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Discovery of a novel spot care cosmetic ingredient and a study of its functions that regulate the expression of SDF-1 in senescent fibroblasts and the dendritic changes of melanocytes caused by nerve fibers

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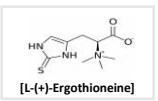
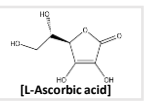
Introduction:

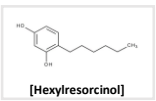
Dark spots or aged spots are hyperpigmented macules of the skin that usually form after long-term exposure to ultraviolet radiation [1], but they can be caused by other things, too. Skin conditions, pregnancy and certain medications or medical conditions may cause dark spots. Skin pigmentation is primary related to melanocytes functionality, and the surrounding cells and extracellular matrix proteins contribute to cutaneous homeostasis [1]. There is increasing evidence of a crucial role of senescent fibroblasts and the senescence-associated secretory phenotype in melanogenesis. A few studies have examined that a large number of senescent fibroblasts accumulated in senile lentigo area and that the loss of SDF1 in aged fibroblasts may lead to skin pigmentation through crosstalk with melanocytes [2]. Also, it has been found that nerve branches extend more toward superficial layers in senile lentigo, and the increased density of the nerve fibers in the SL cutis may contribute to the persistence and regeneration of dark spot [3].

Materials & Methods:

Human primary fibroblast cells (HDFn, C-12300, PromoCell), Murine melanoma cell line (B16-F10, CRL-6475, ATCC) and murine neuroblastoma cell line (Neuro-2A, CCL-131, ATCC) were used. L-(+)-Ergothioneine (EGT, 497-30-3, Sigma) and L-Ascorbic acid (AA, 50-81-7, Sigma) were dissolved in PBS, and hexylresorcinol (HR, 136-77-6, Sigma) was dissolved in DMSO.


[L-(+)-Ergothioneine]


[L-Ascorbic acid]


[Hexylresorcinol]

***In vitro* model of senescent fibroblasts:** HDFn cells were irradiated with UVA light(Bio-Sun system, Vilber Lourmat, Inc.) at a total dose of 50 mJ/cm^2.
Measurement of melanin contents and dendrite legth of B16F10 cells: Conditioned medium from Neuro-2a cells treated with or without skin care ingredients were cultured with B16F10 cells for 3 days. After centrifugation, the pellets were lysed with 100μL of 1N NaOH containing 10% DMSO solution and heated at 60 ℃ for 10 min. Absorbance was then measured at 490nm using an EPOCH2 microplate reader. B16f10 cells were observed using a microscope(DMi1, Leica microsystems), and the dendrite length was measured by a ruler.
Measurement of mRNA expression: RNA extraction and qRT-PCR was performed from the cells and the samples were purified following the manufacturer's instructions. Using a SYBR Green Realtime PCR Master Mix(4367659, Applied Biosystems™) and QuantStudio™ 3 real-time PCR machine.
Gene konckdown(SDF1 siRNA Transfection): Human *SDF1* siRNA(Bionics Co.,) was incubated with lipofectamine RNAi max in serum free DMEM for 5 min following the manufacturer's protocol. HDFn cells were supplemented with the siRNA by directly adding to the cell culture medium followed incubation for 48h.

Results & Discussion:

1. Effects of the skincare ingredients to promote SDF1 in senescent fibroblasts and improve skin pigmentation.
SDF1 can decrease melanogenesis by down-regulating the MITF/MC1R/TYR signaling in melanocytes. Moreover, hexylresorcinol treatment restored the SDF1 expression to levels comparable to control. These findings indicate that hexylresorcinol can restore SDF1 in senescent fibroblasts and correct the uneven pigmentation.

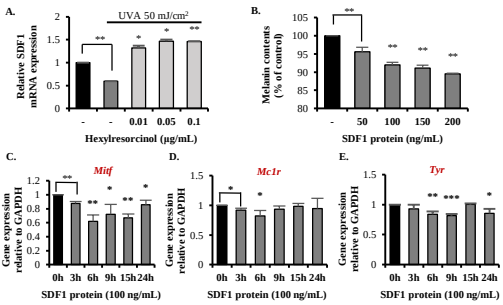


Fig1. Effects of the skincare ingredients to promote SDF1 in senescent fibroblasts and improve skin pigmentation.

Results & Discussion:

2. Effects of the skincare ingredients to inhibit neurosecretion and suppresses melanogenesis.
The amount of some melanocyte activator secreted from N2A cells was suppressed by EGT, AA and HR, and a number of melanin and melanogenesis relative mRNAs produced by melanocytes was reduced. Based on these results, it is expected that EGT, AA and HR can effectively suppress the cause of dark spots from the depths of the skin by reducing the effect of nerves on melanocytes.

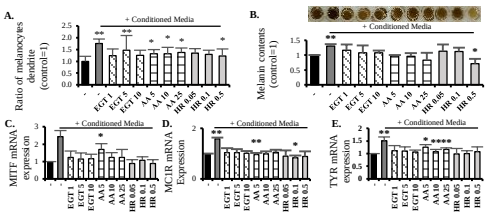


Fig2. Effects of the skincare ingredients to inhibit neurosecretion and suppresses melanogenesis.

3. The skincare ingredients act directly on B16F10 melanocytes to inhibit the effects from N2A cells.
The EGT, AA, HR effects on melanocytes are to prevent dendrite elongation and excessive formation of melanin by N2A cells and also decrease the relative melanogenesis gene expression like MITF, MC1R, and TYR.

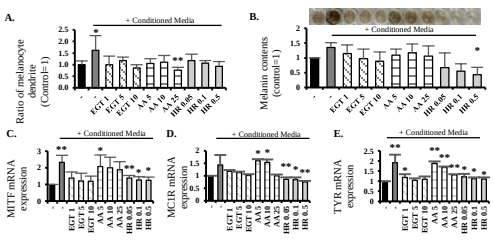


Fig3. The skincare ingredients act directly on B16F10 melanocytes to inhibit the effects from N2A cells.

4. The connection of the ageing marker GDF15 and SDF1 on age-related pigmentation.
In ageing-related pigmentation, senescent fibroblasts exhibit SDF1 deficiency as a result of promoting GDF15, which then finally stimulates melanogenesis-associated gene expression and synthesis melanin in melanocytes. And hexylresorcinol can inhibit GDF15 in senescent fibroblast. They provide further support for the therapeutic potential of hexylresorcinol in restoring SDF1 and decreasing GDF15 to correct skin-related dark spots.

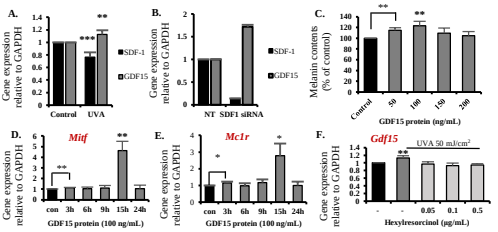


Fig4. The connection between GDF15 and SDF1 on age-related pigmentation.

Conclusions:

This study examines that hexylresorcinol can inhibit the mRNA expression of the aging marker GDF15 and restore the mRNA expression of SDF1 to reduce the effects of senescent fibroblasts on melanocytes. Furthermore, L-(+)-Ergothioneine, L-Ascorbic acid, and hexylresorcinol can prevent the dendrite extension effect by N2A cells and inhibit the expression of melanogenesis related genes of MITF, MC1R, TYR to reduce melanin formation in melanocytes, ultimately suppressing aged related dark spots. Through these effects, as a result, our dark spot care ingredients can reduce the melanin content in dark spot areas and inhibit dark spot regeneration by nerve cells. Thus, this study showed that EGT, AA, and HR have value as stronger dark spot care ingredients.

References:

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