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Original Article

Topical application of 8DSS induces anagen hair growth in telogenic mouse skin

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Abstract: Octuplet repeats of aspartate-serine-serine (8DSS) peptides induce hair regrowth, but the underlying mechanism is largely unknown. Activation of the Wnt/β-catenin pathway in dermal papilla cells (DPCs) is critical for hair regeneration. It is possible that 8DSS affects human hair follicle DPCs via activating the Wnt/β-catenin pathway. This study sought to explore the effects of topical 8DSS on promoting the transition from telogen to anagen during hair regeneration and whether 8DSS activates Wnt/β-catenin signaling in human DPCs. Here, 1% 8DSS was topically applied onto the shaved dorsal skin of telogenic C57BL/6 mice (seven-week-old) daily for 31 days. After 7 days, the biopsies of dorsal skins were double processed with hematoxylin-eosin and immunohistochemical staining. Topical application of 8DSS to C57BL/6 mice induced early telogen-to-anagen transition of hair follicles and increased the number of hair follicles in telogenic mouse skin. The 8DSS-treated mouse skin exhibited earlier induction of β-catenin protein expression. Treatment with 8DSS increased *wnt10b, Lef-1 and c-myc* mRNA levels, nuclear β-catenin protein levels, and GSK3β and Akt phosphorylation levels in human dermal papilla cells. These results demonstrate that 8DSS induced early telogen-to-anagen transition of hair follicles in C57BL/6 mice. Treatment with 8DSS promotes hair follicle regrowth through activating the Wnt/β-catenin signaling pathway in dermal papilla cells. Thus, 8DSS may be a promising agent for hair loss treatment.

Keywords: Octuplet repeats of aspartate-serine-serine (8DSS), dermal papilla cells (DPCs), GSK3β, Wnt/β-catenin

Introduction

Alopecia is a major cosmetic concern because it causes a variety of psychological problems, including depression, anxiety, and social phobias. The prevalence of alopecia in both men and women ranges from 16 to 96%, depending on age and whether mild forms of hair loss are considered [1]. The current therapies for hair loss are limited due to low efficacy and adverse effects, such as dryness and itchiness. Therefore, it is important to develop novel therapeutic agents that promote hair regrowth.

The hair follicle (HF) comprises dermal and epidermal compartments whose close interactions are pivotal to hair-related biological and pathological processes [2, 3]. The dermal papilla (DP), which is the major dermal compartment of the hair follicle, undergoes periodic regeneration through cycles ranging from anagen, to catagen, to telogen [4]. DP plays an important role in hair formation and adult hair cycle control [5-8]. Dermal papilla cells (DPCs) regulate hair growth by stimulating the surrounding epithelial cells to proliferate and differentiate into the hair shaft and inner root sheath [5, 9, 10]. Multiple signaling pathways contribute to the regulation of hair follicle growth and proliferation. DPC-derived growth factors, such as *Igf-1, hgf* and *Tgf-β*, control the development and growth of hair follicles [11-13]. Moreover, inductive signals, including Wnt/β-catenin and PI3K/Akt, are involved in the regulation of hair cycling and growth. Several studies have demonstrated that Wnt/β-catenin signaling plays important roles in hair follicle morphogenesis and regeneration [14-16]. The binding of Wnt pro-

teins to the Frizzled (Fz) receptors leads to stabilization of cytoplasmic β-catenin, which enters the nucleus and interacts with the transcription factor T-cell factor/lymphoid enhancer factor (TCF/LEF) to regulate the expression of downstream target genes. β-catenin accumulates in the nuclei of anagen DPCs and provides growth signals to activate hair follicle progenitors [17, 18]. DP-specific ablation of β-catenin leads to a premature catagen stage [14]. Further, *wnt10b* is the earliest and highest expressed Wnt ligand in hair follicle development and hair cycle induction and is expressed in hair follicles in postnatal skin [19]. Therefore, the Wnt/β-catenin signaling pathway is essential in maintaining the hair-inducing capacity of the dermal papilla and the anagen-phase characteristics [20, 21]. These findings suggest that DPCs are the most likely target cells and that Wnt/β-catenin signaling could serve as an ideal target pathway for the development of drugs that activate hair growth.

Numerous repetitive nucleotide sequences of aspartate-serine-serine within the human dental phosphoprotein are believed to promote the formation of hydroxyapatite more effectively than entire proteins in dental remineralization [22]. Octuplet repeats of aspartate-serine-serine (8DSS) are small peptides that are synthesized based on the human dentin phosphoprotein. These repeats represent the most active peptide that mediates biologically directed mineral deposition [23]. Interestingly, 8DSS induces mouse hair regrowth and nail growth (Pub. No. US2010/0239503 A1). However, whether 8DSS affects the hair cycle and the mechanisms by which it induces hair regrowth remain unknown. In this study, we investigated whether 8DSS stimulates hair regrowth and affects the hair cycle in C57BL/6 mice. Because Wnt/β-catenin signaling is essential in the regulation of hair growth, we also investigated whether 8DSS activates the Wnt/β-catenin pathway in human dermal papilla cells.

Materials and methods

8DSS dermal permeation

C57BL/6 mice were purchased from the Third Military University (Chong Qing, China). Nine-week-old C57BL/6 mice were shaved under anesthesia using animal clippers. Each group received topical 1% green-fluorescence-8DSS, which was provided by C3-Jian Inc (Los Angeles, CA, USA), on the dorsal skin of the shaved area. Skin samples were obtained by biopsy for analysis at one and two hours after the first application. Then, the frozen skin sections (5 μm) were examined under fluorescence microscopy (Olympus, Tokyo, Japan).

In vivo study of 8DSS and hair cycle staging

Seven-week old female C57BL/6 mice were randomly divided into 2 groups (14 mice per group). All hair follicles were shaved with clippers at 7 weeks of age at which all follicles were in telogen stage. The mice were topically treated with 50 μl of 1% 8DSS or phosphate buffered saline (PBS) daily for 31 days. Hair growth promotion was evaluated simply by observing the darkening of the skin color and photographed at 0, 7, 17, 25 and 31 days after depilation, which indicated telogen-to-anagen conversion [4]. Quantitative determination of the darkened skin and hair follicle areas in these sections was recorded by photographs and calculated using Image J software (NIH, US).

Histological examination

Seven days after treatment, 3 mice in each group were euthanized, and the treated region of dorsal skin was collected. Biopsy specimens were fixed in 4% paraformaldehyde, dehydrated, embedded in paraffin, and cut into 4-μm sections. Sections were stained with hematoxylin and eosin (H&E) by routine methods and examined under a microscope (Olympus, Tokyo, Japan) at 200 × magnification.

Immunohistochemistry

Paraformaldehyde-fixed, paraffin-embedded dorsal skins at day 7 after the treatments were cut into 4-μm sections. The monoclonal rabbit anti-mouse β-catenin (1:500, Abcam, Cambridge, MA, USA) and goat anti-rabbit IgG, HRP conjugated (1:200, Comwin, Beijing, China) antibodies were used for detection of the primary antibodies. Visualization was performed using a SP Rabbit HRP Kit (DAB) according to the manufacturer's instructions (Comwin, Beijing, China). The slides were analyzed by light microscopy. The relative levels of target protein expression compared with the internal control were qualified using Image J analysis software (NIH, US).

Isolation and culture of hDPCs

Human scalp specimens were obtained with informed consent from healthy men who were 25 to 45 years old and underwent plastic surgery. The human dermal papilla cells (hDPCs) were isolated with a collagenase IV and dispase digestion method as previously described [24]. DPCs were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 U/mL) and streptomycin (100 mg/ mL) at 37°C. The cells used in our experiments were passaged only 3 times.

Immunofluorescence

The hDPCs were seeded on coverslips in 24-well culture plates. At 80% confluence, the cells were fixed with cold pure acetone at room temperature for 5-10 min, followed by blocking with 10% normal rabbit serum at 37°C for 30 min. The cells were subsequently incubated with a mouse anti-human smooth muscle actin-α antibody (1:100; Boster, Wuhan, China) at 4°C over-night, washed with PBS three times, incubated with Alexa Fluor 488-labeled goat anti-mouse IgG (H+L) (1:250, Comwin, Beijing, China) at room temperature for 30 min, and stained with 4,6-diamidino-2-phenylindole at room temperature for 5 min. Anti-K14 antibody was used as a control to rule out the epidermal cells.

Immunofluorescence was observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Cell Counting Kit-8 assay

The proliferation of hDPCs was evaluated by the Cell Counting Kit-8 (CCK8) assay. Cells (10^4 in 200 μl) were seeded in each well of a 96-well plate, incubated for 6 h, washed with PBS once and cultured for 24 h in 200 μl of serum free DMEM. The cells were then incubated with 8DSS at a final concentration of 0, 12.5, 25, 50, 100, or 200 μmol/L for 72 h. The cells were subsequently incubated with 20 μl of CCK8 after removal of media for 4 h. The optical density was determined at a wavelength of 450 nm using an ELISA reader. All experiments were performed three times with each in triplicate and the mean absorbance was calculated.

RT-qPCR analysis

Briefly, hDPCs (10^5) were seeded in each well of 6-well plates and incubated with 10 μmol/L 8DSS for 72 h. Total RNA was isolated using TRIzol reagent (Invitrogen) according to the manufacturer's instructions followed by a reverse transcription step using reverse transcriptase (TaKaRa, Dalian, China). The following PCR conditions were employed: 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 58°C for 20 s, and 72°C for 30 s. GAPDH was used as an internal control. The primers were designed based on published cDNA sequences as follows: *GAPDH:* Forward: 5'-AGAAGGCTGGGGCTCATTTG-3', Reverse: 5'-AGGGGCCATCCACAGTCTTC-3'; *wnt10b*: Forward: 5'-CTCTGGGATGTGTAGCCTTC-3', Reverse: 5'-GGCTCTGGAGTTGAGAAGTG-3'; *lef-1*: 5'-AGTGACGAGCACTTTTCTCCAG-3', Reverse: 5'-GGATGTTCCTGTTTGACCTGAG-3'; *Igf-1*: Forward: 5'-TCAACAAGCCCACAGGGTAT-3', Reverse: 5'-ACTCGTGCAGAGCAAAGGAT-3'; *hgf:* Forward: 5'-CGAGGCCATGGTGCTATACT-3', Reverse: 5'-ACACCAGGGTGATTCAGACC-3'; *Tgf-β2*: Forward: 5'-GCTTTGGATGCGGCCTATTGCTTT-3', Reverse: 5'-GCATTGTACCCTTTGGGTTCGTGT-3'; *c-myc*: Forward: 5'-TCCGTCCAAGCAGAGGAG-3', Reverse: 5'-ACGCACAAGAGTTCCGTAGC-3'. All experiments were performed thrice in triplicate, and the mean absorbance was calculated.

Western blot

hDPCs were treated with PBS or 10 μmol/L 8DSS for 5, 15, 30, and 60 min and 24 h. NE-PER Nuclear and Cytoplasmic Rxtraction Reagents (Pierce, Rockford, IL, USA) were used to extract nuclear and cytoplasmic proteins. A Total Protein Extraction Kit (Keygen, Naniing, China) was used to extract whole cell proteins according to the manufacturer's instructions. Nuclear (10 μg) or total cell (20 μg) proteins were separated via 10% SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk in PBS at room temperature for 1 h. The membranes with nuclear protein were probed with β-catenin (1:2000, Abcam, USA) and histone H3 (1:2000, Cell Signaling, USA). For the detection of p-Akt and p-GSK3β, the membranes with total cell protein were probed with p-Akt (Ser473, 1:1000, Cell Signaling, USA), p-GSK3β (Ser 90, 1:1000, Cell Signaling, USA) and GAPDH (1:2000, Santa Cruz, USA) at 4°C overnight. The membranes were washed with PBS/0.5% Tween-20 (PBST) thrice and incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:7000, Jackson) for 2 h. The membranes were subsequently washed thrice with PBST, and the bands were detected using

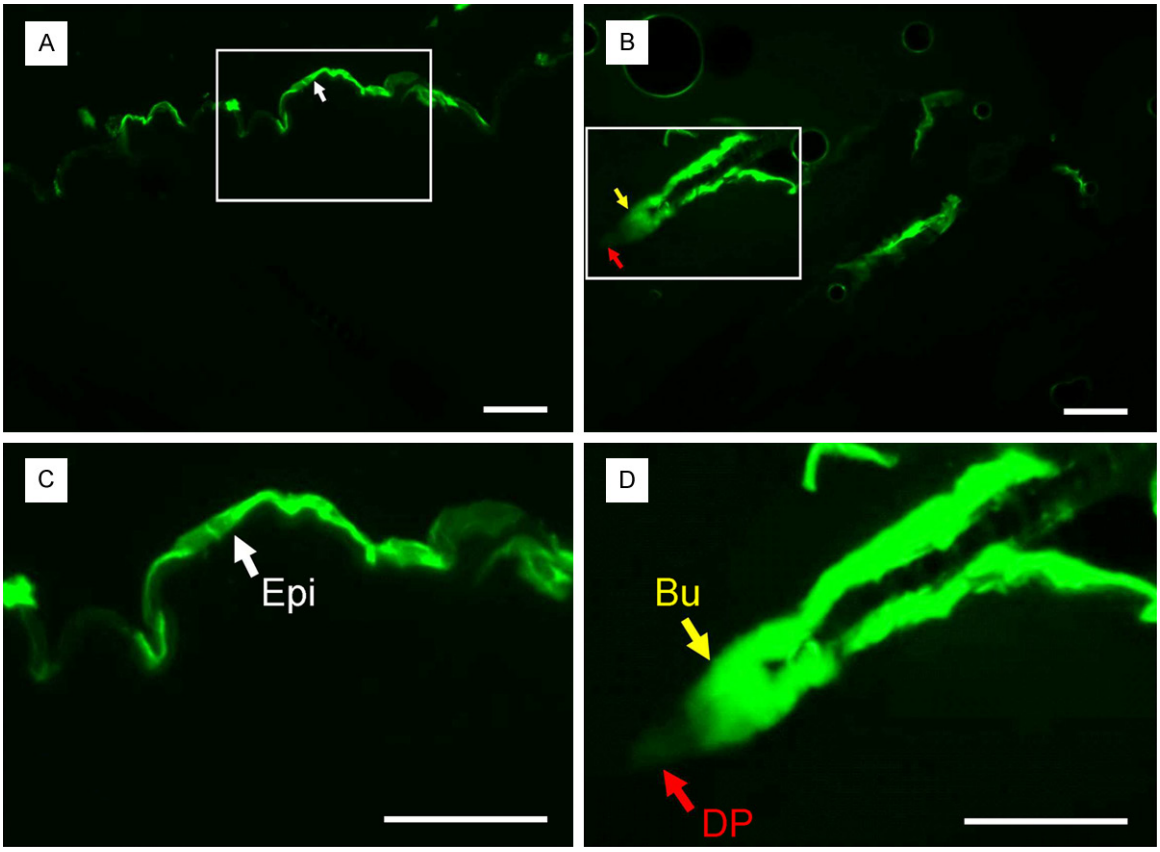

Figure 1. Topical delivery of 8DSS. Penetration of 1% green-fluorescence-8DSS at different time points after topical administration: Fluorescence intensity of the 1% green-fluorescence-8DSS in the stratum corneum layer and follicles after 1 h (A, C) and 2 h (B, D). The white arrows indicate epidermis (Epi), the yellow arrows indicate hair bulge (Bu) and the red arrows indicate dermal papilla (DP). Bar = 50 μm.

Clarity™ Western ECL Substrate (Bio-Rad, USA). The relative levels of target protein expression to the internal control were qualified using Image J analysis software (NIH, US).

Statistical analysis

Statistical analyses were performed with SPSS 13.0 software (SPSS, IL, USA). The experimental data are expressed as the mean ± SD. The significance of differences among groups was analyzed using Student's t-test or the Mann-Whitney u test when the data were normally distributed using the GraphPad Prism (Version 5.0). A value of $P < 0.05$ was considered significantly different.

Results

Efficient topical delivery of 8DSS in mouse skin

We applied 1% 8DSS to the surface of shaved mouse skin, and the distribution of 8DSS was examined by immunofluorescence assay. The fluorescence signal was detected in the epidermis 1 h after drug application (**Figure 1A**). At 2 h, the fluorescence signal was detected in the hair isthmus and hair bulge cells (**Figure 1B**). However, no apparent fluorescence was detected in subcutaneous adipocytes (**Figure 1B**).

Effect of 8DSS on mouse hair regrowth

To investigate whether anagen-phase induction was promoted by 8DSS, six-week-old female C57BL/6 mice were allowed to adapt to their new environment for one week. Then, all dorsal hair of C57BL/6 mice was shaved using an animal clipper at 7 weeks of age, at which a time-synchronized hair growth cycle is noted. The shaved skin of telogen mice is pink and darkens along with anagen initiation. In contrast to the mock-treated group, as shown in **Figure 2A**, most of the 8DSS-treated mice exhibited pink to gray skin at day 7. The black area significantly increased, and hair shafts were visible in more mice on day 17. The mock-treated group

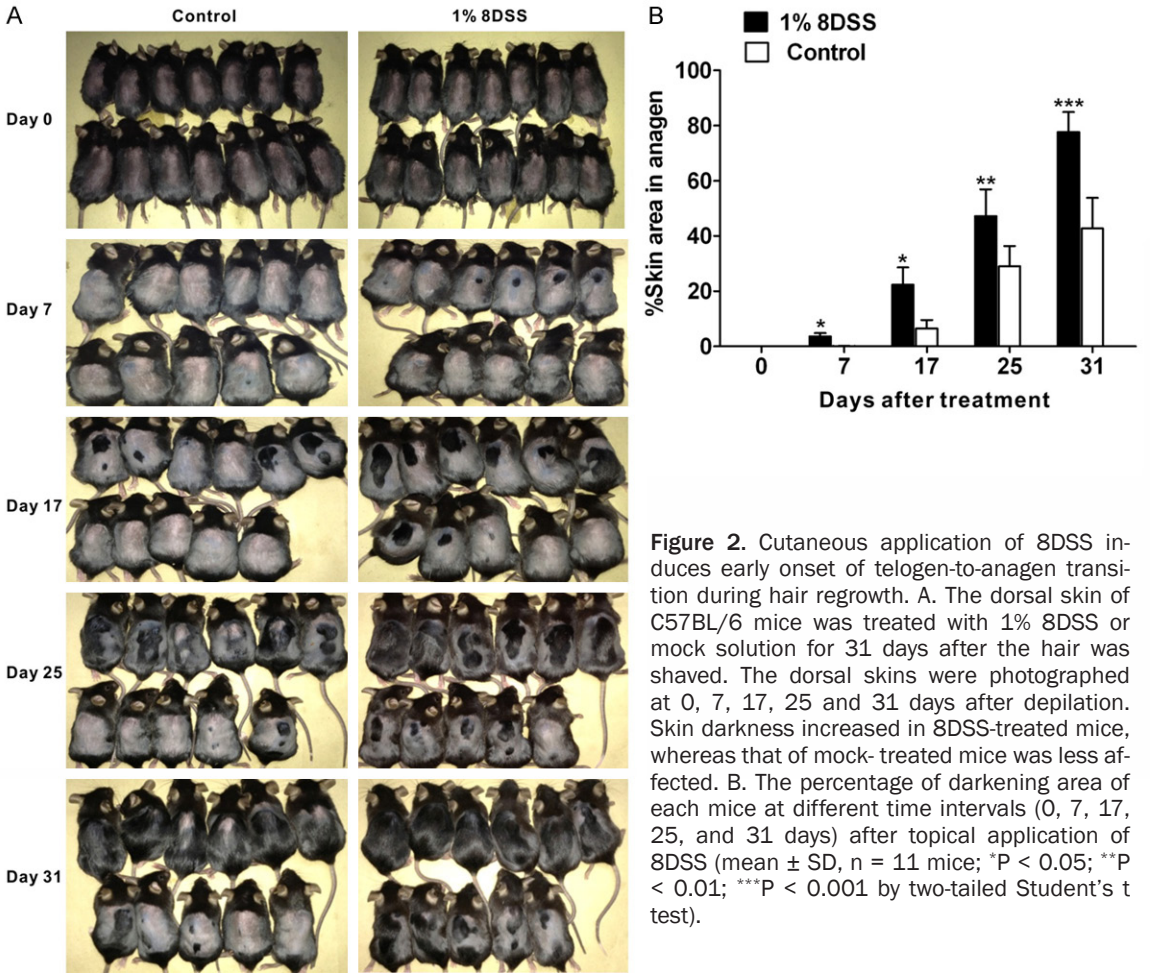

Figure 2. Cutaneous application of 8DSS induces early onset of telogen-to-anagen transition during hair regrowth. A. The dorsal skin of C57BL/6 mice was treated with 1% 8DSS or mock solution for 31 days after the hair was shaved. The dorsal skins were photographed at 0, 7, 17, 25 and 31 days after depilation. Skin darkness increased in 8DSS-treated mice, whereas that of mock- treated mice was less affected. B. The percentage of darkening area of each mice at different time intervals (0, 7, 17, 25, and 31 days) after topical application of 8DSS (mean ± SD, n = 11 mice; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ by two-tailed Student's t test).

exhibited less pigment and hair shafts until day 17, indicating an earlier telogen-to-anagen transition by 8DSS (**Figure 2A**). At day 31, the dorsal skins were in the anagen phase in almost all mice in the 8DSS-treated group. To determine whether 8DSS promoted hair regrowth, the percentage of darkening in each mouse was calculated using Image J software (NIH, US), as shown in **Figure 2B**. To investigate the progression of hair follicles during the hair cycle, histology revealed an increased number and depth of the hair follicles, larger follicles, and denser derma papillae, pigmentation, IRS and hair shafts in 8DSS-treated mice compared with mock-treated mice. Furthermore, almost all hair follicles were in anagen IV in 8DSS-treated mice. In contrast, the mock-treated group exhibited anagen II (**Figure 3**). These results suggested that the 8DSS-treated group could induce an earlier anagen phase and promote hair regrowth.

Effect of 8DSS on the proliferation of cultured DP cells

To investigate the effect of 8DSS on the proliferation of DP cells, fresh hair follicles were isolated, and DP cells were disassociated and cultured in DMEM/10% FBS. The DP cells migrated and projected outwards from the isolated DP (**Figure 4A**). To define the identity of these isolated cells, human hair follicle DP marker smooth muscle actin α (α-SMA) was detected by immunofluorescence assay. We found that α-SMA was strongly expressed in the cultured cells. In addition, K14 (epidermal cell marker) expression was not detected (**Figure 4B**). To investigate the effects of 8DSS on DP cell proliferation, DP cells were treated with various concentrations of 8DSS for 72 h. CCK8 assay revealed that 12.5 μmol/L or lower 8DSS has no effect on DP cell proliferation, whereas 25, 50, 100 and 200 μmol/L 8DSS significantly suppressed DP cell proliferation (**Figure 4C**).

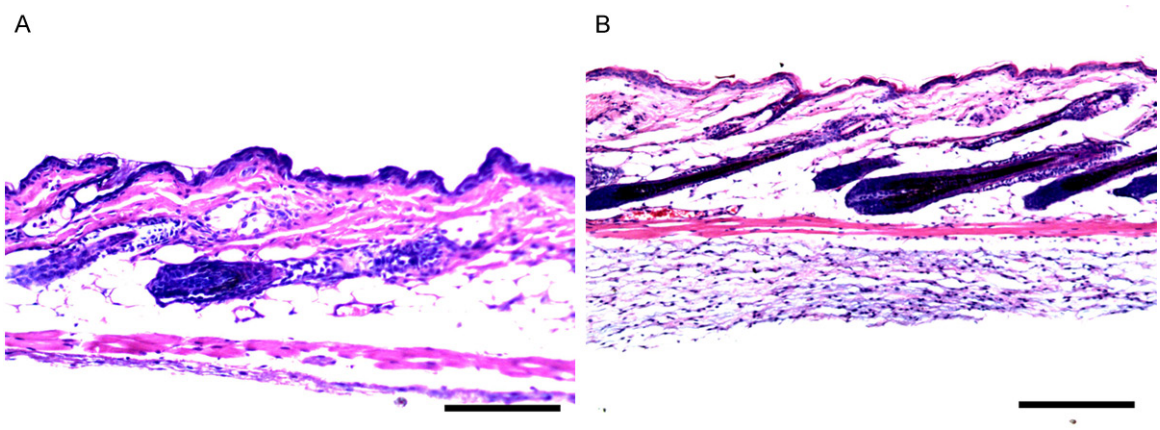

Figure 3. Cutaneous application of 8DSS induced the growth of hair follicle. Effect of 8DSS on hair follicle regrowth was analyzed using hematoxylin-eosin (H&E) staining at the 7th day post treatment (image magnification × 200). Histology revealed an increased number and depth of the hair follicles, larger follicles, and dense derma papillae, pigmentation, IRS and hair shafts. Bar = 50 μm.

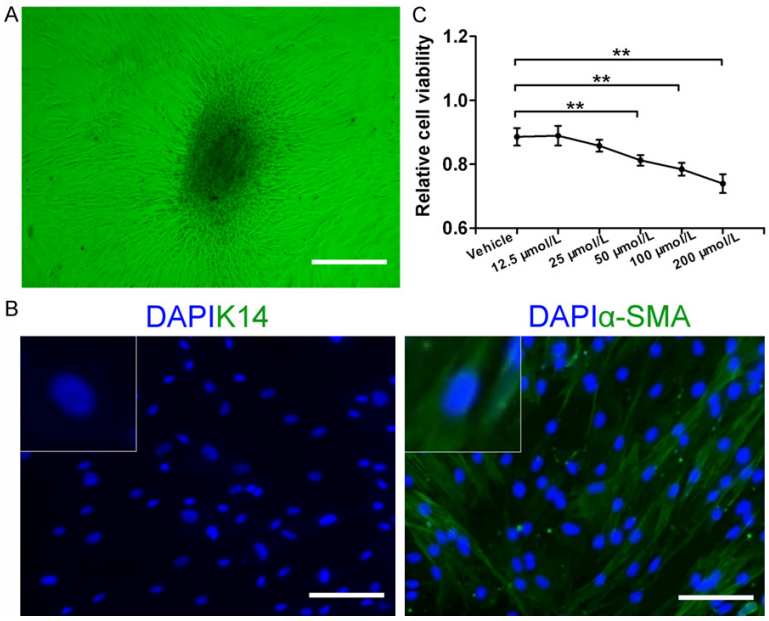

Figure 4. Effect of 8DSS on the proliferation of hDPCs. A. DPCs migrated and spread out from the DP under microscopy. B. Define the identity of the isolated cells. Immunofluorescence assay for α-SMA. Green color, positive α-SMA or K14 staining; blue color, DAPI nuclear staining. Bar = 50 μm. C. CCK8 assay to determine the effects of 8DSS on hDPCs proliferation after 72 h of treatment (mean ± SD, n = 5; $^{**}P < 0.01$ vs. the mock-control group by two-tailed Student's t test).

These results suggest that 8DSS promotes hair growth and hair cycle conversion independently of its regulation of DP cell proliferation.

Effect of 8DSS on the expression of hair-inductive and differentiation related genes in cultured hDPCs

The growth and cycling of the hair follicle is regulated by several factors. To determine the mechanism by which 8DSS affects hair follicle regeneration, the expression of genes related to hair induction and differentiation, such as *wnt-10b, lef-1, Igf-1, hgf, Tgf-β2* and *c-myc*, were analyzed by RT-qPCR. Interestingly, *wnt-10b, Lef-1* and *c-myc* were significantly up-regulated 72 h after treatment with 10 μmol/L 8DSS (**Figure 5**). These results indicated that 8DSS influences *wnt10b, Lef-1* and *c-myc* mRNA expression in cultured hDPCs.

8DSS induces nuclear translocation of β-catenin and GSK3β and Akt phosphorylation in a time-dependent manner

The Wnt/β-catenin pathway plays an important role in hair re-growth regulation. To investigate the effects of 8DSS on the activity of the Wnt/β-catenin pathway, the expression of nuclear β-catenin and phosphorylation of GSK3β, both of which are key factors of the Wnt/β-catenin pathway in DP cells, were examined at different time points following 8DSS treatment by Western blot. Obvious nuclear translocation of β-catenin was observed 60 min after 8DSS treatment (**Figure 6A**). Notably, the phosphorylation GSK3β reached maximal levels 15 min

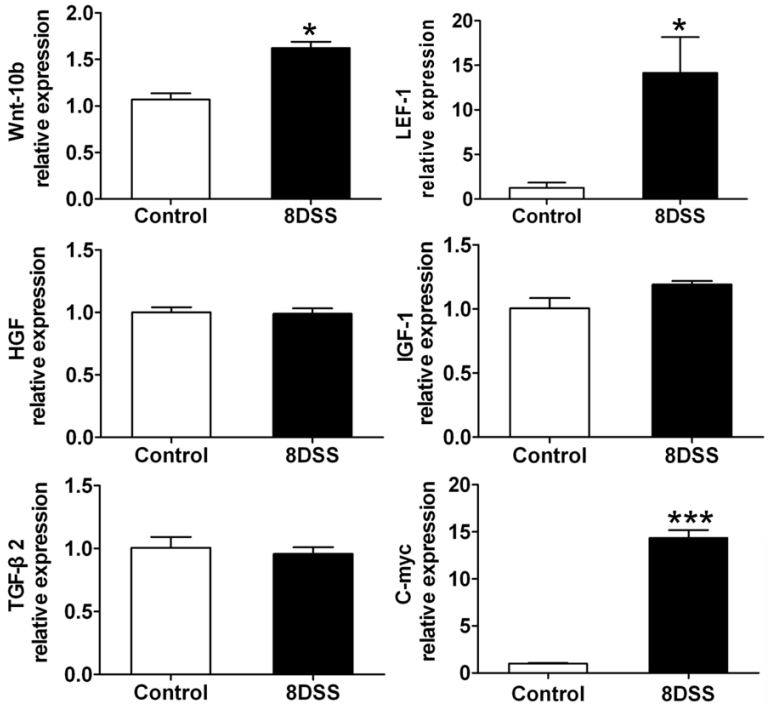

Figure 5. Effect of 8DSS on the expression of *wnt10b, lef-1, Igf-1, hgf, Tgf-β2* and *c-myc* in cultured hDPCs. hDPCs were cultured in DMEM/10% FBS supplemented with or without 8DSS (10 μmol/L) for 72 hours. The expression of *wnt10b, lef-1, Igf-1, hgf, Tgf-β2* and *c-myc* was examined by RT-qPCR, and GAPDH served as the internal control (mean ± SD, n = 3; $^{*}P < 0.05$, $^{***}P < 0.001$ vs. the mock-control group by SPSS 13.0 (SPSS, IL, USA) using the nonparametric Mann-Whitney U-test or two-tailed Student's t test).

after 8DSS treatment (**Figure 6B**). Given that GSK3β phosphorylation is regulated by Akt and that Akt pathway activation is important in maintaining DPCs, we further assessed Akt phosphorylation. As shown in **Figure 6B**, 8DSS treatment resulted in the robust phosphorylation of Akt as early as 5 min. However, Akt and GSK3β phosphorylation returned to basal levels 24 h after 8DSS treatment (**Figure 6B**). In addition, immunohistochemistry analysis showed that nuclear β-catenin levels were significantly increased in DP and the surrounding hair matrix cells on day 7 after 8DSS treatment (**Figure 6E**). These results showed that 8DSS activated the Wnt/β-catenin pathway in hDPCs through stimulating Akt and GSK3β phosphorylation. Quantitative measurements indicated that the levels of nuclear translocation of β-catenin and GSK3β and Akt phosphorylation in hDPCs were significantly up-regulated in a time-dependent manner by 8DSS (**Figure 6C**, **6D**). The relative levels of target protein expression compared with the internal control are presented in **Figure 6F**.

Discussion

In this study, we found that topical application of 8DSS induces hair re-growth. Moreover, we revealed that 8DSS up-regulated Wnt-related genes, such as *wnt10b*, *lef-1* and *c-myc*. This effect was accompanied with increased expression of nuclear β-catenin and phosphorylated GSK3β and Akt in hDPCs. Although 8DSS up-regulated the expression of *wnt10b, lef-1 and c-myc* in cultured hDPCs, it did not affect the expression of *Igf-1*, *Tgf-β2* and *hgf*. These results suggest that 8DSS activates hDPCs through the Wnt/β-catenin pathway.

The critical role of the Wnt/β-catenin pathway in hair follicle morphogenesis and regeneration has been well documented [4, 20, 21]. Wnts have important roles in hair regeneration during adult life [23]. *wnt10b* is a core member of the Wnt/β-catenin signaling pathway, and enhanced expression of *wnt10b* activates the Wnt/β-catenin signaling pathway in the regulation of hair growth. In addition, several studies have demonstrated that *wnt10b* stimulates DPC growth via activating the Wnt/β-catenin signaling pathway, resulting in the proliferation and differentiation of matrix cells next to DP to induce hair shaft growth [24-26]. Our observation that 8DSS up-regulated the expression of *wnt10b* in hDPCs suggests that 8DSS may promote and maintain hDPCs to induce epithelial cell differentiation and control the hair cycle.

Lef-1, the key nuclear mediator of the Wnt/β-catenin signaling pathway, plays an important role in regulating the interaction between the epidermis and dermis in hair follicles. Recent studies indicate that *lef-1* is critical to the differentiation of bulge stem cells. For example, over-expression of *lef-1* in bulge stem cells promotes the translocation of β-catenin into the nucleus to activate downstream genes of the Wnt signaling pathway, resulting in the differen-

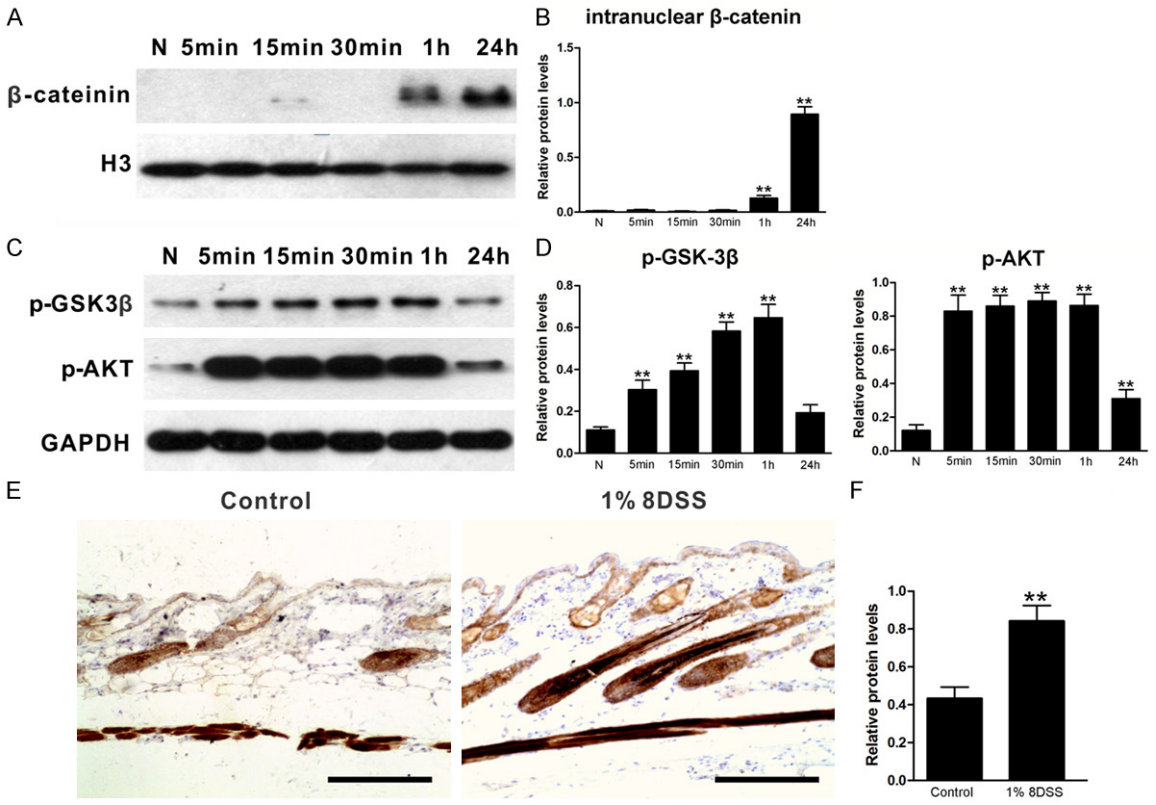

Figure 6. Nuclear β-catenin expression and Akt and GSK3β phosphorylation in hDPCs after 8DSS treatment. A. Phosphorylation of nuclear β-catenin at 60 min after the treatment (nuclear protein) as determined by Western blot. B. Phosphorylation of Akt and GSK3β at 5 min, 15 min, 30 min, 60 min or 24 h after the treatment (total protein) as determined by Western blot. C, D. Quantitative analysis of the relative levels of nuclear β-catenin and Akt and GSK3β phosphorylation in hDPCs (mean ± SD, n = 3; $^{**}P < 0.01$, vs. the control group, by two-tailed Student's t test). E. Induction of β-catenin after topical application of 8DSS for 7 days; β-catenin in longitudinal sections of the dorsal skins was stained by immunohistochemistry (magnification × 200). Bar = 50 μm. F. Quantitative analysis of β-catenin expression in mock-treated group and 8DSS-treated mice (mean ± SD, n = 3; $^{**}P < 0.01$, vs. the control group, by two-tailed Student's t test).

tiation of hair shaft precursor cells in the hair matrix [27]. Similarly, our results showed that 8DSS markedly promoted *lef-1* expression in hDPCs, suggesting that 8DSS may promote the differentiation of the epithelial cell lineage next to DP. We also analyzed the expression of *c-myc* in hDPCs. *c-myc* is an important nuclear transcription factor that is strongly expressed in an anagen hair follicle. Moreover, *c-myc* is the Wnt/β-catenin target gene that regulates cell growth [28, 29]. Several studies have shown that *c-myc* is expressed in the inner root sheath of hair follicle, which indicates a relationship between *c-myc* and the differentiation of the inner root sheath of hair follicle. Other studies demonstrated that *c-myc* is localized to the outer root sheath and plays important roles in regulating stem cell activation [29-31]. However, in our study, 8DSS activated the expression of *lef-1* and *c-myc* in cultured hDPCs. These findings strongly suggest that *lef-1*-mediated signaling transduction determines DP cell fate.

Many other signaling pathways are reported to intersect with Wnt/β-catenin signaling to regulate hDPCs. GSK3β is an important mediator between the Wnt/β-catenin and PI3K/Akt pathways. GSK3β phosphorylates β-catenin and reduces the translocation of β-catenin to the nucleus. GSK3β activity is suppressed when it is phosphorylated at Ser9 by Akt [32]. Recent studies have shown that GSK3β plays a critical role in hair follicle regulation, including proliferation, survival, gene expression, nuclear export, differentiation and apoptosis [33, 34]. Our results demonstrated that 8DSS increased the phosphorylation of both GSK3β and Akt, sug-

gesting that 8DSS activates the Wnt/β-catenin pathway in hDPCs through the Akt signaling pathway.

In summary, we demonstrated that 8DSS induces hair regrowth in C57BL/6 mice through activating the Wnt/β-catenin signaling pathway in hDPCs. The ability of 8DSS to induce anagen hair follicles suggests that 8DSS may delay the progression of follicular miniaturization or promote hair re-growth in patients with hair follicle problems, such as androgenetic alopecia (AGA).

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Disclosure of conflict of interest

None.

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