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Red Ginseng Extract Promotes the Hair Growth in Cultured Human Hair Follicles

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ABSTRACT Ginseng has been shown to promote hair growth in several recent studies. However, its effects on human hair follicles and its mechanisms of action have not been sufficiently elucidated. This study aimed to investigate the hair growth-promoting effects of red ginseng extract (RGE) and its ginsenosides. The proliferative activities of cultured human hair follicles treated with RGE and ginsenoside-Rb1 were assessed using Ki-67 immunostaining. Their effects on isolated human dermal papilla cells (hDPCs) were evaluated using cytotoxicity assays, immunoblot analysis of signaling proteins, and the determination of associated growth factors. We examined the ability of RGE and ginsenosides to protect hair matrix keratinocyte proliferation against dihydrotestosterone (DHT)-induced suppression and their effects on the expression of androgen receptor. The *in vivo* hair growth-promoting effect of RGE was also investigated in C57BL/6 mice. Both RGE and ginsenoside-Rb1 enhanced the proliferation of hair matrix keratinocytes. hDPCs treated with RGE or ginsenoside-Rb1 exhibited substantial cell proliferation and the associated phosphorylation of ERK and AKT. Moreover, RGE, ginsenoside-Rb1, and ginsenoside-Rg3 abrogated the DHT-induced suppression of hair matrix keratinocyte proliferation and the DHT-induced upregulation of the mRNA expression of androgen receptor in hDPCs. Murine experiments revealed that the subcutaneous injection of 3% RGE resulted in more rapid hair growth than the negative control. In conclusion, RGE and its ginsenosides may enhance hDPC proliferation, activate ERK and AKT signaling pathways in hDPCs, upregulate hair matrix keratinocyte proliferation, and inhibit the DHT-induced androgen receptor transcription. These results suggest that red ginseng may promote hair growth in humans.

KEY WORDS: • ***cell growth*** • ***ginseng*** • ***ginsenosides*** • ***proliferation*** • ***signal transduction***

INTRODUCTION

RED GINSENG IS THE steamed root of *Panax ginseng* C. A. Meyer, which has a long history use as a medicinal herb in East Asia.[1,2] Recent studies have reported various effects of red ginseng, including antioxidant, antitumor, antimutagenic, antidiabetic, and immunomodulatory activities.[3,4] Accumulating evidence suggests that the major pharmacologically active ingredients are ginsenosides, which have a four-ring, steroid-like structure with attached sugar moieties.[1,5] *P. ginseng* contains >40 different ginsenosides, including ginsenoside-Rb1, -Rb2, -Rc, -Rd, -Re, -Rf, -Rg1, -Rg2, -Rg3, -Rh1, and -Ro.[5] Of these, ginsenoside-Rb1 is the quantitatively most significant component of red ginseng.[6] Ginsenoside-Rg3 reportedly inhibits the transcriptional activities of androgen receptors and downregulates androgen receptors in a prostate cancer cell line.[7]

Several murine studies support the hypothesis that ginseng and its ginsenosides may promote hair growth.[8–10] The administration of ginseng suppresses apoptosis in hair follicles in irradiated mice.[8] In addition, red ginseng extract (RGE) promotes the growth of cultured murine vibrissal hair follicles in a dose-dependent manner, and ginsenoside-Rb1 has been identified as a potential active ingredient.[9] In addition to these murine studies, a human *in vivo* study by Kim *et al.* reported that the hair density of androgenetic alopecia patients increased following the oral administration of RGE, although the study did not determine if the difference between the ginseng and placebo groups was statistically significant.[11] Regardless, none of

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these studies proposed a mechanism that explains the effects of red ginseng.

In our present study, we evaluated the *in vivo* efficacy of RGE on hair growth in mice. We also investigated the effects of RGE, ginsenoside-Rb1, and ginsenoside-Rg3 on cultured human hair follicles and suggest a possible mechanism for these effects.

MATERIALS AND METHODS

Materials

Korean RGE was manufactured from the fresh roots of 6-year-old ginseng (*P. ginseng*) and supplied by the Korea Ginseng Corporation (Seoul, Korea). High-performance liquid chromatography confirmed that the Korean RGE samples contained ginsenoside-Rb1 (8.27 mg/g), -Rb2 (3.22 mg/g), -Rc (3.90 mg/g), -Rd (1.09 mg/g), -Re (2.58 mg/g), -Rf (1.61 mg/g), -Rg1 (2.01 mg/g), -Rg2(s) (1.35 mg/g), -Rg3(s) (1.04 mg/g), -Rg3(r) (0.67 mg/g), -Rh1 (0.95 mg/g), and other minor ginsenosides.[12] The Korean RGE was dissolved in distilled water to a final concentration of 100 mg/mL just before use. Ginsenoside-Rb1 (Santa Cruz Biotechnologies, Santa Cruz, CA, USA) and ginsenoside-Rg3 (Santa Cruz Biotechnologies) were dissolved in distilled water and methanol, respectively, to a final concentration of 50 mg/mL immediately before use. Dihydrotestosterone (DHT) was purchased from Sigma (St. Louis, MO, USA).

Hair growth in C57BL/6 mice

Six-week-old female C57BL/6 mice were purchased from OrientBio, Inc. (Seongnam, Korea) and allowed to acclimate under specific pathogen-free conditions for 1 week. The dorsal hair was shaved using clippers and anagen development was assessed. To assess the hair growth-promoting activity of RGE, animals were randomly divided into three groups ($n = 4$ mice/group): the treatment group (subcutaneous injection of 3% RGE), the negative control (subcutaneous injection of normal saline), and the positive control (topical application of 0.5% minoxidil solution). One hundred microliters of each reagent was administrated every other day for 7 weeks. Hair growth was assessed by visual scoring once a week for 7 weeks following the first administration (Table 1).[13] In addition, BrdU (Invitrogen, Carlsbad, CA, USA) was intraperitoneally injected into each mouse 2 h before skin biopsy on day 49 following administration. Sections were incubated with anti-BrdU and anti-Ki-67 as further described in the Immunofluorescence Staining section. This experiment was approved by the Institutional Animal Care and Usage Committee and conducted in accordance with the American Physiological Society Guiding Principles in the Care and Use of Animals.

Immunofluorescence staining

To visualize any proliferating cells, paraffin sections (4-μm thick) of the mouse skin were deparaffinized with xylene, rehydrated using graded alcohol solutions, and then incubated in 0.1 N HCl for 20 min at 37°C. Frozen blocks of cultured human hair follicles were cut to a thickness of 7 μm, and the sections were fixed in acetone for 10 min at 4°C. Primary antibodies included the sheep anti-BrdU antibody (Invitrogen) and mouse anti-Ki-67 antibody (Invitrogen). Before the anti-Ki-67 treatment, the sections were blocked with 1.5% normal horse serum in phosphate-buffered saline (PBS). The sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI), washed twice with PBS, and then analyzed using an LSM-710 confocal microscope (Zeiss, Oberkochen, Germany).

Table 1. Visual Scoring Scale Used to Assess Hair Growth in the Study Mice

Score	*Condition*
0	No change
1	< 30% darkening of the affected area
2	30–70% darkening of the affected area
3	> 70% darkening of the affected area or hair growth in < 30% of the affected area
4	> 70% darkening of the affected area and hair growth in 30–70% of the affected area
5	> 70% darkening of the affected area and hair growth in > 70% of the affected area
6	Hair growth in > 90% of the affected area

Isolation and culture of human hair follicles

Nonbalding scalp specimens were obtained from patients undergoing hair transplantation surgery. Hair follicles from these samples were used in the organ culture studies. Hair follicles were isolated and cultured as previously described.[14]

Culturing dermal papilla cells and outer root sheath keratinocytes

Human dermal papilla cells (hDPCs) were isolated as described previously,[15] cultured in Follicle Dermal Papilla Cell Media (PromoCell, Heidelberg, Germany; supplemented with 0.04 mL/mL fetal calf serum, 0.004 mL/mL bovine pituitary extract, 1 ng/mL basic fibroblast growth factor, and 5 μg/mL insulin), and passaged one to three times before use in this study. Human outer root sheath cells (hORSCs) were isolated from the same hair samples. The hair bulb region of each hair follicle was removed to avoid contaminating other cells. The cut hair follicles were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 20% fetal bovine serum. The medium was changed to keratinocyte growth medium (Gibco BRL) after 3 days, and the cells were passaged one to three times before use in this study.

Cytotoxicity assay

The cytotoxic effects of Korean RGE and ginsenoside-Rb1 on proliferating cells were assayed using a Cell Counting Kit-8 (CCK-8; Dojindo Lab, Tokyo, Japan). Briefly, the cells (1×10^4 cells/well) were seeded onto 96-well culture plates and incubated overnight at 37°C. The

medium was removed and various concentrations of the Korean RGE and ginsenoside-Rb1 were added to each well. After 24 h of incubation, CCK-8 (10 μL) was added to each well and incubated at 37°C for 2 h. Absorbance was measured at 450 nm using a microplate reader (Victor X3; Perkin Elmer, Santa Clara, CA, USA). All experiments were performed in triplicate and repeated four times.

Western blot analysis

hDPCs were exposed to various concentrations of the Korean RGE (0, 100, or 300 μg/mL) or ginsenoside-Rb1 (0, 0.01, 10, or 15 μg/mL) for 15 min. Whole-cell extracts were isolated using a lysis buffer (1 mM Na_3VO_4, 1 mM NaF, and a protease inhibitor cocktail). Protein concentrations were measured using the Bradford assay (Bio-Rad, Hercules, CA, USA) and separated on 10% SDS-PAGE gels. Proteins were electrotransferred to Immobilon-P transfer membranes (Millipore, Billerica, MA, USA), incubated overnight with primary antibodies against ERK, phospho-ERK, AKT, phospho-AKT (Cell Signaling Technology, Inc., Danvers, MA, USA), and β-actin (Sigma), and then incubated with horseradish peroxidase-conjugated secondary antibodies. Protein expression was visualized using the Luminata Forte Western HRP Substrate (Millipore). The experiments were repeated thrice.

RNA isolation and reverse transcription–polymerase chain reaction

Total RNA was prepared from the hDPCs using the Trizol reagent (Ambion, Austin, TX, USA) according to the manufacturer's instructions and reverse transcribed to cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD, USA). cDNA was amplified using polymerase chain reaction (PCR) and specific primers (Table 2). The amplified products were separated on 2% agarose gel using ethidium bromide and visualized under ultraviolet light. The experiments were repeated thrices.

DHT treatment of the cultured hair follicles

DHT is a known inhibitor of human hair follicle proliferation and induces apoptosis in hDPCs.[16] To determine the antagonizing effects of RGE, ginsenoside-Rb1, and ginsenoside-Rg3 on the DHT-induced suppression of hair matrix keratinocyte proliferation, isolated human hair follicles were pretreated with the vehicle, 100 μg/mL RGE, 10 μg/mL ginsenoside-Rb1, or 10 μg/mL ginsenoside-Rg3 for 24 h and incubated with 10 nM DHT for 12 h.

Statistical analyses

All statistical analyses were performed using the statistical software R (version 2.15.3; The R Foundation for Statistical Computing, Vienna, Austria), and $P < .05$ was considered statistically significant. The mixed-effect models with Benjamini and Hochberg adjustments were used to analyze the effect of RGE on hair growth in mice, the viability of hDPCs, and the results of western blot analyses and reverse transcription (RT)-PCR.[17]

RESULTS

RGE promotes hair growth in C57BL/6 mice

Increased rates of hair growth due to treatments were confirmed by visual inspection of hair growth in C57BL/6 mice. The injection of 3% RGE started to promote hair growth within 28 days, was able to produce a significantly higher level of hair growth than the administration of normal saline from day 35 ($P = .035$, $< .001$, and .008 at days 35, 42, and 49, respectively), and showed comparable hair growth to that by 0.5% minoxidil until day 49 after starting administration (Fig. 1A). In addition, the double immunofluorescence staining for BrdU and Ki-67 revealed that 3% RGE induced significantly more cellular proliferation within hair follicles than the negative controls (Fig. 1B).

RGE and ginsenoside-Rb1 enhance the proliferation of hair matrix keratinocytes

Isolated human hair follicles were cultured in William's E media and 100 μg/mL RGE or 10 μg/mL ginsenoside-Rb1 was added to the culture media. In addition, 10 nM minoxidil was used as a positive control. After 2 days of culturing, the follicles were stained for Ki-67 immunofluorescence. Immunoreactivity to Ki-67 indicated that both RGE and ginsenoside-Rb1 significantly enhanced the proliferation of hair matrix keratinocytes in follicles in comparison with the negative control (Fig. 2).

Table 2. Specific Sequences of the Forward and Reverse Primers Used in the Reverse Transcription–Polymerase Chain Reactions

Gene	*Forward primer sequence (5′→3′)*	*Reverse primer sequence (5′→3′)*	*Size (bp)*
HGF	GCC TGA AAG ATA TCC CGA CA	TTC CAT GTT CTT GTC CCA CA	523
IGF-1	TGG ATG CTC TTC AGT TCG TG	CCT GCA CTC CCT CTA CTT GC	314
VEGF	AAG GAG GAG GGC AGA ATC AT	TTT CTT GCG CTT TCG TTT TT	380
AR	TCC TTC ACC AAT GTC AAC TC	AAG CGT CTT GAG CAG GAT GT	311
GAPDH	CAC CAT CTT CCA GGA GCG AG	GGA TTC TAA TAC GAC TCA CTA TAG GCT CAC GCC ACA GTT TCC CGG A	398

HGF, hepatocyte growth factor; *IGF-1*, insulin-like growth factor-1; *VEGF*, vascular endothelial growth factor; *AR*, androgen receptor; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

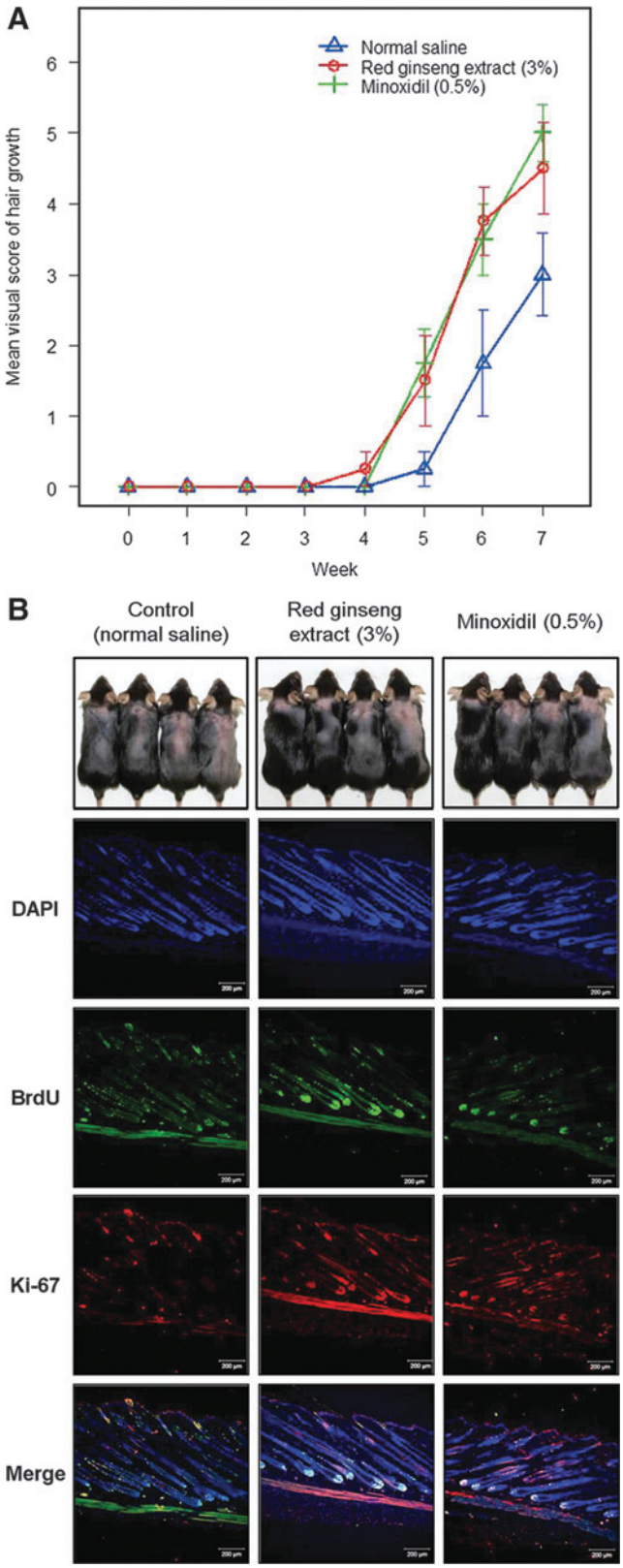

FIG. 1. Red ginseng extract (RGE) promotes hair growth in C57BL/6 mice. **(A)** Mean visual scores for hair growth. Error bars represent standard error of the mean. **(B)** Gross appearance and immunofluorescence staining for 4′,6-diamidino-2-phenylindole (DAPI), BrdU, and Ki-67 (×60 magnification) on day 49 of treatment.

RGE and ginsenoside-Rb1 induce the proliferation of hDPCs

Cytotoxicity assays were used to determine the effects of RGE and ginsenoside-Rb1 on the proliferation of hDPCs and hORSCs. Both RGE and ginsenoside-Rb1 affected the proliferation of hDPCs ($P = .002$ and $< .001$, respectively). RGE tended to increase the survival of hDPCs up to a concentration of 300 μg/mL, and the difference from the control was statistically significant at 300 μg/mL (*P*-values at concentrations of 100, 200, 300, and 400 μg/mL were 0.899, 0.097, 0.049, and 0.187, respectively; Fig. 3A). Ginsenoside-Rb1 significantly promoted the proliferation of hDPCs at concentrations of 5, 10, 15, and 20 μg/mL in comparison with the control (all *P*-values < 0.001, Fig. 3B). However, neither RGE nor ginsenoside-Rb1 resulted in consistent changes in the patterns of hORSC proliferation (data not shown).

RGE and ginsenoside-Rb1 activate both ERK and AKT signaling pathways in hDPCs

To investigate the mechanisms that result in the proliferation of hDPCs, signaling proteins were examined using western blot analysis. Both RGE and ginsenoside-Rb1 treatment increased phospho-ERK expression at 15 min ($P = .002$ and .013, respectively, Fig. 4). In comparison with the control, RGE enhanced the phospho-ERK expression at concentrations of 100 and 300 μg/mL (both *P*-values < 0.001), and ginsenoside-Rb1 at concentrations of 10 and 15 μg/mL (*P*-values at concentrations of 0.01, 10, and 15 μg/mL were 0.070, 0.002, and < 0.001, respectively). In addition, both RGE and ginsenoside-Rb1 treatments increased phospho-AKT expression at 15 min ($P = .031$ and .004, respectively, Fig. 4). In comparison with the control, RGE enhanced the phospho-AKT expression at a concentration of 100 μg/mL (*P*-values at concentrations of 100 and 300 μg/mL were < 0.001 and 0.426, respectively), and ginsenoside-Rb1 at concentrations of 0.01, 10, and 15 μg/mL (all *P*-values < 0.001).

Effects of RGE and ginsenoside-Rb1 on the expression of growth factors in hDPCs

To assess the expression levels of possible paracrine factors in hDPCs, RT-PCR was performed 24 h after administering 100 μg/mL RGE, 10 μg/mL ginsenoside-Rb1, or the vehicle (0.1% methanol for negative control). In the negative control, the RT-PCR did not detect any mRNA expressions of hepatocyte growth factor (*HGF*), insulin-like growth factor-1 (*IGF-1*), or vascular endothelial growth factor (*VEGF*) in hDPCs. Furthermore, their expressions were also nearly undetectable after RGE or ginsenoside-Rb1 treatment (data not shown). Similar results were obtained in three independent experiments, suggesting that other factors might be involved in the proliferation of hDPCs following treatment.

RGE, ginsenoside-Rb1, and ginsenoside-Rg3 abrogate the DHT-induced suppression of hair matrix keratinocyte proliferation

Ki-67 immunostaining demonstrated that treatment with 10 nM DHT clearly decreased cell proliferation in the hair

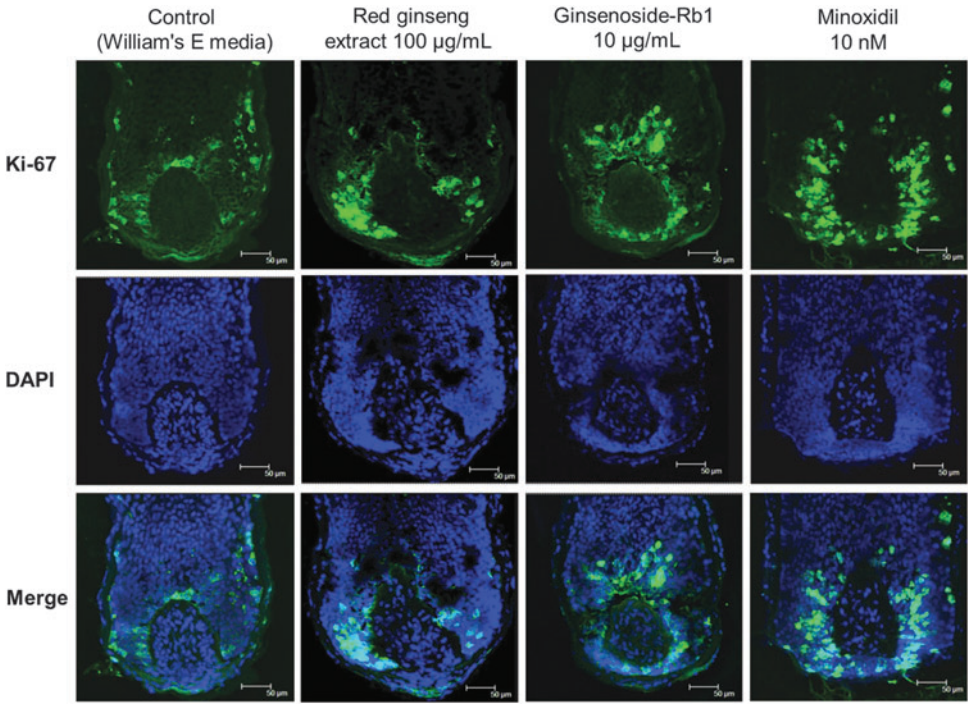

FIG. 2. RGE and ginsenoside-Rb1 induce the proliferation of hair matrix keratinocytes in *ex vivo* organ cultures of human hair follicles. RGE and ginsenoside-Rb1 increased the number of Ki-67-positive hair matrix keratinocytes in comparison with the negative control.

bulb in comparison with the negative control, and pretreatment with 100 μg/mL RGE, 10 μg/mL ginsenoside-Rb1, and 10 μg/mL ginsenoside-Rg3 abrogated the DHT-induced suppression of hair matrix keratinocyte proliferation (Fig. 5).

RGE, ginsenoside-Rb1, and ginsenoside-Rg3 abrogate the DHT-induced upregulation of the mRNA expression of androgen receptor in hDPCs

To explain how RGE, ginsenoside-Rb1, and ginsenoside-Rg3 could abrogate the suppressive effect of DHT on hair growth, the expression level of androgen receptor in hDPCs was assessed using RT-PCR. hDPCs were pretreated with the vehicle (0.1% methanol), 100 μg/mL RGE, 10 μg/mL ginsenoside-Rb1, or 10 μg/mL ginsenoside-Rg3 for 1 h and then incubated with 10 nM DHT for 24 h. The treatments significantly affected the mRNA level of androgen receptor in hDPCs ($P = .001$, Fig. 6). DHT upregulated the expression level of androgen receptor in comparison with the negative control ($P < .001$), and pretreatment with RGE, ginsenoside-Rb1, or ginsenoside-Rg3 abrogated the DHT-induced upregulation of the mRNA expression of androgen receptor (all P-values $< .001$).

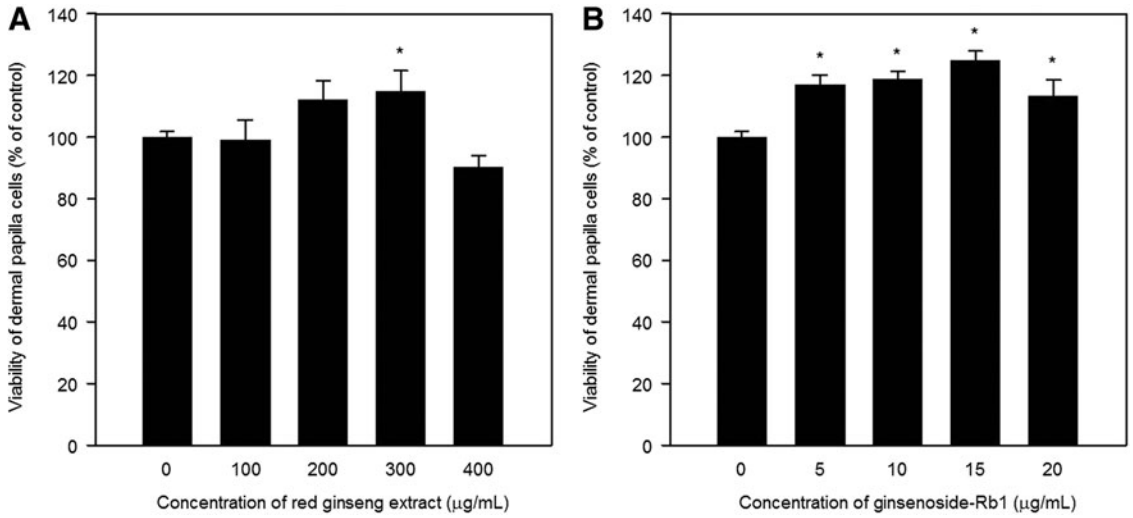

FIG. 3. Both RGE and ginsenoside-Rb1 induce the proliferation of human dermal papilla cells (hDPCs). **(A)** RGE tended to increase the proliferation of hDPCs up to a concentration of 300 μg/mL, and the difference from the control was statistically significant at 300 μg/mL. **(B)** Ginsenoside-Rb1 enhanced the proliferation of hDPCs at concentrations of 5, 10, 15, and 20 μg/mL in comparison with the control. Shown are the columns and error bars to denote the mean and SE of experiments performed in triplicate and repeated four times. *$P < .05$ versus 0 μg/mL.

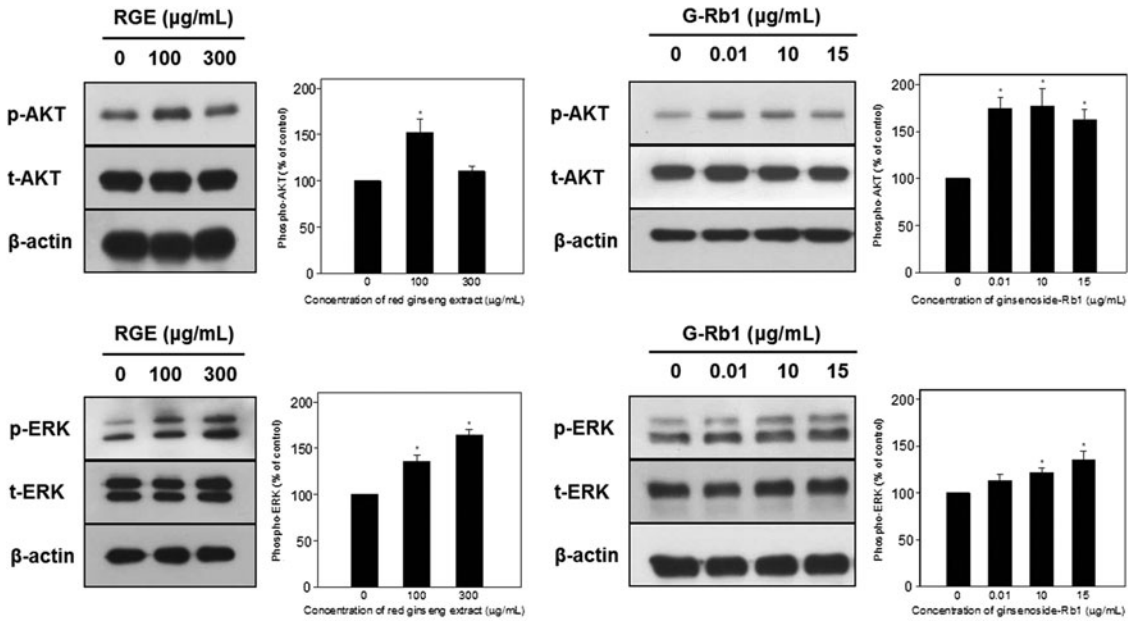

FIG. 4. RGE and ginsenoside-Rb1 (G-Rb1) activate the ERK and AKT signaling pathways in hDPCs. hDPCs were exposed to RGE or G-Rb1 for 15 min. ERK and AKT activations were evaluated using western blot analysis. Both the ratio of phospho-ERK (p-ERK) to total ERK (t-ERK) and that of phospho-AKT (p-AKT) to total AKT (t-AKT) were significantly increased by RGE or G-Rb1. Shown are the representative blots and columns and error bars to denote the mean and SE of the three independent experiments. $*P < .05$ versus 0 μg/mL.

DISCUSSION

Although no definitive evidence has been reported from human *in vivo* studies, several animal and human *in vitro* studies describe the hair growth-promoting effects of red ginseng and its ginsenosides.[8,9,11,18] However, there are no well-established mechanisms that explain these beneficial effects on hair growth. The present study confirmed the hair growth-promoting effects of RGE and ginsenoside-Rb1 through mouse *in vivo* and human *ex vivo* studies. Our cytotoxicity assays also demonstrated that RGE and ginsenoside-Rb1 increase the proliferation of hDPCs in a dose-dependent manner up to concentrations of 300 and 15 μg/mL, respectively. In addition, western blot analyses demonstrated

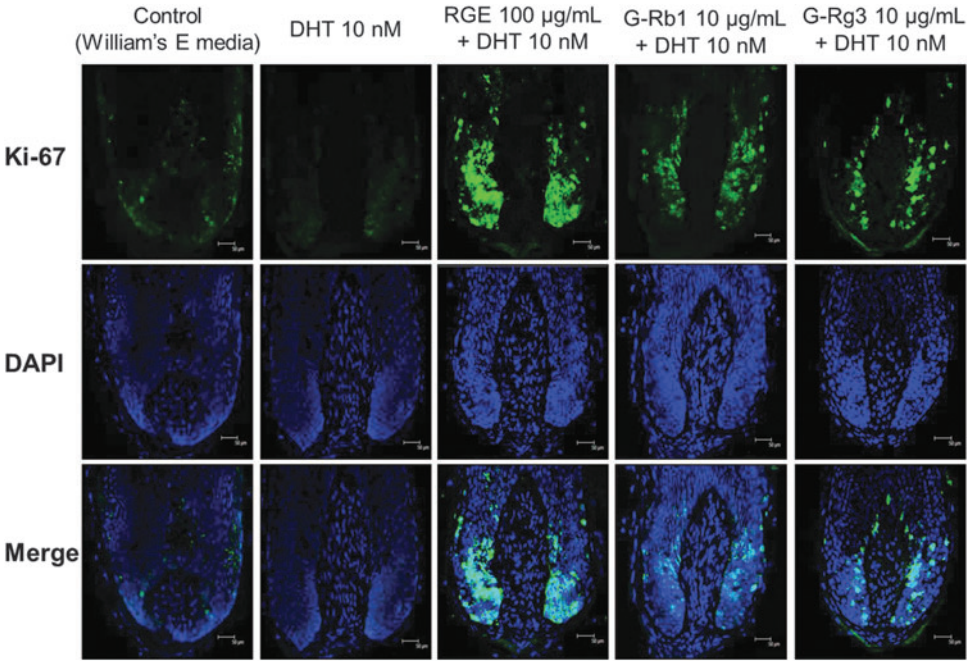

FIG. 5. RGE, G-Rb1, and ginsenoside-Rg3 (G-Rg3) abrogate dihydrotestosterone (DHT)-induced suppressive effects in hair matrix keratinocytes. Ki-67 immunostaining revealed that 10 nM DHT suppresses the proliferation of hair matrix keratinocytes and pretreatment with 100 μg/mL RGE, 10 μg/mL G-Rb1, and 10 μg/mL G-Rg3 effectively prevented its suppressive effects.

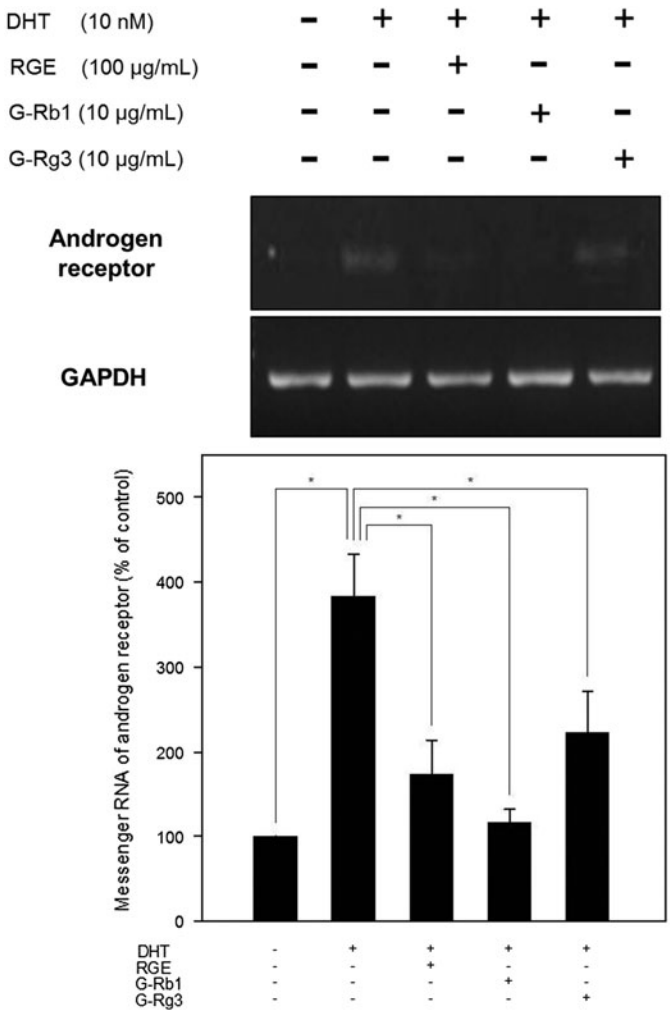

FIG. 6. RGE, G-Rb1, and G-Rg3 abrogate the DHT-induced upregulation of the mRNA expression of androgen receptor in hDPCs. hDPCs were pretreated with the vehicle (0.1% methanol), 100 μg/mL RGE, 10 μg/mL G-Rb1, or 10 μg/mL G-Rg3 for 1 h, and then incubated with 10 nM DHT for 24 h. The expression of androgen receptor in hDPCs was quantified using reverse transcription–polymerase chain reaction and normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). In comparison with the negative control, DHT upregulated the expression level of androgen receptor, and pretreatment with RGE, G-Rb1, or G-Rg3 abrogated the DHT-induced upregulation of androgen receptor. Shown are the representative blots and columns and error bars to denote the mean and SE of three independent experiments. $*P < .05$.

that the effects of RGE and ginsenoside-Rb1 are associated with activated ERK and AKT signaling pathways.

In our animal studies, 3% RGE enhanced hair growth and staining for both BrdU and Ki-67 and increased the proliferation of hair matrix cells. Furthermore, RGE and ginsenoside-Rb1 treatments increased the proliferation of hair matrix keratinocytes, as shown by the Ki-67 immunofluorescent activity of the cultured human hair follicles. However, neither RGE nor ginsenoside-Rb1 enhanced the proliferation of outer root sheath keratinocytes in human hair follicles according to the results of cytotoxicity assays. These results suggest that RGE and ginsenoside-Rb1 do not directly stimulate the proliferation of hair follicular keratinocytes. Instead, RGE and ginsenoside-Rb1 may affect other parts of the hair follicle that mediate the stimuli received by hair matrix keratinocytes.

The dermal papillae comprise the mesenchymal compartment of the hair follicle.[19,20] DPCs play pivotal roles in the induction of hair growth and the maintenance of the hair-producing functions of follicular epithelial cells,[19,21] which may be associated with the secretion of various soluble paracrine factors.[22] Moreover, the sizes of the dermal papillae, which depend on the number of DPCs, have been found to be well correlated with hair growth, and the number of DPCs increases during the growth phase of the hair cycle.[18,23] The proliferation of hDPCs in our current study seems to indicate the hair growth-promoting activities of red ginseng.

The ERK signaling pathway is known to be activated by mitogens in all types of mammalian cells and its key roles in cell growth have been established previously.[24] Furthermore, it was recently demonstrated that ERK activation plays an important role in the proliferation of hDPCs.[25] In our present study, both RGE and ginsenoside-Rb1 activated the ERK signaling pathway. Thus, the proliferation of hDPCs by red ginseng may be mediated by the ERK signaling pathways. AKT mediates critical signals for cell survival and also regulates the survival of DPCs as an antiapoptotic molecule.[26] Therefore, the activation of AKT by RGE and ginsenoside-Rb1 may prolong the survival of hDPCs.

Paracrine factors from hDPCs have been assumed to stimulate or regulate hair growth.[22,27,28] We also speculate that paracrine factors from hDPCs may help induce the proliferation of hair matrix cells due to RGE. However, we did not observe an increase in the expression of *IGF-1*, *HGF*, or *VEGF*, which are well-known mitogenic factors secreted by dermal papillae. As a myriad of types of factors were reported to be secreted by hDPCs,[29] the candidates for the paracrine factors cover a wide range of growth factors and cytokines, including fibroblast growth factor-7,[30] keratinocyte growth factor-2,[31] platelet-derived growth factor,[32,33] macrophage-stimulating protein,[34] angiogenin,[35] leptin,[36] stem cell factor,[37–39] interleukin-1β,[40,41] interleukin-6,[42] IGF binding protein,[39,43] transforming growth factor-β,[44] bone morphogenetic protein,[45] brain-derived neurotrophic factor,[46] and dickkopf 1.[47,39] However, little is yet known about which DPC-derived factors actually play a role in hair growth *in vivo*, and thus, there can be difficulties in identifying the paracrine factors to mediate the hair growth promotion by RGE. Further studies will be required to determine the RGE-induced factors promoting hair growth.

Recently, Murata *et al.* reported that red ginseng and ginsenoside-Rg3 inhibit 5α-reductase and that the topical application of RGE inhibits hair regrowth suppression in testosterone-treated mice.[10] Ginsenoside-Rg3 also inhibits the proliferation of prostate cancer cells by inhibiting the 5α-reduction of testosterone.[48] These results imply that the inhibition of 5α-reductase may promote hair growth.

5α-reductase is an enzyme that converts testosterone to DHT. DHT is the principal mediator of androgen-dependent hair loss,[49] and male patients with a genetic deficiency in 5α-reductase do not develop androgenetic alopecia.[50,51] Furthermore, finasteride, an inhibitor of 5α-reductase, is an efficacious drug for the treatment of androgenetic alopecia.[52]

However, our present study findings raise the possible existence of mechanisms other than the inhibition of 5α-reductase. In our current experiments, RGE, ginsenoside-Rb1, and ginsenoside-Rg3 abrogated the DHT-induced suppression of hair matrix keratinocyte proliferation. DHT is the product of testosterone and does not require the activity of 5α-reductase to affect hair follicles. In addition, the concentrations of RGE, ginsenoside-Rb1, and ginsenoside-Rg3 used in the present study were much lower than the levels that have been shown to inhibit 5α-reductase.[10] Because the inhibitory effect of DHT on hair growth is mediated by the androgen receptor in DPCs,[53] these results suggest that RGE may affect hair growth through the regulation of androgen receptor signaling. We proved this speculation by showing that RGE and its ginsenosides inhibit the DHT-induced upregulation of androgen receptor in hDPCs.

In conclusion, RGE may promote hair growth, possibly through the synergistic effects of its ginsenosides, including ginsenoside-Rb1 and -Rg3. Possible mechanisms include the indirect stimulation to hair follicular keratinocytes, which are possibly mediated by DPCs through the ERK and AKT signaling pathways, and protection against the DHT-induced suppression of hair matrix keratinocyte proliferation through inhibiting DHT-induced upregulation of androgen receptor in DPCs.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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