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Kukui nut oil (*Aleurites moluccanus* seed oil) promotes hair growth by activating the Nrf2/ARE-AKR1C family-PGF2α signaling axis

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Abstract
Hair plays an essential role in protecting the eyes and skin, and hair loss can cause psychological distress. Kukui nut oil (*Aleurites moluccanus* seed (AMS) oil) has been traditionally used to promote hair growth. While it is now widely used as a cosmetic ingredient, its mechanism for promoting hair growth has remained unknown. In this study, we investigated the hair growth–promoting mechanism of AMS oil, primarily focusing on prostaglandin F2α (PGF2α) production. Ex vivo culture of human hair follicles treated with AMS oil demonstrated a significant hair-growth effect. AMS oil elevated PGF2α levels and increased the expression of AKR1C family members, which are key enzymes involved in PGF2α synthesis. In addition, proliferation markers, including Ki67 and cyclins B1, D1, and E1, were upregulated. Transcriptional analysis revealed that AMS oil activates Nrf2 signaling, which leads to AKR1C family gene regulation via antioxidant response elements. Furthermore, a randomized, double-blind, left–right comparison human testing confirmed the efficacy of AMS oil in promoting eyelash growth. These findings suggest that AMS oil holds promise for applications not only in promoting hair and eyelash growth but also in improving health through its antioxidant effects.

Keywords: Kukui nut oil; hair growth; PGF2α; AKR1C; Nrf2 signaling; eyelash growth

Introduction

Hair is an important organ that protects the eye and skin from external stimulation, while contributing to overall appearance and attractiveness. Hair loss leads to psychological morbidity or distress since attractiveness is closely related to self-esteem[1,2]. Some review articles suggest that alopecia is considered the most traumatic side effect of cancer treatment, especially in female cancer patients[3]. Therefore, hair growth not only contributes to an attractive appearance in healthy individuals but is also important for cancer survivors to increase their self-esteem.

There are several mechanisms of hair growth, one of which involves prostaglandin F2α (PGF2α)[4]. PGF2α is converted from prostaglandin E2 (PGE2) and prostaglandin H2 (PGH2) by the aldo-keto reductase family 1 member C1 (AKR1C) via the arachidonic acid cascade[4]. A PGF2α analog was initially used as a treatment drug for glaucoma[5–7]. It was also repositioned as a treatment for eyelash hypotrichosis because it promotes eyelash growth by prolonging the anagen phase[5–7]. Clinical trials have reported hair growth–promoting effects in trichiasis anopia, androgenetic alopecia (AGA), and eyelash alopecia areata (AA)[8–11].

Organic hair-growth cosmetics are in high demand among consumers. *Aleurites moluccanus* seed (AMS) oil has been extensively employed in traditional folk medicine[12]. The hair loss suppression and growth effects of AMS oil have been shown empirically[13]. However, only a few animal studies have reported that AMS oil promotes hair growth, and the mechanism has not been elucidated[14,15]. Therefore, this study aimed to reveal the mechanism of AMS oil-induced hair growth, focusing on PGF2α production and its synthesis pathway. We also investigated its efficacy in human hair growth through a formulation containing AMS oil.

Results

AMS oil promotes hair follicle (HF) elongation in human HF organ culture

To analyze the hair growth effect of AMS oil treatment at the organ level, cultures of human scalp hair follicles were investigated. We found that the hair shaft was significantly elongated in the AMS oil treatment group compared with the control group (Fig. 1A-C). Since AMS oil tended to increase the growth phase and decrease the resting phase, it may promote hair growth by prolonging or maintaining the anagen phase and delaying the transition to the catagen phase (Fig. 1C).

AMS oil induces AKR1C family members and proliferation marker expression

To examine the effect of AMS on hair growth, we focused our analysis on PGF2α synthesis, which has been reported to affect hair growth[5]. First, we performed western blotting (WB) to confirm their expression at the hair follicle organ level. After AMS oil treatment, AKR1C1-3 signals increased (Fig. 2A). Prostaglandin F receptor (PTGFR) expression was not changed by the treatment (Fig. 2 B, C). Previously, expression of PTGFR, the PGF2α receptor, its ligand, PGF2α, and AKR1C1-3, which function as PGF2α synthases, has been reported in the skin, including the outer root sheath and keratinocytes.[4] Therefore, we focused on keratinocyte responses to reveal the mechanism by using a keratinocyte cell line (HaCaT cells). Gene and protein expressions of AKR1C1-3 showed an increasing trend in a dose-dependent manner at each time point. Significant differences in expression were observed for all *AKR1C1-3* genes between the control and the 0.01% AMS oil treatment group (Fig. 2C). The effect of AMS oil on AKR1C1-3 expression was time-dependent in terms of protein expression (Fig. 2D). We further investigated the effect of AMS oil on proliferation. The protein expression levels of the proliferation marker Ki-67 and the cell cycle–related proteins cyclin B1, which is upregulated during the G2/M phase, as well as cyclin D1 and cyclin E1, which are required for the G1/S transition, were increased following AMS oil treatment (Fig. 2E). In addition, AMS oil treatment reduced the expression of inflammation-related genes, including prostaglandin E synthase (PGES), which synthesizes PGE2, suggesting a potential anti-inflammatory effect (Supplementary Fig. S1).

AMS oil increases PGF2α production, which is regulated by *AKR1C1-3*

The investigation of AMS oil as a hair growth promoter was continued by evaluating the effect of AMS oil treatment on PGF2α production in HaCaT cells. As shown in Figure 3, ELISA analysis showed an increase in PGF2α concentration following 0.01% AMS oil treatment in comparison with the control. To investigate which isoforms of the *AKR1C1-3* play an important role in PGF2α production in terms of efficacy and specificity, PGF2α production in each AKR1C1-3 knockdown cell was investigated. siRNA-mediated knockdown of *AKR1C1-3* successfully decreased gene expressions (Fig. 3B). Knockdown of *AKR1C1-3* abolished the increase in PGF2α secretion induced by AMS oil treatment, demonstrating that each *AKR1C1-3* gene contributes to PGF2α (Fig. 3C).

AMS oil activates the antioxidant response element (ARE) resulting in *AKR1C1-3* upregulation

ARE is known as a transcriptional regulator of *AKR1C1-3.*[16] Therefore, we investigated whether AMS oil regulates transcriptional activation of ARE using a luciferase assay in HaCaT cells. AMS oil treatment increased luciferase activity of Nrf2/ARE activity (Fig. 4A).

NRF2 binds to AREs and thereby activates the transcription of downstream target genes. Using siRNA-mediated knockdown of NRF2, we demonstrated that NRF2 regulates a set of ARE-driven genes. AMS oil treatment increased the expression of genes involved in detoxification and antioxidant responses (AKR1C1, AKR1C2, AKR1C3, HMOX1, NQO1, SOD1, SOD2) as well as glutathione metabolism (GCLC, GCLM, SLC7A11, G6PD, GSR), whereas siNRF2 reduced their expression. These results suggest that AMS oil regulates cellular redox homeostasis through activation of the NRF2 pathway (Fig. 4B). Furthermore, the effect of *NRF2* knockdown on PGF2α production was investigated, and it was shown that *NRF2* knockdown significantly decreased PGF2α production (Fig. 4C). This observation suggests that activation of ARE, which promotes coordinated expression of AKR1C1-3, the enzymes responsible for PGF2α production, is essential for PGF2α production.

AMS oil induces eyelash growth in human subjects

To evaluate the efficacy of AMS oil in humans, a randomized double-blind, left–right comparison study was conducted using a serum applied to eyelashes containing 0.1% AMS oil and a control serum. The concentration was determined based on formulation stability, user acceptability, and safety, assuming its use as an eyelash serum. Since we were exploring cosmetic applications for AMS oil, we avoided animal testing for ethical and regulatory reasons.

The study products were self-applied by the participants according to the instructions provided at enrollment. Participants were instructed to apply the test product to the assigned eyelash side and the vehicle to the contralateral side once daily throughout the study period. Compliance with product use was confirmed through participant diaries and interviews at follow-up visits. All participants reported consistent daily application of the assigned products, and no major protocol deviations were observed. The interventions were administered as intended throughout the study period. Treatment with the AMS oil serum resulted in a significant increase in eyelash length compared to the control (Fig. 5A, B). In addition, participant questionnaires administered before and after the treatment period revealed perceived improvements in eyelash length, thickness, and volume (Fig. 5C). No adverse effects, including skin pigmentation, were reported throughout the study. No changes to the study design or protocol occurred after trial commencement.

Discussion

This study revealed that AMS oil promotes human hair growth in vitro and in vivo. AMS oil promotes PGF2α secretion by enhancing the expression of AKR1C1-3 enzymes via the NRF2/ARE pathway. AKR1C1–3 are enzymes that synthesize PGF2α, thus contributing to hair growth. The ARE/NRF2 pathway functions as an antioxidant pathway. It has been reported to suppress cellular senescence and increase cell survival by promoting the expression of downstream molecules with anti-inflammatory and detoxifying effects[17,18]. In hair, it has been reported that activation of the NRF2 pathway by the phytochemical sulforaphane rescues the inhibition of hair elongation under oxidative stress[18]. Therefore, in addition to sulforaphane, other natural compounds that activate NRF2, such as sesaminol, may also be linked to mechanisms related to hair growth[18–20]. In addition, since AMS has been reported to be non-cytotoxic to human conjunctival cells, it can be used not only for hair growth on the head but also as a safe eyelash growth agent[21]. AMS oil primarily comprises linoleic acid (ω6 fatty acid), oleic acid (ω9 fatty acid), and linolenic acid (ω3 fatty acid), while also containing antioxidants including tocopherols and polyphenols[12,22,23]. Tocopherols, linoleic acid, and linolenic acid have been reported to activate the NRF2/ARE pathway[24–26]. These findings suggest that several components of AMS oil could activate the NRF2/ARE pathway. Meanwhile, unsaturated fatty acids such as oleic and linoleic acids are known to be competitive inhibitors of AKRs[27]. Since our results showed that PGF2α was produced by the AKR1C family, it is possible that the activity of the AKR1C family was more dominant than the inhibitory effect of unsaturated

fatty acids on AKRs.

Prostaglandins are closely involved in hair growth[8]. PGD2 inhibits hair growth by enhancing testosterone metabolism, while both PGE2 and PGF2α are known to promote hair growth via PTGFR[8]. Of these, PGF2α derivatives have been reported to be effective in hair growth and regrowth in clinical trials for trichiasis anopia, AGA, and eyelash AA[9–11]. Although PGF2α is known to bind to PTGFR, a GPCR, and prolong the growth phase of hair, the mechanism of this effect was unclear. In this study, we focused on cell proliferation and the cell cycle and found that PGF2α facilitates the G1/S transition, upregulates the G2/M phase of the cell cycle, and increases the expression of the proliferation marker Ki67. PTGFR is a type 1 GPCR, and the PGF2α/PTGFR axis is known to promote human retinal microvascular endothelial cell (HRMEC) proliferation and tube formation via the Gq/CAMK2G/p38/ELK-1/FOS pathway[28]. PGF2α is also known to regulate the cell cycle and thicken the endometrium[29]. These pathways may also be involved in hair growth.

The AKR1C family is also involved in the regulation of sex hormones. Androgenetic alopecia (AGA) is the leading type of scalp hair loss, affecting 60–70% of the population worldwide[30]. One cause of this is excessive 5α-dihydrotestosterone (5α-DHT) production in hair follicles[30]. 5α-DHT is reduced to 5α-androstan-3α, 17β-diol (3α-diol) and 5α-androstan-3β, 17β-diol (3β-diol) by AKR1C2 and AKR1C1, respectively[31]. Thus, AKR1C1 and AKR1C2 may suppress male pattern baldness by causing male hormones to become inactive. The PGF2α-independent response caused by AMS oil is expected to ameliorate AGA.

In human subjects, an AMS oil serum significantly lengthened human eyelashes without causing side effects such as hyperpigmentation reported with PGF2α analogues. One possible reason is the difference in PGF2α concentration between drugs, such as bimatoprost 0.03%, and endogenous PGF2α produced by cells in the body (Fig. 3A)[32]. The effect of AMS oil on melanogenesis remains to be elucidated.

A limitation of this study is that, although we demonstrated AMS-induced, PGF2α-mediated hair growth, it remains unclear whether PGF2α-independent mechanisms also contribute to this process, and the specific AMS components responsible for the observed effects have not yet been identified. In addition, although this study employed a single-dose human trial, evaluation under multiple dosing regimens will be important to better characterize dose–response relationships. Furthermore, to support broader applicability, future studies should assess effects not only on eyelash growth but also on scalp hair. In conclusion, AMS oil increased the expression of AKR1C family members by activating antioxidant responses via the NRF2/ARE pathway and promoted the secretion of PGF2α, which exerts hair growth effects. These findings suggest that AMS oil holds promise for applications not only in promoting hair and eyelash growth but also in improving health through its antioxidant effects.

Materials and methods

AMS oil stock preparation

AMS oil was purchased from Nikko Chemicals (Osaka, Japan). To prepare a stock solution of AMS oil, AMS oil was diluted with a 9× higher weight of DMSO (D2650, Merck, Darmstadt, Germany) to make the oil water soluble. Due to the cytotoxic effects of DMSO, AMS oil concentrations higher than 0.01% could not be used.

Cell culture

The HaCaT human keratinocyte cell line was cultured in Dulbecco's modified Eagle's medium (DMEM; D4629, Merck) containing 10% fetal bovine serum (FBS; 10270-106, Thermo Fisher Scientific, Waltham, MA, USA) and 1% antibiotic-antimycotic (15240062, Thermo Fisher Scientific) in a humidified incubator containing 5% CO_2 at 37°C. The cells were sub-cultivated and harvested at 80% confluency following detachment using 0.25% trypsin EDTA (201-16945, Fujifilm, Tokyo, Japan). Cells were cultured in triplicate and then treated with 0.09% DMSO (control-treatment) and 0.001%, and 0.01% AMS oil in each

plate. The samples were collected after 1, 2, and 3 days of incubation.

Isolation and treatment of hair follicles from the skin

Human skin tissues were obtained with informed consent from Seishin Plastic and Aesthetic Surgery Clinic Co. Ltd. (Tokyo, Japan). Experiments using human skin were approved by the ethics committee of Osaka University (Yakuhito2019-28). All research was performed in accordance with relevant guidelines and regulations. Informed consent was obtained from all participants. Human scalp skin from nonbalding areas was collected during surgery and transported using sterile DPBS (-) (045-29795, Fujifilm).

Human hair follicles were microdissected individually from each skin sample under an M125 stereomicroscope (Leica, Wetzlar, Germany) using sterile equipment and plasticware as previously reported[33]. Intact anagen follicles were dissected from the subcutaneous fat using fine forceps and scissors, taking care not to damage them. Isolated follicles were carefully transferred to an individual well of a 24-well plate (353047, Corning) containing William's E Medium, GlutaMAX™ Supplement (32551020, Thermo Fisher Scientific), which contained 1% antibiotic-antimycotic, 100 ng/mL hydrocortisone (H6909, Merck), and 5 mg/mL insulin (093-06351, Fujifilm) as previously reported[33]. AMS stock solution, DMSO, or 100 nM bimatoprost (029-18441, Fujifilm) were added for the hair follicle culture treatment. The hair follicle samples were maintained free-floating and were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air in a humidified incubator.

Analysis of hair follicle length and hair cycle

The morphology and length of hair follicles were photographed every 2–3 days until day 9 using an Olympus stereomicroscope (SZX16). Hair follicles that failed to grow after three days of culturing were excluded from analysis. Total hair length was measured from the tip of the hair bulb along the hair shaft. The hair cycle was analyzed based on hair bulb morphology as previously reported[34].

ELISA

The culture supernatant was harvested following centrifuging at 300 × g for 5 min at room temperature to remove the debris. The concentration of PGF2α in HaCaT cells was determined using a PGF2α High Sensitivity ELISA kit (ADI-930-069, Enzo Life Sciences, Farmingdale, NY, USA) according to the manufacturer’s instructions. The absorbance was measured using a microplate reader (Glo-Max, Promega, Madison, WI, USA) at 405 nm, and the concentration of PGF2α was calculated from the standard curve.

Reverse transcription-quantitative polymerase chain reaction

RNA was extracted from cells that had been treated with AMS oil. After washing with DPBS, phenolic RNA extraction was performed using NucleoSpin® RNA (740955.50, Macherey-Nagel) or TRI reagent (TR118, Molecular Research Center, Inc., Cincinnati, OH, USA). Precipitation and purification of RNA were performed using isopropanol and 70% ethanol. cDNA was synthesized using a QuantiTect Reverse Transcription Kit (205315, Qiagen, Venlo, Netherlands). The PCR mixture was prepared using SYBR ® Green Realtime PCR Master Mix (QPK-201, Toyobo, Osaka, Japan), and the primer used for RT-qPCR was shown in the Table 1 and Supplementary Table S1. Each RT-qPCR experiment consisted of three temperature change steps.

Table 1 Primer information for qRT-PCR

Gene	Forward Primer(5'->3')	Reverse Primer(5'->3')
AKR1C1	TTGCATGAGGTCTGCCA	GCTGTAGCTTGCTGAAAT
AKR1C2	ACCATATTGATTCTGCACATGTTT	TGGGAATTGCTCCAAAGC
AKR1C3	GAGAAGTAAAGCTTTGGAGGTCACA	CAACCTGCTCCTCATTATTGTATAAATGA
18S	CGGCTACCACATCCAAGGAA	AGCTGGAATTACCGCGGC
HMOX1	TACCACATCTATGTGGCCCTG	TGGCTGGTGTGTAGGGGAT
NRF2	TCAGCGACGGAAAGAGTATGA	CCACTGGTTTCTGACTGGATGT
HMOX1	TACCACATCTATGTGGCCCTG	TGGCTGGTGTGTAGGGGAT
NQO1	GGCAGAAGAGCACTGATCGTA	TGATGGGATTGAAGTTCATGGC
GCLC	AAACCCAAACCATCCTACCC	GCATGTTGGCCTCAACTGTA
GCLM	TGCTGTGTGATGCCACCAGATTTG	GTGCGCTTGAATGTCAGGAATGC
SLC7A11	GCTTTGTCTTATGCTGAATTGG	TGCAGGGCGTATTATGAGGAG
G6PD	AAGCCCATCCCCTATATTTATGGC	GGTGCCCTCATACTGGAAACC
GSR	TGATCCCAAGCCCACAATAGAGGTCAGT	CCATCGCTGGTTATTCCTAAGCTGGCAC
SOD1	AGGGCATCATCAATTTCGAG	ACATTGCCCAAGTCTCCAAC
SOD2	TTGGCCAAGGGAGATGTTAC	AGTCACGTTTGATGGCTTCC

Western Blotting

Protein was harvested from HaCaT cell lysates that had been treated as mentioned above for the cell treatments. The cell extracts were prepared in ice-cold radioimmunoprecipitation assay buffer (RIPA buffer, Santa Cruz Biotechnology, Dallas, TX, USA) containing protease and phosphatase inhibitors (complete mini and PhosSTOP, Roche, Basil, Switzerland) for 10 min on ice. The lysate was centrifuged at 14,000 x g for 15 min at 4°C. The proteins were mixed with sample buffer (Bio-Rad, Hercules, CA, USA) and heated before loading into wells of a sodium dodecyl sulfate-polyacrylamide gel (TGX, Bio-Rad). After the gel electrophoresis was complete, the proteins were transferred onto polyvinylidene difluoride membranes using a semi-dry method (Trans-Blot Turbo, Bio-Rad). Each membrane was then incubated with one of the following primary antibodies: anti-AKR1C1 (ab192785, Abcam, Cambridge, UK), anti-AKR1C2 (DF3757, Affinity Bios, Cincinnati, OH, USA), anti-AKR1C3 (ab209899, Abcam), anti-ki67 (ab16667, Abcam), anti-CyclinB1, D1, E1 (4138, 2978, 4129, Cell Signaling Technology (CST), Danvers, MA, USA), Prostaglandin F2α Receptor/PTGFR (APR-067, Alomone Labs, Jerusalem, Israel) and anti-β-actin (4970, CST) antibodies overnight at 4°C. Subsequently, the membrane was washed with Tris-buffered saline with 0.01% tween20 (TBST) and was incubated with a secondary antibody: horseradish peroxidase-conjugated goat anti-rabbit IgG (CST, 7074S) or anti-mouse IgG (Bio-Rad, 1706516) diluted in 5% skim milk at room temperature for 1.5 h. Finally, the blots were developed using an enhanced chemiluminescence detection (ECL) kit (Cytiva, Marlborough, MA, USA; RPN2236). β actin was used as an internal positive control. Images were acquired using an Amersham Imager 600 (GE Healthcare, Chicago, IL, USA).

RNA interference to knock down AKR1C1, AKR1C2, AKR1C3, and NRF2

The human small interfering RNA experiments were performed using siRNA for AKR1C1 (s56843); AKR1C2 (s194378); AKR1C3 (13535); NFE2L2 (NRF2) (s9493) (Silencer® Select or Silencer®, Themo Fisher Scientific). Silencer® negative Control No. 2 siRNA (cat# AM4613) was used for the control. Cell cultures reaching 50% confluence were transfected using RNAi at a final concentration of 10 nM and Lipofectamine™ RNAiMax (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions in an Opti-MEM reduced serum medium (51985-034, Themo Fisher Scientific) without antibiotics for 24 h. Medium was changed on the following day and AMS oil treatment was started. After 48 h, an mRNA and supernatant were harvested for quantitative real-time-PCR and PGF2α ELISA.

Luciferase assay

HaCaT cells were cultured in a 24-well plate and co-transfected with 0.5 μg NanoLuc®

luciferase reporter (pNL[NlucP/ARE/Hygro] Vector (#CS180902) or pNL[NlucP/minP/Hygro] Vector (#CS188006); Promega) and 0.05 μg firefly loading control reporter (pGL4.54[luc2/TK] vector; Promega) with PEI MAX (24765-2, Polysciences, Warrington, PA, USA) in a Opti-MEM. Cells were cultured for 24 h in DMEM containing 10% FBS and 0.01% AMS oil or DMSO one day after transfection. Luminescence was detected using a Nano-Glo® Dual-Luciferase® Reporter Assay System (N1620, Promega) and a GloMax® Discover Microplate Reader (GM3000, Promega) according to the manufacturer's instructions.

In-vivo test of the eyelash serum in human participants

This study employed a randomized, double-blind, left–right comparison design to evaluate the effects of a serum containing 0.1% AMS oil and a vehicle-matched placebo serum on eyelash growth. The study protocol was approved by the Institutional Review Board of the Mandom Corporation Ethics Committee (Approval No. 108-21055). Written informed consent was obtained from all participants. The trial was registered at UMIN-CTR (UMIN000060798) on March 2nd, 2026. This trial was registered retrospectively because the study was initiated prior to mandatory registration awareness. This study was conducted in accordance with the principles of the Declaration of Helsinki. Fifteen healthy Japanese women aged 27–48 years, with no ocular, eyelash, or skin disorders in the application area, were enrolled (Table 2). The test and placebo serums were applied to the roots of the upper and lower eyelashes. Each participant applied the assigned serums twice daily, once in the morning and once in the evening following their regular skincare routine, using a standardized dosage for each application. Clinical evaluations were performed at baseline and after four weeks of treatment. Eyelash length was quantitatively assessed using an imaging system (ViSIA Generation 7, Canfield Scientific, Parsippany, NJ, USA) and compared with baseline measurements. In addition, a self-assessment questionnaire was administered at the same time point, with each item analyzed as an independent endpoint. No changes to the study design or protocol occurred after trial commencement and no changes to trial outcomes were made after trial commencement. The detail of this study is available in supplemental information 3.

Table 2. Baseline characteristics of study participants

Characteristic	Value
Number of participants	n = 15
Age (years), mean ± SD	40.1 ± 6.53
Sex (female/male)	Female n=15
Baseline eyelash length (mm), mean ± SD	4.9 ± 0.73

Statistical analysis

Statistical analysis was performed using GraphPad Prism, version 7.04 (GraphPad, Boston, MA, USA) and Statcel4 (OMS Publishing, Tokyo, Japan). P-values were adjusted using Holm–Bonferroni method for the multiple comparisons using Holm-Bonferroni sequential correction 1.3.1.xlsx provided by Dr. Justin Gaetano (https://www.researchgate.net/publication/322569220_Holm-Bonferroni_sequential_correction_An_Excel_calculator_13).

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Author contributions

Conceptualization, H.K., N.R.B. and T.E; investigation, H.K., N.R.B., J.F., M.A.S.R., D.R., S.S., R.F., Y.A. and T.H.; writing—original draft preparation, H.K., N.R.B, and M.A.S.R.; writing—review and editing, H.K., N.R.B., J.F., T.E., M.A.S.R., T.H., K.S., T.H. and F.F.; supervision, H.K. and F.F.; funding acquisition, H.K. All authors have read and agreed to the published version of the manuscript.

Data availability statement

Data is contained within the article and supplemental information.

Additional Information

K.S., T.H. and F.F. are employed by Mandom Corporation. The remaining authors declare that the research herein was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics declarations

Ethics declarations are described in the Materials and Methods section.

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Figure legends

Fig. 1. Hair growth induced by *Aleurites moluccana* seed (AMS) oil in hair follicle culture. (A) Representative photos of hair follicles after AMS oil treatment. The total hair length

was measured from the bulb to the top of the elongated part (green arrow symbol). (B) The graph of the total length of the hair (mean ± SEM; subject number = 10; hair follicle numbers = 52, 53, and 50, respectively; *P < 0.05; **P < 0.01; Dunnett's test following a one-way ANOVA). (C) The graph of hair cycle analysis. Bars in the graph describe the percentage of each phase. The total percentage of the growth phase and resting phase for each treatment is 100%. The transitional phase was not detected in all samples. (mean ± SEM, n=10, no significance; Dunnett's test was conducted following a one-way repeated measures ANOVA).

Fig. 2. Effect of *Aleurites moluccana* seed (AMS) oil on AKR1C1–3 expression and proliferation. Protein expression of (A) AKR1C family and PTGFR in hair follicles, (B) PTGFR expression in HaCaT cells. (C) The relative mRNA expression of *AKR1C1*, *AKR1C2*, and *AKR1C3* in HaCaT cells after induction with 0.001% and 0.01% AMS oil (mean ± SEM, n = 10, *P < 0.05, **P < 0.01; Steel test among each day of control sample vs treatment sample following Kruskal-Wallis test). (D) The protein expressions of AKR1C1–3 in HaCaT. (E) Proliferation and cell cycle markers of representative data for the western blot result for HaCaT cells. Red arrows indicated the target bands. Original blotting images are available in supplemental information 2.

Fig. 3. Effect of *Aleurites moluccana* seed (AMS) oil on PGF2α secretion. (A) PGF2α secretion in HaCaT cells after treatment with 0.01% AMS oil for 24, 48, and 72 h (mean ± SEM, n = 6; Dunnett's test for each treatment time). (B) Gene expression of the target genes treated with each respective siRNA after 0.01% AMS oil treatment (mean ± SEM, n = 10; Mann Whiteny's U-test for indicated pairs with Holm–Bonferroni correction). (C) PGF2α secretion after siRNA (mean ± SEM, n = 5; student's t-test between non-treatment and AMS oil treatment in each siRNA treatment group). ns: non-significance, *P < 0.05, **P < 0.01, ***P < 0.001.

Fig. 4. Effect of *Aleurites moluccana* seed (AMS) oil on the activation of Nrf2, and *NRF2* knockdown effect on downstream genes and PGF2α secretion. (A) Luciferase activity in HaCaT cells were transfected with the firefly vector containing *Nrf2/ARE* or an empty vector, *minP*, as the control. Values are relative to firefly luciferase activity (mean ± SEM, n = 5; student's t-test for indicated pairs with Holm–Bonferroni correction). (b, c) HaCaT cells were treated with 0.01% AMS oil following the knockdown of NRF2. (B) Expression of Nrf2 and *Nrf2/ARE* downstream target genes. The bar graph shows the mean values (mean + SEM, n = 10; Mann Whiteny's U-test (*NRF2*, *AKR1C1*, *AKR1C2*, *HMOX1*, *GCLC*, *SLC7A11*, and *G6PD*) or Student's t-test (the others) for indicated pairs with Holm–Bonferroni correction). (C) PGF2α concentration in the supernatant (mean ± SEM, n = 5; student's t-test for indicated pairs with Holm–Bonferroni correction.). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.001.

Fig. 5. *Aleurites moluccana* seed (AMS) oil induces eyelash lengthening in humans. (A) Difference in eyelash length between placebo and AMS oil application after four weeks. (mean + SEM, n = 15; Wilcoxon signed-rank test for indicated pairs with Holm–Bonferroni correction). (B) Representative images of eyelash growth in treatment with AMS oil. (C) Questionnaire results before and after AMS treatment. (mean + SEM, n = 15, Wilcoxon signed-rank test). *P < 0.05, **P < 0.01).

Figure 1
Figure 2
Figure 3

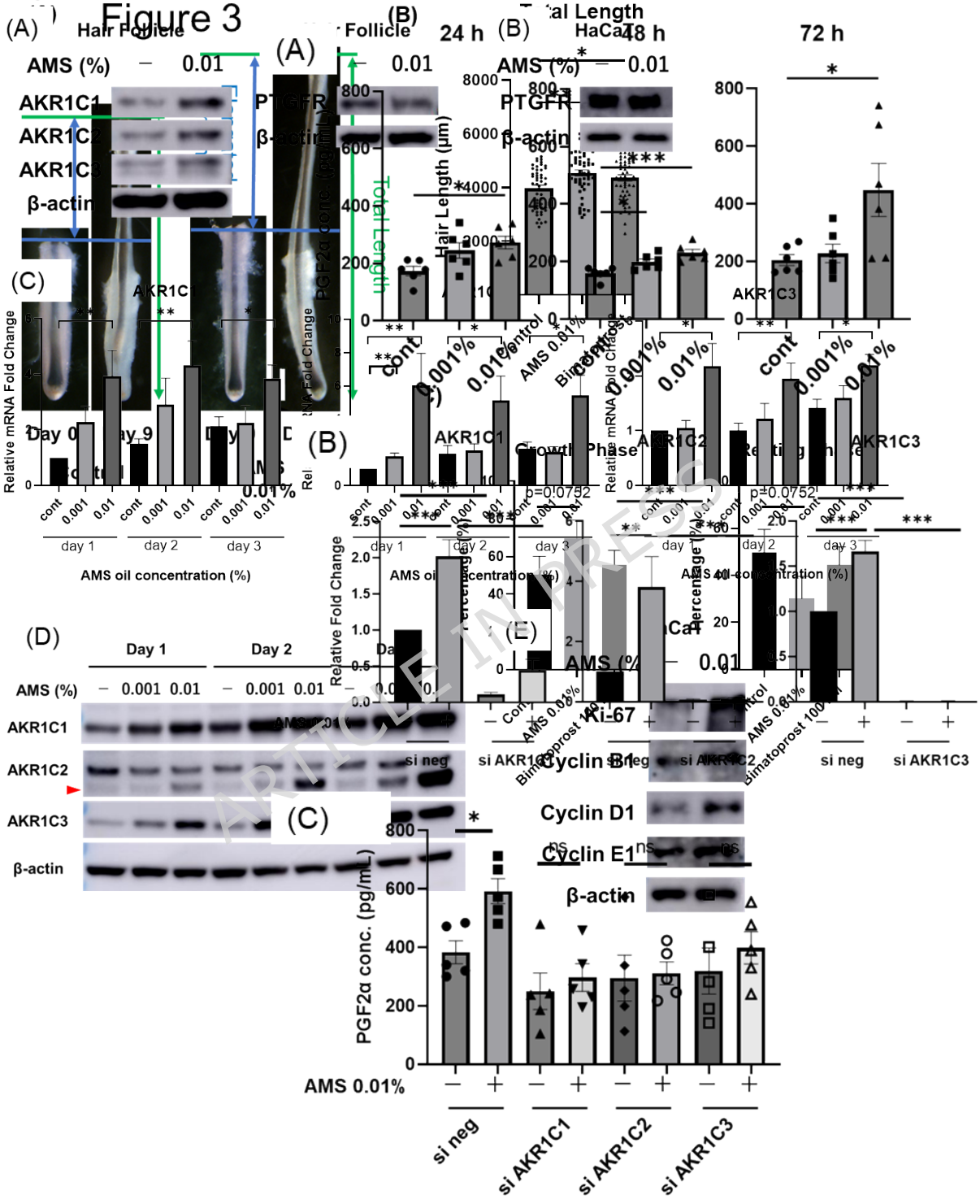

Figure 4
Figure 5