Regenerative Therapy 29 (2025) 184–191

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

The potential of *Spirulina maxima* extract-treated conditioned medium from adipose-derived mesenchymal stem cells for hair growth promoting effect on human dermal papilla cells

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ARTICLE INFO

Article history:
Received 9 September 2024
Received in revised form
5 March 2025
Accepted 18 March 2025

Keywords:
Spirulina maxima
Conditioned medium
Adipose-derived stem cells
Human dermal papilla cells
Hair growth promoting effect

ABSTRACT

Introduction: Therapeutic applications using adipose-derived mesenchymal stem cells (ADMSCs) have been highlighted as an attractive option to treat multiple diseases. It is widely accepted that ADMSCs exhibit their curative effects on hair loss via diverse secretory factors which promote hair follicle growth and remodeling of hair growth cycle. Recently, further studies using marine resources have been carried out to stimulate ADMSCs, enhancing both the quantitative and qualitative secretion to obtain more effective ADMSCs-derived conditioned medium (ACM).

Methods: Our study investigated the effects of *Spirulina maxima* on cell proliferation in ADMSCs and determined optimal treatment condition. Human growth factor array was performed to evaluate the effects of *S. maxima* treatment on the alterations of growth factors secretion in ADMSCs. Subsequently, we examined the effects of *S. maxima*-treated ACM (SACM) on cell proliferation and alkaline phosphatase (ALP) activity in human dermal papilla cells (HDPCs). The underlying mechanisms of hair growth promotion effect of SACM was illustrated by confirming the expression levels of proteins related to mTOR/Akt and Wnt/β-catenin signaling pathway.

Results: S. maxima extract promoted cell proliferation in ADMSCs and stimulated the secretion of fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF). SACM treatment also increased cell proliferation and ALP activity in HDPCs, similar with those of ACM treatment. Furthermore, SACM significantly enhanced in vitro hair growth promotion by activating mTOR/Akt and Wnt/β-catenin signaling pathway.

Conclusion: SACM could be a more effective regenerative therapy for the promotion of hair growth and might have the potential to utilizing in pharmaceutical and cosmeceutical industries compared to non-treated ACM, suggesting the potential of *S. maxima* as an ADMSCs-preconditioning agent.

1. Introduction

Hair is a representative phenotype of mammalian and plays an important role in the body such as protection against pathogen, sensory recognition, thermoregulation, and modulation of skin homeostasis including repairment and regeneration. Hair follicle (HF), which consists of the epithelial cells and dermal papilla cells (DPCs), is regarded as a major regulator of hair growth [1]. The growth of HF is mainly organized with the three cyclic phases:

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Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2025.03.013

anagen (growth), catagen (regression), telogen (quiescence), which consistently repeats circulation for the hair growth and regeneration [2]. Human DPCs (HDPCs), originated from dermal stem cells (DSCs), interact with adjacent epithelial cells and initiate cell proliferation and differentiation, causing the maintenance of the hair cycle homeostasis. These interactions are also affected by multiple growth factors those are located within and outer areas of the HF, enhancing the cell number and function of HDPCs [3].

Cellular therapy using mesenchymal stem cells (MSCs) has been considered as a potent regenerative medicine for the treatment of multiple diseases, especially on the inflammatory and degenerative conditions. MSCs have the capability to self-renew, differentiate into multiple lineages, and secrete growth factors with paracrine potential, which are responsible for their advantageous health benefits [4]. There have been used a wide range of MSCs derived from various physiological components in the human body such as bone marrow, umbilical cord blood, and adipose tissue. Notably, in hair biology, adipose-derived MSCs (ADMSCs) have shown their critical role in the regeneration of HF and optimization of the microenvironment surrounding the HDPCs, leading to the promotion of hair growth [5]. In addition, many previous studies have also reported that the secretory factors from the paracrine actions of ADMSCs could prevent hair loss and promote hair growth by stimulating the growth of HF and cyclic normalization [6–8]. Consistently, it is worth noting that cytokines, growth factors, and extracellular vesicles released from ADMSCs are commonly included in culture medium and can be a promising source for the enhancement of human hair health.

Recently, conditioned medium (CM) derived from ADMSCs (ACM) has attracted a great deal of interest as an alternative to direct cellular therapy. Although MSCs have a wide range of biological activities owing to their multipotency and high regenerative potential, safety issues including side effects and depleted clinical efficacy are still challenged by abnormal immunogenicity, tumorigenic potential, and susceptibility to infections [9]. However, ACM has no risk of the side effects due to its 'cell-free' condition, and ACM-derived cytokines and growth factors can be sufficient ingredients to fulfill its regulatory requirements [10]. Hence, MSCs-derived ACM is an encouraging option to satisfy both safety and effectiveness of the regenerative therapies using stem cells. As the safety of ACM is confirmed, further investigations have been carried out to enhance the efficacy of ACM by stimulating quantitative and qualitative secretion of growth factors from ADMSCs. In a previous study, minoxidil, a representative medicinal agent for hair loss, was used to evaluate the indirect promotion of hair growth via stimulation of the secretory growth factors from ADMSCs [11]. In this way, discovering a novel candidate to enhance the contents and functions of ADMSCs-derived secretome will be required as a strategy to improve degenerative hair loss and abnormal hair growth.

Microalgae are protein-rich biomass, which have been used for various industrial applications such as purification of wastewater, resource for beneficial compounds, and production of renewable energy [12]. In recent years, microalgae have also been considered as food supplements and cosmeceuticals due to their nutritional compositions and natural components [13,14]. Especially, many research has investigated the effects of microalgae extracts on hair growth promotion and hair loss prevention along with their anti-aging and regenerative features [15–17]. *Spirulina maxima* is a type of microalgae belonging to cyanobacterium. *S. maxima* is proved as a safe material for food and pharmaceutical applications, indicating non-cytotoxicity [18]. There have been a variety of studies displaying the therapeutic properties of *S. maxima* such as antioxidant, anti-inflammatory, immunoregulatory, and improving metabolic disorders [19–22]. In addition, a previous study has demonstrated the positive effects of *S. maxima* extract on cell growth as an alternative to fetal bovine serum (FBS), supporting the notion that *S. maxima* can be a potential option to enhance the cell proliferation and paracrine actions of ADMSCs [18]. Furthermore, marine pectin derived from *S. maxima* has shown wound healing and regenerative effects in both in vitro human dermal skin cells and in vivo zebrafish model [23]. There is, however, to date no report that illustrates the potential of *S. maxima* extract as a pre-conditioning agent of ADMSCs that can act as both an inducer of growth factors secretion and an enhancer of hair growth promoting activities.

Therefore, the objective of this study was to investigate the effects of the optimized *S. maxima*-treated ACM (SACM) on hair growth promotion in HDPCs. We applied the extract of *S. maxima* to ADMSCs and evaluated cell proliferation and compared the alterations of the secreted growth factor profiles. We obtained SACM and further examined the effects of SACM on the cell proliferation, ALP activity, and hair growth-related protein expression levels in HDPCs, to determine the potential of SACM that can be utilized as a 'cell-free' hair health therapy and a pre-conditioning agent of ADMSCs.

2. Materials & methods

2.1. Preparation of S. maxima extract

Marine *S. maxima* was kindly provided by Korea Institute of Ocean Science & Technology (Jeju, Korea). Extraction of *S. maxima* was performed according to the method in a previous study [18] with some modifications. In brief, ground *S. maxima* was added into distilled water (DW). The cells were decomposed and extracted by sonication, followed by the treatment of high pressure at high temperature. The extract was then clarified by centrifugation at 10,000×*g* for 20 min to precipitate any particulates and wastes. Supernatant of extract was filtered through 0.2-μm Whatman filter (Whatman, Maidstone, England), and then lyophilized. The lyophilized sample was used as a *S. maxima* extract (SE) sample and stored at 4 °C for the further experiments. The biochemical components of the SE were shown in Table 1 [18]. The SE contained significantly high portions of protein (79.20 %). The pH and salinity of the SE were 7.8 and 0 %, respectively.

2.2. Cell culture

ADMSCs and HDPCs were purchased from ATCC (American Type Culture Collection, Manassas, VA, USA), and CEFO CO., Ltd (Cell Engineering For Origin, Seoul, Korea), respectively. ADMSCs were cultured in Mesenchymal Stem Cell Basal Medium for Adipose, Umbilical, and Bone Marrow-derived MSCs (PCS-500-030™) supplemented with Mesenchymal Stem Cell Growth Kit for Adipose and Umbilical-derived MSCs – Low Serum (PCS-500-040™) and 1 % antibiotics. HDPCs were cultured in CEFOgro ™ Human Dermal Papilla Growth Medium (Basal Medium containing Supplements and Penicilin/Stereptomycin, CEFOgro-HDP). All the cell lines were maintained in a humidity-regulated incubator at 37 °C with 5 % CO_2 condition.

2.3. Preparation of CM

For CM collection, ADMSCs at 6 passages were seeded in a 15-cm culture dish with a density of 5×10^4 cells/mL. After incubation for overnight (confluency about 80 %), cells were washed with phosphate buffered saline (PBS, 1 ×) and replenished with serum-free medium. Cytotoxicity and/or cell proliferative effect of the SE on ADMSCs were determined with a colorimetric CCK-8 assay. According to the optimized dose and period of SE treatment, ADMSCs

Table 1
Biochemical components of *S. maxima* extract (SE)[a].

Organic indices	Components	SE	
		Concentration (mg/L)	Percentage (%)
Biochemical components	Carbohydrate	900.00	13.20
	Protein	5400.00	79.20
	Lipid	300.00	4.40

[a] Information from the previous study [18].

were treated with 500 μg/mL of SE for 72 h, and then supernatant was collected. The supernatant was centrifuged at 3000×*g* for 20 min and filtered with 0.45 μm syringe filter (Advantec, Tokyo, Japan), to clarify cell debris and residues. Prepared non-treated and SE-treated supernatants were used as ACM and SACM, respectively.

2.4. Human growth factor array

Human Growth Factor Array C1 (RayBiotech, Cat# AAH-GF-1) was used to detect 41 growth factors in ACM and SACM. 1 mL of ACM and SACM were collected from 5×10^5 ADMSCs, which were cultured in 10 mL medium. In brief, blocked membranes were incubated with samples for overnight at 4 °C, and then washed three times with wash buffer. After incubation with biotinylated detection antibody cocktail for 2 h at room temperature (RT), washed three times, and then additional incubation was performed with HRP-conjugated streptavidin for 2 h at RT. After further three times washing, the detection buffer mixture was added, and the membranes were incubated for 2 min. The protein expression levels were analyzed with chemiluminescence detection system (FUSION SOLO Vilber Lourmat System, Paris, France) within the exposure time of 10 min. Quantitative analysis of the data was conducted using RayBio Analysis Tool software.

2.5. Cell proliferation assay

Cell proliferation and viability in HDPCs was determined with a colorimetric CCK-8 assay. HDPCs at 7 passages were seeded on a 96-well plate with a density of 5×10^4 cells/mL and incubated overnight. Then, cells were washed with PBS and the cultured medium was replaced with serum-free medium. ACM and SACM were added at various concentrations (5, 10, 25, 50 %) for 48 h, and then CCK-8 substrate was added into each well. The plate was incubated for 2 h at 37 °C, and the absorbance was measured at 450 nm using a microplate reader (Synergy HTX Multi-Mode Reader, BioTeK, Santa Clara, CA, USA).

2.6. ALP assay

HDPCs were seeded on a 6-well plate with a density of 1×10^5 cells/mL and incubated overnight. After washing with PBS, HDPCs were cultured in serum-free medium with or without ACM and SACM for 48 h. Cells were washed twice with PBS and lysed with M-PER™ Mammalian Protein Extraction Reagent (Thermo Scientific, CA, USA). The cell lysate was collected by centrifugation at 10,000×*g* for 10 min at 4 °C, and ALP activities were measured using a colorimetric Alkaline Phosphatase Assay Kit (Abcam, Cambridge, MA, USA) according to the manufacturer's instruction.

2.7. Western blot assay

HDPCs were seeded and treated with indicated concentration (50 %) of ACM and SACM. Cells were then washed twice with cold PBS and lysed with M-PER™ Mammalian Protein Extraction Reagent. Centrifugation was performed to obtain cell lysates, and protein concentrations were determined using the BCA protein assay kit (Thermo Scientific, CA, USA). Western blot analysis was conducted according to our previous study [24]. To elucidate the protein expression levels related to hair growth promotion, primary antibodies, which were purchased from Cell Signaling Technology (Danvers, MA, USA), including mammalian target of rapamycin (mTOR, #2983), protein kinase B (Akt, #4691), p-Akt (#4060), glycogen synthase kinase-3 beta (GSK-3β, #12456), p-GSK-3β (#5558), proliferating cell nuclear antigen (PCNA, #13110), , β-catenin (#9562), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, #5174) were used.

2.8. Statistical analysis

The results are represented as the mean ± standard deviation (SD). The statistical analysis was performed using SPSS software (version 28, IBM, Armonk, NY, USA). The values were evaluated by one-way analysis of variance (ANOVA), supplemented with Duncan's multiple range test. Statistically significant difference was set at $P < .05$.

3. Results

3.1. Effects of SE on cell proliferation in ADMSCs

To evaluate the effects of SE on ADMSCs, we firstly treated various concentrations (31.25–1000 μg/mL) of SE for 48 or 72 h and determined the cytotoxicity and cell proliferation. SE treatment had no significant reduction on cell viability (>90 %) and obviously increased cell proliferation in a dose-dependent manner, without the highest concentration of 1000 μg/mL at both incubation time (Fig. 1A and B), indicating the proliferative effect of SE on ADMSCs. At the condition of 500 μg/mL of SE treatment for 72 h, the proliferative rate of ADMSCs was more than 120 % compared with non-treated CON group (Fig. 1B). Therefore, we determined the optimal condition of SE treatment as the incubation time (72 h) and the concentration (500 μg/mL) for the preparation of ACM and SACM, and the secretory profiles of growth factors were further analyzed.

3.2. Effects of SE on secretion of growth factors in ADMSCs

To identify the stimulating effects of SE on the growth factors secretion from ADMSCs, we then examined alterations of secreted growth factor profiles in SACM using a human growth factor array. By comparing the results of SACM compared to ACM, SE treatment remarkably increased the secretion of basic fibroblast growth factor (bFGF) (3.16-fold), hepatocyte growth factor (HGF) (6.36-fold), and vascular endothelial growth factor A (VEGF-A) (1.2-fold) in ADMSCs (Fig. 2). Previous studies have reported that the production of these cytokines plays a key role in hair growth both in vitro and in vivo [25]. These results suggest that SE enhanced cell proliferation by stimulating the secretion of multiple growth factors in ADMSCs, indicating a potential of SACM on the improvement of hair growth.

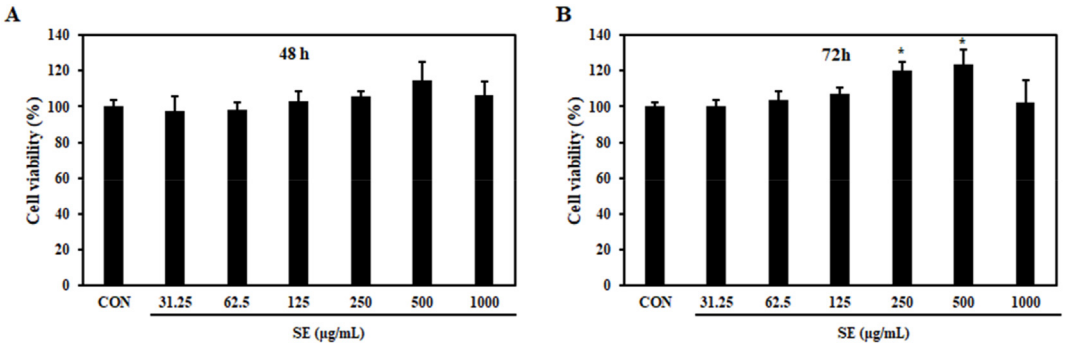

Fig. 1. Effects of *S. maxima* extract (SE) on the cell proliferation in adipose-derived mesenchymal stem cells (ADMSCs). Cell proliferative rate in ADMSCs treated with SE at the concentrations (31.25–1000 μg/mL) for 48 (A) and 72 h (B). Data are expressed as the mean ± SD (n = 3). *$p < 0.05$ indicate significant differences compared with the CON group.

3.3. Effects of CM on cell proliferation and ALP activity in HDPCs

Cell proliferation and ALP activity are the most widely accepted biomarkers determining cellular activities of HDPCs. Proliferation of HDPCs commonly represents upregulated hair growth rate, which is closely related to the activity of ALP as a critical enzyme for hair growth promotion [25]. Therefore, we evaluated the effects of SACM treatment on cell proliferation and ALP activity in HDPCs. At the incubation time of 48 h, there was significant increment of cell proliferation in a dose-dependent manner by both ACM and SACM treatment, but there were no significant effects in SACM-treated cells (Fig. 3A). In contrast, significant proliferative properties were displayed at the condition of 25–50 % SACM treatment at the incubation time of 72 h, similar with ACM treatment (Fig. 3B). These results were consistent with the ALP activities of HDPCs treated with ACM and SACM compared to the non-treated CON group, however, minor elevation of ALP activities was shown in SACM group more than in ACM group (Fig. 3C). Our results implied that SACM could facilitate the cell proliferation and related protein activities more than ACM in HDPCs, suggesting that SE treatment enhanced the effects of ACM on cell proliferative activity.

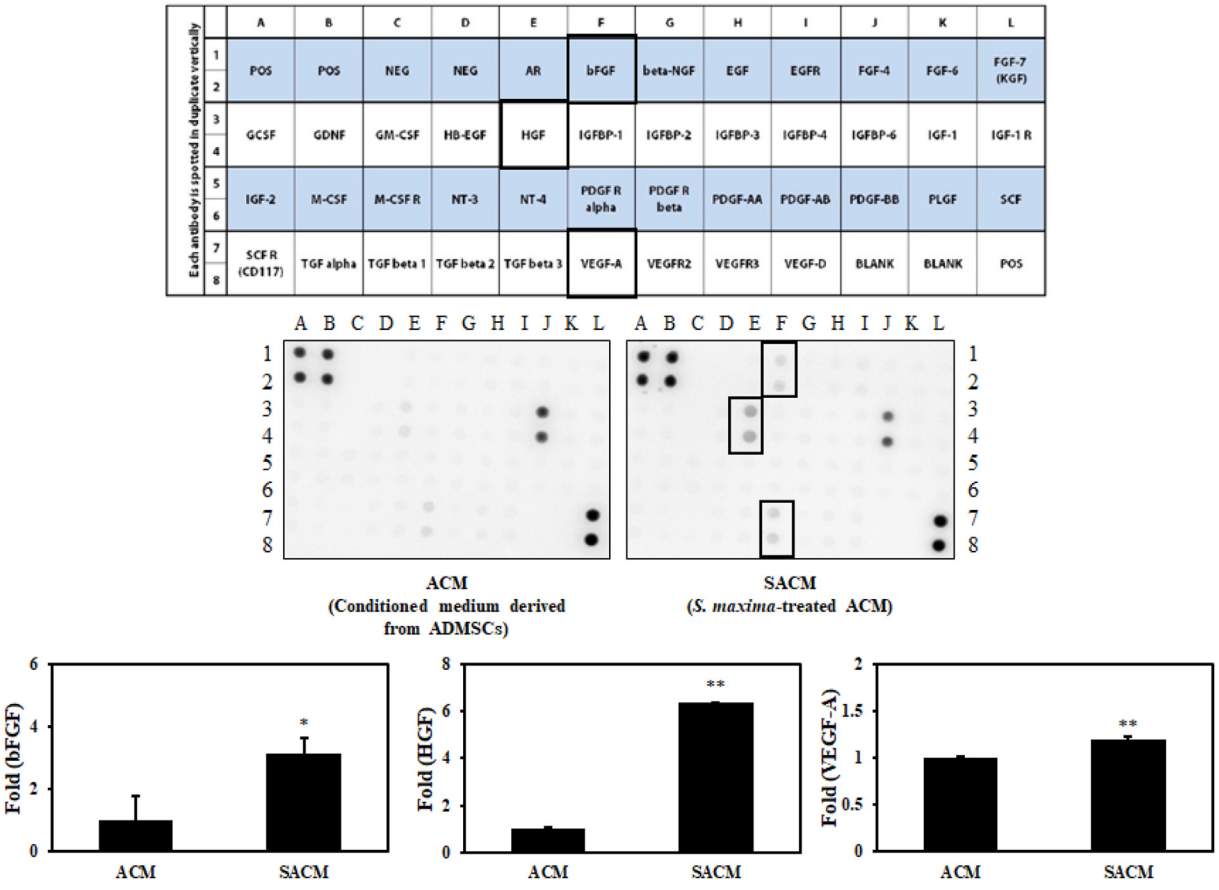

Fig. 2. Effects of SE on the secretion of growth factors in ADMSCs. (A) The intensity analysis of secretory proteins in ACM and SACM were determined using an antibody array. The levels of three growth factors including (B) bFGF, (C) HGF, and (D) VEGF-A were analyzed and quantified.

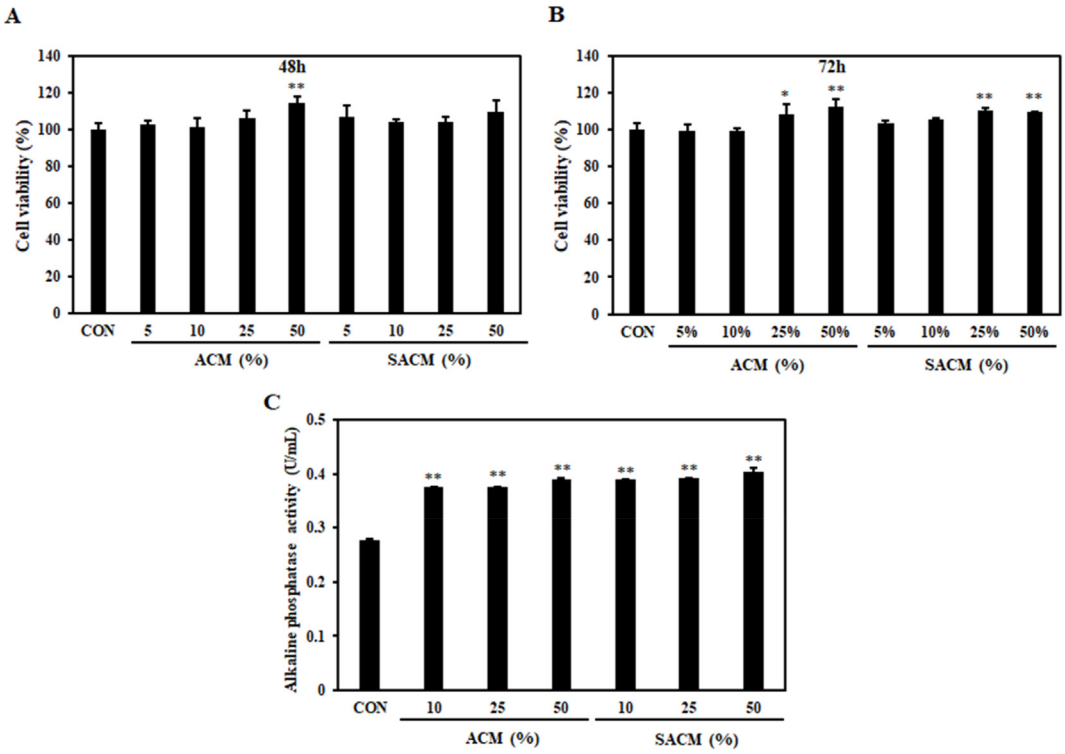

Fig. 3. Effects of CM on cell proliferation and ALP activity in HDPCs. Cell proliferative rates in HDPCs treated with ACM and SACM at the concentrations (5–50 %) for 48 (A) and 72 h (B). (C) ALP activities in ACM and SACM-treated HDPCs. Data are expressed as the mean ± SD (n = 3). *$p < 0.05$, **$p < 0.01$ indicate significant differences compared with the CON group.

3.4. Effects of CM on hair growth-related protein expressions in HDPCs

The activity and growth cycle regulatory mechanism of HDPCs involves mTOR/Akt and Wnt/β-catenin signaling pathway. The mTOR/Akt pathway plays a key role in the beginning phase of hair cycle, and the Wnt/β-catenin pathway is responsible for hair growth and regeneration by activating cell development and proliferation [26,27]. In this study, SACM treatment remarkably increased both the expression level of mTOR and phosphorylation level of Akt, even compared with ACM treatment (Fig. 4). Moreover, Wnt/β-catenin signaling pathway was regulated with the elevation of β-catenin expression and GSK3β phosphorylation in SACM-treated group, and the effects of SACM on the expression level of PCNA was stronger than that of ACM (Fig. 4). In brief, these results altogether suggest that SACM could promote in vitro hair cell proliferation through the regulation of mTOR/Akt and Wnt/β-catenin signaling pathway, indicating that SE treatment promoted the effects of ACM on the protein expressions related to hair growth.

4. Discussion

ADMSCs are promising tissue sources for the development of regenerative medicine, due to the ease of cellular experiments and therapeutic potential based on their multipotency to differentiate into various origins-derived cells [28]. Moreover, ADMSCs-derived secretome including a wide range of cytokines, growth factors, and other extracellular microvesicles, influence the ecology of cell and tissues, and mediate the function of neighboring cells, which can evoke curative outcomes [29,30]. In recent years, many attempts to enhance the effectiveness of cell-based therapy using ACM have been performed by stimulating the function of ADMSCs and their derivatives, especially in dermatological and aesthetic medicine [31]. Consistently, in the research field of human hair health, previous studies have reported various strategies, such as external stimulus, biomolecules preconditioning, and genetic modification, to facilitate the secretion of paracrine factors from ADMSCs, thereby affecting the therapeutic properties of ACM [32]. Hence, our present study prepared conditioned medium by optimizing the treatment conditions of *S. maxima* on ADMSCs and investigated the efficacy of SACM on hair growth promoting activities using HDPCs, when compared with non-treated ACM.

There have been many efforts to utilize marine-derived ingredients for stimulating hair cell cycle and promoting hair cell growth [33]. Especially, various microalgae have been studied for hair growth promoting effects based on their abundant and rich functional components [15,17,34]. However, it is not fully understood about exploring microalgae as a pre-conditioning agent of ADMSCs to enhance hair growth potential of ACM. *S. maxima* is a well-known microalga as evidenced by a wide range of nutritional value and usage in various industrial applications. Notably, a previous study investigated the clinical potential of *Spirulina* supplementation, highlighting its therapeutic uses to improve multiple health outcomes including inflammation, oxidative stress, immune function, and metabolic disorders. In particular, there was an observable study using *S. maxima* as a sufficient alternative source for FBS which is responsible for the cultivation and growth of cellular organisms in many experimental fields [18]. Regarding that *S. maxima* can promote cell proliferation and regulate cell cycle, we prepared SE and confirmed the effects on ADMSCs. Concentration-dependent significant cell proliferative effects were displayed in the SE-exposed ADMSCs at the incubation time of 72h, indicating the potential of SE that can promote the function and homeostasis of stem cells. According to the literature evidences, preconditioning of ADMSCs with pharmacological or chemical agents has been conducted to enhance survival and capability of ADMSCs to

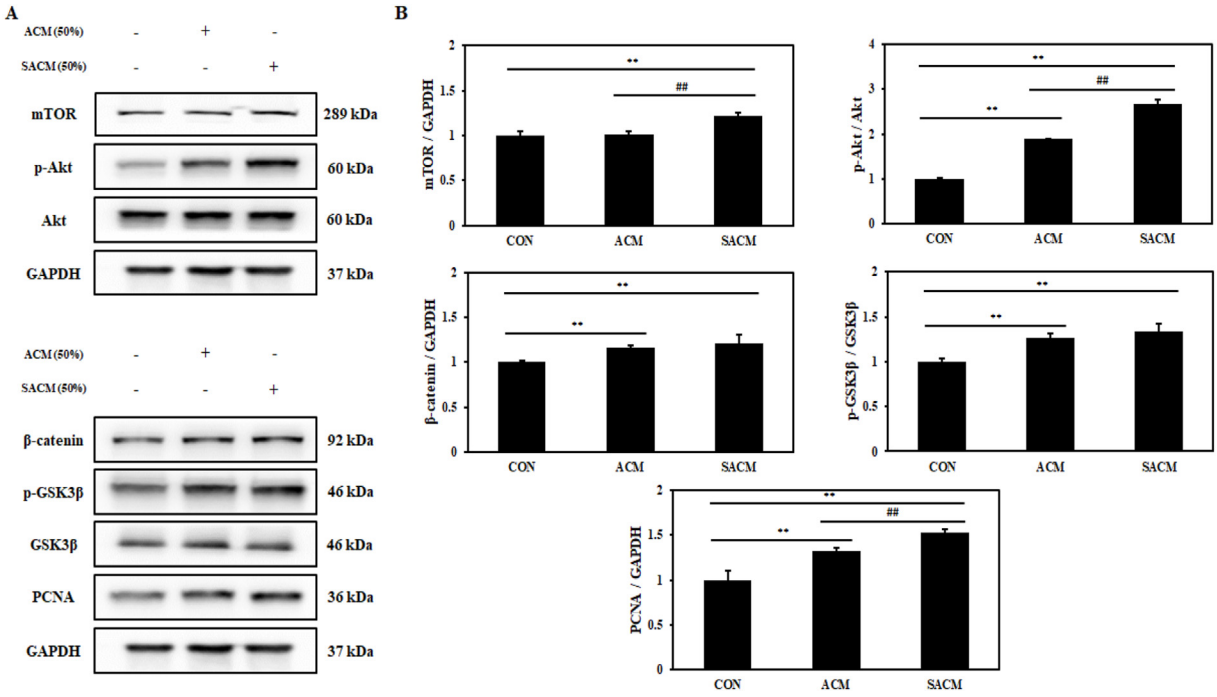

Fig. 4. Effects of CM on the expression levels of hair growth-related proteins in HDPCs. The expression levels of mTOR/Akt and Wnt/β-catenin signaling pathway were analyzed with (A) western blots. (B) The quantitative results are displayed. Data are expressed as the mean ± SD (n = 3). $^{**}p < 0.01$ indicate significant differences compared with the CON group; $^{\#\#}p < 0.01$ compared with the ACM group.

constitute a proliferative and regenerative environment [35]. Moreover, this is of great significance for elucidating the effects of SE on the alteration of secreted growth factors from ADMSCs, which is in line with previous reports demonstrating the association between stem cell proliferation and upregulation of multiple growth factors [36–38]. Indeed, multiple characteristics of the production of SE such as sample size, extract dose, and compositional diversity can affect the reproducibility of SACM, it is reasonable for us elucidating specific compounds with drug-like properties using bioactivity-guided isolation to standardize SE [39,40]. Therefore, further analysis about the effects of SE, obtained in standardized culture and extract condition, on the secretory protein profiles was performed via the comparison of ACM with SACM using human growth factor array.

Therapeutic effects of ADMSCs are closely related to paracrine signaling, the secretion of various cytokines and growth factors that can promote tissue repair and regeneration [41]. Generally, paracrine growth factors from ADMSCs including VEGF, FGF, and HGF play a role in the normalization of hair growth cycle and promotion of hair regeneration [32]. VEGF commonly increases the size of HF and shaft by promoting angiogenesis, and bFGF facilitates the development of HF. HGF also upregulates the proliferation of hair follicle cells, indicating the regulatory effects of growth factors on hair cell survival [42]. Our results from antibody array revealed that the expression levels of bFGF, HGF, and VEGF-A in SACM were obviously elevated compared to non-treated ACM, implying that 500 μg/mL of SE treatment could enhance the secretion of above-mentioned growth factors from ADMSCs. A previous study [43] used bee venom as a stimulator of growth factor release from ADMSCs for hair regeneration, which is similar with our experiments. In addition, many pre-clinical and clinical studies demonstrated that CM obtained from various MSCs revealed their ability for tissue maintenance and regeneration, highlighting the safety and effectiveness of the novel therapeutic method [44]. In this way, we anticipated the safety and stability of SACM and further examined the effects of SACM on in vitro hair growth promotion using representative hair follicle cells.

In vitro models to investigate hair growth promoting effects generally utilize HDPCs, which can be assessed by cell proliferation and related biomarkers such as ALP, mTOR/Akt and Wnt/β-catenin signaling pathway [25]. The present study showed that SACM exposure significantly increased cell proliferation in a dose-dependent manner, which was significant in the concentrations of 25–50 % for 72 h treatment. The results from non-treated ACM group were similar with those from SACM group. ALP is an important enzyme modulating hair growth cycle that reflects survival and proliferation of hair follicle cells [2,45]. ALP activities were gradually elevated by ACM and SACM treatment and significant results were displayed in all the concentrations (10–50 %), when compared with CON group. In addition, SACM-treated group showed that ALP activities were enhanced more than ACM-treated group. To identify underlying mechanisms of the effects of SACM on in vitro hair cell growth, we investigated the regulatory effects on mTOR/Akt and Wnt/β-catenin signaling pathway. Protein mTOR, a critical regulator of various metabolic pathways, regulates hair follicle cycle and activates the phosphorylation of Akt, resulting in cell survival, growth, and proliferation [27]. Consistently, a previous study showed that the inhibition of the mTOR signaling significantly deteriorated hair cell cycle and regeneration in mice model [46]. Wnt-mediated phosphorylation of GSK3β stabilizes β-catenin, which participates in HF development and hair growth and regeneration [26]. Previous research demonstrated that the activation of Wnt/β-catenin signaling pathway plays a role in the proliferation of HDPCs and facilitation of hair growth in mammalian model [26,47]. Especially, a clinical study elucidated the underlying mechanism of VEGF-mediated upregulation of Wnt/β-catenin signaling, which was induced by primed CM derived from MSCs [44]. PCNA is also a representative cell proliferation marker, thereby stimulating hair cell growth [47]. Our results showed that SACM could promote both the expression levels of mTOR, β-

catenin, and PCNA and the phosphorylation levels of Akt and GSK3β in HDPCs. Interestingly, there were significant differences in mTOR/Akt and PCNA expressions even compared with ACM group, indicating that SE treatment could enhance the hair cell regulatory effects. Moreover, the present results were in parallel with the above-mentioned expression levels of 3 growth factors, which are known to be interacted with hair growth modulating biomolecular pathway [27,48,49]. On one hand, it is reasonable to conclude that further studies using hair growth agent such as minoxidil and confirming the direct effects of SE treatment on HDPCs will be required to sufficiently prove the upregulating effects of SACM on mTOR/Akt and Wnt/β-catenin signaling pathway. Taken together, these results elucidated the underlying mechanisms of hair cell regulatory effects of SACM in HDPCs.

In conclusion, the present study demonstrated that the treatment of extract derived from *S. maxima* promoted cell proliferation and secretion of growth factors including bFGF, HGF, and VEGF in ADMSCs. Prepared and optimized SACM significantly enhanced the proliferative effect and intracellular ALP activity in HDPCs. The effects of SACM on hair cell growth were further clarified by portraying the protein expressions related to the promotion of mTOR/Akt and Wnt/β-catenin signaling pathway. Worth mentioning, future studies both the detailed compositional analysis of SE displaying active components and comparative evaluation of hair growth promoting effect using approved agents will be required to corroborate the positive effects of SACM on human hair health. Thus, SACM is a novel cell-free therapy for hair growth promotion and regeneration and could be applied to pharmaceutical and cosmeceutical that can improve hair condition, highlighting the SE as a potent ADMSCs-preconditioning candidate.

Declaration of competing interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

The following authors have affiliations with organizations with direct or indirect financial interest in the subject matter discussed in the manuscript.

Acknowledgements

This research was funded by a research grant from the Korea Institute of Ocean Science and Technology (PEA0315) and the Soonchunhyang University Research Fund.

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