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Hair growth-promoting effect of *Aconiti Ciliare Tuber* extract mediated by the activation of Wnt/β-catenin signaling

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ABSTRACT

Aims: The activation of Wnt/β-catenin signaling pathway plays an important role in hair follicle morphogenesis by stimulating bulge stem cells. This study was to obtain the activator of Wnt/β-catenin signaling pathway from natural products and to determine whether this activator can induce anagen hair growth in mice.

Main methods: To identify materials that activate Wnt/β-catenin signaling pathway, 800 natural product extracts were screened using pTOPFlash assay and neural progenitor cell (NPC) differentiation assay. A selected extract was further tested for its effects on alkaline phosphatase (ALP) activity in human immortalized dermal papilla cell (iDPC) and the proliferation in iDPC and immortalized rat vibrissa DPC (RvDP). Finally, hair growth-promoting effects were evaluated in the dorsal skin of C57BL/6 mice.

Key findings: Aconiti Ciliare Tuber (ACT) extract was one of the most active materials in both pTOPFlash and NPC differentiation assays. It promoted the differentiation of NPC cells even under proliferation-stimulating conditions (basic fibroblast growth factor: bFGF). It also increased ALP activity and proliferation of iDPC in dose-dependent manners, and it stimulated the induction of the anagen hair growth in C57BL/6 mice. These results suggest that ACT extract activates the Wnt/β-catenin signaling pathway by enhancing β-catenin transcription and has the potential to promote the induction of hair growth via activation of the stem cell activity of the dermal papilla cells.

Significance: This is the first report indicating benefits of ACT extract in hair loss prevention by triggering the activation of Wnt/β-catenin signaling pathway and induction of the anagen hair growth in mice.

Introduction

The scalp is a protective layer on the head that contains hair follicles, which are small, dynamic organs that continuously synthesize hair fibers (Ebling, 1987; Stenn and Paus, 2001). Hair growth is cyclic and involves 3 phases: a growth phase (anagen), an involution phase (catagen), and a resting phase (telogen). Hair loss can be caused by cicatricial or non-cicatricial alopecia. Non-cicatricial alopecia can be a consequence of genetic disorders (hypotrichosis), an increase in the induction of the telogen phase (telogen effluvium), genetic follicular miniaturization (androgenetic alopecia), an increase in telogenic depilation with detention of the follicle in early anagen (alopecia areata), and/or the elimination of the hair in anagen (anagenic effluvium). In the case of androgenetic alopecia, decreases in the number and size of anagen hair follicles and the thickness of the hair fibers and an increase in the number of vellus-like hair follicles can be observed (Paus et al., 1999; Barraud and Ralph, 2000; Ellis et al., 2002; Hofmann et al., 1999; Nidal et al., 2005; Ralph, 2007; Park et al., 2011).

To date, 2 drugs—finasteride and minoxidil—have been approved for the treatment of hair loss in men by the Food and Drug Administration (FDA) of the USA. However, these drugs have limited therapeutic uses due to their unsatisfactory cure rates (D'Amico and Roehrborn, 2007). To complement these drugs, some researchers have focused their attentions on medicinal herbs, such as the hair growth-promoting herbs *Polygonum multiflorum* and *Schisandra nigra*, both of which have traditionally been used for a variety of medicinal purposes (Kang et al., 2009; Park et al., 2011).

The Wnt/β-catenin pathway plays an important role in the initiation, development, and growth of hair follicles (DasGupta and Fuchs, 1999; Huelsken et al., 2001; Andl et al., 2002; Stefanie and Stefan, 2006). Furthermore, the transient activation of β-catenin results in hair re-growth in mice. Ablation of β-catenin results in dramatic hair shortening and abnormal regeneration of hair in the dermal papilla of mouse hair

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follicles (Van Mater et al., 2003; Enshell-Seijffers et al., 2010). The expression of β-catenin in the dermal papilla is high in the anagen phase but low in both the catagen and the telogen phases (Bierie et al., 2003; Ouji et al., 2006). An interaction between β-catenin, androgen receptors and keratinocyte growth inhibition through modification of Wnt signaling by androgens has been reported to be related to androgenic alopecia, a common form of hair loss (Singh et al., 2006; Inui et al., 2007; Kitagawa et al., 2009). Another well-known effect of β-catenin stabilization in the Wnt/β-catenin pathway is the differentiation of neural progenitor cells (NPCs) (Raballo et al., 2000; Phiel et al., 2001; Maric et al., 2003; Cho et al., 2004; Hsieh et al., 2004; Bug et al., 2005; Shen et al., 2005).

Therefore, in this study, to identify materials that promote hair follicle morphogenesis, we first screened 800 natural product extracts by using the pTOPFlash assay system, which can measure β-catenin transcription. We then confirmed the results by evaluating the degree of NPC differentiation. *Aconiti Ciliare Tuber* (ACT) extract was identified as one of the most active materials in both systems. ACT extract was selected for further studies regarding the activation of alkaline phosphatase (ALP), the proliferation of human immortalized dermal papilla cells (iDPC), and hair growth in C57BL/6 mice.

Materials and methods

Materials

Wnt-3A, basic fibroblast growth factor (bFGF), and FGF-2 were purchased from R&D Systems (MN, USA). Gaussia luciferase (GLuc) and ALP assay kits were purchased from Biovision Co. (CA, USA). Recombinant peptide DKK1 was purchased from Abcam (UK). The pTOPFlash plasmid was purchased from Upstate (CA, USA), and the pGLuc mini-TK vector was purchased from NEB Co. (MA, USA). Minoxidil and valproic acid (VPA) were purchased from Sigma Aldrich (MO, USA).

Natural product extract library

The 800 natural product extract library, composed entirely of the water extracts of medicinal herbs, was purchased from Bioland Corp. (Cheonan, Korea). These natural product extracts were diluted to 20 and 50 μg/mL and then screened for their abilities to activate the Wnt/β-catenin pathway using the pTOPFlash luciferase reporter gene assay and for the induction of NPC differentiation.

Cell culture

iDPC and immortalized rat vibrissa dermal papilla cells (RvDP) were prepared by Won CH et al. and maintained in Dulbecco's Modified Eagle Medium (DMEM) (PAA Laboratories, Austria) containing 10% (vol/vol) fetal bovine serum (FBS) (PAA Laboratories, Austria) and 1% (vol/vol) penicillin/streptomycin (Gibco BRL, NY, USA) in a humidified atmosphere of 95% air/5% CO_2 at 37 °C (Son et al., 2010; Won et al., 2010). Human embryonic kidney (HEK-293) cells were maintained in DMEM containing 10% (vol/vol) FBS. NPCs were isolated from the cerebral cortex of E14.5 Sprague–Dawley (SD) rats as described previously (Filsell et al., 1994; Enarsson et al., 2002; Lukaszewicz et al., 2002). Briefly, the tissue was gently triturated followed by sedimentation of the meninges and larger cell clumps. The cell suspension was then pelleted and resuspended in N2 medium (Invitrogen Co, CA, USA). Cells were plated onto poly-L-ornithine- and fibronectin-coated tissue culture dishes containing N2 medium with FGF-2 (10 ng/mL). Purified NPCs were used immediately for the differentiation assay.

pTOPFlash luciferase reporter gene assay

To assay the transcriptional activation of β-catenin target genes, we transiently transfected HEK-293 cells with pTOPFlash reporter plasmids. After 24 h of transfection, the cells were incubated for another 24 h with DMEM containing the natural product extracts. The cells were then harvested, lysed, and centrifuged. Supernatants were assayed for luciferase activity by using a Dual Luciferase Assay system (Promega, WI, USA) and a Glomax luminometer (Promega, WI, USA).

Western blot analysis

For western blot analysis, iDPCs were incubated with ACT extract at the indicated concentrations for 12 h. The cells were collected, washed twice with PBS and then lysed using the Mammalian Cell Lysis Kit (Sigma-Aldrich, MO, USA). The protein concentration was determined using BCA protein assay reagent (Thermo scientific, IL, USA). The protein was resolved on 10% SDS-PAGE and blotted with the following antibodies: rabbit monoclonal anti-beta-catenin antibody (Cell Signaling, MA, USA) and mouse monoclonal anti-beta-actin antibody (Santa Cruz Biotechnology, CA, USA).

NPC differentiation assay

NPCs isolated from the cerebral cortex of E14.5 SD rats were plated at a clonal density of 1×10^3 cells/cm^2 on 35-mm poly-L-lysine-coated (Sigma-Aldrich, MO, USA) tissue culture dishes (MatTek Corp., MA, USA). The cells were cultured in N2 medium with or without natural product extracts supplemented with a 2× working stock of B27 additives (Invitrogen Co, CA, USA), and morphological exchanges were observed by a micrograph. NPCs were classified into 3 groups—differentiated, undifferentiated, and dead—based on their micrographic morphologies. We used bFGF (10 ng/mL) as a negative control for inhibition of NPC differentiation (Cavanagh et al., 1997). Na-VPA, a well-known GSK-3β inhibitor and inducer of NPC differentiation in the presence of bFGF, was used as a positive control (Gage, 2000).

Effect of ACT extract on Wnt/β-catenin pathway activation

To confirm the effect of the ACT extract on Wnt/β-catenin pathway activation, we established a stable HEK-293 cell line transfected with a pGLuc mini-TK vector carrying the TCF-binding consensus sequence. To create the cell line, we first separated the β-catenin/TCF target gene from the pTOPFlash vector and inserted it into the pGLuc mini-TK vector. The reconstructed vector was then transfected into HEK-293 cells. The transfected HEK-293 stable cell line was maintained in DMEM containing 10% (vol/vol) FBS supplemented with 0.1 mg/mL G418 (Geneticin; Gibco BRL, NY, USA). Wnt-3A peptide, as a positive control, or different concentrations of ACT extract were added to cells in a 96-well plate at 24 h after seeding and

Table 1
Natural compounds capable of inducing Wnt/β-catenin pathway activation and NPC differentiation.

Natural compounds	Activation of pTOPFlash[a]	Differentiation of NPC[b]
Aconitum Ciliare Tuber	++	††
Ephedrae Radix	+	††
Cibortii Rhizoma	++	†
Mentha piperascens (Malinv.) Holmes	+	†
Evodia officinalis DODE	+	†

[a] "+" denotes statistically significant differences. "++" denotes $p<0.01$ compared with control, "+" denotes $p<0.05$ compared with control.
[b] "†" denotes the ratio of differentiated cells. "††" indicates >30%, and "†" indicates 15–30%.

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incubated for 72 h at 37 °C in a humidified atmosphere under 5% CO_2. The culture media were then transferred to a black clear-bottom 96-well plate (Costar, Corning Co., USA), and 50 μL of GLuc assay solution was added to each well to determine GLuc activity using Flex-station 3 (Molecular devices, CA, USA).

ALP activity assay

iDPCs were plated on 60-mm dishes (2.5×10^4 cells/cm^2). After 48 h of culture, cells were treated with test reagents for 72 h, washed twice with PBS, harvested by scraping after the addition of 300 μL of ALP assay buffer, and then transferred to a new tube. The harvested cells were lysed by sonication and centrifuged to obtain the supernatant. The ALP activity of each sample was measured using an ALP assay kit, and the results were normalized according to the amount of protein in each sample. Wnt-3A peptide and Na-VPA were used as positive controls.

Proliferation assay

The proliferation of iDPC and RvDP was evaluated by measuring their metabolic activities using a 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) assay. The WST-1 assay was performed as follows: dermal papilla cells (1.5×10^3 cells/well of a 96-well microplate) were treated with 5, 10, 20, and 50 μg/mL of the ACT extract. After a 24-h incubation, 10 μL of WST-1 was added to each well, and the cells were then incubated at 37 °C for 2 h. The absorbance was measured at 440 nm with a spectrophotometer (Synergy H2, BioTek., VT, USA). All experiments were performed 3 times, and the mean absorbance values were calculated. The results are expressed as the percentage of the absorbance with ACT extract treatment compared to the absorbance of the untreated controls. Wnt-3A peptide and Na-VPA were used as positive controls.

Animals and in vivo hair growth test

Six-week-old male C57BL/6 mice were purchased from Orient Bio Co. (Seoul, Korea) and allowed to adapt to their new environment for 1 week. After a 7-day acclimation period in a controlled barrier facility, the animals were divided into 3 randomized groups ($n=8$) to study the hair growth-promoting activity of the ACT extract. Minoxidil was used as a positive control. Hair cycles in seven-week-old mice are known to be synchronized in the telogen phase (Müller et al., 2001). All animals were shaved by clipping to observe the anagen phase hair follicle development. One hundred microliters of each reagent (10 mg/mL ACT extract, 20 mg/mL minoxidil) were topically applied daily for 35 days. The final hair weight was determined by clipping and weighing on day 35. All reagents used for the hair-growth test were dissolved in a vehicle containing 50% (v/v) ethanol, 30% water, and 20% propylene glycol. During the experimental period, the mice were cared for in accordance with the American Physiological Society Guiding Principles in the Care and Use of Animals. This study was approved by the Animal Care Committee of AmorePacific Corporation R&D Center.

Statistical analysis

All values are presented as the mean ± standard deviation (SD) of at least three independent experiments performed in triplicate. Statistical comparisons between groups were performed by independent two-sample *t*-tests. Results with p-values<0.05 were considered statistically significant.

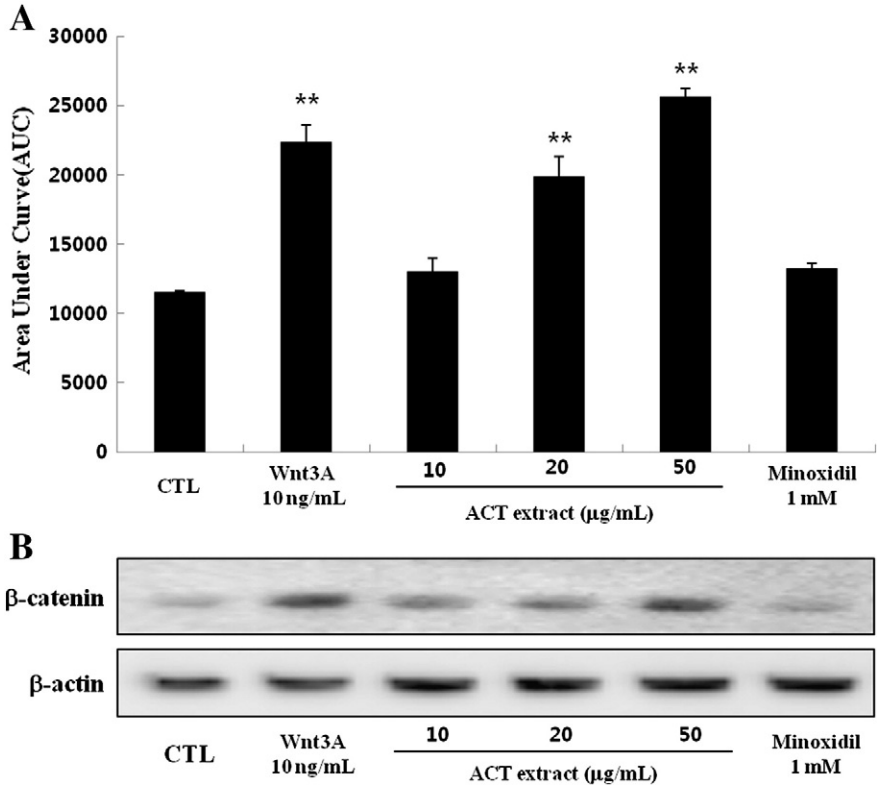

Fig. 1. Effect of ACT extract on Wnt/β-catenin pathway activation. A. Analysis of the activation of the Wnt/β-catenin pathway using a stable HEK-293 cell line transfected with the pGLuc mini-TK vector carrying the TCF-binding consensus sequence (** $p<0.01$ compared with control). B. Western blot analysis of β-catenin.

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Results

Identification of materials that activate the Wnt/β-catenin pathway and induce NPC differentiation

Eight hundred natural product extracts were screened using 2 methods: the pTOPFlash luciferase reporter gene assay and the NPC differentiation assay. The overall top 5 most active extracts are summarized in Table 1. As shown in Table 1, ACT extract was one of the most active materials in both screening systems. Therefore, this extract was selected for further in vitro and in vivo studies.

Effect of ACT extract on β-catenin transcriptional activity in the Wnt/β-catenin pathway

A stable HEK-293 cell line transfected with pGLuc mini-TK vector carrying the TCF-binding consensus sequence was established and used to confirm the effect of ACT extract on the β-catenin stabilization of the Wnt/β-catenin pathway by measuring the increase of β-catenin transcription. ACT extract significantly increased GLuc activity in these cells (Fig. 1A).

The β-catenin protein level in iDPCs treated with ACT extract also increased compared with the control (Fig. 1B). Wnt-3A peptide was used as a positive control (Mohammed et al., 2002).

Effect of ACT extract on NPC differentiation

The effect of ACT extract on NPC differentiation was confirmed and compared with that of the positive control, Na-VPA (Fig. 2). The number of differentiated cells significantly increased after treatment with ACT extract, although this increase was not dose dependent. In addition, the extent of this increase was much less than that induced by treatment with Na-VPA. Nonetheless, ACT extract induced NPC

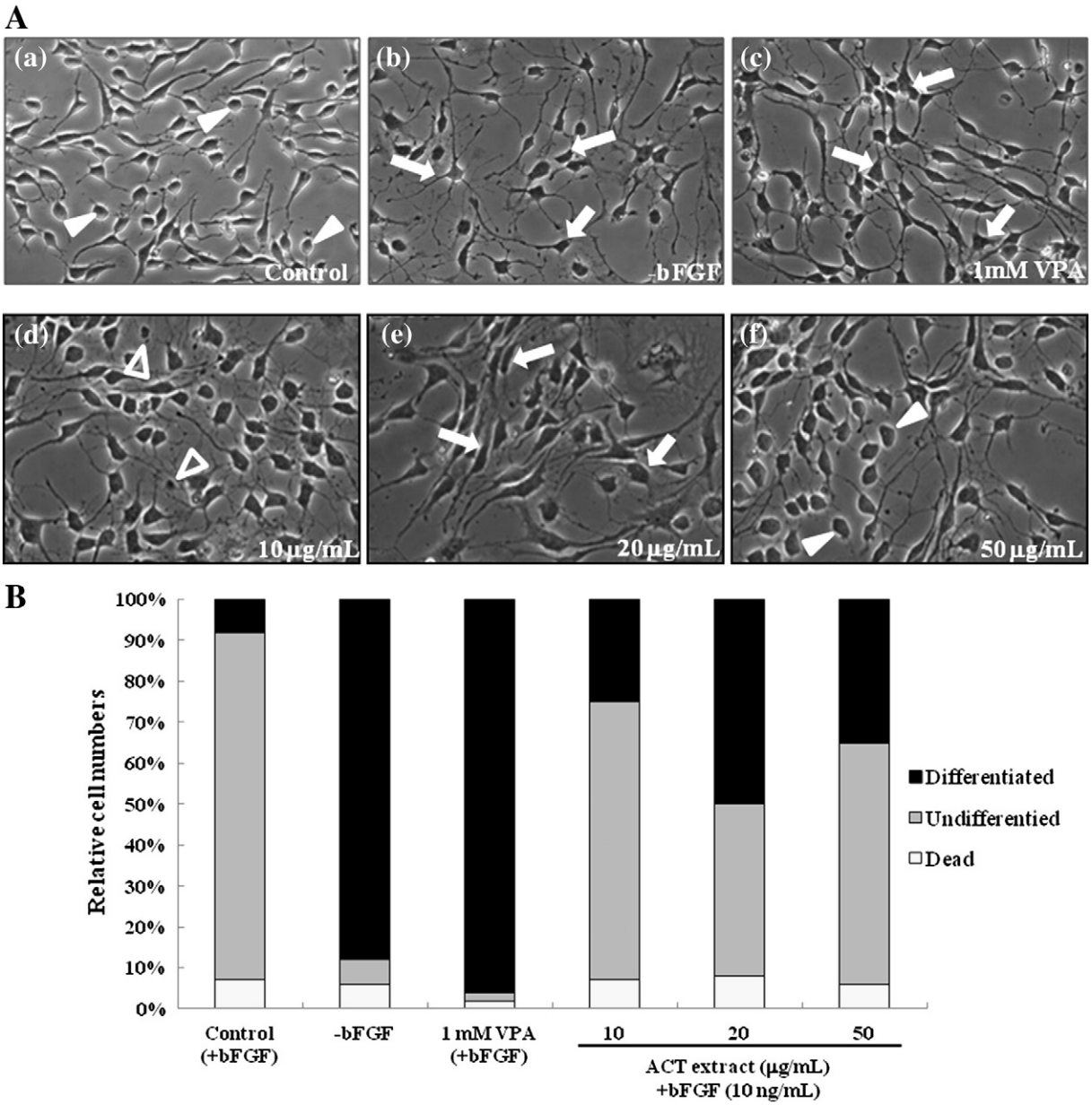

Fig. 2. Effect of ACT extract on NPC differentiation. A. Micrographs of NPC differentiation: (a) negative control treated with 10 ng/mL bFGF; (b) control without bFGF; (c) positive control treated with 1 mM VPA in the presence of 10 ng/mL bFGF; NPCs treated with (d) 10 μg/mL, (e) 20 μg/mL, and (f) 50 μg/mL of ACT extract in the presence of bFGF. Open triangles (△) show dead NPCs which are small and irregularly shaped. Arrows (↑) show differentiated NPCs which have many dendrites, and closed triangles (▲) show undifferentiated NPCs which have no dendrites. B. Analysis of relative numbers of differentiated, undifferentiated, and dead NPCs identified by their micrographic morphologies.

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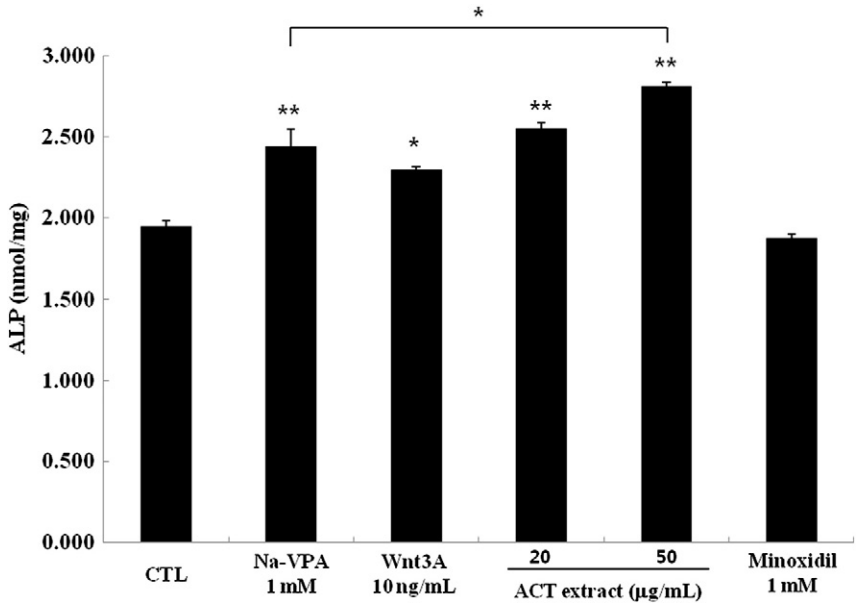

Fig. 3. Effect of ACT extract on alkaline phosphatase activity. Changes in iDPC ALP activity, a well-known biomarker of stem cells, after ACT extract treatment were evaluated using the ALP assay system. All experiments were performed in triplicate (* $p<0.05$, ** $p<0.01$ compared with control unless otherwise indicated).

differentiation by overcoming the anti-differentiation and pro-growth effects of bFGF.

Effect of ACT extract on ALP activity

To confirm the relationship between ACT extract and stem cell activation via activation of the Wnt/β-catenin pathway, we measured the change in ALP activity after treatment with ACT extract. As shown in Fig. 3, ACT extract significantly elevated ALP activity in a dose-dependent manner. Treatment with 50 μg/mL ACT extract significantly increased ALP activity compared with 1 mM Na-VPA or 10 ng/mL Wnt-3A peptide treatments; both Na-VPA and Wnt-3A peptide are well-known stem cell activators (McPherson and Jani, 1995; Jan et al., 2004).

Effect of ACT extract on dermal papilla cell proliferation

Treatment with ACT extract significantly increased iDPC proliferation. The highest degree of proliferation was achieved using 10 μg/mL of ACT extract, and the degree of proliferation decreased with increasing concentrations of ACT (Fig. 4). Changes in the mitogenic activity in RvDP after treatment with ACT extract showed a trend similar to those observed in iDPC, although these changes were not statistically significant.

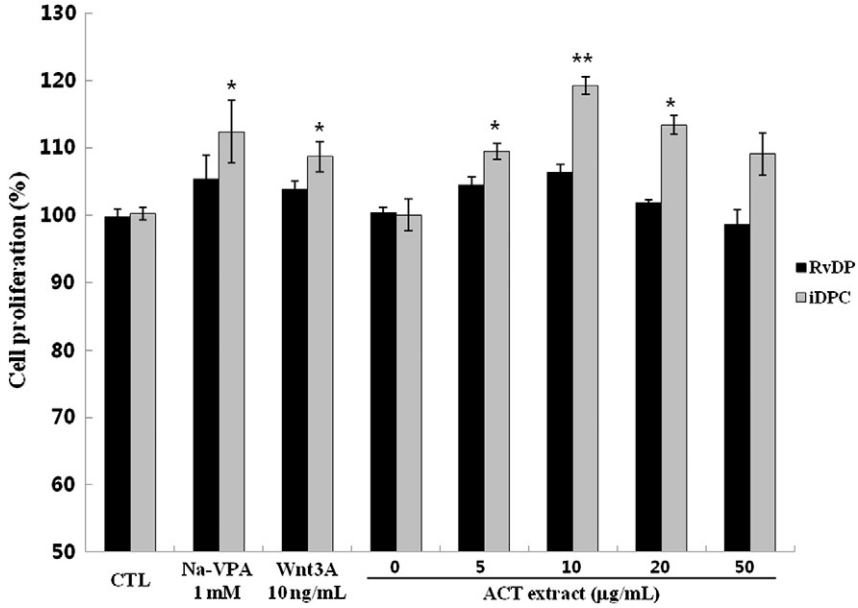

Fig. 4. Effect of ACT extract on dermal papilla cell proliferation. RvDP and iDPC (1.5×10^3 cells/mL) were treated with ACT extract at various concentrations (0, 5, 10, 20, and 50 μg/mL). Cell proliferation was measured by WST-1, and all experiments were performed in triplicate (* $p<0.05$, ** $p<0.01$ compared with the control).

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Effect of ACT extract on anagen induction in C57BL/6 mice

C57BL/6 mice were used to investigate the effect of ACT extract on anagen phase induction. The shaved skin of telogen phase mice is pink and darkens with the initiation of anagen (Müller et al., 2001; Kim et al., 2010). The day after the C57BL/6 mice were shaved using a clipper, the dorsal skin of these mice was treated with 10 mg/mL of ACT extract or 20 mg/mL of minoxidil as a positive control 2 times daily for 5 weeks. ACT extract- and minoxidil-treated groups exhibited gray skin at day 10 after hair-growth induction, and their hair shafts were visible at day 14. The skin color of the vehicle control-treated group remained less pigmented until day 10. This group still exhibited gray skin at day 14, and their hair shafts were not visible until days 15–17 (data not shown). On day 35, the dorsal skin of all the mice, except several control mice, was in the anagen phase, but the amount of hair growth was significantly different between ACT extract-treated and minoxidil-treated groups (Fig. 5). During the experimental period, visual scoring of hair growth was performed weekly based on the scoring guideline shown in Fig. 6, and the results are presented in Fig. 7A. The total hair weight for each group at day 35 is presented in Fig. 7B. Overall, these results indicate that ACT extract induced early telogen-to-anagen phase conversion of hair follicles in the C57BL/6 mice.

Discussion

Here, ACT extract was investigated both in vitro and in vivo for its ability to promote the induction of anagen hair-growth. To the best of our knowledge, this is the first study to show that ACT extract promotes hair growth through the activation of the Wnt/β-catenin pathway by enhancing β-catenin transcription and possibly by a mitogenic effect on dermal papilla cells. Because the Wnt/β-catenin pathway and β-catenin, in particular, play important roles in hair growth and NPC differentiation, we employed the pTOPFlash and NPC differentiation assays to screen materials for their abilities to activate this pathway (Jiro and Robert, 2000; Ito et al., 2007; David et al., 2010). From the results of these assays, the ACT extract was selected for further studies.

ACT is an herbal medicine processed from the dried root tubers of *Aconitum ciliare Decaisne* and other plants of the Ranunculaceae family (Kim et al., 2004). Aconitum plants contain aconitum alkaloid, a toxin whose composition and amount vary with the species and the preparation method (Lee et al., 2007). Despite safety issues regarding aconitum toxicity, pharmacologists have given sizeable amounts of attention to this mysterious oriental herb that is both poisonous and medicinally efficacious. ACT is used in folk remedies and oriental medicine. It is known to possess anti-inflammatory and pain-relieving properties and is also used to aid in digestion and to strengthen the heart (Baek, 2005). Previous studies have found several pharmacological uses of ACT. For example, one study showed that this plant has an inhibitory effect on NF-κB and an anti-histamine effect, thus making it useful in anti-allergy drugs (Kim, 2010). Another study in white mice injected with ACT extract suggested that ACT reduces pain and aids in the recovery of motor skills (Cho et al., 2009). Yet another study on the pain-relieving effects of some herbal medicines suggested that topical application of ACT extract can aid in relieving pain in cancer patients (Lee et al., 2000). Although various studies have identified pharmacological applications for ACT, no other study has investigated its hair growth-promoting activity.

To confirm the effect of ACT extract on the activation of the Wnt/β-catenin pathway, we established a stable HEK-293 cell line transfected with pGLuc mini-TK vector carrying the TCF-binding consensus sequence. Because GLuc is secreted into the media, this method has the advantage of allowing measurement of enzyme activity in the culture media without the need for cell lysis. The ACT extract significantly increased luciferase activity in this cell line to the same extent as the positive control, Wnt-3A peptide (Fig. 1A). Moreover, the increased β-catenin protein level in iDPCs treated with ACT extract confirmed the effect of ACT extract on the activation of the Wnt/β-catenin pathway (Fig. 1B).

Because β-catenin also plays an important role in NPC differentiation, the effect of ACT extract on NPC differentiation was compared with that of the positive control, VPA (Fig. 2). Previous studies have shown that 1 mM VPA induces differentiation and inhibits NPC proliferation by overcoming the effect of bFGF, a factor which inhibits NPC differentiation (Cavanagh et al., 1997; Gage, 2000; Jiro and Robert, 2000; Bug et al., 2005; Jung et al., 2008). Here, we demonstrated that treatment with ACT extract in the presence of bFGF (10 ng/mL) induced differentiation and morphological changes in NPCs, although to a much lesser degree than did treatment with VPA. NPC differentiation induced by ACT extract peaked at a concentration of 20 μg/mL and then decreased at an ACT extract concentration of 50 μg/mL. This may be attributed to a side-effect of the ACT extract, though further studies are needed to confirm this result.

Because of the continuous expression of ALP in dermal papilla cells throughout the hair growth cycle, measurement of ALP expression in the skin has been often used to determine the number and distribution of dermal papilla cells (Handjiski et al., 1994; Eiges et al., 2001; McElwee et al., 2003; Wu et al., 2005; Iida et al., 2007; Ryan et al., 2011). ACT extract significantly elevated ALP activity in iDPCs

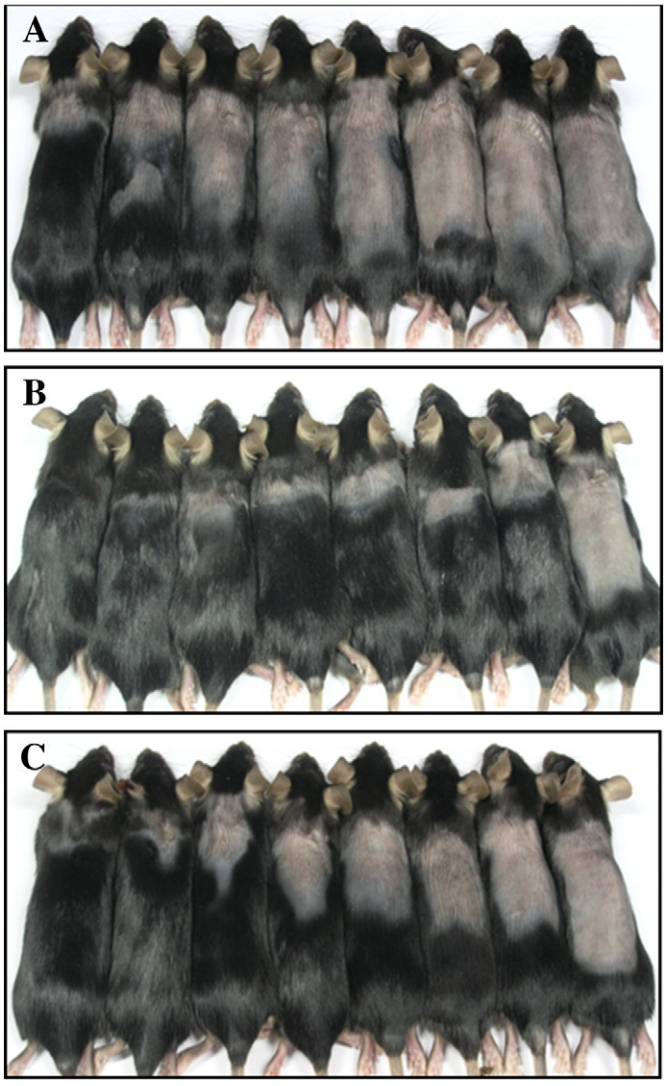

Fig. 5. Effect of ACT extract on anagen phase induction in C57BL/6 mice. After synchronizing the telogen phase, the dorsal skin of C57BL/6 mice was treated with ACT extract, minoxidil, or vehicle. A. Control, vehicle; B. ACT extract, 10 mg/mL; and C. Positive control, minoxidil, 20 mg/mL.

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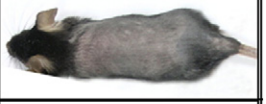
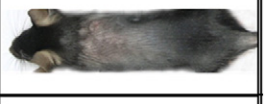
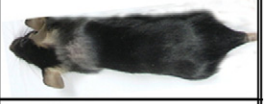
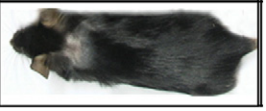
Grade	Condition	Pattern
0	No change	
1	<30% of shaved area shows skin darkening. No hair growth.	
2	30%~70% of shaved area shows skin darkening. No hair growth.	
3	>70% of shaved area shows skin darkening, or <30% shows hair growth.	
4	>70% of shaved area shows skin darkening, and 30%~70% shows hair growth.	
5	>70% of shaved area shows skin darkening, and >70% shows hair growth.	
6	>90% of shaved area shows hair growth.	

Fig. 6. Index of hair growth visual scoring guide in C57BL/6 mice.

(Fig. 3). The efficacy of ACT extract for the induction of proliferation was also evaluated in 2 dermal papilla cell lines, RvDP and iDPC, which are rat and human cell lines, respectively. ACT extract significantly promoted iDPC proliferation, which peaked at a concentration of 10 μg/mL and then decreased at higher concentrations (Fig. 4). The extent of the ACT extract-induced increase in RvDP proliferation was similar to that of the ACT extract-induced proliferation in iDPC, but was not statistically significant.

Taken together, these results strongly suggest that ACT extract induces NPC differentiation, increases ALP activity and proliferation of dermal papilla cells and exerts hair growth-promoting effects through the activation of Wnt signaling. This hypothesis was directly tested by treating the dorsal skin of C57BL/6 mice with ACT extract. Topical application of 10 mg/mL ACT extract significantly promoted induction of the anagen phase compared with 20 mg/mL minoxidil (Figs. 5, 7). Importantly, ACT extract treatment did not induce any specific side effects on the dorsal skin or any internal organs, including the liver, lungs, and heart, according to necropsy results (data not shown). Minoxidil, which was used as a positive control in these experiments, did not exhibit efficacy in the in vitro evaluation systems used in this study, including the Wnt/β-catenin pathway and ALP activity. This finding suggests that the ACT extract promotes induction of the anagen phase by activating the Wnt/β-catenin signal pathway, which is distinctly different from the pathway affected by minoxidil.

Conclusions

In this study, ACT extract was identified from by screening an array of 800 natural product extracts and was notable for its ability to activate the Wnt/β-catenin signaling pathway by enhancing β-catenin transcription and NPC differentiation. ACT extracts also increased ALP

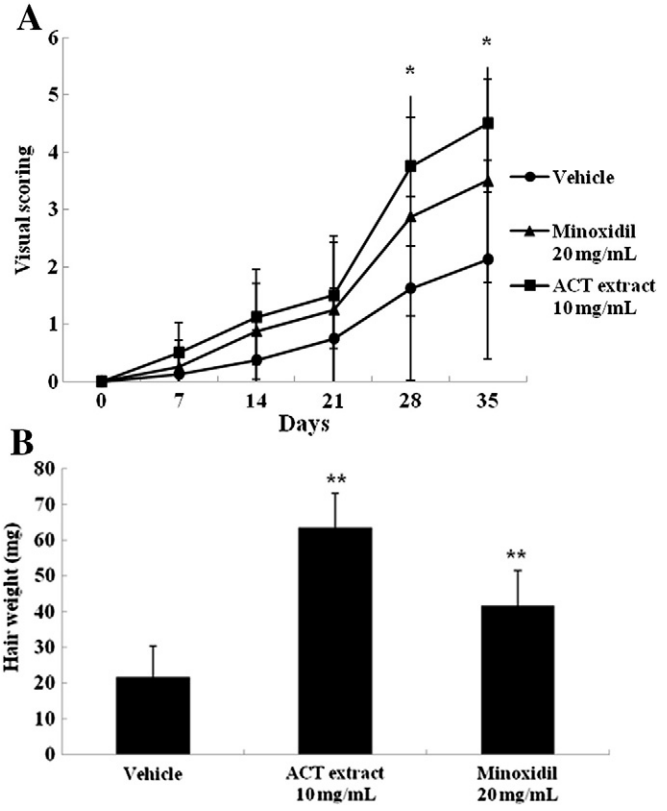


Fig. 7. Effect of ACT extract on hair growth in C57BL/6 mice. A. Visual scoring of the hair growth-promoting effect of ACT extract by using the scoring guideline shown in Fig. 6. B. Hair growth in each group was determined by measurement of total hair weight at day 35 (* $p<0.05$, ** $p<0.01$ compared with the vehicle treated group).

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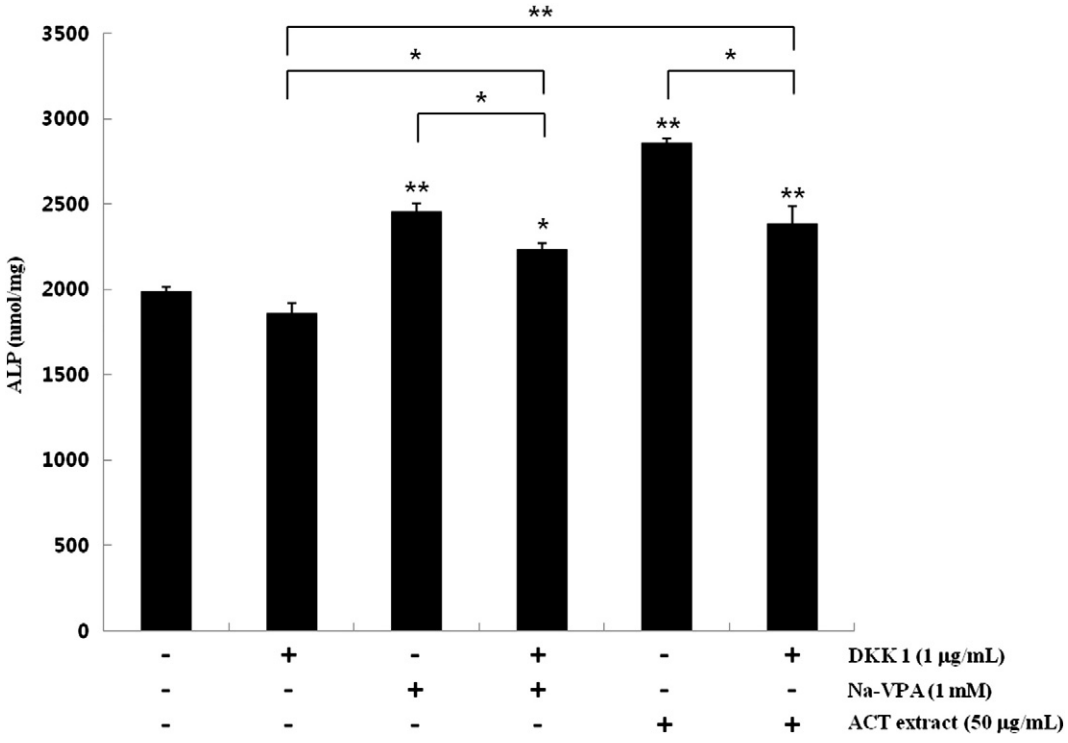

activity and iDPC proliferation and promoted induction of the anagen phase in the dorsal skin of C57BL/6 mice. These findings may lead to the development of new treatments for hair-loss patients.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgments

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.lfs.2012.09.008.

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