IFSCC 2025 full paper (IFSCC2025-611)

"Epidermal retinol prevents aging by protecting epidermal stem cells - The key role of BCO1 –"

Ai Tomiyama [1] and Masato Yoshioka [1,*]

[1] Research & Development Department, SEIWA KASEI CO., LTD., Osaka, Japan

1. Introduction

Retinol has been used in anti-aging cosmetics to prevent or improve skin wrinkles caused by dermal aging [1]. Keratinocytes but not fibroblasts express β-carotene 15,15'-monooxygenase (BCO1), which synthesizes retinol [2]. That localization of BCO1 might be biologically significant. Thus, we hypothesized that BCO1 may help to prevent epidermal aging by supplying retinol to keratinocytes. We previously reported that reactive oxygen species (ROS), which are gener-ated by UVB irradiation, are one of the factors responsible for the downregulation of BCO1 expression in keratinocytes. Furthermore, it has been reported that a decrease in the expression of BCO1 impairs retinol-mediated functions, such as epidermal hyaluronic acid (HA) production and increased AQP3 expression. These results suggest that maintaining the ability to synthesize retinol is important for suppressing epidermal aging.

A typical symptom of epidermal aging is a slower turnover rate of the skin, which may result from a slower proliferation rate of basal keratinocytes. Additionally, a reduction in stem cell keratinocytes may also be related to a slower turnover rate. In the epidermis, HA is present in high concentrations in living cell layers. Generally, HA helps to maintain the water content in tissues and in intercellular spaces and contributes to the diffusion of nutrients and metabolites. In addition, epidermal HA promotes the proliferation of keratinocytes by acting as a ligand for CD44, thus being involved in the turnover [3]. It has been reported that HA in the epidermis is reduced in sun-exposed elderly skin due to changes of basement membrane (BM) structure [4]. Furthermore, the age-dependent loss of collagen XVII (COL17) in basal keratinocytes is as-sociated with the loss of epidermal stem cells due to the induction of vertical cell divisions [5].

In this study, we investigated the relationship between BCO1 and COL17, which are involved in the maintenance of epidermal stem cells, and we demonstrate that the decrease in BCO1 reduces COL17, which may be associated with an elevation of intracellular ROS. Thus, as

one of the solutions to prevent epidermal aging, we examined the effects of 3-*O*-laurylglyceryl ascorbate (VC-3LG), an ascorbic acid derivative that has been confirmed to have a strong antioxidant effect [6].

2. Materials and Methods

2.1. Ex vivo study: BCO1 expression in UVB-irradiated human skin

Excised human skin (purchased from Biopredic, France) was treated with VC-3LG on the surface and was then cultured for 24 h. After removing VC-3LG, the skin was irradiated with 200 mJ/cm² UVB and further cultured for 24 h with VC-3LG applied topically. The changes in BCO1 expression in the epidermis induced by UVB irradiation and VC-3LG application were examined using immunostaining.

2.2. The influence of BCO1 knockdown (KD) on normal human epidermal keratinocytes (NHEKs)

BCO1 was knocked down in NHEKs using siRNA, and NHEKs treated with a non-targeted siRNA were used as a control. Intracellular ROS levels in those cells were measured using a ROS-reactive fluorescent probe. BCO1-KD NHEKs and control NHEKs were cultured in the presence of β-carotene, a substrate for BCO1, for 24 h. The expression of matrix metalloproteinase 1 (MMP-1) and COL17 expression in those cells at the mRNA and protein levels was evaluated by quantification using real-time PCR, and ELISA or western blotting, respectively.

2.3. The effects of VC-3LG on COL17 in NHEKs exposed to UVB

NHEKs were pretreated with VC-3LG for 24 hours and then cultured in the presence of β-carotene after being irradiated with UVB. The expression of COL17 at the mRNA and protein levels was measured using real-time PCR and western blotting, respectively.

3. Results

3.1. BCO1 expression in UVB-irradiated skin

First, we investigated the expression of BCO1 in human skin in response to UVB exposure and the effect of VC-3LG treatment. The results show that BCO1 is predominantly expressed in the epidermis and that UVB exposure significantly reduces BCO1 (Figure 1). On the other hand, pretreatment with VC-3LG caused a significant suppression of the reduction in BCO1 in human skin.

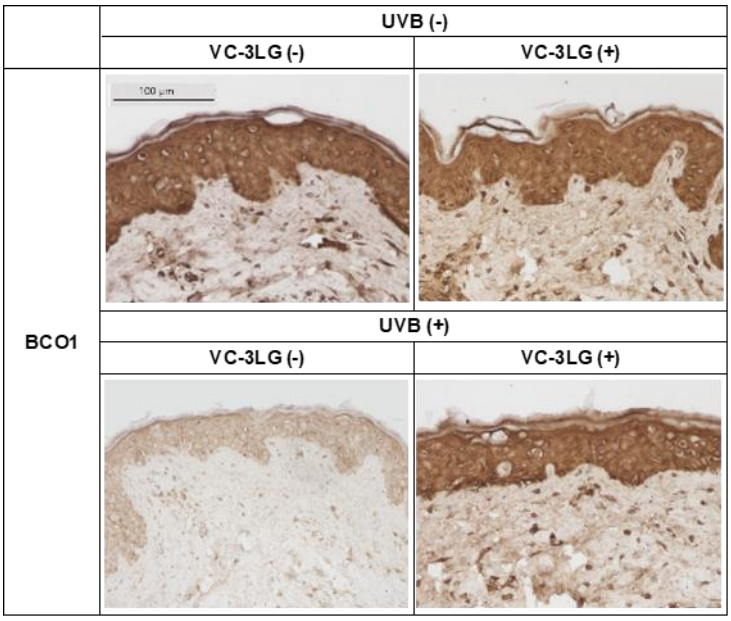

Figure 1. BCO1 in human skin in response to UVB exposure and the effect of VC-3LG treatment. Immunostaining of BCO1 in UVB-irradiated human skin; scale bar; 100 μm.

3.2. The influence of BCO1 KD on NHEKs

Since intracellular ROS levels are known to increase with age and retinol is considered to function as an antioxidant based on its chemical structure, we measured intracellular ROS levels in BCO1-KD NHEKs. The results indicate that intracellular ROS levels were significantly increased in BCO1-KD NHEKs (Figure 2).

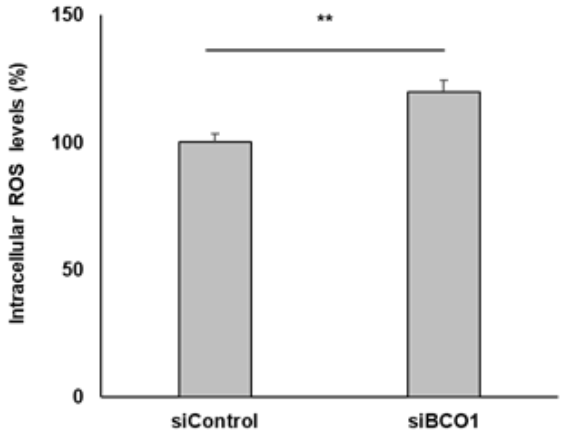

Figure 2. Intracellular ROS levels in BCO1-KD NHEKs; bars indicate means ± S. D. of percentages compared to the control (n=3); significance **p<0.01 (Student's T-test).

Furthermore, the increased expression of COL17 and the decreased expression of MMP-1 were confirmed at both the mRNA and protein levels (Figure 3).

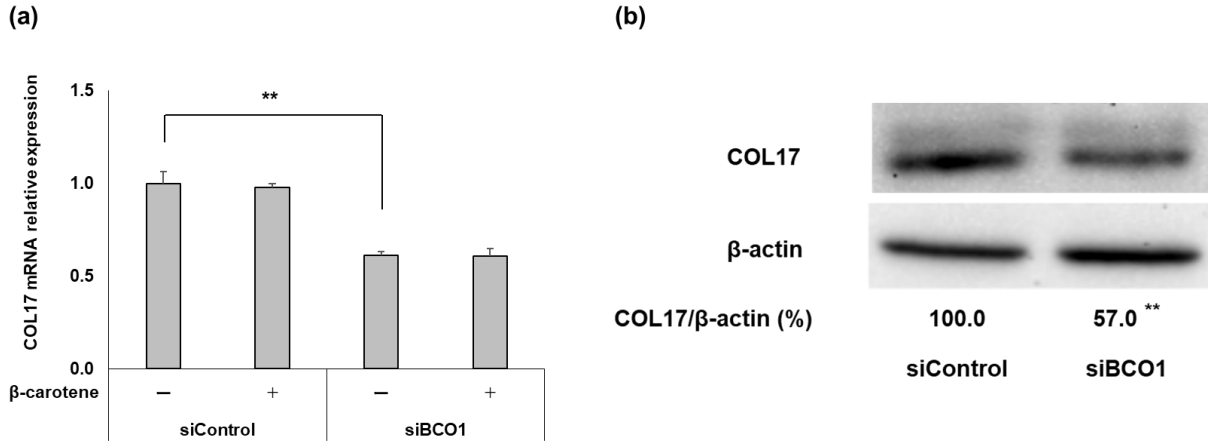

Figure 3. The expression of COL17 and MMP-1 in BCO1-KD NHEKs. (a) mRNA expression levels of COL17 in BCO1-KD NHEKs; (b) Protein expression levels of COL17 in BCO1-KD NHEKs; bars indicate means ± S. D. of percentages compared to the control (n=3); **p<0.01 indicates a significant difference vs. siControl (Student's T-test).

3.3. The preventive effect of VC-3LG on retinol-mediated functions in UVB-irradiated NHEKs

We previously reported that treatment with UVB and H2O2 reduces BCO1 expression in NHEKs. Furthermore, in this study, we observed that treatment with UVB decreases BCO1 in excised human skin. Thus, to investigate the impact of BCO1 on COL17 expression and the role of VC-3LG, we examined the changes in COL17 expression in UVB-irradiated NHEKs in the presence or absence of β-carotene and VC-3LG. The results show that UVB reduces COL17 expression, and the decreased COL17 expression was rescued by the presence of VC-3LG, but not by β-carotene (Figure 4).

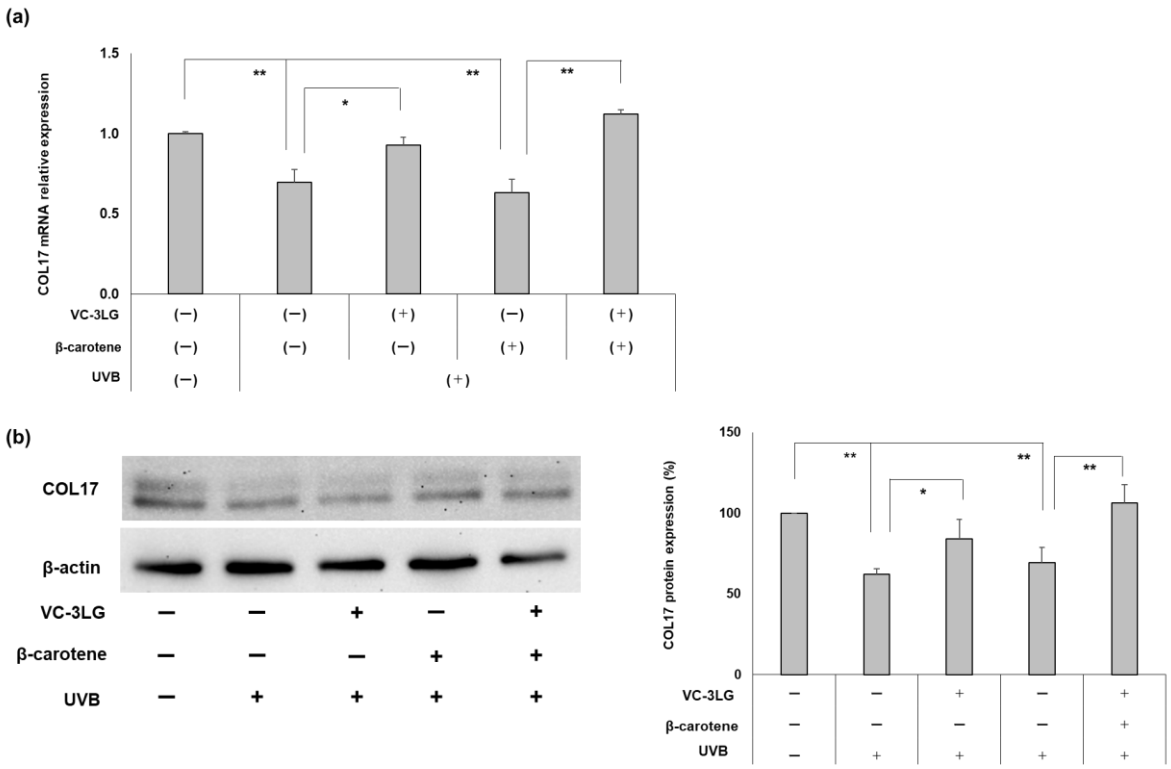

Figure 4. The effect of VC-3LG on the decrease in COL17 expression in UVB-irradiated NHEKs. (a) mRNA expression levels of COL17 in UVB-irradiated NHEKs; (b) Protein expression levels of COL17 in UVB-irradiated NHEKs; bars indicate means ± S. D. of ratios compared to the control (n=3); significance *p<0.05, **p<0.01 (Student's T-test).

4. Discussion

Epidermal aging is defined by the following characteristics: thinning of the living cell layers of the skin from the basal to the granular layer, a reduction in the number of layers in the granular layer, a decrease in keratohyalin granules in granular cells, and an increase in the number of layers in the stratum corneum due to a slower turnover rate. These histological alterations are associated with impaired barrier and moisture-retaining functions [7]. When discussing skin aging, although changes in the dermal structure are mainly emphasized, it is also important to pay attention to epidermal aging.

The epidermal structure is maintained by the balance between the supply of basal keratinocytes through cell division and their subsequent differentiation. Recent studies have highlighted the significance of the direction of cell division in basal keratinocytes in maintaining thefunction and structure of the skin. The direction of division in basal keratinocytes is categorized into symmetric divisions, which occur horizontally, and asymmetric divisions, which occur vertically. Symmetric divisions help maintain the number of epidermal stem cells, whereas asymmetric divisions lead to the loss of those cells [8, 9]. It is presumed that the loss of epidermal stem cells leads to atrophy, similar to that seen in aged epidermis. Thus, to prevent epidermal aging, it is important to maintain the symmetric divisions of basal keratinocytes in order to preserve the number of epidermal stem cells.

Recent studies have demonstrated that the expression of COL17, which is a hemidesmosomal protein, in basal keratinocytes is important for maintaining symmetric divisions. Basal keratinocytes with low levels of COL17 lose in cell competition against basal keratinocytes with high levels of COL17, leading to asymmetric divisions, and eventually, they are directed towards differentiation and are desquamated from the epidermal layer. Thus, the decrease in COL17 suggests the depletion of epidermal stem cells. Additionally, differences in the expression levels of COL17 among individual stem cells are caused by genomic stress and oxidative stress. In fact, it has been reported that the levels of COL17 are decreased by UV exposure or with age. Taken together, these findings indicate that COL17 is a key protein in epidermal aging. Therefore, we investigated the relationship between BCO1 and COL17 in the context of epidermal aging.

In our previous study, we reported that UVB irradiation reduced BCO1 expression in NHEKs at both the mRNA and protein levels. Thus, we first confirmed whether the results of that in vitro study are reproduced in UVB-irradiated skin in an ex vivo study. The results showed that UVB exposure of the skin reduced the protein expression level of BCO1, which is localized in epidermal cells (Figure 1). The results suggest the possibility that exposure to sunlight suppresses the retinol-mediated function in the epidermis. We then characterized BCO1-KD NHEKs to clarify the role of BCO1 in epidermal aging. Intracellular ROS levels were higher in BCO1-KD NHEKs than in control NHEKs (Figure 2). Furthermore, BCO1-KD NHEKs showed changes in the expression of COL17 at both the mRNA and protein levels, and that behavior

was not affected by the addition of β-carotene (Figure 3). These results indicated that BCO1 is involved in intracellular redox and has a function in maintaining the reduced state in NHEKs. It is possible that retinol synthesized from β-carotene by BCO1 stimulates an antioxidant defense system to maintain the reduced state in cells, as retinoic acid has been reported to activate Nrf2 signaling [10]. In addition, the changes in the expression of COL17 and MMP-1 observed in BCO1-KD NHEKs could be attributed to oxidative stress resulting from retinol depletion, as they were not rescued by β-carotene. Furthermore, UVB, which decreases BCO1 expression in NHEKs, also reduces COL17 levels at both the mRNA and protein levels (Figure 4). Taken together, these results indicate that BCO1 plays a critical role in maintaining the reduced environment in keratinocytes, thereby contributing to the maintenance of COL17 levels in NHEKs. From these findings, it could be deduced that the stable expression of BCO1 constitutes one of the mechanisms involved in the prevention of epidermal aging.

A previous study demonstrated that the UVB-induced decrease in BCO1 is caused by ROS, and that VC-3LG, which enhances the intracellular antioxidant system through the activation of Nuclear factor erythroid 2-related factor 2 (Nrf2) signaling, rescues that decrease. This ex vivo study also confirmed that VC-3LG suppresses the UVB-induced decrease in BCO1 (Figure 1). These findings suggest that VC-3LG may help prevent epidermal aging by maintaining COL17 levels. In fact, VC-3LG rescued the UVB-induced decrease in COL17 at both the mRNA and protein levels. Furthermore, the presence of β-carotene tended to enhance this rescue effect (Figure 4). Based on these results, we concluded that VC-3LG protects BCO1, which is reduced by UVB exposure, and thus contributes to the prevention of epidermal aging.

On the other hand, we previously reported that reduced BCO1 expression leads to decreased HA production in NHEKs, and that VC-3LG maintains HA levels by protecting BCO1 from UVB-induced reduction. Epidermal HA, which is mainly localized around cells in the basal layer and spinous layer, has been reported to be involved in differentiation, proliferation, and turnover of the epidermis [11, 12]. Thus, maintaining epidermal HA is important for maintaining the balance of basal keratinocytes between proliferation and differentiation. However, in sun-exposed skin, the amount of epidermal HA decreases due to the lower expression of hyaluronan synthases 1 and 3 and the degradation of the BM [13, 14], which may eventually contribute to the progression of epidermal aging. These facts suggest that BCO1 plays a critical role in preventing epidermal aging by expressing retinol-mediated function. Furthermore, since VC-3LG suppresses the UVB-induced decrease in BCO1, these results suggest that VC-3LG prevents epidermal aging through retinol-mediated functions.

5. Conclusion

Based on the results of this study, we propose that retinol-mediated functions contribute to the modulation of intracellular redox levels and are involved in epidermal aging, based on investigations conducted in the absence of BCO1. The results indicate that VC-3LG, an enhancer of the intracellular antioxidant system, prevents epidermal aging by maintaining BCO1 levels reduced by UVB.

6. References

1. Yuan S, Tianyuan H, Gary JF, John JV, Taiho Q, et al. (2017) Molecular basis of retinol anti-aging properties in naturally aged human skin in vivo. Int J Cosmet Sci 39(1):56-65.

2. Roos TC, Jugert FK, Merk HF, et al. (1998) Retinoid metabolism in the skin. Pharmacol Rev 50:315-333.
3. Bourguignon LY, Ramez M, Gilad E, Singleton PA, Man MQ, Crumrine DA, Elias PM, Feingold KR, et al. (2006) Hyaluronan–cd44 interaction stimulates keratinocyte differen-tiation, lamellar body formation/secretion, and permeability barrier homeostasis. J Invest Dermatol 126:1356-1365.
4. Iriyama S, Nishikawa S, Hosoi J, Amano S, et al. (2021) Basement membrane helps maintain epidermal hyaluronan content. Am J Pathol 191(6);1010-1019.
5. Liu N, Matsumura H, Kato T, Ichinose S, Takada A, Namiki T, Asakawa K, Morinaga H, Mohri Y, Arcangelis AD, Geroges-Labouesse E, Nanba D, Nishimura EK, et al. (2019) Stem cell competition orchestrates skin homeostasis and ageing. Nature 568(7752):344-350.
6. Katsuyama Y, Tsuboi T, Taira N, Yoshioka M, Masaki H, et al. (2016) 3-O-Laurylglyceryl ascorbate activates the intracellular antioxidant system through the contribution of PPAR-g and Nrf2. J Dermatol Sci 82(3):189-196.
7. Hara M, Kikuchi K, Watanabe M, et al. (1993) Senile xerosis: functional, morphological, and biochemical studies. J. Geriatr Dermatol 1:111-120.
8. Williams SE., Beronja S, Pasolli HA, Fuchs E, et al. (2011) Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 470:353-358.
9. Poulson ND, Lechler T, et al. (2012) Asymmetric cell divisions in the epidermis. Int Rev Cell Mol Biol 295:199-232.
10. Lai X, Wu A, Bing Y, Liu Y, Luo J, Yan H, Zheng P, Yu J, Chen D, et al. (2023) Retinoic acid protects against lipopolysaccharide-induced ferroptotic liver injury and iron disorders by regulating Nrf2/HO-1 and RARβ signaling. Free Radic Biol Med 20:205;202-213.
11. Tammi R, Agren UM, Tuhkanen AL, Tammi M, et al. (1994) Hyaluronan metabolism in skin. Prog Histochem 29:1-81.
12. Tzellos TG, Klagas I, Vahtsevanos K, Triaridis S, Printza A, Kyrgidis A, Karakiulakis G, Zouboulis CC, Papakonstantinou E, et al. (2009) Extrinsic ageing in the human skin is associated with alterations in the expression of hyaluronic acid and its metabolizing en-zymes. Exp Dermatol 18:1028-1035.
13. Tammi RH, Tammi MI, Hascall VC, Hogg M, Pasonen S, MacCallum DK, et al. (2000) A preformed basal lamina alters the metabolism and distribution of hyaluronan in epidermal keratinocyte "organotypic" cultures grown on collagen matrices. Histochem Cell Biol 113:265-277.
14. Yoshida H, Nagaoka A, Komiya A, Aoki M, Nakamura S, Morikawa T, Ohtsuki R, Sayo T, Okada Y, Takahashi Y, et al. (2018) Reduction of hyaluronan and increased expression of HYBID (alias CEMIP and KIAA1199) correlate with clinical symptoms in photoaged skin. Br J Dermatol 179;136-144.