SUPPLEMENTARY INFORMATION

Title. Keratin is not only a Structural Protein in Hair: Keratin-mediated Hair Growth
Short title: Keratin-mediated Hair Growth

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SUPPLEMENTARY METHODS

Human Hair Keratin Extraction

Briefly explaining, human hair was washed using general detergent and delipidized with chloroform (JUNSEI CHEMICAL, 28560-0350):methanol (Merck Millipore, 106009) (2:1, v/v) for 24 hr at room temperature. The delipidized hair was oxidized with 2(w/v) % peracetic acid (Sigma-Aldrich, 269336) for 12 hr at 37°C. The hair was reacted with Shindai solution (5M urea (Sigma-Aldrich, U5378), 2.4M thiourea (Sigma-Aldrich, T7875), 5% 2-mercaptoethanol (Sigma-Aldrich, M6250), 24mM trizma base (Sigma-Aldrich, T1503), pH 8.5) for 72 hr at 50°C. After reaction, The mixture was centrifuged at 3,500 rpm for 20 min and supernatant was dialyzed (12-14 kDa cut-off, Spectra/Por 4 dialysis membrane, 132706) against deionized water for 5 days with three changes in water a day. Solution of hair keratin was centrifuged at 3,500 rpm for 20 min and supernatant was lyophilized by freeze-dryer.

Human Hair Keratin-mediated Hair Growth Test in Mice

For in vivo studies, six-week-old C57BL/6 male mice were purchased from Orientbio Inc. (Gyeonggi-do, Korea). This study was performed within the animal facility area in Gyeonggji bio center, and the animals were housed in a room that was maintained at a temperature of 23 ± 3 °C and a relative humidity of 55 ± 15 %, with artificial lighting from 08:00 to 20:00, 150-300 Lux of luminous intensity. Throughout the experimental period, the temperature and humidity of animal room were measured every hour with a computer-based automatic sensor, and as a result of measurements, there were no deviations to have adverse effect to the result of study. Animals were offered irradiation-sterilized pellet food for lab animal (Teklad certified irradiated global 18 % protein rodent diet; 2918C, Envigo, UK). Underground water disinfected by ultraviolet sterilizer and ultrafiltration was given via water bottle, ad libitum. Examination of water was performed by an authorized Gyeonggido Institute of Health & Environment (Suwon, Gyeonggi-do, Republic of Korea), and there were no factors that could affect results. All animals experiment were performed based on standard operating procedure of Chemon.Inc and the Animal experimentation policy of Gyeonggji bio center (experimentation number: 2018-08-011).

To examine the hair growth promoting effect of keratin according to keratin concentration 0.5(w/v)% and 1.0 (w/v)% keratin in phosphate buffered saline (PBS; Gibco, 10010023) was used. Animals were anesthetized by intraperitoneally administration of zoletyl and rumpoon mixture (4:1, v/v) at the dose of 1 mL/kg. The dorsal area was shaved with an animal clipper. Upon shaving the mice all of the hair follicles were synchronized in the telogen stage, showing pink color. The wound-free animals were selected and weighed. The selected animals were distributed in a randomized manner so that the average weight of each group was distributed as uniformly as possible according to the weighted weight. All animals were randomized into 5 groups based on different topical applications: normal control, DPBS administration, 0.5% keratin administration, 1% keratin administration, and 3% minoxidil as a positive control. The test substance was administered intradermally, the clinically planned route, and the positive control substance was applied tropically. The keratin groups were administered once at day 1, and the positive control substance was administered once/day, 5 times/week, and 4 weeks. The intradermal administration was divided into 2 sites in the dorsal part of animals inhaled with isoflurane using a 0.3 mL insulin syringe (31G), and divided into 75 μL at 1 site. Topical administration was applied to the back of the animal, which was corrected for 0.15 mL using a 1 mL syringe, rubbed 10 times with a glass rod, and applied evenly. The mice were sacrificed after 24 weeks.
Interaction Assay of DP Cells with Keratin on Matrigel

For DP cell condensation assay on matrigel, the matrigel was diluted at 1:2 volume ratio in ice cold serum free DMEM media (CORNING, 10-013-CV), and gelation was done by incubating at 37°C for 30 minutes. DP cells were seeded at a seeding density of 2x10⁴ cell/cm² on top of matrigel, and the DP cells on matrigel were adjusted to be stable for 1 day in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂ prior to keratin treatment. After 1 day of adjustment, DP cells were cultured in human dermal papilla growth medium containing 1.0(w/v)% keratin or not. Cell viability of DP cells on matrigel was visualized using two-colour fluorescence cell viability assay, EthD-1/Calcein AM live/dead assay (Invitrogen, L3224), which was done according to the manufacturer’s instruction, and the morphological change and live/dead stage of DP cells on matrigel in the presence of keratin was observed under inverted fluorescent microscopy (Olympus IX71).

RNA Extraction and Sequencing

To perform transcriptome sequencing (RNA-Seq) analysis of DP cells and ORS cells, total RNA was extracted from the ORS and DP cells in the absences of keratin and in the presence of keratin. Quality and integrity of the extracted total RNA was assessed by BioAnalyzer and the standard illumina sequencing system protocol (TruSeq Stranded mRNA LT Sample Prep Kit) have been used to make libraries for RNA-Seq. Around 300 bp fragments were isolated using gel electrophoresis, amplified by PCR and sequenced on the Illumina HiSeq 2500 in the paired-end sequencing mode (2x10¹ bp reads).

RNA-Seq Read Processing and Differential Gene Expression Analysis

Quality of the raw sequencing reads were assessed, and qualified raw sequencing reads were aligned to the human genome reference hg19 using TopHat alignment tool (v2.1.0) [PMID: 23618408]. Uniquely and properly mapped read pairs have been used for further analysis. Gene annotation information was downloaded from Ensembl (release 75) biomart (http://www.ensembl.org/). To evaluate expression levels of genes, the RPKM (reads per kilobase of exon per million mapped reads) measurement unit was used [PMID: 18516045] and the fold change between two samples (untreated and treated with keratin) was calculated based on the calculated RPKM. DESeq2 R package [PMID: 20979621] was used to identify differentially expressed genes between undifferentiated neural stem cell and the differentiated dopaminergic neuron. Differentially expressed genes were defined as those with changes of at least 2-fold between samples at a false discovery rate (FDR) of 5%.

DP Cell Spheroid Formation and Maintenance assay of the replated DP Cell Spheroids

200 µL of cell suspension (1 x 10⁶ cells per mL) was spread on PEG microwells mounted on a glass slide, and undocked cells were removed by gentle washing with PBS after 30 mins of incubation. DP cell spheroid was formed within PEG microwell by incubating the cell-docked microwell in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂ for 1 day. The formed DP cell spheroids were retrieved from PEG microwell by gentle agitation, and then replated on 12 well and 6 well tissue culture plate (SPL LIFE SCIENCES, 30012, 30006). The replated DP cell spheroids were adjusted to adhere on tissue culture plate for 1 day in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂ prior to keratin treatment. After 1 day of adjustment, DP cells were cultured in human dermal papilla growth medium containing 1.0(w/v)% keratin or not. The morphological change of DP cell
spheroids in the presence of keratin was observed under inverted fluorescent microscopy (Olympus IX71).

**TGFβ2-mediated ORS Cell Apoptosis and Co-culture with DP Cells**

To evaluate DP cell condensation in direct co-culture of DP cells and TGFβ2-treated ORS cells, DP cells were stained with a cell tracker (Red CMTPX, Invitrogen, C34552) according to manufacturer’s instruction. The stained DP cells were seeded at a density of 2x10^4 cell/cm^2 on confluent TGFβ2-treated ORS cell layer which cultured for 5 days prior to co-culture, and cultured in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5% CO₂. After 1 and 2 days of co-culture, DP cell condensation was observed under inverted fluorescent microscopy (Olympus IX71).

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To evaluate DP cell condensation under conditioned media from TGFβ2-treated ORS cell layer, the conditioned media were collected from TGFβ2-treated ORS cell layer after 5 days of culture. DP cells were seeded at a density of 2x10^4 cell/cm^2 on 12 well and 6 well non-treated tissue culture plate (SPL LIFE SCIENCES, 32012, 32006). The DP cells were adjusted to be stable for 1 day in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5% CO₂. After 1 day of adjustment, DP cells were cultured under various culture media; human dermal papilla growth medium, human outer root sheath cell growth medium containing 100ng/ml TGFβ2, conditioned medium collected from TGFβ2-treated ORS cell layer and human dermal papilla growth medium containing (w/v)% keratin. After 1 and 2 days of culture, DP cell condensation was observed under inverted fluorescent microscopy (Olympus IX71).

**Immunodepletion Study**

To evaluate DP cell condensation under keratin-removed conditioned media, DP cells were seeded at a density of 2x10^4 cell/cm^2 on 12 well and 6 well non-treated tissue culture plate (SPL LIFE SCIENCES, 32012, 32006). The DP cells were adjusted to be stable for 1 day in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5% CO₂. After 1 day of adjustment, DP cells were cultured under various culture media; human dermal papilla growth medium, conditioned medium collected from TGFβ2-treated ORS cell layer, conditioned medium collected from TGFβ2-treated ORS cell layer and incubated with guinea pig normal IgG-conjugated beads, conditioned medium collected from TGFβ2-treated ORS cell layer and incubated with guinea pig anti-Type I+II Hair Keratins antibody-conjugated beads, and human dermal papilla growth medium containing 1(w/v)% keratin. After 1 and 2 days of culture, DP cell condensation was observed, and the number of condensed DP cell aggregates was counted using inverted fluorescent microscopy (Olympus IX71).

To evaluate P-cadherin expressing germ formation of ORS cells under keratin-removed conditioned media, ORS cells were seeded at a density of 2x10^5 cell/cm^2 on 12 well tissue culture plate (SPL LIFE SCIENCES, 30012) to make confluent ORS cell layer. The ORS cells were adjusted to be stable for 1 day in human outer root sheath cell growth medium (CEFO, CB-CBORS-GM) at 37°C in a humidified atmosphere containing 5% CO₂. After 1 day of adjustment, ORS cells were cultured under various culture media; human outer root sheath cell growth medium, conditioned medium collected from ORS cell layer, conditioned medium collected from TGFβ2-treated ORS cell layer, conditioned medium collected from TGFβ2-treated ORS cell layer and incubated with guinea pig normal IgG-conjugated beads, conditioned medium collected from TGFβ2-treated ORS cell layer and incubated with guinea pig anti-Type I+II Hair Keratins antibody-conjugated beads, and human outer root sheath cell growth medium.
growth medium containing 1(w/v)% keratin. After 3 days of culture, P-cadherin expressing germ formation of ORS cells was characterized by immunocytochemical staining.

**In Vitro Caspase-6 Gene Silencing Study**

Caspase-6 siRNA duplex (Bioneer, 839-1) or negative control siRNA duplex (Bioneer, SN-1012) was diluted in 250 µl Opti-MEM (Gibco, 31985062) to make a final concentration of 100 nM. 3.5 µl Lipofectamine 2000 (Invitrogen, 11668-030) was mixed in 250 µl Opti-MEM, and the mixture was incubated for 5 min at room temperature. The diluted caspase-6 siRNA duplex and diluted Lipofectamine 2000 were mixed and incubated for 20 min at room temperature. Before transfection, ORS cells were seeded at a density of 2x10⁵ cell/cm² on 12 well tissue culture plate (SPL LIFE SCIENCES, 30012) to make confluent ORS cell layer. The ORS cells were adjusted to be stable for 1 day in human outer root sheath cell growth medium (CEFO, CB-ORS-GM) at 37°C in a humidified atmosphere containing 5 % CO₂. After 1 day of adjustment, human outer root sheath cell growth medium (CEFO, CB-ORS-GM) was changed with fresh same medium without serum. The capase-6 siRNA / Lipofectamin 2000 mixture or negative control siRNA / Lipofectamine 2000 mixture was added to ORS cell culture, and incubated for 5 hr at 37°C. After transfection, the medium was changed with a fresh medium containing serum, and the transfected ORS cells were cultured in the presence of 100ng/ml TGFβ2 or in the absence of TGFβ2 for 5 days.

To evaluate DP cell condensation in direct co-culture of DP cells and TGFβ2-treated / caspase-6 silenced ORS cells, DP cells were stained with a cell tracker (Invitrogen, C34552) according to manufacturer’s instruction. The stained DP cells were seeded at a density of 2x10⁴ cell/cm² on confluent TGFβ2-treated / caspase-6 silenced ORS cell layer which cultured for 5 days prior to co-culture, and cultured in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂. After 2 days of co-culture, DP cell condensation was observed under inverted fluorescent microscopy (Olympus IX71).

To evaluate DP cell condensation under conditioned media from TGFβ2-treated / caspase-6 silenced ORS cells, the conditioned media were collected from TGFβ2-treated / caspase-6 silenced ORS cell culture after 5 days. DP cells were seeded at a density of 2x10⁴ cell/cm² on 12 well and 6 well non-treated tissue culture plate (SPL LIFE SCIENCES, 32012, 32006). The DP cells were adjusted to be stable for 1 day in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂. After 1 day of adjustment, DP cells were cultured under various culture media; human dermal papilla growth medium, human outer root sheath cell growth medium containing 100ng/ml TGFβ2, conditioned medium collected from TGFβ2-treated / negative control siRNA transfected ORS cell layer, conditioned medium collected from TGFβ2-treated / negative control siRNA transfected ORS cell layer containing additional 100ng/ml of TGFβ2, and conditioned medium collected from TGFβ2-treated / caspase-6 silenced ORS cell layer. After 1 and 2 days of culture, DP cell condensation was observed under inverted fluorescent microscopy (Olympus IX71).

**In Vitro KRT31/KRT34 Gene Silencing Study**

KRT31 siRNA duplex (Bioneer, 3881-1) and KRT34 siRNA duplex (Bioneer, 3885-1) or negative control siRNA duplex (Bioneer, SN-1002) were diluted in 250 ul Opti-MEM (Gibco, 31985062) to make a final concentration of 100 nM. 3.5 ul Lipofectamine 2000 (Invitrogen, 11668-030) was mixed in 250 ul Opti-MEM, and the mixture was incubated for 5 min at room temperature. The diluted caspase-6 siRNA duplex and diluted Lipofectamine 2000 were mixed and incubated for 20 min at room temperature. Before transfection, ORS cells were seeded at a density of 2x10⁵ cell/cm² on 12 well tissue culture plate (SPL LIFE SCIENCES,
30012) to make confluent ORS cell layer. The ORS cells were adjusted to be stable for 1 day in human outer root sheath cell growth medium (CEFO, CB-ORS-GM) at 37°C in a humidified atmosphere containing 5% CO₂. After 1 day of adjustment, human outer root sheath cell growth medium (CEFO, CB-ORS-GM) was changed with fresh same medium without serum. The KRT31/KRT34-siRNA / Lipofectamine 2000 mixture or negative control siRNA / Lipofectamine 2000 mixture was added to ORS cell culture, and incubated for 5 hr at 37°C. After incubation, the medium was changed with a fresh medium containing serum, and the transfected ORS cells were cultured in the presence of 100ng/ml TGFβ2 for 5 days, and morphological change was observed under inverted fluorescent microscopy (Olympus IX71).

**Apoptosis and Growth Factor Antibody Array**

For apoptosis array analysis, cell lysates were prepared as follows; cells on culture plate were washed with PBS three times. After washing, cells were incubated with the 1X Lysis buffer at 4°C for 30 min, and lysates were centrifuged at 12,000 RPM for 10 min to remove the debris. After centrifuge, the supernatant was collected and used for the apoptosis array analysis. Following process of apoptosis antibody array was done according to manufacturer’s instructions. Dot blots on membrane were analyzed using the plugin Protein Array Analyzer (http://image.bio.methods.free.fr) on ImageJ (https://imagej.nih.gov/ij/).

For growth factor antibody array analysis, in immunodepletion study, conditioned media were collected from TGFβ2-treated ORS cell culture and prepared as follows; the collected conditioned media were centrifuged to remove debris, and supernatant was collected. The supernatant was concentrated about 20 times by Amicon Ultra centrifugal filter units (Millipore, Z717185). The concentrated conditioned media were used for growth factor antibody array analysis and following process of growth factor antibody array was done according to manufacturer’s instructions. Dot blots on membrane were analyzed using the plugin Protein Array Analyzer (http://image.bio.methods.free.fr) on ImageJ (https://imagej.nih.gov/ij/).

**Western Blot Analysis**

Proteins from various conditioned medium were concentrated about twentyfold by Amicon Ultra centrifugal filter units (Millipore, Z717185), and cells were lysed on ice for 30 min in 100 µl ice-cold RIPA lysis buffer (Millipore, 20-188) containing a protease inhibitor cocktail (Roche, 4693116001), and then lysates were centrifuged at 12,000 rpm to remove debris. Samples were denatured at 70°C for 10 min in LDS sample buffer (Invitrogen, B0007), and equal amounts of denatured samples were loaded in pre-casted 4-12% Bis-Tris Plus Gels (Invitrogen, NW04120BOX), and the electrophoresis was done by running at 200 V for 22 min. After electrophoresis, the proteins in the gel were transferred to PVDF membranes (Bio-Rad, 1620174) using electrophoretic transfer cell (Bio-Rad, 1703930). Immunoblotting for the membranes was carried out as follows; the membranes were incubated in TBS containing 5% skim milk (bioWORLD, 30620074-1) at room temperature for 60 min. The membranes were further incubated in TBS containing 1% skim milk and primary antibodies for overnight at 4°C. The primary antibodies used in western blot analysis were as follows; rabbit Anti-KRT34 (LifeSpan BioSciences, LS-B15620, diluted 1:1000), guinea pig Anti-Type I+II Hair Keratins (PROGEN, GP-panHK, diluted 1:1000) and Mouse Anti-GAPDH (Abcam, ab8245, diluted 1:5000). After incubation with primary antibodies, the membranes were incubated with following secondary antibodies in TBS containing 1% skim milk at room temperature for 2 hr; HRP conjugated goat anti-guinea pig IgG (Abcam, ab97155, diluted 1:10000), HRP conjugated mouse anti-rabbit IgG (Santa Cruz Biotechnology, sc-2357, diluted 1:5000) and HRP conjugated goat anti-mouse IgG (Santa Cruz Biotechnology, sc-2005, diluted 1:5000). At each
process, the membranes were washed three times with TBS containing 0.1% Tween 20 (Duchefa Biochemie, P1362.1000) for 10 min. The membranes were treated with ECL substrate (Bio-Rad, 1705061) to visualize signal, and the signals on the membranes were transferred to X-ray film (AGFA, CP-BU New).

**Real Time Quantitative Polymerase Chain Reaction (RT-qPCR)**

The gene expressions indicative of DP cell’s intrinsic property were evaluated by RT-qPCR. Total RNA was extracted from DP cells and DP Cell spheroids using Hybrid-R (GeneAll, 305-101). cDNAs were synthesized by reverse transcription reaction using a CycleScript RT PreMix (Bioneer, K-2044). After cDNA synthesis, cDNAs were mixed with SYBR kit (PhileKorea, QS105-10) and primers (Table1). The mixture was reacted by thermal cycler (QIAGEN, Rotor-Gene Q) and analyzed by the program provided (Rotor-Gene Q Series Software, V1.7). The whole process was carried out according to the manufacturer's instructions.

TGFβ2 gene expressions of DP cell spheroids and the replated DP cell spheroids were evaluated by RT-qPCR. DP cell spheroids were generated by PEG microwell-mediated condensation as previously described. The formed DP cell spheroids were retrieved from PEG microwell, and then replated on 6 well tissue culture plate (SPL LIFE SCIENCES, 30006). The replated DP cell spheroids were cultured in human dermal papilla growth medium (CEFO, CB-HDP-GM) at 37°C in a humidified atmosphere containing 5 % CO₂. After 0, 1, 3, 5 and 7 days of culture, total RNA was extracted from DP cell spheroid, and RT-qPCR was done by previously described same method. The primer used in this experiment was noted in Table S1.

**Indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA)**

Replated DP spheroids cultured in the presence of keratin were harvested and lysed with RIPA lysis buffer (Millipore, 20-188) containing a protease inhibitor cocktail (Roche, 4693116001). Lysates were centrifuged for 15 min at 12,000 rpm to remove the debris, and the supernatant was collected. The lysates were diluted to a final concentration of 20 μg/ml in DPBS (Dulbecco's Phosphate-Buffered Saline; Gibco, 14190-144). Antigens were coated on wells in a 96 well ELISA plate (Invitrogen, 44-2404-21) by loading 50 μl of the diluted lysate, and the wells were incubated at 4°C for 18hr. The plate was washed three times with 200 μl of DPBS, and non-specific binding of antibodies was blocked by treating with DPBS containing 5(w/v)% bovine serum albumin (BSA; Sigma-Aldrich, A9418) for 2 hr at room temperature. Primary antibodies such as rabbit anti-β-actin (Abcam, ab8227, diluted 1:1000), rabbit anti-β-catenin (Abcam, ab16051, diluted 1:1000), rabbit anti-FGF7 (Santa Cruz Biotechnology, sc-7882, diluted 1:200), Goat anti-FGF10 (Santa Cruz Biotechnology, sc-7375, diluted 1:200) and goat anti-BMP6 (Santa Cruz Biotechnology, sc-7406, diluted 1:200) were diluted in DPBS containing 1(w/v)% BSA, and added 100 μl to each well. The plate was incubated at room temperature for 2 hr for the reaction of antibodies with antigens. After incubation with primary antibodies, the wells were washed with DPBS, and 100 μl of HRP conjugated secondary antibodies (anti-rabbit IgG-HRP (Santa Cruz Biotechnology, sc-2004) or anti-goat IgG-HRP (Santa Cruz Biotechnology, sc-2033)) was added to each well, and then was incubated for 2hr at room temperature. After incubation with secondary antibodies and following washing with DPBS. 100 μl of TMB substrate solution (Thermo Scientific, N301) was added to each well and incubated for 30 min at room temperature. The reaction was stopped by 100μl of the stop solution (2M sulfuric acid; Sigma-Aldrich, 258105), and the absorbance of samples was measured at 450 nm on a microplate spectrophotometer reader (Bio-Rad, Benchmark Plus, BR170-6930).
RT-qPCR for In Vivo KRT31/KRT34 Gene Silencing Study

Total RNA was extracted from the cells using Hybrid-R (GeneAll, 305-101). cDNAs were synthesized by reverse transcription reaction using a CycleScript RT PreMix (Bioneer, K-2044). After cDNA synthesis, cDNAs were mixed with SYBR kit (PhileKorea, QS105-10) and primers (Table S2). The mixture was reacted by thermal cycler (QIAGEN, Rotor-Gene Q) and analyzed by the program provided (Rotor-Gene Q Series Software, V1.7). The whole process was carried out according to the manufacturer's instructions.

Immunocytochemical staining

DP cells and ORS cells were fixed for 10 min in 3.7% paraformaldehyde (Sigma-Aldrich, F8775), and were permeabilized in 0.2% Triton X-100 (Sigma-Aldrich, T9284). Non-specific binding was blocked by treating with 4% BSA (Sigma-Aldrich, A9418). Cells were incubated in primary antibody diluents (GBI Labs, E09-500) containing the following primary antibodies for overnight at 4°C; rabbit anti-β-catenin (Abcam, ab16051, diluted 1:100), rabbit anti-SOX2 (Cell Signaling Technology, sc-59829, diluted 1:50), rabbit anti-P-cadherin (Cell Signaling Technology, 2189S, diluted 1:50), mouse anti-β1-integrin (Santa Cruz Biotechnology, sc-59829, diluted 1:50), rabbit anti-P-cadherin (Cell Signaling Technology, 2189S, diluted 1:50), mouse anti-E-cadherin (Abcam, ab1416, diluted 1:100), mouse anti-RUNX1 (Santa Cruz Biotechnology, sc-365644, diluted 1:50), rabbit anti-CD34 (Abcam, ab81289, diluted 1:100), rabbit anti-KRT34 (LifeSpan BioSciences, LS-B15620, diluted 1:100), rabbit anti-CD34 (Abcam, ab81289, diluted 1:100), rabbit anti-CD34 (Abcam, ab81289, diluted 1:100), rabbit anti-Annexin V (Abcam, ab14196, diluted 1:100), rabbit anti-caspase-3 (Abcam, ab13847, diluted 1:100), rabbit anti-caspase-6 (Abcam, ab52951, diluted 1:100). After incubation with primary antibodies, cells were washed three times with DPBS and were incubated with the following secondary antibodies for 1 hr at room temperature; Alexa Fluor 488 conjugated goat anti-rabbit IgG (Invitrogen, A-11034, diluted 1:200), Alexa Fluor 594 conjugated goat anti-rabbit IgG (Invitrogen, A-11012, diluted 1:200), Alexa Fluor 594 conjugated goat anti-mouse IgG (Invitrogen, A-11032, diluted 1:200) and Alexa Fluor 488 conjugated goat anti-mouse IgG (Invitrogen, A-11001, diluted 1:200). With secondary antibodies, actin was stained using rhodamine phalloidin (Invitrogen, R415, diluted 1:400), and tunel staining was performed using the Turner Enzyme (Roche, 11767305001) and Tunel Label (Roche, 11767291910). Briefly, cells were washed twice with DPBS (Gibco, 14190-144) and incubated at 37°C for 1 hr with 200 μl of TUNEL reaction mixture (Turner Enzyme: Tuner Label, 1:9, v/v). After incubation with secondary antibodies, phalloidin and tunel reaction mixture, cells were washed with DPBS three times and counterstained with DAPI (Sigma-Aldrich, D9542). Finally, stained cells were observed under an inverted fluorescence microscope (OLYMPUS, IX71) and captured.

Immunohistochemical Staining

Paraffin embedded tissue sections were deparaffinized by treating xylene three times for 5 min and rehydrated in graded ethanol (100%, 95%, 90%, 80% and 50%). Antigen retrieval step was performed by incubating the sections in sodium citrate buffer (10mM Sodium Citrate; Biosesang, C2004, 0.05% Tween 20; Duchefa Biochemie, P1362.1000, pH 6.0) at sub-boiling temperature for 20 min. Non-specific binding of antibody was blocked by treating with blocking buffer containing 1% normal horse serum (Abcam, ab7484) and 5% BSA (Sigma-Aldrich, A9418) for 30 min at room temperature. After blocking, tissue sections were incubated in primary antibody diluents (GBI Labs, E09-500) containing the following primary antibodies
for overnight at 4°C; goat anti-P-cadherin (R&D Systems, AF761, diluted 1:50), rabbit anti-β-catenin (Abcam, ab16051, diluted 1:50), rabbit anti-KRT34 (LifeSpan BioSciences, LS-B15620, diluted 1:50) and guinea pig anti-type I+II hair keratins (PROGEN, GP-panHK, diluted 1:50). After washing tissue sections with DPBS three times, were incubated with the following secondary antibodies for overnight at 4°C; donkey Alexa Fluor 488-conjugated anti-goat IgG Alexa Fluor 488 (Invitrogen, A-11055, diluted 1:200), goat Alexa Fluor 594-conjugated anti-rabbit IgG (Invitrogen, A-11012, diluted 1:200), goat Alexa Fluor 488-conjugated anti-rabbit IgG (Invitrogen, A-11034, diluted 1:200) and goat Alexa Fluor 647-conjugated anti-guinea pig IgG (Abcam, ab150187, diluted 1:200). After incubation with secondary antibodies, tissue sections were washed with DPBS three times, counterstained with DAPI (Sigma-Aldrich, D9542) and mounted with mounting medium (Sigma-Aldrich, F4680). Finally, images were acquired using an inverted fluorescence microscope (OLYMPUS, IX71).
## SUPPLEMENTARY TABLES

### Table S1. Primers used in realtime-qPCR

<table>
<thead>
<tr>
<th>Forward</th>
<th>Reverse</th>
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<tbody>
<tr>
<td>CD133 CAGAGTACAACGCAAACCA</td>
<td>CD133 AAATCACGATGAGGGTCAGC</td>
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<tr>
<td>Vesican GGGATTGAAGACACACAGACACG</td>
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<td>Shh CACCTCTGAGTTCACGCTG</td>
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<tr>
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<td>Gapdh TTTTCTAGACGAGCGAGTCAG</td>
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<tr>
<td>TGFβ2 AAATGGACGTAGGCGACAG</td>
<td>TGFβ2 GACCAACCUGCGGAAG</td>
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### Table S2. Primers used in *in vivo* KRT31/KRT34 silencing study

<table>
<thead>
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<th>Forward</th>
<th>Reverse</th>
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<td>mKrt31 CAAGCAATGCATGCGGCAA</td>
<td>mKrt31 CGTACAAAGGAGTTGATGCC</td>
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<tr>
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<td>mKrt34 GAACCAATTTGATTGCCC</td>
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<tr>
<td>mGapdh GAGAGTTTCCCTCGTCCC</td>
<td>mGapdh ATGAAGGGGTCGATTGATGC</td>
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</table>
Supplementary Figure 1 | Intradermal injection of human hair-derived keratin with different concentrations induced hair follicle formation and improved hair growth. A: Images of hair growth on the back skin of mice at day 1, day 14 and day 28 after injection of keratin. B: Histological images of the back skin of mice at 4 weeks after injection of keratin. C and D: Graphical representation and quantification of hair follicles with different hair cycle stages in skin sections of mice (n = 12 sections, in 6 mice; mean ± standard deviation (s.d.)). *P < 0.05, indicates a significant difference between control and keratin injected groups. Scale bars, 200μm.
Supplementary Figure 2 | Hair keratin treatment upregulated DP cell property-related gene expressions and induced DP cell condensation. A: Image of DP cell and DP cell condensation on day 1 after keratin treatment by immunofluorescent staining at low magnification; DAPI, blue; SOX2, ALPase, FGF10, BMP6, red; β-catenin, CD133, FGF7, green. Scale bars, 200μm. B: Quantification of DP cell growth in the presence of keratin. *P<0.01, indicates a significant difference between control and keratin treated. (n=6; mean ± standard deviation (s.d.)). C: Time lapsed images of DP cell condensation in the presence of keratin. Scale bars, 100μm.
Supplementary Figure 3 | Hair keratin treatment induced DP cell condensation at different cell density. A: Images of DP cell condensation on day 3 after keratin treatment and graphical representation of DP cell condensation at different cell seeding densities. *P<0.01, indicates a significant difference between control and keratin treated. Scale bars, 200μm. (n=4; mean ± standard deviation (s.d.)). Arrows indicate the condensed DP cell aggregates. B: Images of DP cell condensation at different cell density in the presence of keratin by immunofluorescent staining; DAPI, blue; SOX2, red; β-catenin, green. Scale bars, 100μm.
Supplementary Figure 4 | Hair keratin treatment induced DP cell condensation on matrigel. Images of DP cell condensation on matrigel in the presence of keratin and live/dead assay. Scale bars, 100μm.
**Supplementary Figure 5** | DP cell condensation at different keratin concentrations. A: Images of DP cell condensation on day 3 after treatment with different keratin concentrations, and graphical representation of DP cell condensation at different keratin concentrations. *P<0.01, indicates a significant difference between control and keratin treated. Scale bars, 200µm. (n=4; mean ± standard deviation (s.d.)). B: Images of DP cell condensation at different keratin concentrations by immunofluorescent staining; DAPI, blue; β-catenin, green. Scale bars, 100µm.
Supplementary Figure 6 | Differentially expressed genes in DP cells upon on the keratin treatment. RNA-Seq data were acquired from the DP cells in the absence and cell in the presence of keratin. Differential expression test has been performed using DESeq2 method and the differentially expressed genes were defined as those with changes of at least 2-fold between samples at a false discovery rate (FDR) of 5%. A: Upregulated genes in the DP cells treated with keratin. B: Downregulated genes in the DP cells treated with keratin.
Supplementary Figure 7 | Hair-derived keratin induced P-cadherin expressing germ formation of ORS cells. *In vitro* germ formation of ORS cells by immunofluorescent staining of in the presence of keratin (low magnified images); DAPI, blue; phalloidin, integrin β1, red; P-cadherin, β-catenin, green; Control confluent, ORS cell culture at confluent cell density under ORS culture medium; Keratin treated confluent, ORS cell culture at confluent cell density in the presence of keratin. Scale bars, 100μm.
Supplementary Figure 8 | Gene expression profiles of keratin family genes in ORS cells in the absence and presence of keratin. A: RNA-Seq method had been used to measure the gene expression levels of keratin genes. OC_GC: the number of raw sequencing read mapped on the keratin gene in the ORS cell in the absence of keratin. OK_GC: the number of raw sequencing read mapped on the keratin gene in the ORS cell in the presence of keratin. FC: fold change between two samples using normalized expression value (RPKM, see the method section). B: Graphical representation of KRT34 and β-catenin molecular expressions of ORS cells in the presence of keratin: western blot assay.
Supplementary Figure 9 | Images of apoptosis array of ORS cells and TGF-β2 treated ORS cells.
Supplementary Figure 10 | DP cell condensation in co-culture with TGFβ2-treated ORS cells and in conditioned medium collected from TGFβ2-treated ORS cell culture. A: Images of DP cell condensation on TGFβ2-treated ORS cell layers. Co-culture of cell tracker-treated DP cells (red) with TGFβ2-treated ORS cell layers. Immunofluorescent image; cell tracker-treated DP cells, red; E-cadherin, KRT34, green; DAPI, blue. Scale bars, 100μm. B: Images of DP cell condensation in conditioned medium collected from TGFβ2-treated ORS cell culture, and DP cell condensation by immunofluorescent staining; alkaline phosphatase, red; β-catenin, green; DAPI, blue; Control-DP medium, DP culture medium; Control-ORS Medium-TGFβ2, basic ORS medium including TGFβ2; CM from TGFβ2 treated ORS, conditioned medium collected from TGFβ2-treated ORS cell culture; Keratin treatment, DP medium containing 1(w/v)% keratin. Scale bars, 100μm.
Supplementary Figure 11 | Low magnified images of ORS cells, ORS cell culture in the presence of TGFβ2 and caspase 6-silenced ORS cell culture in the presence of TGFβ2 by immunofluorescent staining; Phalloidin, E-cadherin, red; KRT34, tune, caspase 6, green; DAPI, blue. Scale bars, 200μm.
**Supplementary Figure 12** | Images of growth factor array of the conditioned medium and the keratin-eliminated conditioned medium collected from TGFβ2-treated ORS cell culture.
Supplementary Figure 13 | The released keratin in conditioned medium from TGFβ2-treated ORS cell culture was not efficient to induce P-cadherin expressing germ formation. Germ formation of ORS cells in conditioned medium from TGFβ2-treated ORS cell culture and keratin-eliminated conditioned medium of TGFβ2-treated ORS cell culture using a column containing anti-human hair keratin antibody-conjugated beads by immunofluorescent staining; DAPI, blue; phalloidin, red; P-cadherin, green: ORS media, ORS culture medium; ORS CM, conditioned medium from ORS cell culture; TGFβ2-ORS CM, conditioned medium collected from TGFβ2-treated ORS cell culture; TGFβ2-ORS CM (IgG-column), conditioned medium collected from TGFβ2-treated ORS cell culture and then treated with normal IgG-conjugated beads; TGFβ2-ORS CM (keratin Ab-column), keratin-eliminated conditioned medium collected from TGFβ2-treated ORS cell culture; 1% Keratin, DP medium containing 1(w/v)% keratin. Scale bars, 100μm.
Supplementary Figure 14 | Images of time-course P-cadherin expressing germ formation of ORS cells in the presence of TGFβ2 by immunofluorescent staining; DAPI, blue; phalloidin, RUNX1, red; P-cadherin, caspase 6, KRT34, green. Scale bars, 100 μm.
Supplementary Figure 15 | A: Low magnified immunohistochemical images of the back skin of mice on day 7 after KRT31/KRT34 siRNA transfection; Control, mice injected with negative control siRNA-loaded lipofetamine; siRNA, KRT31/KRT34 silenced mice; siRNA+KRT, KRT31/KRT34 silenced and hair keratin injected mice; β-catenin, type I+II hair keratin, red; KRT34, green; DAPI, blue. Scale bars, 50µm. B: Molecular distributions of caspase 6, P-cadherin and KRT 34 in developing hair follicles of the back skin of mice at day 7. Scale bars, 20µm.
Supplementary Figure 16 | TGFβ2 expression was found to be downregulated during DP cell condensation and to be rapidly upregulated during the dispersion of condensed DP cells. A: Images of DP cell condensation via spontaneous self-aggregation within microwells and time-dependent dispersion of the replated DP cell spheroids. Scale bars, 200μm. B: Graphical quantification of TGFβ2 mRNA expressions according to the dispersion of condensed DP cells; real time-qPCR. *P<0.01, indicates a significant difference between control and replated DP cell spheroid culture; Control, DP cells; Day 0, DP cell spheroid within microwells; Day 1-7; replated DP cell spheroid culture on tissue culture plates for 1-7 days (n=4; mean ± standard deviation (s.d.)).