on the anagen-to-catagen transition in the hair cycle. Exp Dermatol 2017;26:785–91. https://doi.org/10.1111/exd.13287.
[10] Bai X, Lei M, Shi J, Yu Y, Qiu W, Lai X, Liu Y, Yang T, Yang L, Widelitz RB, et al. Roles of gasderminA3 in catagen-telogen transition during hair cycling. J Invest Dermatol 2015;135:2162–72. https://doi.org/10.1038/jid.2015.147.
[11] Rishikaysh P, Dev K, Diaz D, Qureshi WM, Filip S, Mokry J. Signaling involved in hair follicle morphogenesis and development. Int J Mol Sci 2014;15:1647–70. https://doi.org/10.3390/ijms15011647.
[12] Enshell-Seijffers D, Lindon C, Kashiwagi M, Morgan BA. beta-catenin activity in the dermal papilla regulates morphogenesis and regeneration of hair. Dev Cell 2010;18:633–42. https://doi.org/10.1016/j.devcel.2010.01.016.
[13] Gao C, Cao W, Bao L, Zuo W, Xie G, Cai T, Fu W, Zhang J, Wu W, Zhang X, et al. Autophagy negatively regulates Wnt signalling by promoting Dishevelled degradation. Nat Cell Biol 2010;12:781–90. https://doi.org/10.1038/ncb2082.
[14] Fan Q, Yang L, Zhang X, Ma Y, Li Y, Dong L, Zong Z, Hua X, Su D, Li H, et al. Autophagy promotes metastasis and glycolysis by upregulating MCT1 expression and Wnt/β-catenin signaling pathway activation in hepatocellular carcinoma cells. J Exp Clin Cancer Res 2018;37:9. https://doi.org/10.1186/s13046-018-0673-y.
[15] Jing Q, Li G, Chen X, Liu C, Lu S, Zheng H, Ma H, Qin Y, Zhang D, Zhang S, et al. Wnt3a promotes radioresistance via autophagy in squamous cell carcinoma of the head and neck. J Cell Mol Med 2019;23:4711–22. https://doi.org/10.1111/jcmm.14394.
[16] Glick D, Barth S, Macleod KF. Autophagy: cellular and molecular mechanisms. J Pathol 2010;221:3–12. https://doi.org/10.1002/path.2697.
[17] Doherty J, Baehrecke EH. Life, death and autophagy. Nat Cell Biol 2018;20:1110–7. https://doi.org/10.1038/s41556-018-0201-5.
[18] Rubinsztein DC, Mariño G, Kroemer G. Autophagy and aging. Cell 2011;146:682–95. https://doi.org/10.1016/j.cell.2011.07.030.
[19] Murrow L, Debnath J. Autophagy as a stress response and quality control mechanism: implications for cell injury and human disease. Annu Rev Pathol 2013;8:105–37. https://doi.org/10.1146/annurev-pathol-020712-163918.
[20] Kim JK, Shin KK, Kim H, Hong YH, Choi W, Kwak YS, Han CK, Hyun SH, Cho JY. Korean Red Ginseng exerts anti-inflammatory and autophagy-promoting activities in aged mice. J Ginseng Res 2021;45:717–25. https://doi.org/10.1016/j.jgr.2021.03.009.
[21] Choi W, Kim HS, Park SH, Kim D, Hong YD, Kim JH, Cho JY. Syringaresinol derived from *Panax ginseng* berry attenuates oxidative stress-induced skin aging via autophagy. J Ginseng Res 2022;46:536–42. https://doi.org/10.1016/j.jgr.2021.08.003.
[22] Hou J, Jeon B, Baek J, Yun Y, Kim D, Chang B, Kim S, Kim S. High fat diet-induced brain damaging effects through autophagy-mediated senescence, inflammation and apoptosis mitigated by ginsenoside F1-enhanced mixture. J Ginseng Res 2022;46:79–90. https://doi.org/10.1016/j.jgr.2021.04.002.
[23] Chai M, Jiang M, Vergnes L, Fu X, de Barros SC, Doan NB, Huang W, Chu J, Jiao J, Herschman H, et al. Stimulation of hair growth by small molecules that activate autophagy. Cell Rep 2019;27:3413–21. https://doi.org/10.1016/j.celrep.2019.05.070. e3.
[24] Parodi C, Hardman JA, Allavena G, Marotta R, Catelani T, Bertolini M, Paus R, Grimaldi B. Autophagy is essential for maintaining the growth of a human (mini-)organ: evidence from scalp hair follicle organ culture. PLOS Biol 2018;16:e2002864. https://doi.org/10.1371/journal.pbio.2002864.
[25] Yoshii SR, Mizushima N. Monitoring and measuring autophagy. Int J Mol Sci 2017;18:1865. https://doi.org/10.3390/ijms18091865.
[26] Yamamoto A, Tagawa Y, Yoshimori T, Moriyama Y, Masaki R, Tashiro Y. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. Cell Struct Funct 1998;23:33–42. https://doi.org/10.1247/csf.23.33.
[27] Greco V, Chen T, Rendl M, Schober M, Amalia Pasolli H, Stokes N, Dela Cruz-Racelis J, Fuchs E. A two-step mechanism for stem cell activation during hair regeneration. Cell Stem Cell 2009;4:155–69. https://doi.org/10.1016/j.stem.2008.12.009.
[28] Koh E, Jang OH, Hwang KH, An YN, Moon BK. Effects of steaming and air-drying on ginsenoside composition of Korean ginseng (*Panax ginseng* C.A. Meyer). J Food Process Preserv 2015;39:207–13. https://doi.org/10.1111/jfpp.12412.
[29] Joo KM, Lee JH, Jeon HY, Park CW, Hong DK, Jeong HY, Lee SJ, Lee SY, Lim KM. Pharmacokinetic study of ginsenoside Re with pure ginsenoside Re and ginseng berry extracts in mouse using ultra performance liquid chromatography/mass spectrometric method. J Pharm Biomed Anal 2010;51:278–83. https://doi.org/10.1016/j.jpba.2009.08.013.
[30] Kim AD, Kang KA, Kim HS, Kim DH, Choi YH, Lee SJ, Kim HS, Hyun JW. A ginseng metabolite, compound K, induces autophagy and apoptosis via generation of reactive oxygen species and activation of JNK in human colon cancer cells. Cell Death Dis 2013;4:e750. https://doi.org/10.1038/cddis.2013.273.
[31] Mai TT, Moon JY, Song YW, Viet PQ, Phuc PV, Lee JM, Yi TH, Cho M, Cho SK. Ginsenoside F2 induces apoptosis accompanied by protective autophagy in breast cancer stem cells. Cancer Lett 2012;321:144–53. https://doi.org/10.1016/j.canlet.2012.01.045.
[32] Zhuang J, Yin J, Xu C, Mu Y, Lv S. 20(S)-Ginsenoside Rh2 induce the apoptosis and autophagy in U937 and K562 cells. Nutrients 2018;10:328. https://doi.org/10.3390/nu10030328.
[33] Andl T, Reddy ST, Gaddapara T, Millar SE. WNT signals are required for the initiation of hair follicle development. Dev Cell 2002;2:643–53. https://doi.org/10.1016/s1534-5807(02)00167-3.
[34] Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. Mol Cell Biol 2002;22:1172–83. https://doi.org/10.1128/MCB.22.4.1172-1183.2002.
[35] Liu J, Xiao Q, Xiao J, Niu C, Li Y, Zhang X, Zhou Z, Shu G, Yin G. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther 2022;7:3. https://doi.org/10.1038/s41392-021-00762-6.
[36] Steinhart Z, Angers S. Wnt signaling in development and tissue homeostasis. Development 2018;145:dev146589. https://doi.org/10.1242/dev.146589.
[37] MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. Dev Cell 2009;17:9–26. https://doi.org/10.1016/j.devcel.2009.06.016.
[38] Chien AJ, Conrad WH, Moon RT. A Wnt survival guide: from flies to human disease. J Invest Dermatol 2009;129:1614–27. https://doi.org/10.1038/jid.2008.445.
[39] Truong VL, Keum YS, Jeong WS. Red ginseng oil promotes hair growth and protects skin against UVC radiation. J Ginseng Res 2021;45:498–509. https://doi.org/10.1016/j.jgr.2020.12.008.
[40] Lee JH. Wnt signaling in hair follicle development. Asian J Beauty Cosmetol 2017;15:242–6. https://doi.org/10.20402/ajbc.2016.0141.
[41] Kishimoto J, Burgeson RE, Morgan BA. Wnt signaling maintains the hair-inducing activity of the dermal papilla. Genes Dev 2000;14:1181–5.
[42] Premanand A, Rajkumari BR. Androgen modulation of Wnt/β-catenin signaling in androgenetic alopecia. Arch Dermatol Res 2018;310:391–9. https://doi.org/10.1007/s00403-018-1826-8.
[43] Kim SN, Kim S, Hong YD, Park H, Shin SH, Kim AR, Park BC, Shin SS, Park JS, Park M, et al. The ginsenosides of Panax ginseng promote hair growth via similar mechanism of minoxidil. J Dermatol Sci 2015;77:132–4. https://doi.org/10.1016/j.jdermsci.2014.12.007.
[44] Lee Y, Kim SN, Hong YD, Park BC, Na Y. Panax ginseng extract antagonizes the effect of DKK-1-induced catagen-like changes of hair follicles. Int J Mol Med 2017;40:1194–200. https://doi.org/10.3892/ijmm.2017.3107.
[45] Higgins CA, Richardson GD, Ferdinando D, Westgate GE, Jahoda CAB. Modelling the hair follicle dermal papilla using spheroid cell cultures. Exp Dermatol 2010;19:546–8. https://doi.org/10.1111/j.1600-0625.2009.01007.x.
[46] Higgins CA, Chen JC, Cerise JE, Jahoda CAB, Christiano AM. Microenvironmental reprogramming by three-dimensional culture enables dermal papilla cells to induce de novo human hair-follicle growth. Proc Natl Acad Sci U S A 2013;110:19679. https://doi.org/10.1073/pnas.1309970110. –88.
[47] Oh JW, Kloepper J, Langan EA, Kim Y, Yeo J, Kim MJ, Hsi TC, Rose C, Yoon GS, Lee SJ, et al. A guide to studying human hair follicle cycling in vivo. J Invest Dermatol 2016;136:34–44. https://doi.org/10.1038/JID.2015.354.
[48] Kloepper JE, Sugawara K, Al-Nuaimi Y, Gáspár E, van Beek N, Paus R. Methods in hair research: how to objectively distinguish between anagen and catagen in human hair follicle organ culture. Exp Dermatol 2010;19:305–12. https://doi.org/10.1111/j.1600-0625.2009.00939.x.