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Titrated extract of *Centella asiatica* increases hair inductive property through inhibition of STAT signaling pathway in three-dimensional spheroid cultured human dermal papilla cells

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Dermal papilla (DP) is a pivotal part of hair follicle, and the smaller size of the DP is related with the hair loss. In this study, we investigated the effect of titrated extract of *Centella asiatica* (TECA) on hair growth inductive property on 3D spheroid cultured human DP cells (HDP cells). Significantly increased effect of TECA on cell viability was only shown in 3D sphered HPD cells, not in 2D cultured HDP cells. Also, TECA treatment increased the sphere size of HDP cells. The luciferase activity of STAT reporter genes and the expression of STAT-targeted genes, SOCS1 and SOCS3, were significantly decreased. Also, TECA treatment increased the expression of the hair growth-related signature genes in 3D sphered HDP cells. Furthermore, TECA led to downregulation of the level of phosphorylated STAT proteins in 3D sphered HDP cells. Overall, TECA activates the potential of hair inductive capacity in HDP cells.

Key words: titrated extract of *Centella asiatica*; hair growth; cell culture; dermal papilla cells; STAT

Hair is one of the most complex integrated systems, in which there are several morphological components that act as a unit.[1,2)] Hair basically have biological protective function from its external environments as well as influence social interaction.[3,4)] Therefore, hair loss or baldness, correctively known as alopecia, directly distresses self-confidence affecting our daily life.[5)] The growing and shape of hair fiber is strictly controlled and generated by the specialized mini-organ, hair follicle (HF) anchoring each hair into the skin.[6)] HF is composed of the root sheath (coat region of HF), bulge (terminal part of the HF) and papilla (the base region of the hair bulb) and matrix (around region of the papilla).[7)] In healthy condition, HF undergoes cycles of growth (anagen), regression (catagen), and rest (telogen). In the patients with androgenic alopecia, the commonest type of alopecia, it has revealed that the progressive hair loss is caused by disruption of hair growth cycle by the miniaturization of the HF.[8)]

The dermal papilla (DP), a specialized mesenchymal population of HF, plays a pivotal role in providing instructive signals required to activate hair follicle morphogenesis and cycling (1). DP cells are characterized by aggregative behavior and distinct gene expression to regulate hair growth cycle. Research revealed that the hair-inductive ability of DP cells is shown in not only embryonic development and postnatal skin, suggesting that it is possible to generate DP cells for treatment of hair loss.[9,10)] Molecular studies further revealed that Wnt signaling to DP cells maintains hair inductive activity.[11,12)] Also, inhibitor of JAK/STAT activation promotes hair inductivity of DP cells, indicating that targeting the JAK/STAT signaling might be a potential treatment for alopecia.[13,14)] Additionally, the hair inductivity of DP cells is managed by the culture system. Monolayer-cultured or 2D cultured DP cells retain the ability to induce HF neogenesis when transplanted. However, DP cells lose their ability to HF neogenesis and expression of DP signature genes, such as alkaline phosphatase (ALP), versican (VCAN), bone morphogenetic protein 2 (BMP2), and Noggin (NOG), during passaging in the 2D culture system.[15,16)] ALP is highly conserved allosteric enzymes that is able to hydrolyze

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and phosphorylates a wide range of compounds. Although the exact physiological role of ALP in DP is still unknown, the activity of ALP has been used as a marker to detect the presence of DP and as an indicator for hair inductivity.[16] VCAN is a large chondroitin sulfate proteoglycan, one of the major components of the extracellular matrix and plays an essential role in hair follicle formation.[16] BMPs are members of the transforming growth factor-β superfamily regulating a large variety of biologic responses in various cells and tissue. BMP-2 is one of BMP family that essential for DP cell functions.[16] NOG is an inhibitor of BMP proteins, and its expression is upregulated BMP protein in the DP cells.[16] Recent study found the existence of a negative feedback loop between BMPs and the regulator NOG in the DP, indicating that BMP signaling is tightly regulated by its negative regulator.[17] Previously, methods to rescue the inductive ability of DP are developed using spheroid cell culture.[18–20] Three-dimensional spheroid DP cells restored their transcriptional signature and capability to induce *de novo* hair follicle.[20]

Centella asiatica (*C. asiatica*), also known as gotu kola or Indian pennywort, is an herbal medical plant, and has been also used as folk medicine in various tropical regions.[21] This plant contains large quantities of pentacyclic triterpene derivatives, known as centelloids.[22] The major triterpenoid components of *C. asiatica* are asiatic acid (30%), madecassic acid (40%), and asiaticoside (30%), and the reconsistuted mixture is commercially marketed as a titrated extract of *Centella asiatica* (TECA).[23] Basically, *C. asiatica* has been known to provide dermatological benefits on the treatment of various dermatoses and skin lesions, such as wounds, burns, and hypertrophic scar.[21,23] Studies have found that TECA stimulates the production of type I collagen and fibroblast migration in scar region and decreases the inflammatory reaction.[24] Also, studies have found that TECA has beneficial in antiphotoaging in skin, mainly due to increases type 1 collagen and metabolism of lysine and proline, the amino acids building the collagen molecules.[23,25] Further research showed that TECA has a UVB protective effect on human dermal fibroblasts and keratinocytes.[26,27] Although numerous studies have revealed the pharmacological and dermatological effects of TECA; however, there is no attempt to investigate the effect of TECA on hair inductive property. This is first report to elucidate the promoting effect and cellular mechanisms of TECA-mediated hair inductive potential in 3D spheroid cultured human dermal papilla (HDP) cells.

Materials and methods

Cell culture, plasmids, and reagents. The human hair follicle dermal papilla cells (HDPs; Innoprot, Bizakaia, spain) were maintained in mesenchymal stem cell medium (MSCM; Innoprot) supplemented with 5% (v/v) fetal bovine serum (FBS) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. HEK293 cells (American Type Culture Collection, Manassas, VA, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific Inc., Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS). TCF/LEF, STAT3, and STAT5 luciferase reporter plasmids were obtained from Promega (Madison, WI, USA). Titrated extract of *Centella asiatica* (TECA) was purchased from Bayer HealthCare (Berlin, Germany).

Three-dimensional (3D) culture of DP cells. Three-dimensional culture procedure was followed as previously reported.[28] Briefly, HDP cells were seeded onto an ultra-low attachment culture plate (Corning Incorporated, Corning, NY, USA) to produce one spherical structure. To unify and optimize sphere size, HDP cells were seeded onto 96-well clear round-bottom ultra-low attachment microplate (Corning Incorporated) with seeding densities of 5×10^4 cells per well. The diameters of spheres were quantified by phase contrast images at 48 h after TECA treatment.

Water-soluble tetrazolium salt (WST-1) assay. To evaluate the effect of TECA on the viability of HDP cells, the cells were seeded on 96-well plates (1×10^4 cells/well) and maintained for 24 h in complete medium. Cell viability was evaluated using the WST-1 assay (EZ-Cytox cell viability assay kit; ITS-Bio, Seoul, Korea) according to the manufacturer's protocol. Briefly, the cells were treated with the indicated doses of TECA for 48 h; WST-1 solution was subsequently added to each well and the cells were incubated for 0.5 h. Cell viability was determined by measuring absorbance at 450 nm using an iMark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA).

Luciferase reporter assay. HEK293 cells stably transfected with TCF/LEF1 (TOPFLASH; EMD Millipore, Temecula, CA, USA), STAT3 or STAT5 luciferase reporter plasmids (Promega) in combination with the pSV-β-galactosidase (pSV-β-gal) plasmid were seeded in a 96-well plate and were subsequently treated with TECA or in combination with IL-6 (PeproTech, Rocky Hill, NJ, USA). After 48 h of post-treatment, the cell lysates were prepared using Passive Lysis Buffer (Promega), and luciferase activity was measured using a Glomax 96 Microplate Luminometer (Turner BioSystems, Sunnyvale, CA, USA) by adding d-Luciferin (Sigma-Aldrich) into each sample. β-gal activity was analyzed using the Luminescent β-galactosidase Detection Kit II (Clontech Laboratories Inc., Mountain View, CA USA). Relative luciferase activity was determined by normalizing the levels to β-gal activity. The results are shown as mean ± SD from three independent experiments.

Western blotting. Total cell lysates were prepared and protein extracts were subjected to sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE), followed by immunoblotting. The primary antibodies used for immunoblotting analysis were follows: Antibodies targeting NCAM (ab75813), ALPL

(ab108337), and STAT5 (ab16276) were purchased from Abcam (Cambridge, UK). Antibodies against p-STAT3 (#9145), STAT3 (#9139), STAT5 (#9363), and p-STAT5 (#4322) were purchased from Cell Signaling Technology (Danvers, MA, USA). Following incubation with the primary antibodies, the membranes were incubated with goat anti-mouse and goat anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Cell Signaling Technology). The protein bands were visualized using the Pierce ECL Western Blotting Substrate (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's protocol.

Quantitative RT-PCR (qRT-PCR) analysis. Total RNA was extracted from DP spheres using a Trizaol reagent (Invitrogen; Thermo Fisher Scientific). cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Invitrogen; Thermo Fisher Scientific). Quantitative RT-PCR (QRT-PCR) was carried out using a HOT FIREPol EvaGreen qPCR Mix Plus (Solis BioDyne, Tartu, Estonia) with a Step OnePlus Real-Time PCR System (Applied Biosystems-Thermo Fisher Scientific). All the above steps were performed according to the manufacturer's protocol. The following primers used to amplify human *Alkaline phosphatase* (*ALPL*): 5′-ATTGACCACGGGCACCAT-3′ (forward) and 5′-CTCCACCGCCTCATGCA-3′ (reverse); *Versican* (*VCAN*): 5′-GGCAATCTATTTACCAGGACCTGAT-3′ (forward) and 5′-TGGCACACAGGTGCATACGT-3′ (reverse); *Bone morphogenetic protein 2* (*BMP2*): 5′-GAGGTCCTGAGCGAGTTCGA-3′ (forward) and 5′-TCTCTGTTTCAGGCCGAACA-3′ (reverse); *Noggin* (N*OG*): 5′-CTGGTGGACCTCATCGAACA-3′ (forward) and 5′-CGTCTCGTTCAGATCCTTTTCCT-3′ (reverse); *Wnt family member 5A* (*WNT5A*): 5′-TCCACCTTCCTCTTCACACTGA-3′ (forward) and 5′-CGTGGCCAGCATCACATC)-3′ (reverse); *Suppressor of cytokine 1* (*SOCS1*): 5′-TTTTCGCCCTTAGCGTGAAG-3′ (forward) and 5′-CATCCAGGTGAAAGCGGC-3′ (reverse); *Suppressor of cytokine 3* (*SOCS3*): 5′-GACCAGCGCCACTTCTTCAC-3′ (forward) and 5′-CTGGATGCGCAGTTCTTG-3′ (reverse). Each mRNA expression level was calculated using the $2^{-\Delta\Delta Ct}$ method and were normalized to the expression level of the *β-actin* housekeeping gene. The following primers were used to amplify human *β-actin*: 5′-GGATTCCTATGTGGGCGACGA-3′ (forward) and 5′-CGCTCGGTGAGGATCTTCATG-3′ (reverse).

Statistical analysis. All data are expressed as mean ± standard deviation (SD) of three independent experiments. The statistical analyses were conducted using SPSS 10.0.5 software for Windows (SPSS Inc., Chicago, IL, USA). Normally distributed data were evaluated using a two-tailed Student's *t*-test as indicated in the figure legends. Statistical significance is considered $p < 0.05$.

Results

Treatment with TECA increases viability of 3D spheroid cultured HDP cells

We firstly compared the effect of TECA on viability between 2D cultured and 3D spheroid cultured HDP cells. Cells were seeded and incubated to be grown in a monolayer. After incubation, the cells were treated with the various indicated doses of TECA for 48 h. As shown in Fig. 1(A), treatment with TECA up to 50 μg/mL does not impact the viability, however, higher doses of TECA significantly decrease the viability of 2D cultured HDP cells. To test the effect of TECA on viability in 3D spheroid cultured HDP cells, the cells were seeded into hydrogel-coated low attachment round surface plate and incubate to be self-assembled for sphere formation. As shown in Fig. 1(B), treatment with TECA up to 25 ug/mL significantly increased cell viability compared with the value in DMSO-treated control. Of note, cell viability is dose-dependently and significantly increased by the treatment of TECA (~ 25 μg/mL). These results indicate that TECA has a potential proliferative effect on 3D spheroid cultured HDP cells.

Treatment with TECA increases DP sphere formation

The sphere formation of cultured HDP cells enhances their hair growth inductivity [19]. Therefore, we next investigated the effect of TECA on the hair growth inductivity by comparing the sphere size of HDP cells *in vitro*. HDP cells were incubated in the 3D spheroid culture system, then 25 μg/mL of TECA was treated for 48 h. As shown in Fig. 2(A), TECA treatment increased the sphere size of HDP cells compared with the size of DMSO-treated control HDP cells. Fig. 2(B) showed the bar chart for the mean of $n = 3$ independent experiments, indicating that TECA enhances the formation of DP spheroids.

Treatment with TECA does not activate Wnt/β-catenin signaling pathway in 3D spheroid cultured HDP cells

Since Wnt/β-catenin signaling is one of important pathways for maintaining hair growth inductivity of DP cells,[11,12] we therefore determined whether the enhanced activity of TECA on DP sphere formation relates to the Wnt/β-catenin signaling pathway in HDP cells. To demonstrate that TECA treatment activates the Wnt/β-catenin signaling, we used the TOPFLASH (β-catenin-TCF/LEF reporter plasmid) luciferase reporter system. Unexpectedly, the luciferase activity in the transfected cells did not changed by the TECA stimulations, as compared with the value in DMSO-treated cells (Fig. 3(A)). Also, we analyzed the expression of Wnt/β-catenin-target genes including WNT5A and LEF1 at the transcriptional level in 3D spheroid cultured HDP cells. As shown in Fig. 3(B) and (C), we found that expression of WNT5A and LEF1 was not significantly changed in TECA-treated 3D spheroid cultured HDP cells compared with those levels in DMSO-treated control cells. Therefore, those results indicate that TECA-mediated growth regulation of 3D spheroid cultured HDP cells is independent of the Wnt/β-catenin signaling pathway.

Treatment with TECA lead to inhibition of STAT activation in 3D sphered HDP cells

It has been reported that the hair inductivity of DP cells is also induced by the inhibition of JAK/STAT

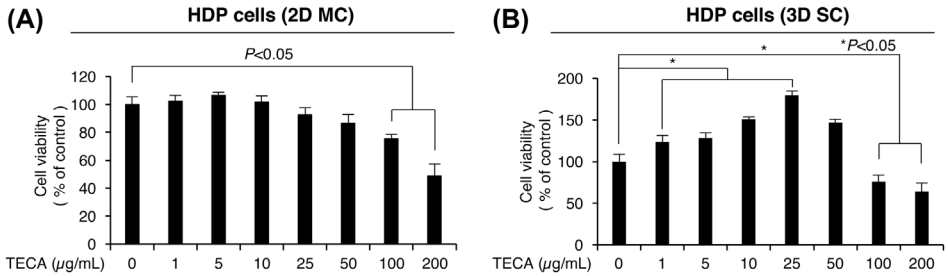

Fig. 1. Effect of TECA treatment on the viability of 2D monolayer cultured (A) and 3D spheroid cultured HDP cells (B).

Notes: Cells were treated with the indicated doses of TECA for 48 h followed by a WST-1 assay. Data are shown as mean ± SD of results from three independent experiments. Values of $p < 0.05$ were considered to be statistically significant. 2D MC, two-dimensional monolayer cultured; 3D SC, three-dimensional spheroid cultured.

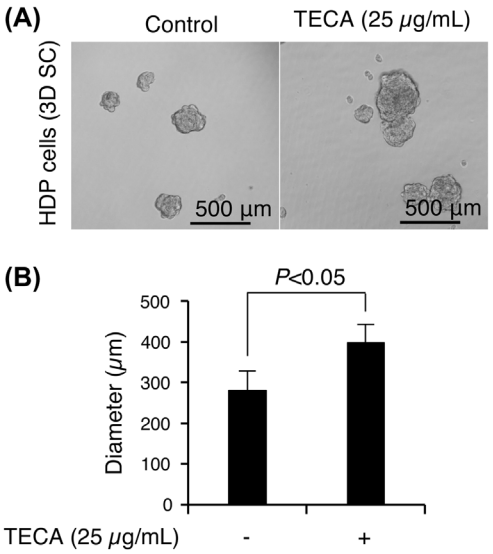

Fig. 2. Comparison of spheroid formation of control and TECA-treated HDP cells.

Notes: (A) Phase images of spheroids of control and TECA-treated HDP cells. Cells were seeded into low attachment culture plate to induce spheroids. Images were captured after 48 h of TECA treatment. The diameters of spheroids were quantified (B). Data are shown as mean ± SD of results from three independent experiments. Values of $p < 0.05$ were considered to be statistically significant.

signaling.[13,14] We therefore used the STAT5 and STAT3 luciferase reporter systems to monitor the activity of JAK/STAT signaling pathway. As shown in Fig. 4(A) and (B), TECA treatment caused significant inhibition of STAT5 and STAT3 reporter luciferase activities in a dose-dependent manner. At the transcriptional level, we further confirmed that TECA treatment significantly downregulated the expression of SOCS1 and SOCS3, target genes of STAT5 and STAT3, respectively, in 3D spheroid HDP cells. Using immunoblotting analysis, we confirmed that TECA treatment induced STAT5 and STAT3 inactivation (Fig. 5(B)). Taken together, those results indicate that TECA-mediated growth regulation of 3D spheroid cultured HDP cells relates to the inhibition of JAK/STAT signaling.

Treatment with TECA increases the expression of DP signature genes in 3D sphered HDP cells

Lastly, we tried to investigate whether TECA treatment causes the upregulation of DP signature genes including ALP, VCAN, BMP2, and NOG, since those genes are important regulators for promoting hair inductivity of HDP cells.[15,16] HDP cells were incubated in the 3D spheroid culture system, then 25 μg/mL of TECA was treated for 48 h. We performed an qRT-PCR analysis to analyze the expression of those genes. We found that TECA treatment resulted in a significant upregulation in the DP signature genes' expressions at the transcriptional level in 3D spheroid cultured HDP cells (Fig. 5(A)). Also, these results were validated by immunoblotting analysis using their specific antibodies at the protein level (Fig. 5(B)). Importantly, TECA treatment inactivated STAT5 and STAT3 proteins by inducing dephosphorylation in 3D spheroid cultured HDP cells (Fig. 5(B)). These results

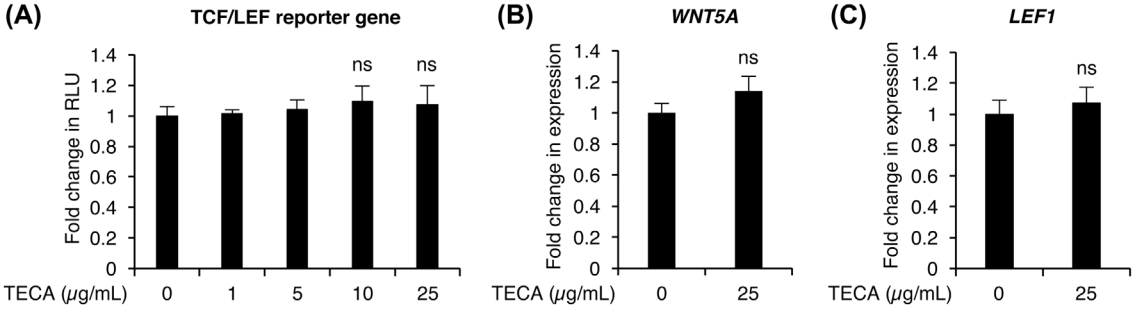

Fig. 3. Effects of TECA treatment on Wnt/β-catenin signaling activity in 3D spheroid cultured HDP cells.

Notes: (A) The luciferase activity of TCF/LEF reporter plasmids (TOPFLASH) in TECA-treated cells. HEK293 cells were co-transfected with the reporter constructs and β-galactosidase plasmids. After 48 h of TECA treatment, luciferase activity was evaluated by normalizing the levels to β-galactosidase activity. The expression levels of target genes of Wnt/β-catenin signaling, *WNT5A* (B) and *LEF1* (C), were measured using qRT-PCR. Data are shown as mean ± SD of results from three independent experiments. Values of $p < 0.05$ were considered to be statistically significant.

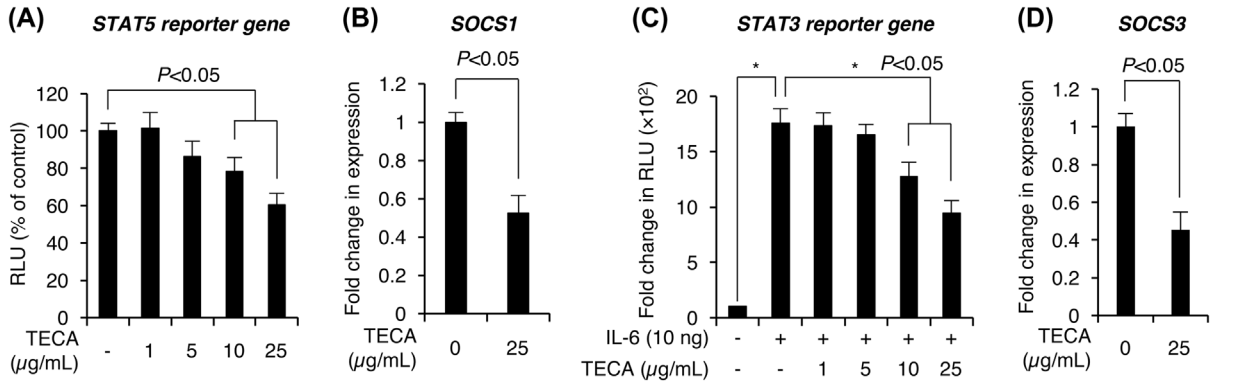

Fig. 4. Effects of TECA treatment on JAK/STAT signaling activity in 3D spheroid cultured HDP cells.

Notes: The luciferase activity of STAT5 (A) and STAT3 (C) reporter plasmids in TECA-treated cells. HEK293 cells were cotransfected with the reporter constructs and β-gal plasmids. After 48 h of TECA treatment, luciferase activity was evaluated by normalizing the levels to β-gal activity. The expression levels of SOCS1 (B) and SOCS3 (D) mRNAs were measured using qRT-PCR. Data are shown as mean ± SD of results from three independent experiments. Values of $p < 0.05$ were considered to be statistically significant.

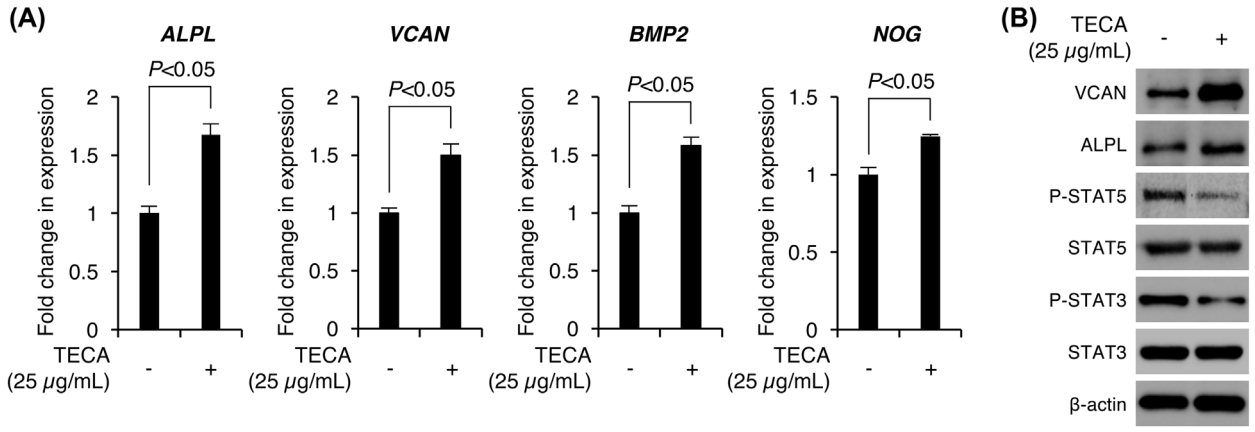

Fig. 5. Effects of TECA treatment on the expression levels of DP signature genes in 3D spheroid cultured HDP cells.

Notes: (A) The expression levels of DP signature genes including ALPL, VCAN, BMP2, and NOG were measured using qRT-PCR with their specific primers in control and TECA-treated HDP cells. Data are shown as mean ± SD of results from three independent experiments. Values of $p < 0.05$ were considered to be statistically significant. (B) The protein levels of DP signature genes and phosphorylated STAT proteins in TECA-treated HDP cells. HDP cells were grown in 3D cultured system followed by TECA treatment for 48 h. Cells were lysed and the indicated protein levels were examined using immunoblotting with their specific antibodies. β-actin was used as a loading control.

demonstrated that TECA treatment is effective for reinforcement of the hair inductive property by inactivation of STAT proteins in 3D spheroid cultured HDP cells.

Discussion

Unfortunately, with age and undefined factors, the incidence of baldness or alopecia is growing in the worldwide.[29] This dermatological disease relates to important medical and psychosocial matters, because the hair loss affects individual emotions and social perceptions.[30] Therefore, it is important to investigate to understand the molecular mechanisms of hair loss and develop candidates to prevent it. It has been scientifically demonstrated that TECA is useful in dermatologic medical purposes, especially in wound healing and collagen synthesis in skin.[21] In this study, we found the novel physiological function of TECA using *in vitro* 3D spheroid cell culture model. We observed that TECA increased the viability and spheroid formation of 3D cultured HDP cells. Also, these results related to the enhancement of hair inductive property of HDP cells through inhibition of STAT signaling and upregulation of DP signature genes' expressions. It has been revealed that TECA showed inhibitory effect on ROS-induced cellular senescence,[31] which is also observed in DP cells of alopecia patients.[32] Thus, these data indicate that TECA could be candidate for scalp alopecia.

There are two scientific approaches to overcome the alopecia: how to inhibit further hair loss, and how to regrow lost hair. The well-studied and major type of hair loss in both male and females is androgenic alopecia (AGA).[5] The mechanism studies revealed that AGA is thought to be sensitive to hormones, such as androgens (typically testosterone).[5] When the hormone binds to the androgen receptor, it triggers activation of 5α-reductase enzyme to convert that testosterone into 5α-dihydrotestosterone (5α-DHT).[5] Overproduction of 5α-DHT causes the downregulation of growth factors, and that results in damaging hair production by shortening anagen growth phase.[33] Therefore, inhibiting this mechanism is the major strategy for inhibiting scalp hair loss. Minoxidil and finasteride are currently available and FDA-approved drugs that have roles in

inhibiting the AGA mechanism.[34] Minoxidil has function in prolongation of the anagen phase,[35,36] and finasteride inhibits the conversion of testosterone to 5α-DHT.[37] However, these drugs are effective on relative small portion of patients (35% and 48%, respectively) and have several side effects including irritation, itchiness, abnormal ejaculation, and abnormal sexual function.[34,38,39] Therefore, recent studies have tried to focus on herbal plants, and many phytochemical reagents have been found to have an inhibitory effect on 5α-reductase enzyme activity.[5,39] Interestingly, one study revealed that the extract of *Centella asiatica* did not reduce the percentage of 5α-DHT formation in HDP cells, indicating that *Centella asiatica* has no 5α-reductase inhibitory activity in HDP cells.[39] However, we showed that TECA increased the viability in 3D spheroid cultured HDP cells, not in 2D cultured cells. This effect related to the significant enhancement of the 3D sphere formation which is directly connected with the hair growth inductivity of HDP cells.[20] Indeed, recent studies showed that 3D spheroid cultured HDP cells enable to induce *de novo* hair follicle growth.[18,20,28] Furthermore, in AGA, the hair shaft becomes smaller (miniaturization), and this reduction in the size of the hair shaft is related with a reduction in the size of the DP.[40–42] Therefore, these results indicate that TECA works better to induce hair inductivity than inhibits 5α-DHT formation in its hair loss prevention effect.

One etiological characteristic of hair loss progression is inability of hair follicle to enter the anagen growth phase after being arrested in the telogen phase.[13] It has been reported that Wnt/β-catenin signaling relates to maintain anagen-phase gene expression and hair inductive activity in DP cells.[11] Canonical Wnt/β-catenin signaling causes to stabilization of β-catenin and allows it to translocate into the nucleus, where it binds to TCF/LEF transcription factor to activate the transcription of downstream target genes, including WNT5A and LEF1.[43] WNT5A is known as one of DP signature genes and its expression is the highest in anagen phase.[44,45] LEF1 is a key regulator of the Wnt/β-catenin signaling, and known to regulate dermal–epidermal interactions in hair follicle development.[46,47] However, our results showed that TECA-mediated sphere formation of HDP cells did not relate to the Wnt/β-catenin signaling. Luciferase-based TCF/LEF reporter assay showed that there is no change in both control and TECA-treated HDP cells. Also, WNT5A and LEF1 expressions were not changed by TECA treatment, indicating that TECA-mediated hair inductive potential in HDP cells is not dependent on the Wnt/β-catenin signaling, but rather dependent on other signals relating hair growth. Two research groups propose that the active phosphorylated STAT5 is upregulated in the DP of follicles in late catagen.[13,48] Further studies revealed that JAK/STAT inhibition relates to promotion of the inductivity of 3D spheroid cultured HDP cells.[13,14] Using hair patch assay, HDP spheres, which was cultured with JAK inhibitor (tofatinib), induced larger and significantly greater numbers of HFs, indicating that inhibition of JAK/STAT signaling promotes inductivity of HDP cells.[13] Also, the group confirmed that the JAK/STAT inhibition-mediated inductivity of HDP cells enhanced the inductive molecular signature.[13] Our results also showed that TECA-mediated hair inductive potential is related to the inhibitory effect of JAK/STAT signaling in HDP cells. STATs are bifunctional proteins, signal transducers and activator of transcription, and play pivotal roles in development and cellular function.[49] Therefore, we conducted the luciferase-based STAT reporter assay, and found that TECA treatment significantly decreased the luciferase activity in HDP cells. Also, the expressions of STAT-targeted genes and the protein levels of active phosphorylated STATs were downregulated by TECA treatment. Furthermore, we confirmed that TECA treatment in HDP cells leaded to upregulation of hair inductivity-responsible DP signature genes at the transcriptional levels. The obtained results indicate that TECA could be effective treatment for hair loss via inhibition of the JAK/STAT signaling and activation of cell proliferation and sphere formation in HDP cells. However, further validation of this mechanism requires additional in-depth studies in the future, because the exact molecular mechanism(s) of TECA-mediated dephosphorylation of STATs are still undiscovered yet. Also, further studies are needed to better understand the effect of TECA treatment on hair induction.

In summary, TECA exerts promoting effects on 3D DP sphere formation through inhibiting STAT activation in HDP cells. This conclusion indicates that TECA treatment may provide useful strategy in promoting hair growth and treating alopecia in human.

Author contributions

Yeong Min Choi and Sungkwan An carried out the experiments and drafted the manuscript. Junwoo Lee, Jae Nam Lee, and Young Sam Kim participated in the design of the study and performed the statistical analysis. Jae Ho Lee, Kyu Joong An, and In-Sook An analyzed and interpreted the data. Seunghee Bae conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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