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# promote skin hydration and barrier function

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## 1. Introduction

Fermentation is a process that has long been used to produce a variety of valuable ingredients utilized across multiple industries, such as pharmaceuticals, cosmetics, and food [1]. By leveraging metabolic control, different materials are combined with various strains of bacteria to yield products with specific, beneficial properties. Among the many fermentation tecniques, the combination of rice and yeast has proven particularly effective, as seen in traditional practices such as Japanese sake brewing. This relationship between rice and yeast has inspired the development of rice fermentation liquid, a unique cosmetic ingredient rich in naturally derived components such as amino acids, sugars, nucleic acids, and polyamines [2]. These components are produced through the fermentation of rice using a specialized yeast strain, *Saccharomyces veronae*, selected from a pool of 280 yeast strains.
In previous studies [3], the beneficial effects of rice fermentation liquid on skin hydration and barrier function have been explored, particularly the impact of 2-ketoglutaric acid, one of its key components. This study builds on that foundation, focusing on two other significant components of rice fermentation liquid: nucleic acids and sugars. Nucleic acids have been recognized for their potential to enhance skin function, especially in terms of hydration and protection against UV damage. The sugars present in the fermentation liquid are well-known for their moisturizing properties. This research specifically aims to assess the effects of these components on human skin cells, particularly keratinocytes, to determine their role in promoting skin health and combating signs of aging. Through this study, we hope to further understand the underlying mechanisms by which rice fermentation products can enhance skin hydration, improve barrier function, and potentially provide anti-aging benefits.

## 2. Materials and Methods

### *2.1. Test substance*

Guanosine (Apollo Scientific), uridine, cytosine, cytidine (FUJIFILM Wako Pure Chemical Corporation), cytidine 5'-monophosphate (CMP, Combi-Blocks) and D-galacturonic acid hydrate (GalA, ChromaDex) were used for this study.

### *2.2. Cell culture*

Normal human epidermal keratinocytes (NHEKs, KURABO) were cultured in keratinocyte basal medium (KBM) or keratinocyte growth medium (KGM) containing insulin,

hydrocortisone, gentamycin/amphotericin B, and growth additives such as bovine pituitary extract and human epidermal growth factor at 37°C under an atmosphere of 5% $CO_2$ in air.

### *2.3. Measurement of prostaglandin (PG) $E_2$*

NHEKs were seeded on a 48-well plate and cultured in KGM overnight. The cells were then incubated with KBM without hydrocortisone for 24 hrs. The medium was exchanged to an aspirin-containing KGM without hydrocortisone and incubated for 4 hrs. The cells were then exposed to 60 mJ/cm$^2$ ultraviolet (UV) B irradiation under a thin layer of PBS(-). After further incubation for 24 hrs post-treatment with or without test substances, amounts of $PGE_2$ in the supernatants were determined using $PGE_2$ EIA Kit (Cayman Chemical).

### *2.4. Measurement of intracellular total glutathione (GSH)*

NHEKs were seeded on a 48-well plate and cultured in KGM for 48 hrs. The cells were then incubated with or without test substances for 24 hrs, and the intracellular total GSH content was measured by the glutathione reductase recycling method [4].

### *2.5. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)*

NHEKs were cultured with or without test substances for the times noted in the text. Total RNA was isolated from NHEKs using ISOGEN II (NIPPON GENE) according to the manufacturer's instructions, and cDNAs were synthesized with PrimeScript RT Master Mix using TaKaRa PCR Thermal Cycler Dice Touch (TaKaRa Bio). RT-qPCR for transglutaminase-1 (*TGM1*), serine palmitoyltransferase (SPT) long chain base subunit 2 ( *SPTLC2*), aquaporin 3 (*AQP3*) and GAPDH genes was performed with TB Green Fast qPCR Mix using Thermal Cycler Dice Real Time System III (TaKaRa Bio), and the primer sets were purchased from Takara Bio. The mRNA expressions were normalized to GAPDH expression and expressed as percentages relative to the expression in control cells.

### *2.6. ELISA*

NHEKs were treated with or without test substances for the times noted in the text. The supernatants after the treatment were used for ELISA. Amounts of laminin-332 in the supernatants were measured using anti-human laminin 5 monoclonal antibody (P3E4) (Santa Cruz) as the primary antibody. Amounts of hyaluronic acid (HA) in the supernatants were measured through a sandwich ELISA, using standard curves constructed from purified HA (Sigma) and HA-binding protein (Hokudo) following a standard operating method. The absorbance of ABTS was measured at 405 nm using a Varioskan Microplate Reader (Thermo Fisher). The HA content was determined from the standard curve and normalized to the value of WST-8 assay. The data are expressed as percentages of control cells.

### *2.7. Statistical Analysis*

All data are expressed as the mean ± standard error of the mean (SEM). The data were statistically analyzed using Dunnett's test by BellCurve for Excel (Social Survey Research Information, Japan). A $p$-value of less than 5% was considered to be statistically significant.

## 3. Results

### *3.1. Inhibitory effects on UVB-induced $PGE_2$ production*

To examine whether test substances have anti-inflammatory effects, amounts of $PGE_2$ induced by UVB in keratinocytes were measured. As shown in **Figure 1**, treatment with uridine, cytosine, and D-galacturonic acid (GalA) significantly suppressed $PGE_2$ levels compared to the UVB(+) control. Specifically, uridine and cytosine reduced $PGE_2$ production with statistical significance (** $p<0.05$, ** $p<0.01$*), and GalA showed similar inhibitory effects, demonstrating their potential in mitigating UVB-induced inflammation.

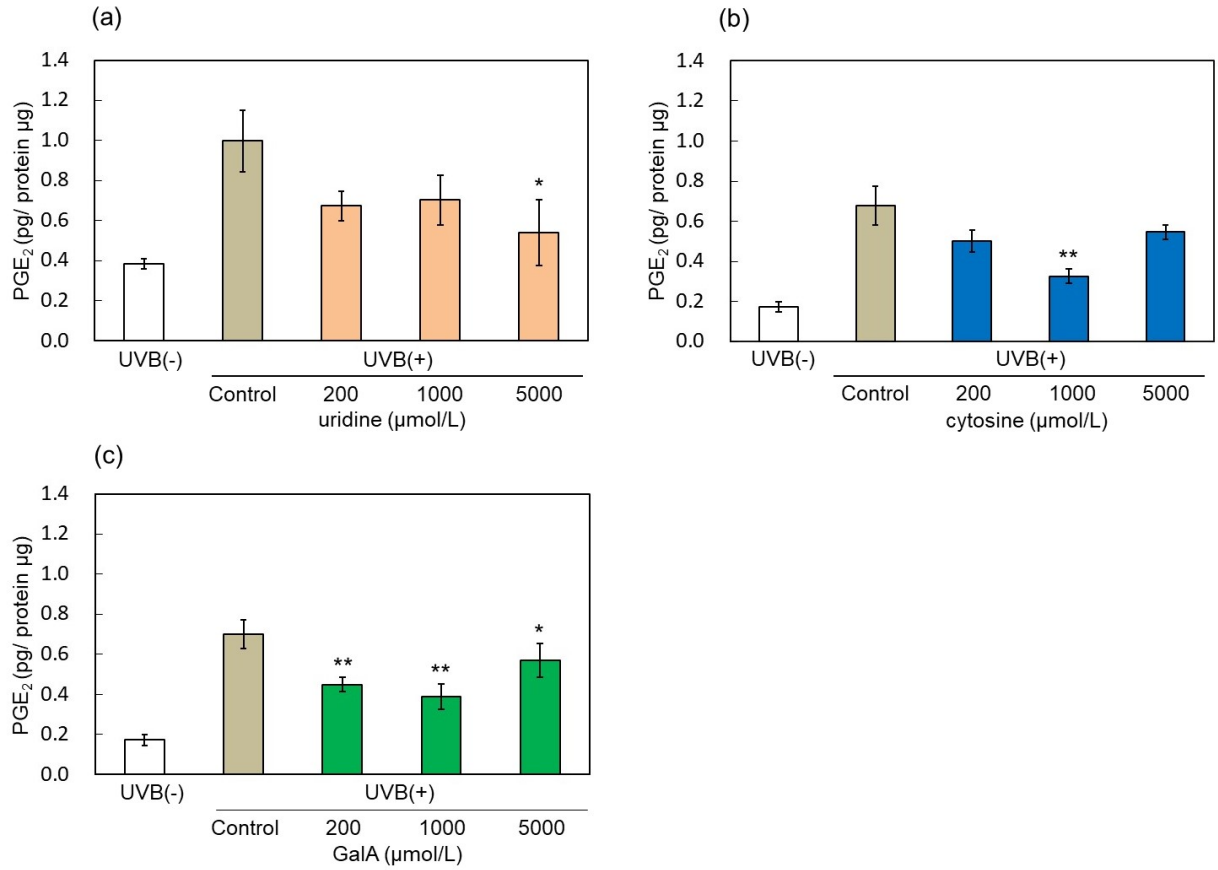


**Figure 1**. Inhibitory effects of uridine, cytosine and GalA on UVB-induced $PGE_2$ production.
NHEKs were treated with or without test substances for 24 hrs after UVB irradiation. Amounts of $PGE_2$ in the supernatant per protein of cells treated with (a) uridine, (b) cytosine, (c) GalA were measured. Each bar indicates the mean ± SEM, n=4, * $p$<0.05, ** $p$<0.01, vs. UVB(+) control using Dunnett's test.

### *3.2. Promoting effects on laminin-332, HA and GSH production*

Next, we investigated whether test substances have anti-aging effects. As shown in **Figure 2** , cytidine 5'-monophosphate (CMP) significantly enhanced laminin-332 secretion ( *** p<0.01* ), a crucial component of the basement membrane associated with epidermal-dermal integrity. **Figure 3** illustrates that uridine, cytidine, CMP, and GalA significantly promoted hyaluronic acid (HA) production in NHEKs after 3 days of treatment. Each compound showed a notable increase in HA levels, suggesting improved skin hydration and anti-wrinkle properties.

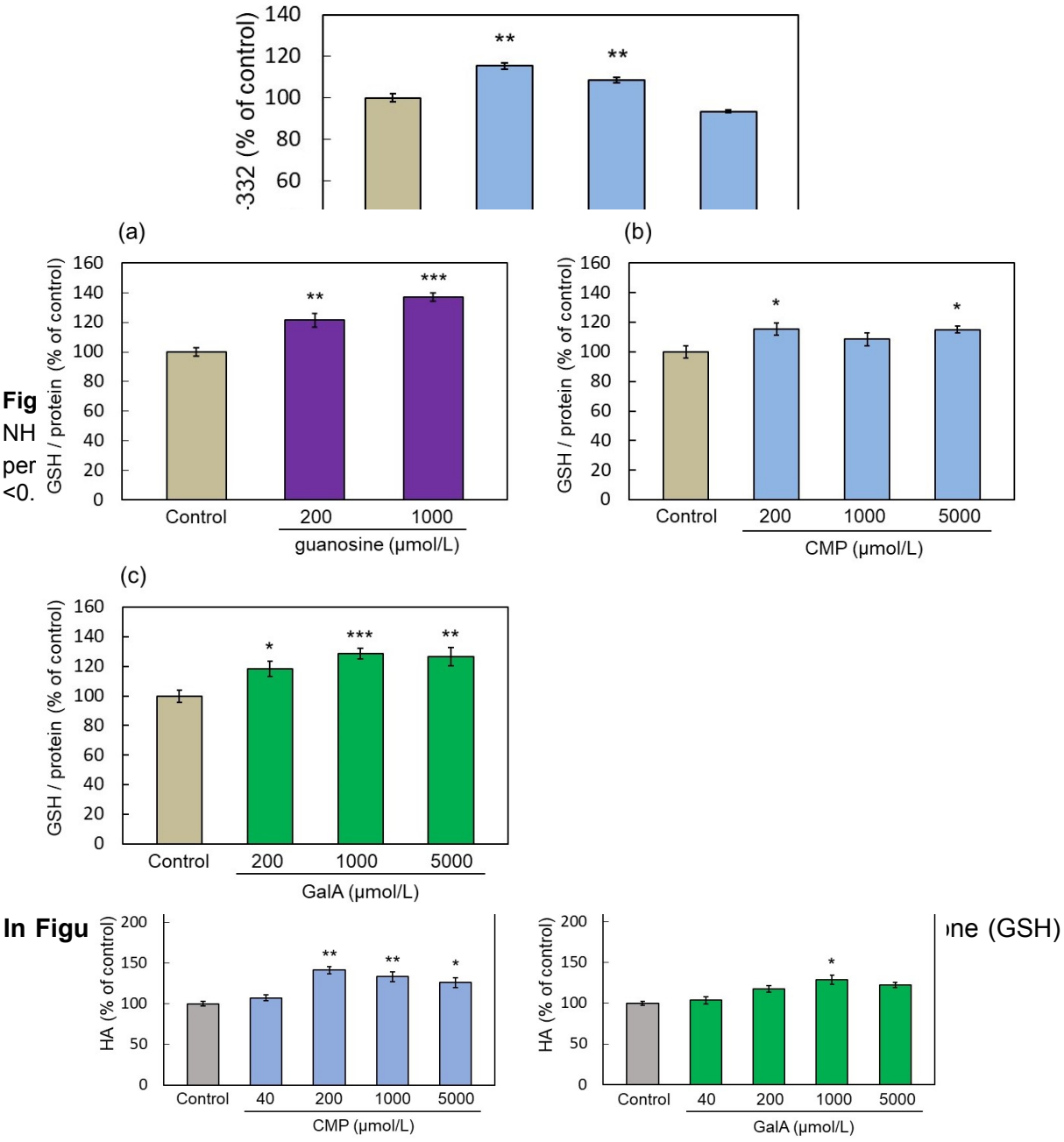


**Fig** NH per <0.

**In Figu** ıne (GSH)

**Figure 3**. Promoting effects of uridine, cytidine, CMP and GalA on epidermal HA production. NHEKs were treated with or without test substances for 3 days. Amounts of HA in the supernatant of cells treated with (a) uridine, (b) cytidine, (c) CMP and (d) GalA were measured. Each bar indicates the mean ± SEM, n=5, * $p$<0.05, ** $p$<0.01, vs. control using Dunnett's test.

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ls, with all showing statistically significant differences from the control ( ** p<0.05, ** p<0.01, *** p<0.001*). These results highlight their capacity to enhance antioxidant defense in keratinocytes, supporting skin protection against oxidative stress.

### *3.3. Promoting effects on mRNA expressions of TGM1, SPTLC2 and AQP3*

To further investigate whether test substances have effects on moisture retention and barrier function, the mRNA expressions of *TGM1*, *SPTLC2* and *AQP3* in keratinocytes were analysed using RT-qPCR. As shown in **Figure 5**, cytosine significantly upregulated the mRNA expression of both *TGM1* and *SPTLC2* ( ** p<0.05, ** p<0.01*), while guanosine significantly increased *SPTLC2* and *AQP3* expression levels, and tended to upregulate *TGM1* expression. These genes are closely related to cornified envelope formation, ceramide synthesis, and water/glycerol transport, respectively, indicating the compounds' beneficial effects on skin barrier integrity and moisture retention.

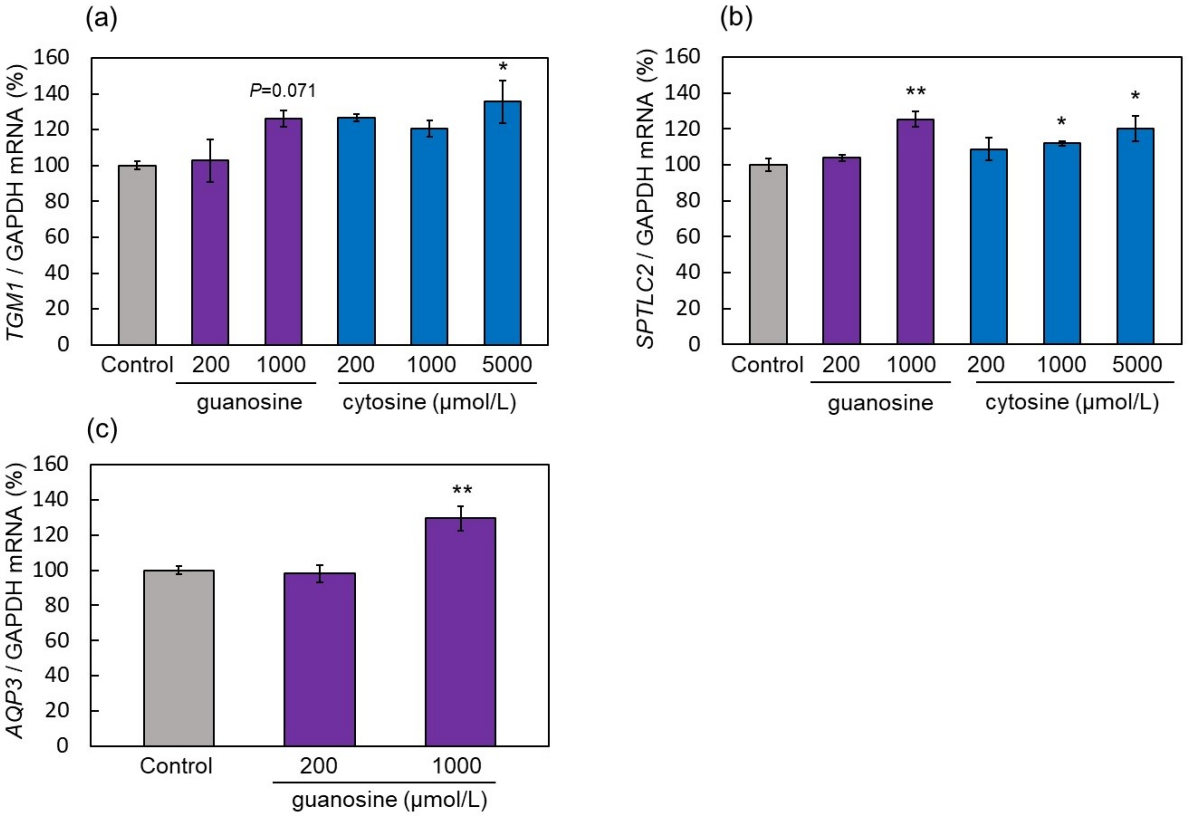


**Figure 5**. Promoting effects of guanosine and cytosine on mRNA expressions of *TGM1*, *SPTLC2* and *AQP3*.

NHEKs were treated with or without test substances for 24 hrs. The mRNA expressions of (a) *TGM1*, (b) *SPTLC2* and (c) *AQP3* was analysed using RT-qPCR. Each bar indicates the mean ± SEM, n=3, * $p$<0.05, ** $p$<0.01, vs. control using Dunnett's test.

## Discussion

To date, approximately 500 components in rice fermentation liquid have been identified. Among them, this study focused on the efficacy of components of nucleic acids (nucleotides , nucleosides, nucleobases) and GalA, a type of sugar. Nucleic acids have long been used

as cosmetic ingredients because of their UV absorption and moisturizing effects. The components of nucleic acids, nucleotides, nucleosides, and polydeoxyribonucleotides, are known to activate the proliferation of fibroblasts and keratinocytes [5,6]. Sugar is also a well-known cosmetic ingredient for moisturizing skin. However, few reports are available on the research of nucleic acids and sugars for the maintenance and/or improvement of human skin functions *in vitro*. Thus, we investigated their efficacy in keratinocytes.

As a result of an anti-inflammatory evaluation, uridine, cytosine and GalA exhibited significant inhibition of UVB-induced $PGE_2$ production. $PGE_2$ is an important inflammatory mediator involved in skin erythema and inflammation [7,8]. $PGE_2$ is also known to stimulate melanocyte dendrite formation [9]. Therefore, these compounds are expected to exert anti-inflammatory and brightening effects via the inhibition of UVB-induced $PGE_2$ production.

Next, we evaluated the promoting effects on the production of laminin-332, HA and GSH, related to anti-aging. Laminin-332 (Laminin-5) is one of the components of basement membrane (BM), which plays important roles in maintaining a healthy epidermis and dermis. The degradation of BM components in UVB-exposed skin may be associated with increases in BM damage, accelerating the aging process [10,11]. Promotion of the synthesis of BM components, such as laminin-332, is important for BM reinforcement. From this point, CMP, significantly promoted laminin-332 production, could be effective for anti-aging. HA is a high-molecular-weight polysaccharide involved in skin moisture having unique capacity in retaining water. Therefore, the control of HA synthesis is very important for skin moisture. Recently, it has been reported that the role of epidermal HA is to enhance the epidermal proliferation and diferentiation and to regulate the morphogenesis and homeostasis [12]. Furthremore, an inducer of epidermal HA production is known to exert anti-wrinkle effects [13]. Thus, uridine, cytidine, CMP and GalA, significantly promoted epidermal HA production, are suggested to be effective as anti-aging materials. GSH is a tripeptide that serves as an endogenous antioxidant and provides protection against oxidative stress-induced cellular damage [14, 15]. Therefore, guanosine, CMP and GalA, showing promotion of intracellular GSH production, could protect the skin from external stress.

We also found promoting effects on mRNA expressions of *TGM1*, *SPTLC2* and *AQP3*. TGM1 is a catalytic membrane-bound enzyme that functions in the formation of the epidermal cornified cell envelope, which acts as a mechanical barrier to protect against water loss and infectious agents [16, 17]. SPT is the rate-limiting enzyme in the *de novo* synthesis of ceramides which are main components of intercellular lipids in the stratum corneum (SC) and plays an essential role in skin barrier function [18, 19]. AQP3, which transports both water and small neutral solutes, such as glycerol and urea, is intensely expressed in epidermis and plays an important role in SC hydration, permeability barrier function, and wound healing [20, 21]. Hence, guanosine and cytosine, significantly up-regulated mRNA expressions of *TGM1*, *SPTLC2* and *AQP3*, are expected to promote skin hydration and barrier function.

In conclusion, six compounds necessary for vital activities included in rice fermented liquid were found to be anti-inflammatory, anti-aging, and moisturizing. Five compounds except GalA are nucleotides or their precursors (nucleosides and nucleobases), which are the building blocks of nucleic acids, may have assisted cellular functions.

## 5. Conclusion

In conclusion, the rice-derived compounds from fermentation with a special yeast show significant potential in enhancing epidermal functions and improving skin health. These compounds exhibit anti-inflammatory, anti-aging, and moisturizing effects, contributing to skin protection against oxidative stress, promoting a radiant complexion, and enhancing hydration. The upregulation of key skin components like laminin-332, HA, and glutathione

supports skin integrity, moisture retention, and antioxidant defense. Additionally, the promotion of mRNA expressions related to skin barrier function further emphasizes their ability to improve skin hydration and overall resilience, making them promising ingredients for anti-aging skincare.

**References**

1. Majchrzak, *et al.*, Biological and cosmetical importance of fermented raw materials: an overview. *Molecules*, **27**(15), 4845 (2022)
2. Yang, F, *et al.*, Biologically Active Components and Skincare Benefits of Rice Fermentation Products: A Review. *Cosmetics*, **12**(1), 29 (2025)
3. Yang, F, *et al.*, The study of skin hydration, anti-wrinkles function improvement of anti-aging cream with alpha-ketoglutarate. *Journal of Cosmetic Dermatology*, **21**(4), 1736-1743 (2022)
4. Rahman, I, *et al.*, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nature Protocols*, **1** (6), 3159-3165 (2006)
5. Thellung, S, *et al.*, Polydeoxyribonucleotides enhance the proliferation of human skin fibroblasts: Involvement of $A_2$ purinergic receptor subtypes. *Life Sciences*, **64** (18), 1661-1674 (1999)
6. Lee, K-S, *et al.*, Analysis of Skin Regeneration and Barrier-Improvement efficacy of polydeoxyribonucleotide isolated from Panax Ginseng (C.A. Mey.) adventitious root. *Molecules*, **28**, 7240 (2023)
7. Kabashima, K, *et al.*, Prostaglandin $E_2$ is required for ultraviolet B-induced skin inflammation via EP2 and EP4 receptors. *Laboratory Investigation*, **87**, 49-55 (2007)
8. Kawahara, K, *et al.*, Prostaglandin $E_2$-induced inflammation: Relevance of prostaglandin E receptors. *Biochimica et Biophysica Acta*, **1851**, 414-421 (2015)
9. Scott, G, *et al.*, Prostaglandin $E_2$ regulates melanocyte dendrite formation through activation of PKCζ. *Experimental Cell Research*, November 1; **313** (18), 3840-3850 (2007)
10. Amano, S, Possible involvement of basement membrane damage in skin photoaging. *Journal of Investigative Dermatology Symposium Proceedings*, **14**, 2-7 (2009)
11. Inomata, S, *et al.*, Possible involvement of basement membrane damage in skin photoaging. *Journal of Investigative Dermatology*, **120** (1), 128-134 (2003)
12. Endo, Y, *et al.*, Accelerated human epidermal turnover driven by increased hyaluronan production. *Journal of Dermatological Science*,**101**, 123-133 (2021)
13. Endo, Y, *et al.*, Antiwrinkle efficacy of 1-ethyl-β-N-acetylglucosaminide, an inducer of epidermal hyaluronan production. *Skin Research and Technology*, **28**, 58-65 (2022)
14. Cui, X, *et al.*, Topical glutathione amino acid precursors protect skin against environmental and oxidative stress. *Journal of the European Academy of Dermatology and Venereology*, **38**,
(Suppl. 3), 3-11 (2024)
15. Lohan, S. B., *et al.*, ROS production and glutathione response in keratinocytes after application of β-carotene and VIS/NIR irradiation. *Chemico-Biological Interactions*, **280**, 1-7 (2018)
16. Eckert, R. L.,*et al.*, Involucrin - Structure and role in envelope assembly. *Journal of Investigative Dermatology*, **100**, 613-617 (1993)
17. Nemes, Z, *et al.*, Involucrin cross-linking by transglutaminase 1. *Journal of biological chemistry*, **274** (16) ,11013-11021 (1999)

18. Weiss, B, *et al.*, Human and murine serine-palmitoyl-CoA transferase cloning, expression and characterization of the key enzyme in sphingolipid synthesis. *European journal of biochemistry*, **249**, 239-247 (1997)
19. Tanno, O, *et al.*, Nicotinamide increases biosynthesis of ceramides as well as other stratum corneum lipids to improve the epidermal permeability barrier. *British Journal of Dermatology*, **143**, 524-531 (2000)
20. Nakahigashi, K, *et al.*, Upregulation of aquaporin-3 is involved in keratinocyte proliferation and epidermal hyperplasia. *Journal of Investigative Dermatology*, **131**, 865-873 (2011)
21. Qin, H, *et al.*, Aquaporin-3 in keratinocytes and skin: its role and interaction with phospholipase D2. *Archives of biochemistry and biophysics*, April 15; **508** (2), 138-143 (2011)