

Hair Growth-Promoting Mechanisms of Red Ginseng Extract through Stimulating Dermal Papilla Cell Proliferation and Enhancing Skin Health

Van-Long Truong and Woo-Sik Jeong

Food and Bio-industry Research Institute, School of Food Science & Biotechnology, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41566, Korea

ABSTRACT: This study aimed to investigate the underlying mechanisms of red ginseng extract (RGE) on regulating hair growth and hair follicle development. Results from *in vitro* studies showed that RGE treatment simultaneously enhanced viability and inhibited apoptosis in human hair dermal papilla cells. Moreover, RGE administration promoted telogen-to-anagen transition, prolonged anagen in hair follicular cycling, and increased the size of hair follicles and skin thickness in a C57BL/6 mouse model. Furthermore, RGE treatment significantly upregulated the expression of β -catenin, phospho-glycogen synthase kinase 3 β , cyclin D1, cyclin E, and Bcl-2, phospho-extracellular signal-regulated protein kinase, and phospho-Akt, which are associated with promoting hair growth. In addition, RGE enhanced skin health by activation of antioxidant defense systems. Our data demonstrates that hair regenerative mechanisms of RGE may be mediated by stimulating dermal papilla cell proliferation and enhancing skin functions.

Keywords: alopecia, β -catenin, dermal papilla cell, hair growth, red ginseng extract

INTRODUCTION

Hair, a vital skin appendage, stems from hair follicles and has diverse functions, including thermoregulation, protection, sensory, and tactile activities, and social interactions (Schneider et al., 2009). Hair follicles are complex regenerative organs that undergo repetitive cycles, with phases including anagen (growth), catagen (regression), and telogen (rest). Its function and cycling depend on effective interactions between different cell populations. Hair shafts are produced in anagen and their lengths corresponds to the duration of the anagen phase (Cotsarelis and Millar, 2001; Schneider et al., 2009).

Hair loss (alopecia or baldness) is a common condition that affects both men and women. Recently, the incidence of hair loss has dramatically increased in young people. Although alopecia is not life-threatening in humans, it causes psychological stress. Hair loss can be attributed to a wide range of factors, such as genetics, disease, nutritional insufficiency, aging, hormone change, and stress, and results in alterations to hair follicle cycles, including shortening of the anagen phase, augmenting the number of telogenic hair follicles, and changes to hair follicle mor-

phology (Cotsarelis and Millar, 2001). It has been suggested that effective strategies to combat hair loss include extending anagen, stimulating telogen-to-anagen transition of follicles, reversing miniaturization, and enhancing dermal papilla cell viability to stimulate new follicle generation. Minoxidil (MNX) and finasteride are current hair loss treatments for patients with alopecia in many countries. However, their therapeutic benefits are limited due to their transient and adverse effects (Park et al., 2011). Therefore, alternative strategies are necessary to develop new agents capable of preventing and/or treating hair loss.

Red ginseng (*Panax ginseng* Mayer) has long been used in traditional medicine in Asian countries, including China, Korea, and Japan. Recent studies have reported that red ginseng extract (RGE) contains a wide variety of ginsenosides, such as ginsenosides-Rb1, -Rb2, -Rc, -Rd, -Rg3, -Rg1, and -Ro, which are primarily responsible for its biological activities, including antioxidant, antitumor, antidiabetic, anti-inflammatory, and hepatoprotective effects (Yun, 2001; Lü et al., 2009; Lee and Kim, 2014). Previous reports have indicated that *P. ginseng* may stimulate hair growth, possibly due to action of its ginseno-

Received 20 April 2021; Revised 21 May 2021; Accepted 30 May 2021; Published online 30 September 2021

Correspondence to Woo-Sik Jeong, Tel: +82-53-950-5775, E-mail: wsjeong@knu.ac.kr
Author information: Van-Long Truong (Researcher), Woo-Sik Jeong (Professor)

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sides ginsenoside-Rb1 and -Rg3, in cultured mouse vibrissal hair follicles (Matsuda et al., 2003; Shin et al., 2014a), and in cultured human hair follicles (Park et al., 2015). Ginsenoside F2, an intestinal microorganism-mediated metabolite of ginsenoside-Rb1, has been reported to trigger telogen-to-anagen transition of hair follicles and hair regrowth via modulating Wnt/ β -catenin signal pathways in mouse (Shin et al., 2014c). Furthermore, ginsenoside F2 has been shown to reduce hair loss in dihydrotestosterone (DHT)-treated *in vitro* and *in vivo* models through preventing apoptosis (Shin et al., 2014b). In addition, ginseng rhizome and ginsenoside-Ro have been shown to exhibit inhibitory activity against 5 α -reductase and enhance hair regrowth in a mouse model of androgenic alopecia (Murata et al., 2012). Although clinical trials have found that consumption of Korean RGE improves hair density and thickness in patients with alopecia (Kim et al., 2009; Oh and Son, 2012), the molecular mechanisms of red ginseng on anagen induction have not been fully elucidated.

The study aimed to investigate the possible molecular mechanisms of RGE in promoting hair growth in human hair dermal papilla cells and a mouse model.

MATERIALS AND METHODS

Materials

Formaldehyde, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), hematoxylin, and eosin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). β -Catenin, vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), cyclin E, β -actin, cyclin D1, p-extracellular signal-related kinase (ERK), p-Akt, p-glycogen synthase kinase 3 β (GSK-3 β), Bcl-2, Bax, peroxidase-conjugated anti-rabbit, and peroxidase-conjugated anti-goat antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) or Cell Signaling Technology (Boston, MA, USA). MNX (3%) was purchased from Hyundai Pharm. Co., Ltd. (Chungnam, Korea). All other reagents used were of the analytical grade commercially available.

Preparation of RGE

RGE was kindly given by Korea Ginseng Corporation (Daejeon, Korea). Briefly, six-year-old red ginseng powders were extracted with ten volumes of potable water at 98°C for 48 h. After filtering, filtrates were condensed under reduced pressure to obtain darkish brown-red ginseng extracts.

Cell culture

Human hair dermal papilla cells (HHDPC) were purchased from ScienCell Research Laboratories, Inc. (Carls-

bad, CA, USA) and maintained in mesenchymal stem cell medium containing 5% fetal bovine serum, 1% mesenchymal stem cell growth supplement, and 1% penicillin/streptomycin at 37°C in a 5% CO₂ incubator. Cell culture medium was replenished every 2 days.

Cell viability assays

HHDPC was seeded onto 96-well microplate (3×10^4 cells/well) and incubated with different RGE concentrations (100, 200, 300, and 400 μ g/mL) or water (control) for 24 h. MTT solution (5 mg/mL) was added to each well and cells were incubated at 37°C for 4 h in the dark. Purple formazan product, a metabolite of MTT, was then solubilized with 100 μ L of dimethyl sulfoxide. The absorbance at 570 nm was measured using a microplate reader (BioTek Instruments, Winooski, VT, USA).

Animal experiments

Six-week-old male C57BL/6 mice (18~20 g) were obtained from Hyochang Science (Deagu, Korea). Mice were maintained under a standard condition, according to Institutional Animal Care and Use Guidelines of Inje University, who approved the mouse experiments with the approval number 2015-05 (Gimhae, Korea). After a week for acclimation, dorsal hairs were removed with hair clippers and hair removal cream from the 7-week-old mice (Veet, Oxy Reckitt Benckiser, Chartres, France). The control group received the vehicle (normal saline) alone, the RGE group received orally administered 50 mg/kg RGE, and the MNX group (positive control) received 3% MNX (topically, 200 μ L per mouse) every day for up to 21 days. The dorsal skin of the mice was photographed at 0, 7, 14, 17, and 21 days after treatment. Mice were subsequently sacrificed and skin tissues were harvested and stored at -80°C for further experiments.

Histological analysis

Dorsal skin tissues were collected and subjected to hematoxylin and eosin staining by using standard techniques. The stained sections were observed and analyzed through an optical microscope. Bulb diameter of hair follicles in the deep subcutis were measured at the level of the largest hair diameter of the hair bulbs with clearly visible dermal papilla. Skin thickness was measured from the epidermis to subcutaneous fat. Digital microimages were taken from representative areas using a digital camera (PAXcam, PAX-it, Villa Park, IL, USA).

Alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (γ -GT), and antioxidant enzyme activity assays

ALP activity assays were performed using an Alkaline Phosphatase Assay kit, according to manufacturer's instructions (BioAssay Systems, Wien, Austria). γ -GT activity assays were conducted using an Activity Colorime-

tric Assay kit (BioVision Inc., Milpitas, CA, USA). Catalase (CAT) activity was determined according to the method of Aebi (1984). One unit of CAT was described as the amount of enzyme required to decompose 1.0 μM of H_2O_2 in a minute. Glutathione peroxidase (GPx) activity assays were conducted following the method of Bogdanska and colleagues (2003). The amount of enzyme that oxidizes 1 nM of nicotinamide adenine dinucleotide phosphate hydrogen per min was defined as a unit of GPx. Manganese-superoxide dismutase (Mn-SOD) was measured by the method of Oyanagui (1984). One unit of SOD was defined as the amount of enzyme needed to inhibit 50% of the superoxide radicals.

Western blot analysis

Proteins were extracted from skin tissues or cultured (human hair dermal papilla cells) HHDPCs by homogenizing cells with radioimmunoprecipitation assay buffer. The protein content of each sample was measured using a BCA protein assay kits (Pierce Manufacturing, Appleton, WI, USA). To analyze protein expression, an equal amount of proteins from each group were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and transferred onto polyvinylidene fluoride membranes using a semidry transfer system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked with 5% non-fat milk and then hybridized with proper primary antibodies overnight at 4°C. After washing with 0.1% Tween-20 in phosphate-buffered saline, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 3 h at 4°C. Protein bands were visualized using enhanced chemiluminescence Western blotting reagents (Santa Cruz Biotechnology, Inc.).

Statistical analysis

Results are presented as mean \pm standard deviation (SD). Student's *t*-tests were used to analyze significant differences between control and treatment groups. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Effect of RGE on viability and apoptosis of HHDPC

To evaluate the effect of RGE on survival of HHDPCs, cells were incubated with different concentrations of RGE and cell viability was determined using MTT assays. RGE treatment significantly enhanced cell survival compared with the vehicle-treated control cells (Fig. 1A). RGE at 200 $\mu\text{g}/\text{mL}$ showed the greatest effect on cell viability, even higher than MNX, a positive control. However, the highest dose of RGE (400 $\mu\text{g}/\text{mL}$) was unlikely to significantly increase the viability of HHDPCs. Apoptosis directly affects cell viability and disturbs hair follicle cycling. RGE treatment upregulated expression of anti-apoptotic Bcl-2 and downregulated expression of apoptotic Bax protein. A similar result was observed for MNX-treated HHDPCs (Fig. 1B).

Effect of RGE on the Wnt/ β -catenin pathway in HHDPCs

We next examined the role of RGE in regulating the Wnt/ β -catenin pathway, which plays a central roles in hair development. Treatment with RGE and MNX significantly upregulated expression of β -catenin in HHDPCs compared with vehicle-treated control cells (Fig. 2). Moreover, the amount of p-GSK-3 β was enhanced by treatment with RGE and MNX. These data suggest that phosphorylation-induced inactivation of GSK-3 β by RGE results in an increase of β -catenin proteins in HHDPC.

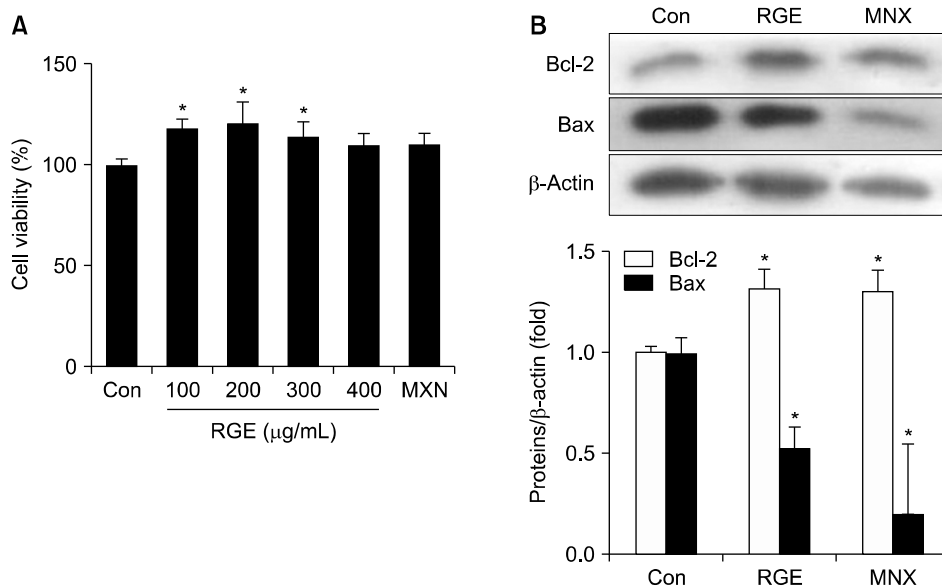


Fig. 1. Effect of RGE on HHDPC proliferation and apoptosis. HHDPCs were treated with various concentrations of RGE or MNX (100 μM) for 24 h. (A) Cell viability was measured using MTT assays, added as a percentage of the vehicle-treated control. (B) Cells were treated with RGE (100 $\mu\text{g}/\text{mL}$) or MNX (100 μM) for 24 h and protein expressions of Bcl-2 and Bax were analyzed by Western blotting. Data show the mean \pm SD of at least three independent experiments. * $P < 0.05$ values are considered significantly different. HHDPC, human hair dermal papilla cells; MNX, minoxidil; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; RGE, red ginseng extract.

Hair growth-promoting activity of RGE in C57BL/6 mice

Hair regenerative activity of RGE was further examined in an *in vivo* model. Mice were orally administered RGE or MNX (positive control) once daily for 21 days. Changes in back skin color, which is bright pink in telogen and turns gray/black in anagen, were used to evaluate hair growth. RGE administration significantly stimulated hair

growth and induced premature progression of hair follicles in the anagen phase of the hair cycle compared with vehicle-treated mice (Fig. 3A). Mice treated with both RGE and MNX exhibited gray skin by day 10 of treatment and dark skin with visible hair shafts by day 14, whereas large areas of dorsal skins remained without pigmentation in the control group. Dorsal skins of mice in both

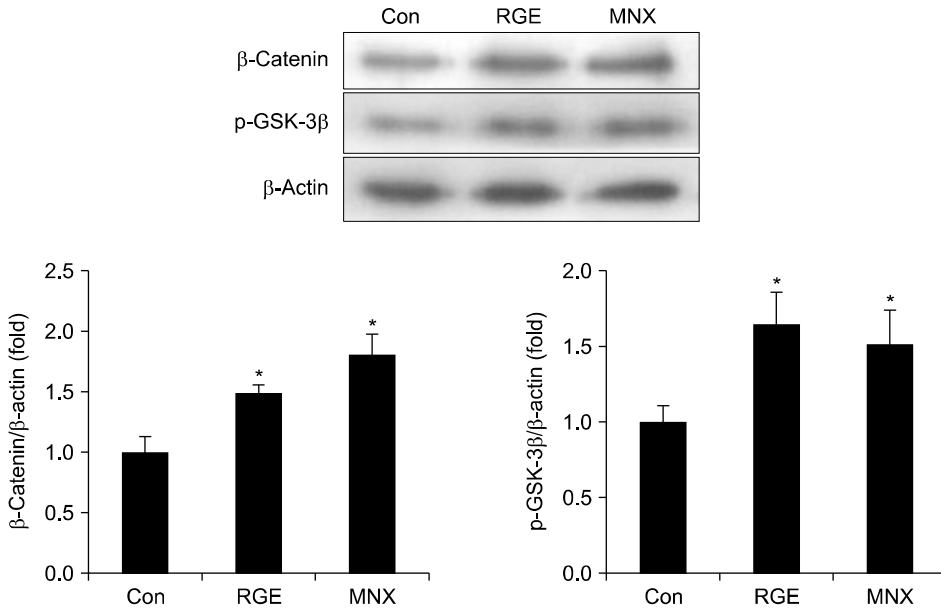


Fig. 2. Effect of RGE on the Wnt/β-catenin pathway in HHDP cells. HHDP cells were incubated with RGE or MNX (100 μM) for 24 h. Expressions of β-catenin and p-GSK-3β were detected using Western blotting. Data show the mean±SD of at least three independent experiments. **P*<0.05 values are considered significantly different. HHDP, human hair dermal papilla cells; MNX, minoxidil; p-GSK-3β, phosphoglycogen synthase kinase 3 beta; RGE, red ginseng extract.

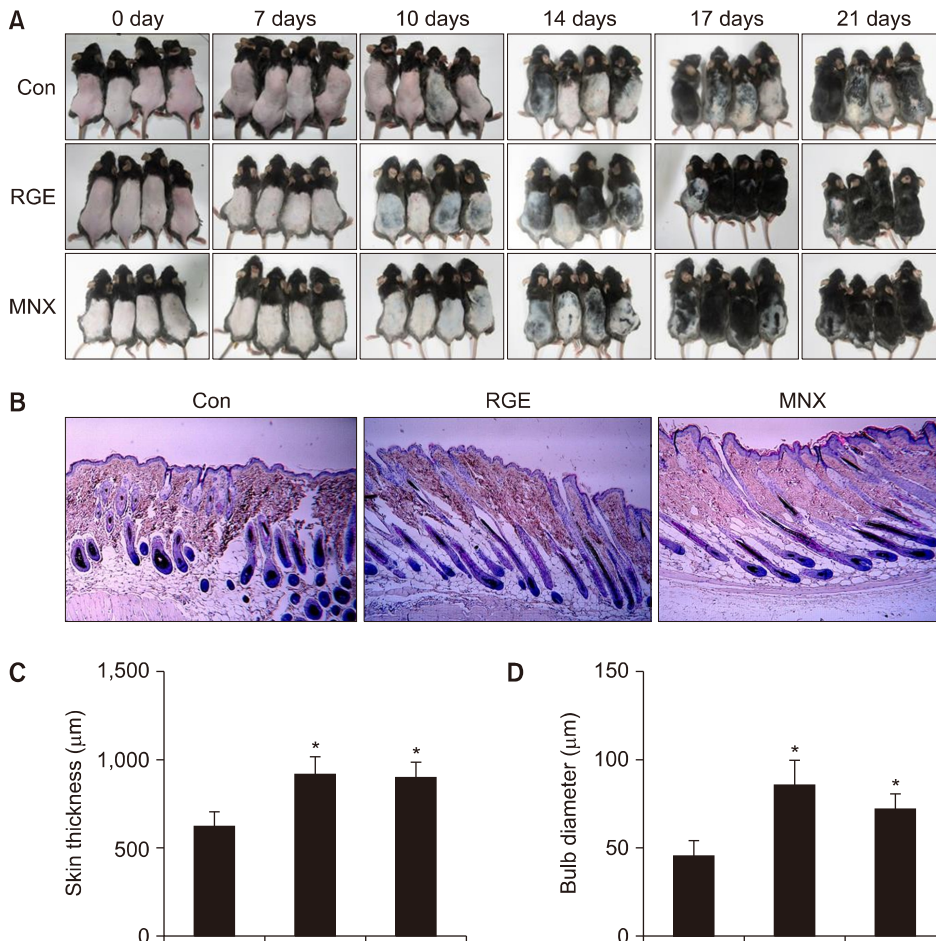


Fig. 3. Hair growth-promoting activity of RGE in C57BL/6 mice. After shaving, mice were treated daily with RGE (50 mg/kg) or 3% MNX for 21 consecutive days. (A) Dorsal skins were photographed at 0, 7, 14, 17, and 21 days. After treatment, dorsal skins were collected and subjected to H&E staining. Representative micro-images of (B) skin sections, (C) skin thickness and (D) hair follicle bulb diameters. Data show the mean±SD (n=4). **P*<0.05 values are considered significantly different. H&E, hematoxylin and eosin; MNX, minoxidil; RGE, red ginseng extract.

the RGE and MNX groups were covered with long hair shafts at day 17. Moreover, hair growth-promoting efficacy of RGE was comparable with that of MNX after 21 days of treatment.

Effects of RGE on development and structure of hair follicles

Histological analysis showed premature telogen-to-anagen conversion of hair follicles in both the RGE and MNX groups compared with the control group. Almost all hair follicles in the RGE and MNX groups appeared at anagen V/VI with hair shaft erupting out of the epidermis. However, some of hair follicles in the control group entered anagen IV/V of the hair cycle (Fig. 3B). In addition, RGE administration significantly increased skin thickness compared with administration of the vehicle (Fig. 3C). Furthermore, the sizes of the hair bulbs of mice in the RGE-treated group were greater than those of both the vehicle-treated and MNX-treated groups (Fig. 3D). These data suggest that RGE may induce early telogen-to-anagen transition of hairs and promote development of hair follicles, thereby stimulating hair regrowth.

Effect of RGE on levels of growth factors and hair growth indicators

Growth factors are involved in regulation of hair development and differentiation. After 21 days of treatment, RGE significantly increased VEGF levels in dorsal skin tissues compared with the vehicle. Likewise, IGF-1 protein expression was upregulated by RGE administration

(Fig. 4A), and ALP and γ -GT, typical biomedical markers of anagen in growing hair, were significantly elevated in mice treated with RGE (Fig. 4B and 4C). Furthermore, γ -GT activity was greater in the RGE-treated group than in the MNX-treated group (Fig. 4C).

RGE modulates the Wnt/ β -catenin pathway in mice

To explore possible hair regenerative mechanisms of RGE, we analyzed expression of hair induction-associated genes in skin tissues of the mice. The Wnt/ β -catenin pathway is involved in initiating and maintaining the anagen phase of the hair cycle (Enshell-Seijffers et al., 2010). Therefore, we surmised that RGE may stimulate hair regrowth by triggering the Wnt/ β -catenin pathway. RGE-treated mice exhibited a significant increase in the level of β -catenin compared with vehicle-treated mice. GSK-3 β , a β -catenin cytosolic suppressor, was inactivated by enhanced phosphorylation in mice treated with RGE (Fig. 5A). Similar results were observed for mice treated with MNX. In addition, Wnt target genes, including cyclin D1 and cyclin E, were upregulated by both RGE and MNX (Fig. 5B).

Effect of RGE on antioxidant status and apoptosis in mice

Oxidative stress may inhibit hair growth, cause skin and hair aging, and delay onset of the anagen phase of the hair cycle (Prie et al., 2015). Enhanced antioxidant defense systems in skin helps inhibit hair aging and promotes hair regeneration. We explored the effect of RGE on the antioxidant status of skin tissues by evaluating

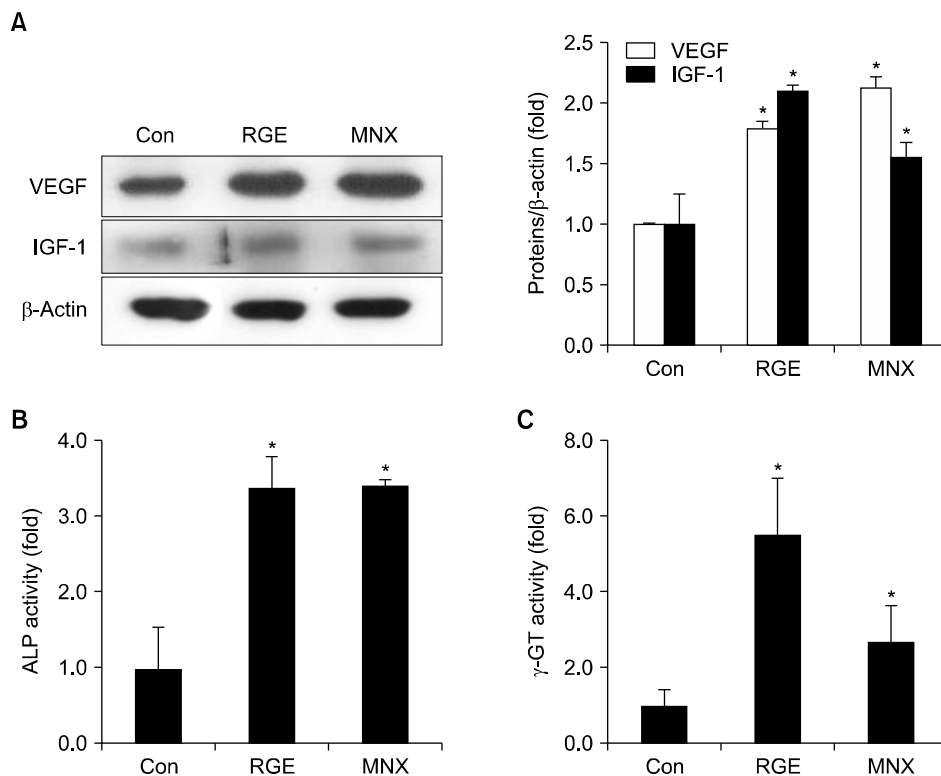


Fig. 4. Effect of RGE on levels of growth factors and hair growth indicators in C57BL/6 mice. (A) Expression of IGF-1 and VEGF in skin tissues, detected by Western blotting. Levels of ALP (B) and γ -GT (C) in skin tissues determined using commercial kits. Data show the mean \pm SD (n=3). * P <0.05 values are considered significantly different. ALP, alkaline phosphates; γ -GT, gamma-glutamyl transpeptidase; IGF-1, insulin-like growth factor; RGE, red ginseng extract; VEGF, vascular endothelial growth factor.

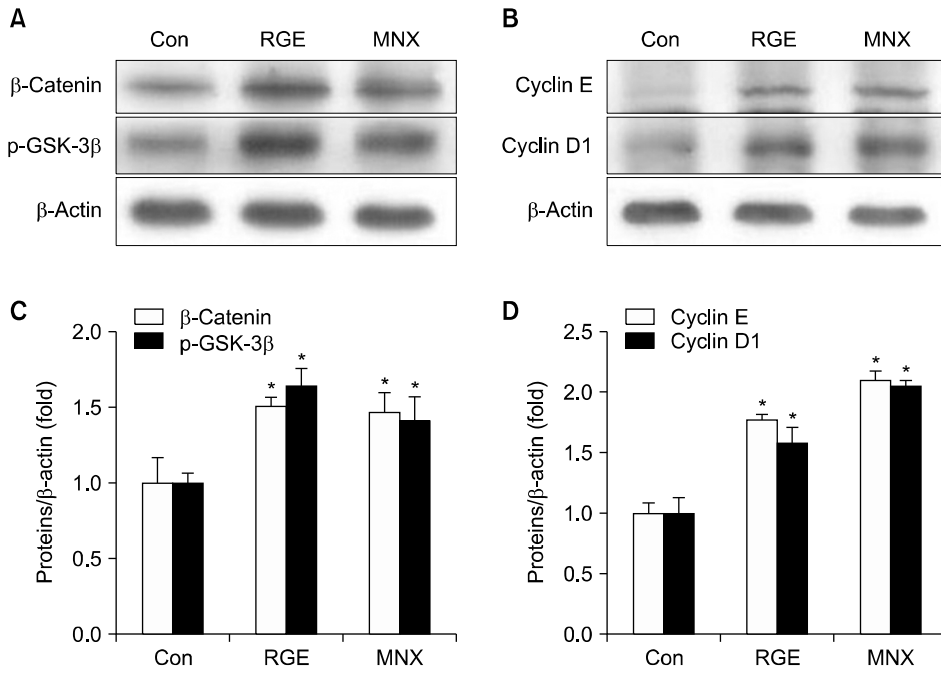


Fig. 5. Effect of RGE on the Wnt/β-catenin pathway in C57BL/6 mice. Expression of (A) p-GSK-3β and β-catenin, and (B) cyclin D1 and cyclin E in skin tissues were measured by Western blotting. Data show the mean±SD (n=3). **P*<0.05 values are considered significantly different. p-GSK-3β, phospho-glycogen synthase kinase 3 beta; RGE, red ginseng extract.

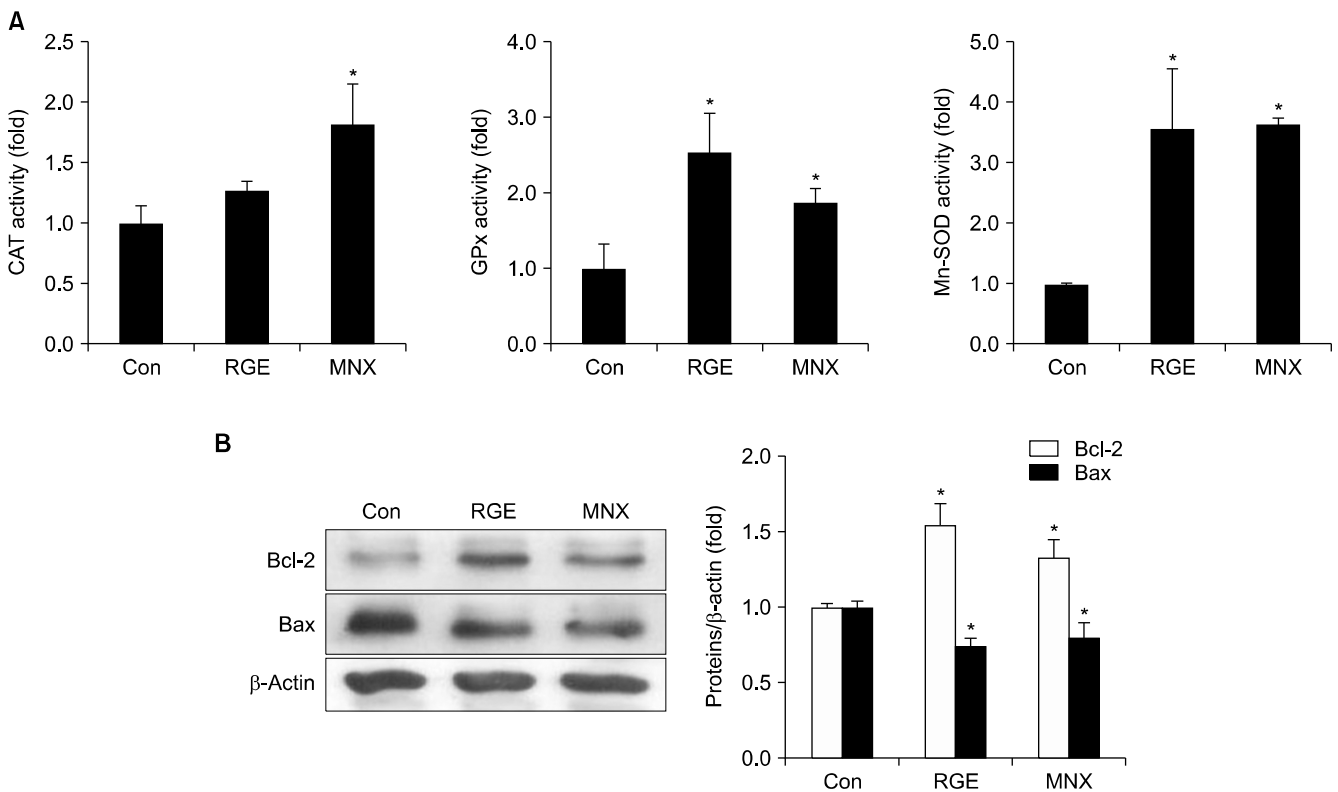


Fig. 6. Effect of RGE on antioxidant status of and apoptosis in C57BL/6 mice. (A) Activities of CAT, GPx, and Mn-SOD enzymes in skin tissues were measured as mentioned in the Materials and Methods. (B) Protein expressions of Bcl-2 and Bax in C57BL/6 mouse skin tissue were analyzed by Western blotting. Data show the mean±SD (n=3). **P*<0.05 values are considered significantly different. CAT, catalase; GPx, glutathione peroxidase; Mn-SOD, manganese-superoxide dismutase; RGE, red ginseng extract.

activity of antioxidant enzymes, such as CAT, GPx, and SOD-2. Activities of GPx and SOD-2 in RGE-treated mice were markedly increased. Although not significant, CAT activity was also slightly enhanced by RGE (Fig. 6A). Therefore, RGE treatment may enhance antioxidant defense system in mouse skin by increasing endogenous

antioxidant enzyme activity. Moreover, apoptosis that suppresses hair growth can be regulated by the ratio of Bcl-2 and Bax during the apoptosis-driven involution phase of the hair cycle. To examine whether RGE blocks apoptosis in hair follicles, we elucidated expression of two key proteins, Bcl-2 and Bax, in skin tissues. Results

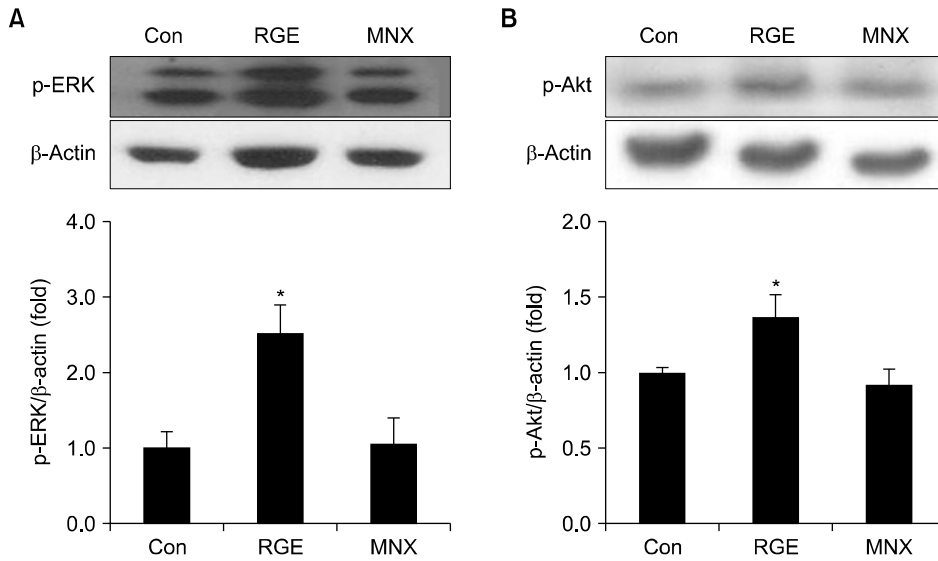


Fig. 7. Effect of RGE on kinase signaling pathways in C57BL/6 mice. Phosphorylation levels of ERK (A) and Akt (B) were detected by Western blotting. Data show the mean \pm SD (n=3). * P <0.05 values are considered significantly different. ERK, extracellular signal-related kinase; RGE, red ginseng extract.

indicated that treatment with RGE or MNX increased anti-apoptotic Bcl-2, but decreased pro-apoptotic Bax in skin tissue (Fig. 6B). These findings suggest that enhanced skin health induced by increased antioxidant status and regulation of apoptosis may, in part, contribute to the hair growth-promoting ability of RGE.

Effect of RGE on kinase signaling pathways in mice

Mitogen-activated protein kinase/ERK and phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathways are involved in the regulation of dermal papilla cell proliferation in hair follicles (Yun et al., 2005; Zhang et al., 2013). To investigate whether RGE activates intracellular signaling pathways, phosphorylated levels of ERK and Akt were determined in skin tissues. Results indicated that RGE significantly increased phosphorylation of ERK compared with the vehicle (Fig. 7A). Likewise, Akt phosphorylation was elevated by treatment with RGE (Fig. 7B).

DISCUSSION

Hair follicles are constituted by multiple layers of epithelial cells and a mass of dermal papilla cells. Hair dermal papilla cells that inhabit the dermal papilla anchored at the bottom of hair roots are necessary for hair formation, growth, and cycling (Yang and Cotsarelis, 2010). Dermal papilla cells are responsible for induction and maintenance of epithelial cell growth and differentiation by instructing surrounding epithelial cells to proliferate and differentiate into the multiple layers of the outgrowing hair shaft and the channel surrounding the hair shaft (Millar, 2002; Driskell et al., 2011). Moreover, the quantity of dermal papilla cells in the follicles correlates with the size and shape of the hair, with degradation of the dermal papilla population causing several hair thinning and loss conditions (Chi et al., 2013). Therefore, stimu-

lation of dermal papilla cell proliferation helps promote hair growth. Our study indicated that RGE may enhance proliferation of HHDPCs.

In addition, apoptosis affects both hair cell survival and hair growth. Activation of signaling pathways that trigger apoptosis in hair cells occurs throughout hair follicle regression. Bcl-2 family proteins, such as anti-apoptotic Bcl-2 and pro-apoptotic Bax, play roles as master regulators of apoptotic process in almost all cell types (Lindner et al., 1997). Treatment of HHDPCs with cisplatin induces production of reactive oxygen species and decreases of Bcl-2/Bax ratios, leading to HHDPC apoptosis and massive hair loss (Luanpitpong et al., 2011). Tea polyphenol epigallocatechin-3-gallate (Kwon et al., 2007) and MNX (Han et al., 2004) enhanced proliferation and inhibited apoptosis (by increasing Bcl-2/Bax ratios) of dermal papilla cells, thereby stimulating hair growth. In mice, 6-gingerol in ginger suppresses cultured human hair elongation and delays telogen-to-anagen transition of hair follicles by inhibitory and pro-apoptotic activities on dermal hair cells (Miao et al., 2013). Furthermore, DHT-mediated hair loss may arise from triggering of apoptotic cell death in hair follicles (Kwack et al., 2008). In our study, we demonstrated that RGE simultaneously upregulated Bcl-2 and downregulated Bax in both HHDPCs and in mouse skin, indicating enhanced survival of dermal cells in hair follicles.

Telogen-to-anagen transition of hair follicle is mediated by effective interactions among various signaling molecules and pathways (Rishikaysh et al., 2014). The Wnt/ β -catenin pathway is one of the most important signaling pathways producing a signal for hair follicle induction (Millar et al., 1999; Kishimoto et al., 2000). Usually, transcription factor β -catenin is anchored in the cytoplasm by phosphorylation of GSK-3 β , subsequently leading to proteasomal degradation of β -catenin (Ikeda et al., 2000). However, activation of upstream signal transduc-

tion cascades induces release of β -catenin from this inhibitory complex. Excessive β -catenin is translocated into the nucleus, where it cooperates with other transcription factors to activate target genes contributing to initiation of the transition from the late G1 to S phases of the cell cycle, including cyclin D1, cyclin E, or hair growth-promoting ALP (Behrens et al., 1996; Tetsu and McCormick, 1999). Recent reports have demonstrated that deletion of β -catenin abolishes direct Wnt/ β -catenin-mediated target gene cyclin D1 and decreased hair regeneration, and eventually causes hair loss (Huelsen et al., 2001; Choi et al., 2013). In contrast, activation of β -catenin in the epidermis promotes expansion of hair follicles during development (Zhang et al., 2008). In addition, inactivation of GSK-3 β by phosphorylation leads to liberation of β -catenin from inhibitory complexes, which enhances expression of β -catenin-mediated target genes crucial for dermal papilla cells growth and proliferation and promotes hair follicle regeneration and hair growth (Yamauchi and Kurosaka, 2009; Soma et al., 2012). In addition to induction of the telogen-to-anagen transition, Wnt/ β -catenin signaling helps prolong and maintain anagen (Kishimoto et al., 2000; Shimizu and Morgan, 2004). Blockage of the β -catenin gene within the dermal papilla of mature hair follicles attenuated proliferation of progenitors and their immediate progeny that produce the hair shaft, and prematurely induced the destructive phase (catagen) of the hair cycle (Enshell-Seijffers et al., 2010). Our findings show that RGE treatment upregulates β -catenin, p-GSK-3 β , cyclin D1, and cyclin E in HHDPs and mouse skin, suggesting that RGE may stimulate transition of telogenic hair follicles to the anagen phase through activating β -catenin signaling pathway, thereby promoting hair regrowth.

Hair regeneration is a complex process that involves growth factors, cytokines, and hormones (Peus and Pittelkow, 1996; Chi et al., 2013). Induction of angiogenesis is essential to satisfy the increased nutritional need of hair follicles during the anagen phase of rapid cell division. VEGF has been identified to induce hair follicle progression into anagen phase of the hair cycle and to enhance hair shaft elongation through stimulating vasculogenesis and angiogenesis (Yano et al., 2001). IGF-1 is an important growth factor that regulates cellular proliferation and differentiation, tissue remodeling and the hair cycle during development of hair follicles (Weger and Schlake, 2005). Loss of IGF-1 induces resting in the telogen phase and retards the onset of a second anagen phase. In the present study, we observed significant increases in VEGF and IGF-1 levels in RGE-treated mouse skins, indicating that RGE may promote hair regrowth, to some extent by inducing growth factors such as VEGF and IGF-1 in skin tissues. Moreover, stimulation of ERK and PI3K/Akt signaling pathways was also observed in

mice treated with RGE. These pathways regulate cell survival, cell proliferation, transcription, and cell migration (Hayashi et al., 2013). A previous study showed that RGE and its ginsenoside Rb1 enhances proliferation of human dermal papilla cells through activating ERK and Akt signaling pathways (Park et al., 2015). VEGF and IGF-1 have shown to signal through cooperation with several different signaling transduction pathways, such as ERK and Akt (Zachary, 2003; Hayashi et al., 2013). Furthermore, some studies have demonstrated that ERK and Akt pathways contribute to activation of transcription factors including β -catenin, resulting in enhanced hair follicular cell survival and proliferation, thereby promoting hair growth (Yun et al., 2005; Zhang et al., 2013).

Overall, this study demonstrates the role of RGE in promoting hair growth and suggests plausible underlying mechanisms for RGE-mediated activity. Our findings show that RGE induces early progression of hair follicles into the anagen phase and stimulates hair regrowth through enhancing proliferation of dermal papilla cells, activating Wnt/ β -catenin pathway, and inducing growth factors. Therefore, RGE could be a potent therapeutic agent for prevention and/or treatment of hair loss.

FUNDING

We are grateful to the Korea Ginseng Corporation for the preparation of RGE. This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIT) (NRF-2014R1A2A1A11050006, 2020R1F1A1073595, and 2021R1A2C2006745).

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Van-Long Truong: Methodology; Formal analysis; Writing-original draft. Woo-Sik Jeong: Project administration; Supervision.

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