Communication to the Editor

Dietary *Eriodictyon angustifolium* Tea Supports Prevention of Hair Graying by Reducing DNA Damage in CD34+ Hair Follicular Keratinocyte Stem Cells

Nobuhiko Taguchi, a,b Takumi Homma, b Hitomi Aoki, a and Takahiro Kunisada a,d

a Department of Tissue and Organ Development, Regeneration and Advanced Medical Science, Gifu University Graduate School of Medicine; Gifu 501–1194, Japan; and b General Research & Development Institute, Hoyu Co., Ltd.; Aichi 480–1136, Japan. Received May 28, 2020; accepted July 15, 2020

Hair follicular keratinocyte stem cells (HFKSC) which provide a functional niche for melanocyte stem cells (MSC) are the primary target of hair graying. However, little research has been done on anti-hair graying medicines targeting HFKSC. We focused on *Eriodictyon angustifolium* (Ea), which reduces human hair graying when applied topically. To investigate the protective effect of dietary Ea tea (EaT) on hair pigmentation, we used an acute mouse model of hair graying that mimics X-ray-induced DNA damage associated with age-related hair graying. Our results suggest that dietary EaT maintained the niche HFKSC function against X-ray-induced DNA damage and hair graying. These results indicate that dietary EaT may prevent age-related hair graying and serve as an anti-hair graying herbal medicine.

Key words hair graying; *Eriodictyon angustifolium*; hair follicular keratinocyte stem cell; sterubin; flavonoid; herbal medicine

INTRODUCTION

Depletion of follicular melanocyte stem cells (MSC) causes persistent loss of functional melanocytes from the hair matrix, 1–3) without hair graying recovery. Age-related hair graying correlates with a decrease in the number of MSC per follicle. 4,5) It has been demonstrated that the cause of mouse hair graying due to X-ray radiation was not a direct effect on MSC, but a loss of their niche function from hair follicular keratinocyte stem cells (HFKSC). 4,5) Therefore, HFKSC comprising the niche for MSC 4,5) may constitute an additional cause of hair graying. The number of phosphorylated H2AX (γH2AX)-positive cells with damaged DNA was significantly reduced in the epidermis of *Kitl* transgenic mice, in which *Kitl* is expressed in basal layer keratinocytes and confers a radiosensitive, anti-gray hair effect on follicular MSC. 5,6) These findings indicate that maintenance of the niche function of HFKSC might protect against hair graying.

*Yerba Santa* (*Eriodictyon angustifolium*: Ea; *Eriodictyon californicum*: Ec) is a plant that grows in the west coast of North America and has been used for many years as a traditional medicinal herb by the indigenous population. Flavonoids in Ea and Ec have anti-inflammatory properties. 7,8) We have shown that Ea rather than Ec exert protective effects on normal human epidermal keratinocytes (NHEK) against X-ray-induced DNA damage and cell death. Using functional assays of various flavonoid species, we previously identified sterubin as the active ingredient in Ea, which has 48.3–66.7 times more sterubin than Ec. 9,10) We also reported that topical application of Ea but not Ec enhances melanin production in melanocytes *in vitro* and reduces human hair graying. 10) Topical application of sterubin and Ea but not Ec extract may help maintain the niche function of follicular HFKSC, as evidenced by decreasing age-related hair graying. 8,9)

We investigated in mice the underlying mechanisms of action of dietary Ea tea (EaT), which has been shown to maintain the HFKSC niche function against X-ray-induced DNA damage and hair graying, to determine whether dietary EaT may serve as an anti-hair graying herbal medicine.

MATERIALS AND METHODS

*Yerba Santa* (Ea and Ec) leaves and stems were kindly supplied by Ichimaru Pharcos Co. Ltd. (Gifu, Japan). They (3 g) were soaked in 100 mL water at 80 °C for 30 min, then filtered. The flavonoid concentration in each tea was determined by ultra-performance liquid chromatography (UPLC), as described elsewhere. 8)

All animal experiments were approved by the Animal Research Committee of Graduate School of Medicine, Gifu University. C57BL/6 mice were housed in standard animal rooms with food *ad libitum* under controlled humidity and temperature (22 ± 2 °C) conditions. EaT and water (control) were given freely from 3 weeks after birth until X-ray irradiation. Water and EaT were replaced every other day (Fig. 1A). There was no significant difference regarding the amount of EaT or water consumed among the mice (EaT − 0.28 ± 0.06 mL/g/d versus water − 0.32 ± 0.05 mL/g/d). Dorsal telogen hair (7–8 weeks after birth) was plucked from the mice fed with dietary EaT for a month. One day after plucking, the mice were exposed to X-radiation with MBR-1520 (Hitachi Medical, Tokyo, Japan) operating at 5 Gy, 50 kV, 20 mA with a 2.0 mm Al filter, and a dose rate of 0.4 Gy/min −1. Six hours after the exposure, tiny skin tissue portions were dissected and fixed by overnight immersion in 10% formalin. Incisions were carefully stitched. Methods for hair plucking, radiation, dissection and immunohistochemical analysis have been described elsewhere. 6,8)

Briefly, Histofine Kit (Nichirei Biosciences, Tokyo, Japan) was used following the manufacturer’s protocol for detecting anti-phospho histone H2AX, using a rabbit antibody (1 : 500, Cell Signaling Technology, MA, U.S.A.) and anti-mouse CD34, a marker for HFKSC, (1 : 500, eBioecience, CA, U.S.A.). The secondary antibodies used in this study were goat anti-rat immunoglobulin G-fluorescein isothiocyanate (IgG-FITC) (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and donkey anti-rabbit IgG-Alexa Fluor® 594 (Invitrogen, Carlsbad, CA, U.S.A.) at a 1/1000 dilution for 60 min at room temperature. Hair lightening was quantitatively measured using hair color spectrometry (CM-700d, Konica Minolta, Tokyo, Japan). 9,10) All data were analyzed by t-test.

*To whom correspondence should be addressed. e-mail: tkunisad@gifu-u.ac.jp

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RESULTS AND DISCUSSION

The flavonoid concentrations in EaT and Ec tea (EcT) are shown in Table 1. Compared to EcT, EaT contained 26.3 times more sterebin; 3.4 times more luteolin; 7.0 times more hydroxygenkwanin (HGK); and 1.27 times more homoeriodictyol. The most abundant flavonoid in both teas was homoeriodictyol. The amounts of others were not noticeably different between the two teas (Table 1). Because EaT contained large amounts of sterebin, which are known to have anti-hair graying effects, we focused on studying EaT further.

To investigate the hair pigment protective properties of dietary EaT, we used X-ray and an acute mouse model of hair graying that mimics DNA damage related to age-related hair graying. Irradiation of 5 Gy during the telogen stage of hair cycle caused DNA damage in HFKSC, comprising the niche for MSC, and subsequent production of non-pigmented hairs during the hair cycle following exposure. Ea has protective effects against X-ray-induced DNA damage in vitro and reduces human hair graying by topical application, possibly by circumventing loss of the niche function of HFKSC. Dietary EaT (Fig. 1A) showed a significant protective effect.
against X-ray-induced hair graying (Figs. 1B, C). Furthermore, dietary EaT significantly decreased X-ray-induced DNA damage as indicated by the reduced γH2AX signals from CD34+ HFKSC (Figs. 1D, E). While dietary EaT decreased hair graying by reducing X-ray-induced DNA damage in CD34+ HFKSC, the exact mechanism was still unknown; i.e., whether these effects took place directly via an increase of skin flavonoids content or indirectly through a systemic effect on the vascular system. However, these results were consistent with our previous in vitro results showing that pre-exposure of NHEK to Ea and sterubin decreased the number of phosphorylated H2AX foci per cell, indicating less DNA damage as indicated by the reduced X-ray-induced DNA damage in hair follicles. 

13) Moreover, sterubin, luteolin, and HGK may serve as active herbal ingredients for preventing human hair graying.

Conflict of Interest This study received funding from Hoyu Co., Ltd. N.T. and T.H. are employees of Hoyu Co., Ltd. The other authors declare no conflict of interest.

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