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Efficacy of derinat as a treatment for murine and androgenetic alopecia (AGA) patients

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Abstract

Background Androgenetic alopecia (AGA), one of the most common types of hair loss, is associated with oxidative stress, inflammation and aging. Derinat, a transient receptor potential canonical channels (TRPCs) inhibitor, restrains TRPCs-mediated increase intracellular Ca^{2+} signaling, which initiates the skin aging process with intracellular reactive oxygen species (ROS) accumulation. This study investigated whether Derinat protected skin from oxidative stress-induced damage and aging, thus inhibiting AGA pathogenesis.

Methods The lifespan of *Caenorhabditis elegans* was measured to examine the capacity of Derinat to oppose the oxidative stress induced-aging process, which drives the hair cycle from anagen to catagen phase. The experiments that used BALB/c-nu and C57BL/6 mice determined the effects of Derinat on hair cycle and oxidative stress in skin. To further apply Derinat to clinical study, the resulting relationship between AGA pathogenesis and TRPCs-regulated oxidative stress was confirmed using the bioinformatics approach. We consequently used the parameters of hair density, hair diameter, hair recovery and quality of life index to evaluate the effect of Derinat treatment on AGA subjects.

Results Derinat restrained the oxidative stress induced-aging process sufficiently to extend the lifespan of worms. Derinat also changed the hair growth patterns of mice by maintenance of the hair cycle at the anagen phase. This efficacy was due to reduction of TRPCs-mediated ROS accumulation. Because the bioinformatics analysis found that AGA pathogenesis is associated with TRPCs-regulated oxidative stress and inflammation, treatment with Derinat in AGA subjects increased positive outcomes of oral medication while mitigating the impairment of AGA subjects' quality of life.

Conclusions Derinat restrains AGA pathogenesis and may provide a new therapeutic approach for treating AGA. ClinicalTrials.gov Identifier NCT05450861, https://register.clinicaltrials.gov, date of registration 07/11/2022.

Trial registration ClinicalTrials.gov Identifier NCT05450861, https://register.clinicaltrials.gov, date of registration 07/11/2022

Keywords Transient receptor potential canonical channels, Oxidative stress, Androgenetic alopecia, Hair cycle, Derinat

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Introduction

Androgenetic alopecia (AGA) is one of the most common hair loss problems, and is characterized by progressive hair loss. There are particular patterns of scalp hair deficiency in both females and males [1]. Subsequent hair loss that is induced by AGA contributes to physiological issues and psychological stress that profoundly influences the quality of life for patients with AGA [2]. The clinical mainstay of treatments for AGA include topical Minoxidil and systemic anti-androgen medications [3]. Patients who receive long-term and sustained treatments to achieve clinical improvement may experience side effects and safety issues associated with these treatments. For instance, treatment with oral anti-androgens is accompanied by common adverse side effects such as breast tenderness, or increased libido in females and decreased libido in males. Minoxidil, a topical agent that is safer to administer as a topical treatment, may result in hypertrichosis and local irritation [4]. Although the minoxidil can provide some relief for AGA patients, treatment based on alternatives to the modulation of androgens is warranted and there is a large research gap to be explored. Recently, there have been new insights into the pathophysiology of hair loss and studies have been undertaken for developing novel approaches in treatment.

Oxidative stress, inflammation and aging can cause dysregulation of complex hair follicle biology in AGA [5]. AGA is caused by the gradual miniaturization of hair follicles, which are the target of androgens mediated by dihydrotestosterone (DHT) [6]. The accumulation of DHT in AGA-prone hair follicles induces hair cycle delay and hair growth deceleration, potentially through oxidative stress–associated senescence in dermal papilla cells (DPCs) and hair follicle stem cell (HFSCs) inactivation [7–9]. DPCs generate instructive signals to induce bulge HFSC activation during the hair cycle; thus, oxidative stress of the DPCs might restrain HFSCs activation as well as the entire hair cycle process [10], and potentially is implicated in AGA pathogenesis.

Our previous study showed that transient receptor potential canonical channels that are (TRPCs)-mediated increase intracellular Ca^{2+} signaling, which initiates the skin aging process. This contributes to intracellular reactive oxygen species (ROS) accumulation, activation of DNA damage response (DDR) and senescence inflammatory response (SIR) [11]. Derinat, a TRPCs inhibitor [11, 12], contains DNA sodium salt isolated from the soft roes of *Acipenser gueldenstaedtii*, (a species of sturgeon fish), which is then depolymerized using ultrasound in 0.1% sodium chloride solution to particles with the molecular weight of 270–500 KDa [13]. Thus, blockage of TRPCs activity by TRPCs inhibitor Derinat restrains skin damage and aging [12]. It has been hypothesized that treatment with Derinat potentiates inhibition of AGA pathogenesis; accordingly, this study investigated the effect of Derinat on hair follicle (HF) cycle progression in mice and its efficacy in AGA patients with the desired outcome of validating the potential for application of Derinat in AGA treatment.

Methods

Caenorhabditis elegans culture

Caenorhabditis elegans (*C. elegans*, Bristol strain N2) was obtained from the Caenorhabditis Genetics Center (CGC), cultivated on nematode growth medium (NGM) agar plates, and fed with UV-killed *Escherichia coli OP50* (*E. coli*). Post-ultraviolent management was aimed to prevent the potential confounding effects of bacterial metabolism. *C. elegans* was then disintegrated by alkaline bleach solution to collect eggs, which were then hatched on NGM plates with *E. coli* at 20 °C for 48 h to achieve age-synchronized *C. elegans* larvae.

Lifespan assay

To measure worm lifespan, the age-synchronized worms (L4 stage) were transferred onto a lawn of 6 cm NGM plates and were marked as day 0 [14]. The worms were supplemented daily with Derinat (Pharm Pack, Moscow, Russia) and then were moved to new agar plates daily during the first 4–5 days to prevent confusion of the different generations. Furthermore, the worms that showed no response to gentle prodding with a platinum wire and no pharyngeal pumping were considered to have expired. The numbers of surviving worms were recorded every day. Three replications were performed and a total of 120 worms were used.

Derinat treatment protocol for mice

BALB/c-nu (6 weeks of age) and C57BL/6 (8 weeks of age) male mice were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). The treatment protocol for use of Derinat on BALB/c-nu mice is based on Zhang et al.'s study [15]. Briefly, BALB/c-nu mice at the initial hair-existing phase, were treated daily with Derinat hydrogel or pure hydrogel (SOMAPEX BIOTECH. CO., Kaohsiung, Taiwan) by placing treatment or control on dorsal skin for 3 h. After 7 days of treatment (at the end of hair-existing phase), the mice were sacrificed and their skin biopsies were harvested from the dorsal area, fixed with 2% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA), and embedded in paraffin for further study.

The treatment protocol for use of Derinat on C57BL/6 mice is based on Wang et al.'s study [16]. 13 weeks of age C57BL/6 mice were daily treated Derinat by subcutaneous injection into the dorsal skin of mice from P91

to P154. The skin biopsies were collected at P105 (anagen VI phase) and P154 (telogen phase), respectively, and then also fixed with 2% formaldehyde and embedded in paraffin for further study. The animal experiments were performed under an affidavit of approval of animal use protocol at Kaohsiung Medical University (IACUC approval number: 101119 and 109195).

Cell culture

Human primary keratinocytes and dermal fibroblasts were purchased from Lonza (Morrisville, NC, USA), and human dermal papilla cells were purchased from Cell Applications (San Diego, CA, USA). Keratinocytes were grown in serum-free keratinocyte growth medium supplemented with human recombinant epidermal growth factor, bovine pituitary extract, human insulin-like growth factor I and hydrocortisone (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). Dermal fibroblasts were grown in Dulbecco's modified Eagle's medium DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco). Dermal papilla cells, DPCs were grown in follicle dermal papilla cell growth medium (CELL, San Diego, CA, USA). All cells were incubated at 37 °C in humidified in a 5% CO_2-in-air atmosphere.

Calcium imaging

TRPCs-activated intracellular Ca^{2+} responses were induced by applying adenosine triphosphate (ATP) (Sigma Aldrich), in the same manner as the previous study [12]. Briefly, cells were pretreated with 60 μg/ml of Derinat at 37 °C for 30 min and then stained with 1 μM Fluo-4-AM (Molecular Probes, OR, USA) at 37 °C for 20 min. Prior to the experiments, cells were washed with a balanced salt solution (BSS) buffer (5.4 mM KCl, 5.5 mM D-glucose, 1 mM MgSO4, 130 mM NaCl, 20 mM HEPES pH 7.4, and 2 mM $CaCl_2$). Intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) were calculated from the ratio of fluorescence intensities (excitation/emission wavelength 488 nm/525 nm) by using an Olympus Cell^R IX81 fluorescence microscope.

Gene expression omnibus database analysis

The Gene Expression Omnibus (GEO) is a gene expression profiling database (https://www.ncbi.nlm.nih.gov/geo) [17]. A portion of this database, specifically accession number GSE90594, was used during analysis of the expression of *TRPCs*, oxidative stress-related genes, and inflammation-related genes in the scalps of healthy donor and AGA patients. A different portion of the GEO data base, accession number GSE101451, was used during analysis of oxidative stress-related genes, inflammation-related genes and expression of *TRPCs* specific to bulb and bulge portions of hair follicles in AGA patients. RNA sequencing of accession number GSE93766 of the GEO database was employed in the analysis of oxidative stress-related genes, inflammation-related genes and expression of *TRPCs* found in balding and non-balding human DPCs derived from AGA patients.

Immunohistochemistry staining

Antibody against prostaglandin-Endoperoxide Synthase 2 (PTGS2, Abcam, Cambridge, UK) was used to detect target molecules. Immunoreactivity was visualized after incubation with 3,3′-diaminobenzidine (DAB) substrate-chromogen system (Dako Omnis) according to the manufacturer's protocol (Agilent, Santa Clara, CA, USA).

Clinical study design

This study was a single-center, randomized, single-blind, parallel-group, placebo-controlled 8-week intervention study in Taiwan. Patients were randomized (2:1 ratio) to Derinat and placebo groups. Patients were blinded to treatment allocation until study completion, or until patient withdrawal from the study. During the execution of the clinical study, male subjects received an oral medication, Dutasteride, which is widely used to inhibit 5α-reductase, a blocker for DHT production. Female subjects received an oral medication, Spironolactone, which functions as a competitive aldosterone antagonist and inhibits the interaction of testosterone and DHT with intracellular androgen receptors in target tissues. Participants in the Derinat group applied a scalp conditioning solution containing Derinat once a day in addition to oral medication. Participants in the placebo group took oral medication and applied the conditioning solution without Derinat once a day.

Study participants

In order to be eligible for the study, subjects had to be in the age range of 20 to 75, and had to have androgenetic alopecia. Subjects had to maintain the same hair color and style for the study duration. Exclusion criteria included history of cancer, use of any topical medication (such as minoxidil or any other hair growth solution), laser therapy or chemotherapy within the past 4 weeks, or being pregnant, breastfeeding or planning to become pregnant during the study. Additional exclusion criteria were hair loss not caused by AGA, scarring of the scalp including prior hair transplantation or scalp reduction, or any other condition/disease of the scalp/hair. Those using medications known to cause hair thinning or with existing scalp conditions were also excluded from the study. The protocol was approved by Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-F(I)-20190142, and this study was also registered at ClinicalTrials.gov (NCT05450861). Each patient provided written informed consent before study procedures.

Assessments

In order to understand whether the scalp conditioning solution containing Derinat could increase the therapeutic effects of oral drugs, the study examined changes in hair density and hair diameter after 4 weeks and 8 weeks of use of scalp conditioning solution. Hair density and hair diameter were detected by a scalp hair analysis device API 202 (ProgenProbe). We also compared the changes in the exposed areas of the scalp using photographs taken from 15 cm above the head. All assays were performed by the same researcher, and the researcher was blinded to the group to which the participants were assigned. Additionally, we also used the Dermatology Life Quality Index (DLQI) questionnaire to assess the impact of AGA on the patients' quality of life [18]. DLQI is a commonly used life quality index questionnaire in dermatology, and a higher score represents greater impairment of the patients' quality of life. When subjects were recruited into the study, the mean DLQI score for both groups was approximately 8.3.

DLQI is a self-explanatory survey that includes ten questions. The items of the questionnaire analyze the following six aspects: symptoms and feelings, daily life, leisure, work and school, personal relationships, and therapy. Questions are scored on a four-point scale (Not at all or Not relevant = 0, A little = 1, A lot = 2 and Very much = 3), and the total score is 0 to 30. Higher total score represents a greater impairment of the patients' quality of life. Among them, 0–1 = no effect at all, 2–5 = small effect, 6–10 = moderate effect, 11–20 = very large effect, 21–30 = extremely large effect. We slightly modified the description of the questions in the questionnaire to fit the hair loss situation (Supplementary Figure S2).

Statistical analyses

GraphPad Prism (La Jolla, CA, USA) was used to generate bar charts; error bars indicate standard deviations (SD). A one-way, two-tailed analysis of variance (ANOVA) was also utilized to compare the means of each group. A p-value of less than 0.05 for differences between groups was considered statistically significant. Hair density, hair diameter, and DLQI questionnaires were used to assess the effects of pre- and post-treatment with conditioning solutions on AGA subjects. The statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, IL, USA) for Windows in the clinical study section. Quantitative data were expressed as mean ± SD, while independent sample t-test was used to compare differences in values between the two groups (Derinat group vs. placebo group). Pre- and post-treatment values (4 weeks vs. baseline and 8 weeks vs. baseline) in the same group of patients were compared using paired t-tests. Categorical variables were analyzed using the chi-square test. Likewise, $p < 0.05$ was considered statistically significant.

Results

Derinat alters murine hair growth patterns potentially through restraining oxidative stress induced-aging process

C. elegans was firstly utilized to screen the feasible concentrations of Derinat for further application to murine and other clinical experiments. Because the HF is a complex mini-organ and its cycle progression can be regulated by oxidative stress [19, 20], *C. elegans* was used as a model to comprehensively understand the anti-oxidative stress properties of Derinat. We evaluated the lifespan of worms which is altered by oxidative metabolism, especially ROS accumulation [21]. Our results revealed that application of Derinat to *C. elegans* at concentrations from 1.5 μg/ml to 150 μg/ml significantly lengthened average worm lifespans compared to the control group (0 μg/ml) (Fig. 1A and B), thus suggesting that Derinat has some ability to inhibit the oxidative stress induced aging process. Treatment with Derinat at the concentration of 1.5 μg/ml in *C. elegans* resulted in the highest average survival days compared to other concentrations (Fig. 1B).

To further detect the ability of Derinat to impact hair growth, male BALB/c nude mice in the initial stage of the initial hair-existing phase were treated daily with three different concentrations of Derinat-containing hydrogels and a control hydrogel with no Derinat. The following concentrations, control 0 (vehicle), 3.5 μg/cm^2 (15 μg/ml) and 14 μg/cm^2 (60 μg/ml) were applied to a 3-cm diameter circular chip which in turn was applied to mice in the study for 3 h per day (Fig. 1C and D). As shown in Fig. 1E, Derinat-treated dorsal skin displayed dense, thicker and longer hair, but vehicle-treated control mouse skin revealed an extremely sparse hair coat. Comparison of hair cycles between vehicle and Derinat groups also resulted in different patterns. Derinat treatment maintained anagen hair morphogenesis compared with that of controls at the catagen phase. Similar results were observed for C57BL/6 mice treated with Derinat. C57BL/6 mice treated daily with a concentration of 60 μg/ml Derinat were able to maintain the hair cycle at the anagen stage compared with that of controls at the telogen phase (Fig. 2). These results indicated that Derinat possessed potential anti-oxidative stress efficacy that extended hair growth patterns and maintained the hair cycle at the anagen phase.

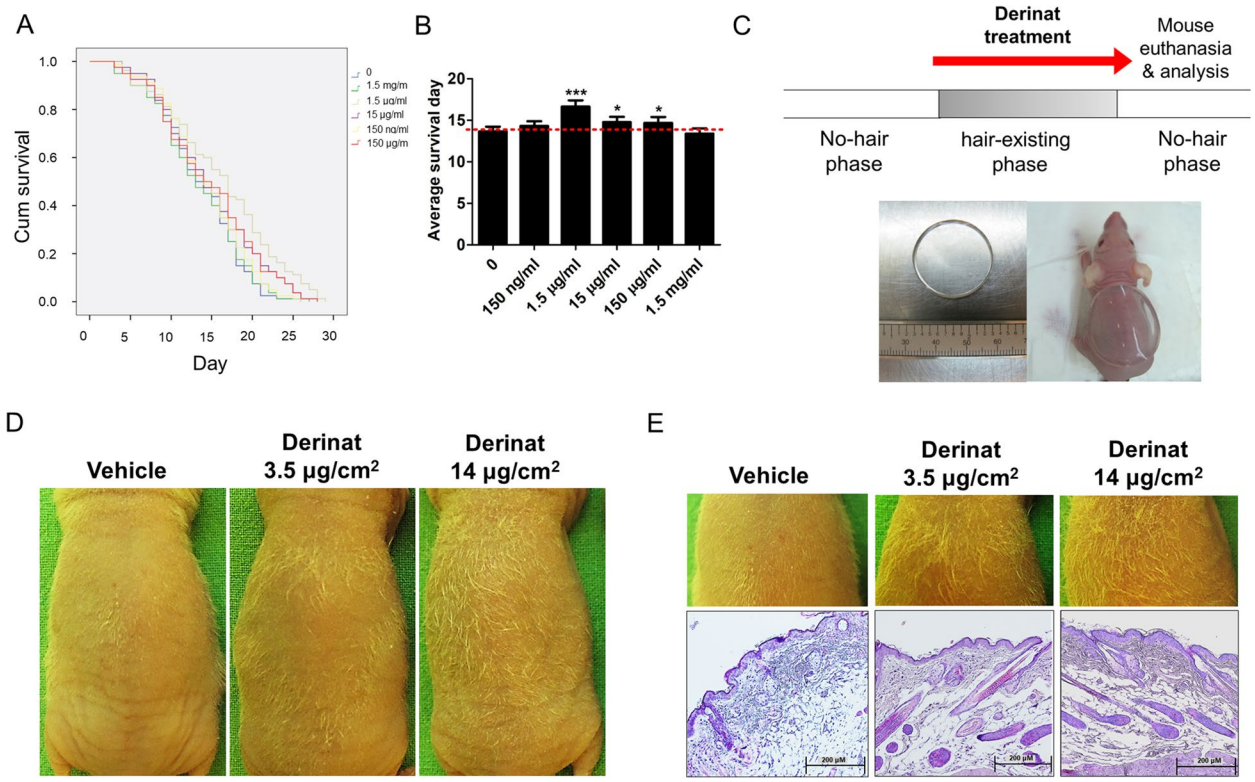

Fig. 1 Effect of Derinat on hair growth pattern in BALB/c-nu mice. **A** Derinat accelerates worms' survival. Kaplan–Meier curves with univariate analyses for survival with different concentrations of Derinat treatment. **B** The average survival day. ($n = 120$, mean ± SD; *, $p < 0.05$; ***, $p < 0.001$). **C** A schematic showing the experimental design with Derinat treatment. Down panel: the Derinat-containing hydrogel forms a 3-cm diameter circular chip and is put on the dorsal skins of mice. **D** Image of nude mice hair on dorsal skin surface, treated with vehicle control and Derinat. **E** Histological features of the skin specimen of vehicle control- and Derinat-treated mice

Derinat extension of the hair cycle at the anagen phase is related to a decreased level of TRPCs-mediated intracellular ROS accumulation

Because of our understanding that intrinsic ROS drives HF cycle progression through DDR and apoptosis and works with macrophage polarization to promote the hair follicle cycle [22], we were able to confirm the effects of Derinat on intracellular ROS accumulation in HFs. The level of intracellular ROS production was increased in the dorsal skin of vehicle-treated (control) mice compared with that of Derinat treatment (Fig. 3A). The intracellular ROS accumulation of the skin cells was significantly restrained by Derinat in a dose-dependent manner (Fig. 3B). The apoptotic hair bulb at catagen phase could be induced by ROS accumulation [23]. Derinat as a TRPCs inhibitor facilitates inhibition of intracellular ROS accumulation as produced by mitochondrial Ca^{2+}-overloading [12]. The effect of Derinat on TRPCs activity in skin cells, keratinocytes, dermal fibroblasts and dermal papilla cells was explored and Derinat was found to restrain ATP-induced TRPCs activity in these cells (Fig. 3C). The intracellular ROS accumulation, which was induced by mitochondrial Ca^{2+}-overloading, was also significantly inhibited with Derinat treatment in keratinocytes, dermal fibroblasts and dermal papilla cells (Fig. 3D and E). Therefore, blockage of TRPCs-mediated ROS accumulation by Derinat in skin cells potentiated extension of the anagen phase.

AGA pathogenesis is associated with TRPCs-regulated oxidative stress and inflammation

ROS accumulation in scalp tissue regulates the progression of inflammation results in AGA pathology. We subsequently detected the relationship between AGA pathogenesis and TRPCs-regulated oxidative stress and inflammation. Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, we also confirmed several inflammatory molecules related to ROS, such as prostaglandin-endoperoxide synthase 1 (PTGS1), prostaglandin-endoperoxide synthase 2 (PTGS22), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). These were all involved in TRPCs and ROS signal regulated inflammation. ROS signal molecules, superoxide dismutase (SOD) family, and

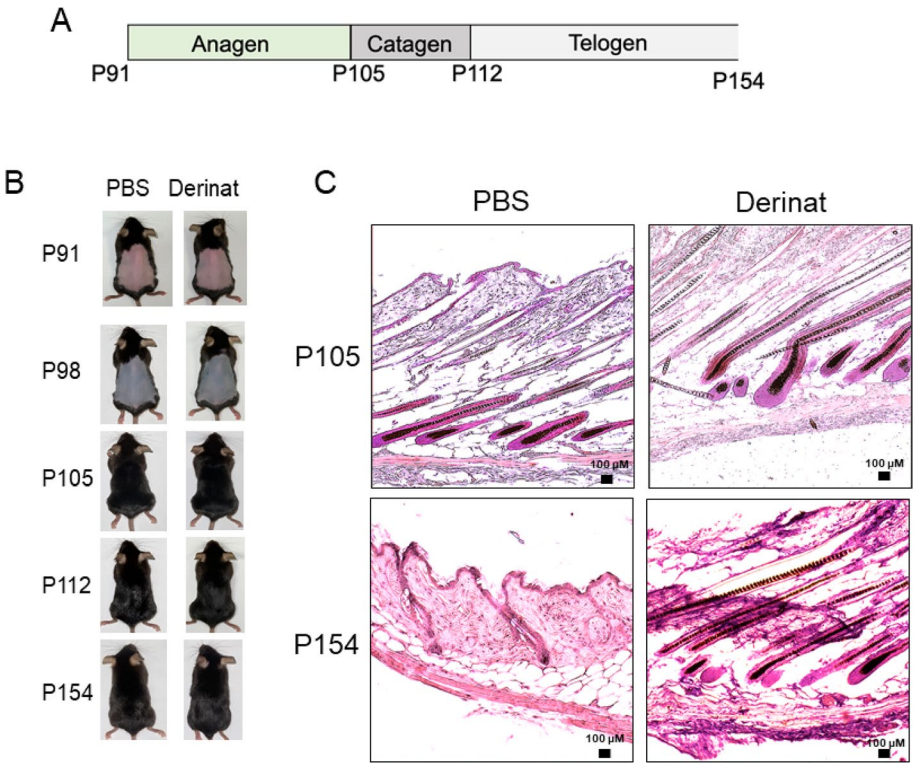

Fig. 2 Effect of Derinat on hair growth pattern in C57BL/6 mice. **A** Schematic showing the experimental design. **B** Mice were shaved at Day 0 and daily spread with Derinat; the dorsal skins of mice were observed at the indicated time points. **C** Skins were harvested and stained with H&E at Day105 and Day 154

forkhead box class O 3a (FOXO3a) are also associated with Ca^{2+} signals and inflammation.

When we compared the expression of *TRPCs*, oxidative stress-related genes, and inflammation-related genes in scalps of healthy donor and AGA patients to the GEO database, we found that *TRPCs*, *SOD1*, *SOD3*, *PTGS1*, *PTGS2*, *IL-1β*, *IL-6* and *TNF-α* were increased in AGA patients (Fig. 4A). We then compared analyses of *TRPCs*, oxidative stress-related genes, and inflammation-related genes from the bulge and bulb areas of hair follicles in non-balding areas and balding areas of AGA patients to the GEO databank (Fig. 4B). We found that for the majority of *TRPCs*, oxidative stress-related genes and inflammation-related genes had decreased in the bulge and bulb of balding areas (Fig. 4B). We also noted *PTGS1* and *PTGS2* had increased levels in the bulb regions of hair follicles of balding areas. When we further explored the expression of *PTGS1* and *PTGS2* in the DPCs, we observed that not only *PTGS1*, *PTGS2* but also *IL-1β* and *IL-6* were higher in balding areas than that in non-balding areas (Fig. 4C). Similar results were found for values of *TRPC1*, *TRPC4*, *SOD1*, and *SOD2*, but there was a surprisingly decreased level of *FOXO3a* in DPCs, which implicates ROS accumulation as the mediator for activation of DDR and SIR during the aging process (Fig. 4C).

Interestingly, higher levels of *PTGS1* and *PTGS2* were expressed in scalp, bulb and DPCs of AGA patients according to GEO database analyses. Prostaglandin D2 (PGD2) treatments, produced by the enzymatic action of PTGS1 and PTGS2, restrained mouse and human hair growth [24]. Increased PGD2 pathway activity has been observed in balding scalp areas of patients with AGA [25]. Our findings also indicated that treatment with Derinat restrained the expression of PTGS2 in the epidermis of nude mice (Supplementary figure S1), which indicates that Derinat may provide benefits for treatment of balding in AGA. KEGG pathways and GEO database analyses indicate that AGA pathogenesis is caused by oxidative stress and inflammation, while Ca^{2+} signal dependent PGD2 pathway activity (PTGS1 and PTGS2), inflammatory cytokines and ROS signal molecules are involved in regulating AGA pathogenesis (Fig. 4D). Consequently, inhibiting TRPCs-induced oxidative stress and inflammation may help to prevent AGA pathogenesis.

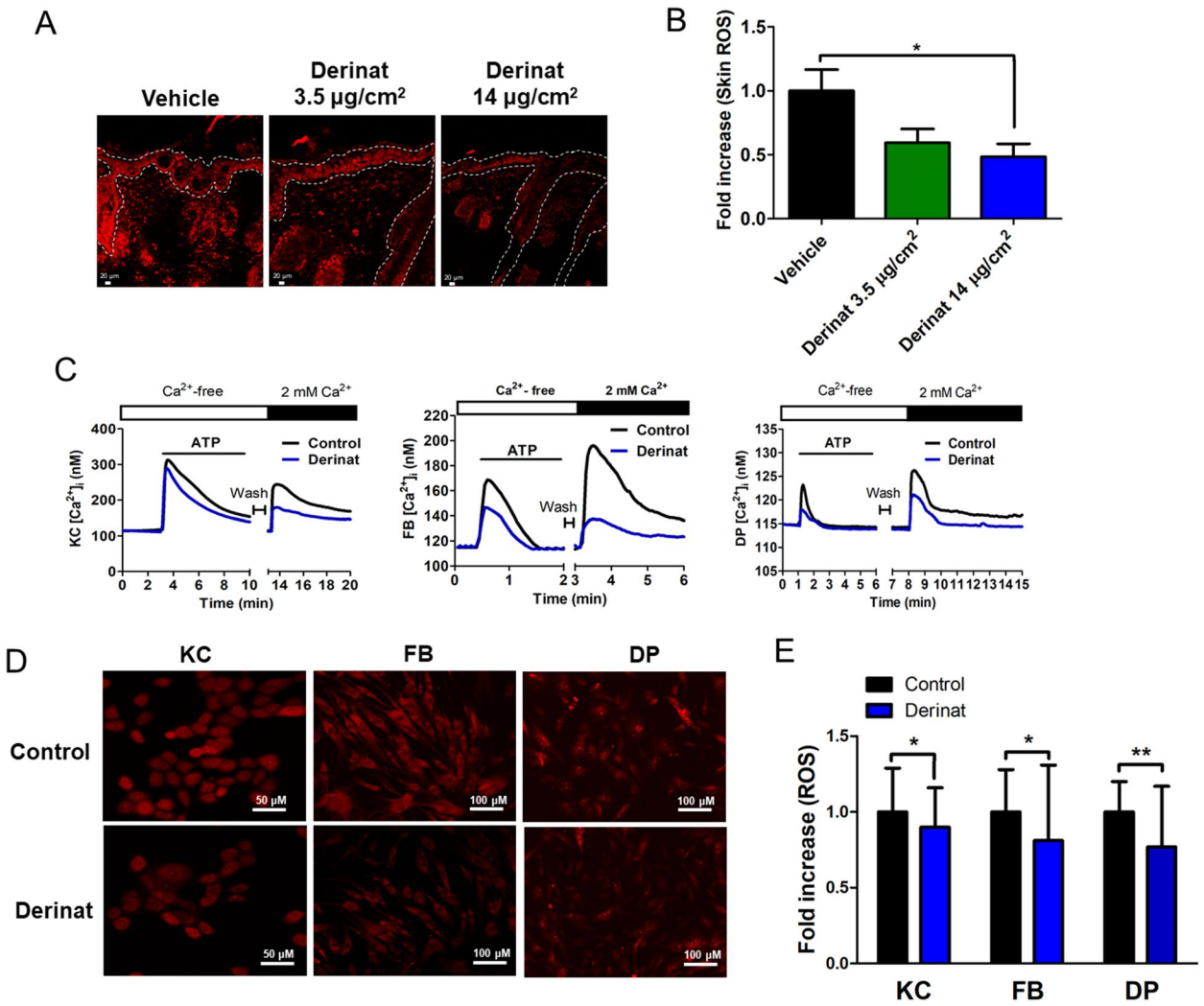

Fig. 3 Treatment with Derinat affects HF cycle progression due to a decrease in intracellular ROS production. **A** Effect of Derinat on skin ROS production. The intracellular ROS production in dorsal skin and HFs was analyzed by DHE staining. **B** Quantification of intracellular ROS production from (**A**) (mean ± SD; *, $p < 0.05$). **C** Effect of Derinat on TRPCs activity in skin cells. After the application of ATP (small black bars) to activate TRPCs via the PLC pathway in Ca^{2+}-free balanced salt solution, extracellular $CaCl_2$ was added (large black bar) in keratinocytes (KC), fibroblasts (FB), and dermal papilla cells (DP). The $Ca.^{2+}$ signals represent the mean value of 30 cells. **D** Effect of Derinat on intracellular ROS production in skin cells. The intracellular ROS production level with an average fluorescence intensity of more than 100 skin cells from (**D**) is quantified in (**E**) (mean ± SD; *, $p < 0.05$; **, $p < 0.01$)

Derinat improves the value of hair density and hair diameter in AGA subjects

We next explored the effect of Derinat treatment on AGA symptoms in AGA subjects. Firstly, the basal characteristics of the AGA subjects were collected and summarized in Table 1. There was no statistically significant difference in basal characteristics between the two groups. We further analyzed the treatment efficacy of Derinat in AGA subjects. Hair density and hair diameter were detected at three indicated times: baseline (before trial), 4 weeks (treatment for 4 weeks) and 8 weeks (treatment for 8 weeks), while AGA subjects also combined treatment with Dutasteride (for males) or Spironolactone (for females). At the 4th week of clinical evaluation, Derinat treatment in AGA subjects had significantly enhanced the value of hair density, which had dramatically increased from 86.4 ± 34.0 hairs/cm^2 to 99.7 ± 31.8 hairs/cm^2, with a following increase to 112.2 ± 38.8 hairs/cm^2 in the final evaluation trial (Table 2 and Fig. 5). Similar results were also shown in hair diameter: Despite the smaller increased value of hair density and hair diameter in the placebo group, there was no statistically significant difference between each time point of evaluation.

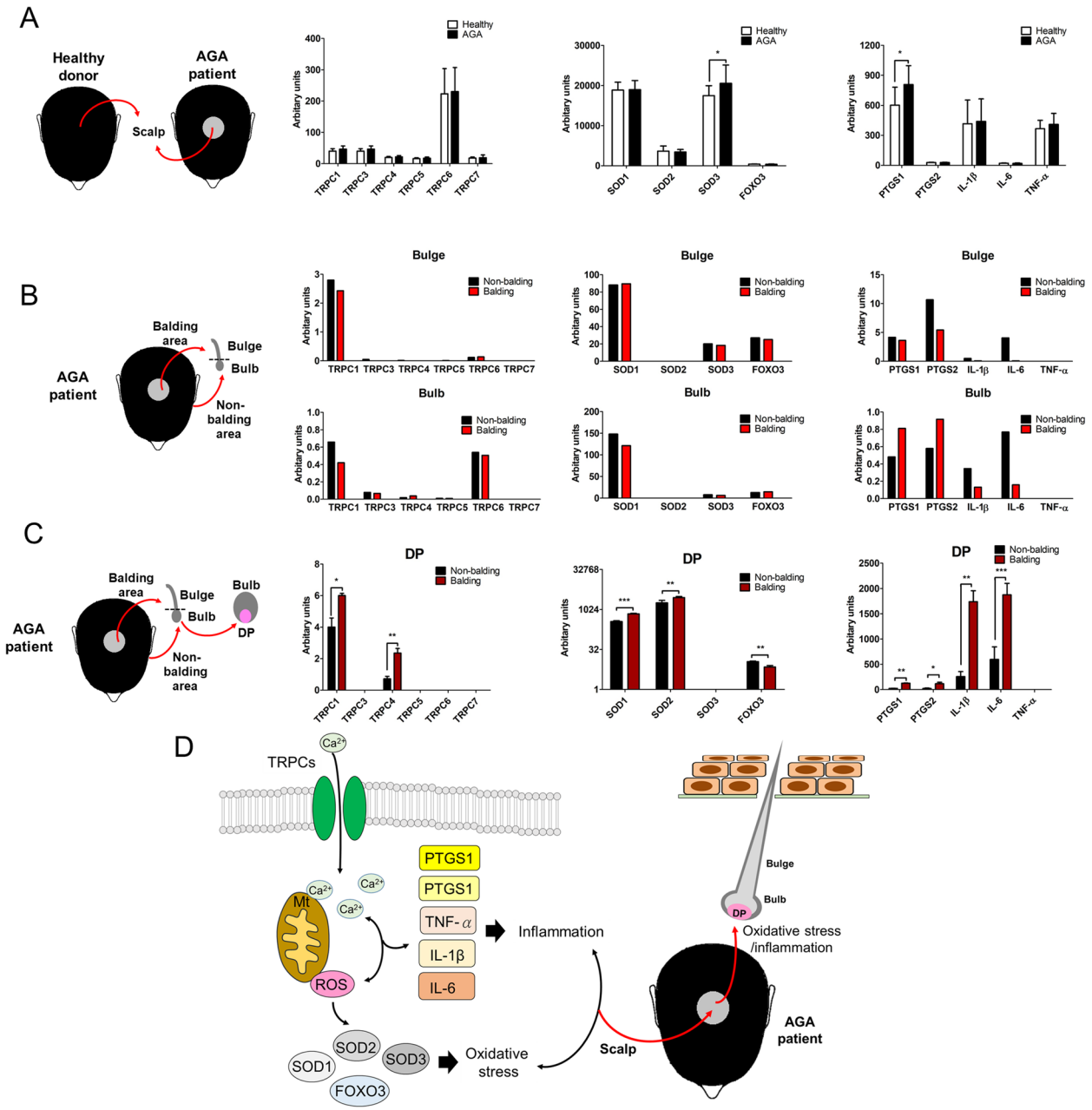

Fig. 4 AGA features TRPCs-activated Ca^{2+} signals, ROS accumulation and inflammation. **A** Gene Expression Omnibus (GEO) database analysis of *TRPCs*, oxidative stress-related genes, and inflammation-related genes in the scalp of healthy donor and AGA patients. Comparison of *TRPCs*, oxidative stress-related genes, and inflammation-related genes in (**B**) the bulge, bulb and (**C**) DP (papilla cells) between non-balding area and balding area of AGA patients (mean ± SD; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). **D** TRPCs-activated Ca^{2+} signals govern the inflammation and oxidative stress KEGG pathways in AGA

Figure 5A and B display the analysis for scalp hair density and hair diameter in AGA subjects. When comparing hair density and hair diameter between placebo and Derinat groups, treatment with the Derinat program improved AGA pathology with an increased value for hair density and hair diameter, especially after treatment for 8 weeks. The value of hair density in the Derinat group was more than that in the placebo group, although there was no statistically significant difference. A statistically significant difference ($p = 0.012$) in hair diameter was revealed, showing 0.061 ± 0.009 mm in the placebo group and 0.071 ± 0.012 mm in the Derinat

Table 1 Basal characteristics of study population

	Placebo ($n=16$)	Derinat ($n=34$)	p value
Age, year	36.6 ± 9.9	34.0 ± 10.5	0.420
Range	26–64	22–66	
Gender			0.266
Female (%)	14 (87.5)	25 (73.5)	
Age at which hair loss began	32.8 ± 11.0	27.7 ± 9.8	0.115
Range	15–64	15–50	
Family history			0.061
Yes	8 (50.0)	26 (76.5)	
No	8 (50.0)	8 (23.5)	

Table 2 The mean hair density, hair diameter, and the dermatology life quality index (DLQI) scores in two groups

	Placebo ($n=16$)	Derinat ($n=34$)	p value[a]
Hair density (Hairs/cm^2)			
Baseline	84.1 ± 29.4	86.4 ± 34.0	0.828
4 weeks	88.3 ± 24.9	99.7 ± 31.8	0.226
8 weeks	90.4 ± 28.1	112.2 ± 38.8	0.058
p value[b]	0.521	**0.010***	
p value[c]	0.170	**< 0.001***	
Hair diameter (mm)			
Baseline	0.060 ± 0.015	0.063 ± 0.016	0.581
4 weeks	0.065 ± 0.016	0.068 ± 0.015	0.515
8 weeks	0.061 ± 0.009	0.071 ± 0.012	**0.012***
p value	0.227	**0.002***	
p value	0.642	**0.006***	
DLQI			
Baseline	8.3 ± 5.1	8.3 ± 5.2	0.981
4 weeks	7.7 ± 4.5	7.0 ± 5.8	0.682
8 weeks	7.9 ± 6.6	5.9 ± 5.3	0.273
p value	0.454	0.071	
p value	0.748	**0.013***	

[a] Derinat vs. Placebo, t test.; [b]4 weeks vs. Baseline, paired t-test. [c]8 weeks vs. Baseline, paired t-test.; *$p < 0.05$

group. Consequently, Derinat augmented the efficacy of oral medication to improve hair density and hair diameter in AGA subjects.

Derinat administration enhances hair recovery and decreases DLQI questionnaire scores

The value of hair density and hair diameter was promoted by Derinat, indicated by a demonstrable change in hair recovery observed and measured in AGA subjects. As shown in Fig. 5C and D, the placebo group had slight reduction in the balding area compared to the baseline at the 8th week of clinical evaluation, but the Derinat group had a significantly decreased balding area.

Furthermore, we used the dermatology life quality index (DLQI) questionnaire to assess the impact of AGA on the patients' quality of life (Table 2). Our results indicated that hair loss had moderate effects on the subjects' quality of life. DLQI scores in the placebo group decreased slightly after drug treatment but were not significantly different from baseline. The score reduction was 7.9 ± 6.6 after 8 weeks of treatment, but it was still in the score range of moderate effects. On the other hand, the DLQI score in the Derinat group decreased to 7.0 ± 5.8 after 4 weeks of treatment. After the completed 8 weeks of treatment, DLQI scores were significantly lower than Baseline (5.9 ± 5.3 vs. 8.3 ± 5.2, $p=0.013$). The above results indicated that the impairment of hair loss on quality of life improved from moderate effects to small effects. Additionally, Derinat had lower DLQI scores than the placebo group after completing the clinical study procedure, but there was no statistically significant difference. In summary, Derinat improved hair density, hair diameter and hair recovery in AGA subjects while mitigating the impairment of AGA subjects' quality of life.

Discussion

Our study demonstrated the efficacy of Derinat through extension of the hair cycle at the anagen phase, and this is related to a decreased level of TRPCs-mediated intracellular ROS accumulation. Intrinsic ROS that drives HF cycle progression through DDR and apoptosis during the hair cycle transition from anagen to catagen [22]; it could be that Derinat which restrains intracellular ROS accumulation in DPCs maintains HF cycle at the anagen phase. Furthermore, the local microenvironment of skin can also affect hair follicle biology to determine HFSC niche [26]. Our results revealed that Derinat inhibited intracellular ROS accumulation and inflammation in keratinocytes and fibroblasts, potentially following influence of DPC-regulated HFSC niche. These could maintain hair follicles at the anagen phase. However, microenvironmental oxidative stress, especially TRPCs-regulated ROS accumulation and ROS regulated-inflammatory progression have been implicated in the pathogenesis of AGA. Unlike murine, anagen phase in the human scalp lasts for several years [27], once microenvironmental or intracellular oxidative stress occurrence in HFs may affect HF cycle progression through change HFSC niche. Derinat as a TRPCs inhibitor, which possesses efficacy for alleviating anti-oxidative stress, was applied in our clinical study and was able to improve the value of hair density, hair diameter and hair recovery in AGA subjects while attenuating the impairment of AGA subjects' quality of life. The efficacy of JAK inhibitors is

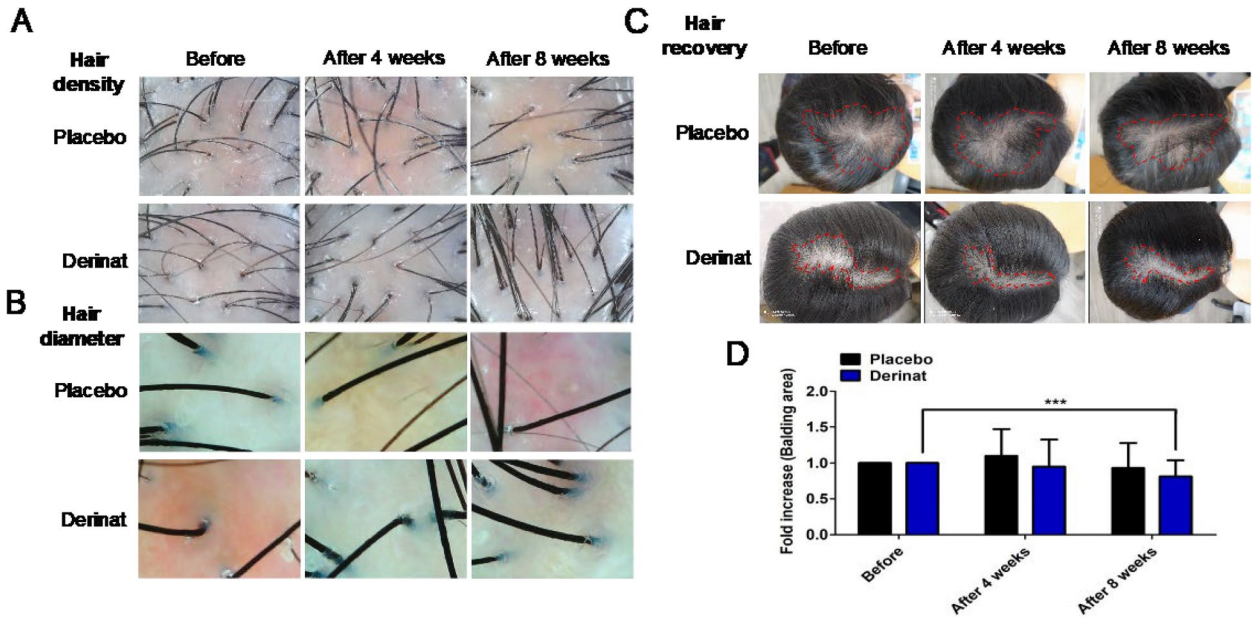

Fig. 5 Treatment with Derinat for 8 weeks improves AGA. **A** Hair density (Magnification: 60×) and (**B**) hair diameter (Magnification: 200×) in AGA patients with placebo or Derinat treatment were evaluated by hair scalp analysis device API 202. **C** Evaluation of hair recovery in AGA patients with placebo or Derinat treatment at the indicated times. **D** Quantification of hair recovery from the areas of (**C**) with red dotted line labeling (mean ± SD; ***, $p < 0.001$)

similar to our study with extension of the hair cycle at the anagen phase in murine also improves AGA pathogenesis [16, 28].

Nevertheless, the placebo group also saw an increase in the value of subjects' hair diameter during the 4th week, but then had a decreased level during the 8th week. This could be due to the inability of oral medication to maintain sustained hair growth for a short period of time (Table 2). Spironolactone not only inhibits the interaction of testosterone and DHT with androgen receptors but weakly inhibits androgen synthesis as well. Previous studies have reported that spironolactone treatment stabilizes or improves hair loss during a follow-up period of 7 to 20 months [29]. Thus, oral medication improves AGA symptoms when taken for long-term treatment, so a combination of Derinat treatment in AGA patients may improve the effects of oral medication and was demonstrated to significantly inhibit AGA pathogenesis during administration for 4 to 8 weeks. However, our study did not conduct planned follow-up of changes after subjects stopped using Darinat. This is a limitation of our research program. AGA, a chronic degenerative disease, becomes more severe with aging, while appropriate therapeutic intervention could slow the progression of hair loss. According to this case, once treatment intervention is stopped, AGA potentially return to the normal disease process. In addition, we did not restrict subjects' treatment options after the trial ended. Some subjects increased their use of other treatments or continued using Derinat. This may also interfere with subsequent evaluation of treatment effects. The well-planned follow-up of AGA symptoms changes after discontinuing Darinat will be further investigated in future work.

Additionally, the majority of our subjects were female (Table 1), meaning that females may pay more attention to AGA-induced hair loss problems than male subjects. We also analyzed the data from these female participants and the basic demographic characteristics are shown in supplementary Table S1. Although comparison of hair density, hair diameter and DLQI between placebo and Derinat groups possessed no statistically significant difference, the value of each item with Derinat treatment had considerable improvement (Supplementary Table S2). Notably, compared to baseline, the value of hair density and hair diameter was significantly increased in subjects treated with Derinat. Additionally, the DLQI questionnaire score was also reduced at the 4th week and 8th week of clinical evaluation (Supplementary Table S2). To date, there is no effective treatment to deal with AGA in women, and the mechanism of AGA in females might be different from males. Our study pointed out that the pathogenesis of AGA in women was correlated with TRPCs-mediated intracellular ROS accumulation. Treatment via the Derinat program improved AGA pathogenesis in female subjects.

Some risk factors for AGA are increasing age and family history [30, 31]. Although the majority of our AGA subjects had relevant family history, increasing age seemed to promote initiation of AGA pathogenesis. Since AGA is a potentially age-associated disease, anti-aging strategies such as quenched ROS accumulation or inflammation disruption might provide novel therapeutic insight for AGA. A paradigm shift in hair loss treatment is necessary, including a switch from single focus to multiple focus therapeutic approaches. Our study supports this new insight for AGA treatment.

Conclusions

This study demonstrated Derinat has some hair restoration influence on AGA patients. Derinat also demonstrated its worth as a potentially effective anti-oxidative stress therapeutic in two studies: facilitating an increase in worms' lifespan and maintenance of the murine hair cycle at the anagen phase with decreased levels of TRPCs-mediated ROS accumulation. Intracellular ROS accumulation has also been implied in the pathogenesis of AGA.

Treatment with Derinat in AGA subjects improved the value of hair density, hair diameter and hair recovery in AGA subjects, while mitigating the impairment of their quality of life. Although oral medication improved AGA symptoms when taken for long-term treatment, Derinat treatment, in combination with oral treatment, promoted the efficacy of oral medication and significantly inhibited AGA pathogenesis within 8 weeks. Our study supports that a multi-focus therapeutic approach for AGA treatment is more efficient than oral medication alone.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41231-023-00159-3.

Additional file 1: Figure S1. Treatment with Derinat decreases PTGS2 expression in the epidermis of nude mice. Down panel: quantification of PTGS2 expression (mean ± SD; *, $p < 0.05$; **, $p < 0.01$). **Table S1.** Basal characteristics of female study population. **Table S2.** The mean hair density, hair diameter, and DLQI scores between two groups in females. **Figure S2.** Dermatology Life Quality Index.

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Authors' contributions
Conceptualization: C.Y.W., W.C.C., W.L.H. Data curation: W.C.C., W.L.H. Funding acquisition: C.Y.W., W.L.H. Investigation: C.H.H., Y.F.L., W.J.L., H.S., W.Y.W., T.Y.C., Y.C.C., H.H. Methodology: C.Y.W., W.C.C., W.L.H. Writing – original draft: C.Y.W., W.C.C. Writing – review & editing: W.C.C., W.L.H.

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Availability of data and materials
All of the relevant data and materials are freely available to any investigator upon request.

Declarations

Ethics approval and consent to participate
The protocol was approved by Institutional Review Board of Kaohsiung Medical University Hospital (KMUHIRB-F(I)-20190142, and this study was also registered at ClinicalTrials.gov (NCT05450861).

Consent for publication
All authors have agreed to publish this manuscript.

Competing interest
The authors would like to declare the following patent associated with this research: "COMPOSITION AND METHOD FOR TREATING HAIR LOSS AND INDUCING HAIR GROWTH" received from Taiwan (I715838) and Japan (6996775). This does not alter our adherence to the *BioMed Research International* policies on sharing data and materials.

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References

1. Ho CH, Sood T, Zito PM. Androgenetic Alopecia. In: StatPearls. Treasure Island (FL); 2022.
2. Gupta S, Goyal I, Mahendra A. Quality of life assessment in patients with androgenetic alopecia. Int J Trichology. 2019;11:147–52.
3. Ashique S, Sandhu NK, Haque SN, Koley K. A systemic review on topical marketed formulations, natural products, and oral supplements to prevent androgenic alopecia: a review. Nat Prod Bioprospect. 2020;10:345–65.
4. Giltay EJ, Gooren LJ. Potential side effects of androgen deprivation treatment in sex offenders. J Am Acad Psychiatry Law. 2009;37:53–8.
5. Sadick NS, Callender VD, Kircik LH, Kogan S. New insight into the pathophysiology of hair loss trigger a paradigm shift in the treatment approach. J Drugs Dermatol. 2017;16:s135–40.
6. Ceruti JM, Leiros GJ, Balana ME. Androgens and androgen receptor action in skin and hair follicles. Mol Cell Endocrinol. 2018;465:122–33.
7. Jung YH, Chae CW, Choi GE, Shin HC, Lim JR, Chang HS, Park J, Cho JH, Park MR, Lee HJ, Han HJ. Cyanidin 3-O-arabinoside suppresses

DHT-induced dermal papilla cell senescence by modulating p38-dependent ER-mitochondria contacts. J Biomed Sci. 2022;29:17.
8. Abdin R, Zhang Y, Jimenez JJ. Treatment of androgenetic alopecia using PRP to target dysregulated mechanisms and pathways. Front Med (Lausanne). 2022;9: 843127.
9. Upton JH, Hannen RF, Bahta AW, Farjo N, Farjo B, Philpott MP. Oxidative stress-associated senescence in dermal papilla cells of men with androgenetic alopecia. J Invest Dermatol. 2015;135:1244–52.
10. Rahmani W, Abbasi S, Hagner A, Raharjo E, Kumar R, Hotta A, Magness S, Metzger D, Biernaskie J. Hair follicle dermal stem cells regenerate the dermal sheath, repopulate the dermal papilla, and modulate hair type. Dev Cell. 2014;31:543–58.
11. Hsu WL, Tsai MH, Wu CY, Liang JL, Lu JH, Kahle JS, Yu HS, Yen CJ, Yen CT, Hsieh YC, et al. Nociceptive transient receptor potential canonical 7 (TRPC7) mediates aging-associated tumorigenesis induced by ultraviolet B. Aging Cell. 2020;19: e13075.
12. Hsu WL, Lu JH, Noda M, Wu CY, Liu JD, Sakakibara M, Tsai MH, Yu HS, Lin MW, Huang YB, et al. Derinat protects skin against Ultraviolet-B (UVB)-induced cellular damage. Molecules. 2015;20:20297–311.
13. Sviatkina OI, Balashov VP, Balykova LA, Shchukin SA. Anti-arrhythmia activity of derinat in an experiment. Eksp Klin Farmakol. 2004;67:22–4.
14. Chen TC, Chao HR, Wu CY, Lai YR, Chen CH, Yoshioka T, Hsu WL, Tsai MH. Effect of 9,12-Octadecadiynoic acid on neurobehavioral development in Caenorhabditis elegans. Int J Mol Sci. 2021;22:8917.
15. Zhang D, Gu L, Li J, Li Z, Wang C, Wang Z, Liu L, Lee M, Sung C. Exogenous stimulations change nude mouse hair cycle pattern. J Dermatolog Treat. 2012;23:90–6.
16. Wang ECE, Dai Z, Ferrante AW, Drake CG, Christiano AM. A subset of TREM2(+) dermal macrophages secretes oncostatin M to maintain hair follicle stem cell quiescence and inhibit hair growth. Cell Stem Cell. 2019;24(654–669): e656.
17. Clough E, Barrett T. The Gene Expression Omnibus Database. Methods Mol Biol. 2016;1418:93–110.
18. Finlay AY, Khan GK. Dermatology Life Quality Index (DLQI)–a simple practical measure for routine clinical use. Clin Exp Dermatol. 1994;19:210–6.
19. Schneider MR, Schmidt-Ullrich R, Paus R. The hair follicle as a dynamic miniorgan. Curr Biol. 2009;19:R132-142.
20. Trueb RM. Oxidative stress in ageing of hair. Int J Trichology. 2009;1:6–14.
21. Giorgi C, Marchi S, Simoes ICM, Ren Z, Morciano G, Perrone M, Patalas-Krawczyk P, Borchard S, Jedrak P, Pierzynowska K, et al. Mitochondria and reactive oxygen species in aging and age-related diseases. Int Rev Cell Mol Biol. 2018;340:209–344.
22. Liu M, Liu X, Wang Y, Sui Y, Liu F, Liu Z, Zou F, Zuo K, Wang Z, Sun W, et al. Intrinsic ROS drive hair follicle cycle progression by Modulating DNA damage and repair and subsequently hair follicle apoptosis and macrophage polarization. Oxid Med Cell Longev. 2022;2022:8279269.
23. Zhao J, Li H, Zhou R, Ma G, Dekker JD, Tucker HO, Yao Z, Guo X. Foxp1 regulates the proliferation of hair follicle stem cells in response to oxidative stress during hair cycling. PLoS One. 2015;10: e0131674.
24. Garza LA, Liu Y, Yang Z, Alagesan B, Lawson JA, Norberg SM, Loy DE, Zhao T, Blatt HB, Stanton DC, et al. Prostaglandin D2 inhibits hair growth and is elevated in bald scalp of men with androgenetic alopecia. Sci Transl Med. 2012; 4:126ra134.
25. Nieves A, Garza LA. Does prostaglandin D2 hold the cure to male pattern baldness? Exp Dermatol. 2014;23:224–7.
26. Chen CL, Huang WY, Wang EHC, Tai KY, Lin SJ. Functional complexity of hair follicle stem cell niche and therapeutic targeting of niche dysfunction for hair regeneration. J Biomed Sci. 2020;27:43.
27. Halloy J, Bernard BA, Loussouarn G, Goldbeter A. Modeling the dynamics of human hair cycles by a follicular automaton. Proc Natl Acad Sci U S A. 2000;97:8328–33.
28. Yale K, Pourang A, Plikus MV, Mesinkovska NA. At the crossroads of 2 alopecias: androgenetic alopecia pattern of hair regrowth in patients with alopecia areata treated with oral Janus kinase inhibitors. JAAD Case Rep. 2020;6:444–6.
29. Famenini S, Slaught C, Duan L, Goh C. Demographics of women with female pattern hair loss and the effectiveness of spironolactone therapy. J Am Acad Dermatol. 2015;73:705–6.
30. Chumlea WC, Rhodes T, Girman CJ, Johnson-Levonas A, Lilly FR, Wu R, Guo SS. Family history and risk of hair loss. Dermatology. 2004;209:33–9.
31. Olsen EA. Female pattern hair loss. J Am Acad Dermatol. 2001;45:S70-80.

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