IFSCC 2025 full paper (IFSCC2025-1724)

"AI-based retinol signature: Identification of new best-in-class anti-aging ingredients"

Robin Terrisse[1,*], Jean-Yves Bruxer[2] , Yuye Zhou[3] , Emmanuelle Gaillard[4], Laetitia Vandoorn[1], Yong Yang[3], Mitsuko Kojima[5] and Gaelle Saint-Auret [1]

[1] Genel, Grenoble, France ; [2] Sethic, HongKong, China ; [3] Sethic (Guangzhou) Research & Development Center, Guangzhou, China ; [4] Laboratoires Sethic Innovations, Paris, France; [5] Sethic Innovation Labo Japan, Tokyo, Japan

1. Introduction

The skin is a complex organ composed of various cells—including keratinocytes, melanocytes, fibroblasts, immune cells, and adipocytes—organized into distinct layers. **[1]** These cells communicate to maintain homeostasis and respond to stress. **[1]** Skin aging is a natural and complex process influenced by a variety of intrinsic and extrinsic factors. Intrinsic aging, also known as chronological aging, is driven by genetic factors and the passage of time. Extrinsic aging, on the other hand, is caused by external factors such as ultraviolet (UV) radiation, pollution, smoking, and other environmental stressors. **[2]** Twelve interconnected hallmarks—telomere attrition, genomic instability, autophagy reduction, loss of proteostasis and extracellular matrix, chronic inflammation, senescence/apoptosis/proliferation, epigenetic alterations, stem cells exhaustion, mitochondria dysfunction and increased oxidative stress, nutrient sensing, altered intercellular communication and finally dysbiosis—are responsible for aging signs. **[7]** These signs such as hyperpigmentation, uneven texture, wrinkles, and skin dryness can greatly impact our self-esteem. **[3]** Therefore, developing active ingredients that aim at combating skin aging signs could be beneficial in improving both the physical and psychological changes associated with aging, thereby enhancing our quality of life. These products work by delaying the formation of wrinkles and age spots through mechanisms such as promoting cellular renewal, enhancing collagen production, and shielding the skin from environmental stressors. Retinol is considered the gold standard in reducing aging hallmarks. **[4,5]** Its topical application enhances cell renewal, reduces hyperpigmentation, and smooths coarse wrinkles, resulting in a more radiant and even complexion. However, retinol's effectiveness comes with a downside—it can cause significant irritation, making it unsuitable for sensitive skin. **[4]** Additionally, EU safety regulations have imposed restrictions on the concentration of retinol in skincare formulations. **[6]** This has sparked a surge in the development of alternatives and complementary ingredients to harness similar benefits without the associated drawbacks.

In this study, we created an aging skin database aggregating the gene expression of 50 genes that control the 12 interconnected aging hallmarks. These 50 genes were experimentally quantified across multiple donors of both young and aged fibroblasts treated or not by retinol. Then, we adopted a data-driven approach powered by artificial intelligence to develop a state-of-the-art retinol signature. Through rigorous classification by a machine learning algorithm, we identified a retinol signature composed of 8 genes within the 50 biomarkers. This signature allows us to define an age score. The latter based on the expression level of the 8 genes predicts the anti-aging potential of products. Therefore, we evaluated the anti-aging potential of 2 products; Astragaloside 4, a plant compound renowned in the Chinese Pharmacopoeia for its anti-aging properties and a novel metabiotic ingredient produced via a multi-fermentation process and known to promote longevity, **[8,9]**.

2. Materials and Methods

1- Cell culture

Human primary fibroblasts from five Caucasian donors (63, 62, 23and 28 years old) were grown in specific medium: DMEM - Dulbecco's Modified Eagle Medium (with pyruvate, glutamax, 4,5 g/l glucose; GIBCO Cat.No.31966-021) from Life Technologies™ supplemented with 10% FBS (Fetal bovine serum, GIBCO Cat.No.10500-064) from Life Technologies™ and 1% PS (Penicillin-streptomycin, GIBCO, Cat.No.15140122). Cells underwent extensive quality controls such as negative results for HIV-1, Hepatitis-B, Hepatitis-C. Cultures were maintained in an incubator equilibrated with 5% CO2 at 37°C. We observed the expected morphology of healthy normal fibroblasts.

2- Tested products

Retinol (Sigma-Aldrich, R7632) is derived from vitamin A and is known for its powerful effects on cell regeneration and reducing signs of aging.

Astragaloside IV (Millipore, 74777) was obtained through a patented manufacturing process. It is a bioactive compound extracted from the root of *Astragalus membranaceus*, benefiting from the unique immunomodulatory, anti-inflammatory and antioxidant properties.

GLYULT1 was obtained according to a patented manufacturing process. It is a polysaccharide complex extracted from *Aphanothece sacrum* and red algae, benefiting from the diversity and bioactivity of glycan structures in the polysaccharides.

3- Treatment

The human primary fibroblasts of 63, 62 years old were treated once a day for 2 days with Retinol (R) at 2 µM, with Astragaloside (AS4) at 30 µM, with GLYULT1 at 1% or left untreated. The human primary fibroblasts of 28 and 23 years old were left untreated.

4- RNA extraction and RT-qPCR

Total RNA with miRNAs from fibroblasts were extracted and purified using a RNeasy Plus Mini Kit (QIAGEN, 74134), following the manufacturer’s instructions. Quality control and total RNA quantitation were performed using Agilent RNA Pico kit and Analysis Agilent 2100 Bioanalyzer. For mRNA target quantitation, total RNA was reverse transcribed with the Superscript VILO

cDNA Synthesis Kit (ThermoFisher) according to the manufacturer's instructions. Quantitative PCR was performed with a Platinum Quantitative PCR SuperMix-UDG Kit (Invitrogen) according to the manufacturer's instructions using the CFX-connect (Biorad). The results were normalized to endogenous control GAPDH expression. Targets primers and GAPDH primers were computationally designed and bought from our knowledgeable suppliers.

5- Aging signature

The expression of 45 genes and 5 miRNAs were quantified with qPCR. A machine learning model was trained to discriminate between old samples on one side, and young and Retinol-treated old samples on the other side. A Lasso regression was then used to select genes with the best predictive potential. 8 genes (and no miR) showed good predictive potential and were selected for the downstream model. A SVM model with RBF kernel was used to discriminate the samples using only the 8 selected genes. The decision function of the model was then extracted to compute a score. This signature score is computed from the expression of our 8 genes-signature and can be used to evaluate the ability of an active to restore the characteristics of a young cell, in the same way as Retinol does.

3. Results

1. Retinol effect on aged human primary fibroblasts

63 and 62-aged fibroblasts were treated with retinol. We assessed the expression levels of 50 genes that control various mechanisms responsible for aging. Their expression in two young donors was also evaluated. We use gene heatmap algorithm to group genes according to their expression. Figure 1 shows hierarchical clustering of gene expression and samples. Each column indicates the expression of one gene and each row represents one condition. Aging is known to increase transcriptional noise. **[10]** Thus, in Figure 1, we observed that young fibroblasts cluster together in the same branch, while older donors are far apart. Treatment with retinol brings the two older donors closer to each other and closer to the younger donors. This suggests that retinol treatment may reduce transcriptional noise due to age.

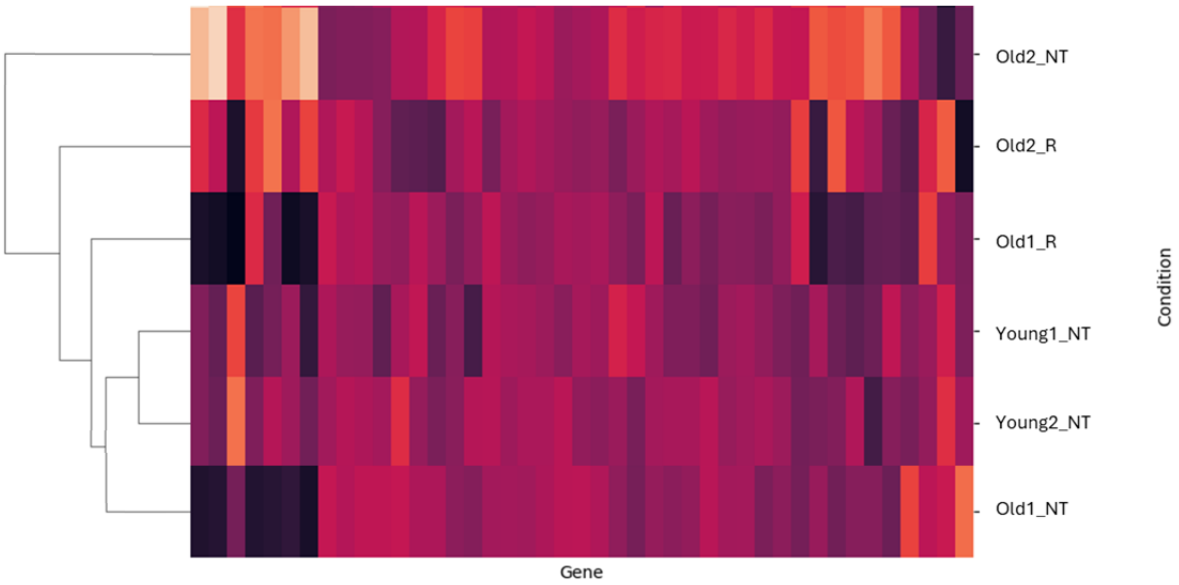

Figure 1. *Visualization of gene expression profiles for the different conditions. The qPCR expression values are shown for each of the 50 genes. Expression values are normalized to housekeeping gene expression. Values are shown in CT units: higher values mean lower gene expression. Cells from three aged donors (Old1, Old2) were treated with Retinol (R), for comparison with untreated (NT), and untreated younger donors (Young1 and Young2). Hierarchical clustering was then performed using the distances between gene expression profiles.*

However, not all genes are regulated by retinol and these genes weaken the classification. We therefore decided to use training methods to identify, among these genes, the ones specific to the rejuvenating action of retinol: the retinol signature.

2. Model building and training for retinol signature

2.1. Retinol based Anti-aging signature building

Following the initial exploration of the gene expression profiles. Samples were randomly divided into a training dataset and a test dataset. Using a dedicated deep learning algorithm combined with a Lasso regression analysis, we constructed a retinol signature composed of 8 markers. These markers are not disclosed in this paper because the patent is in progress. Figure 2 presents hierarchical clustering of gene expressions and samples. Each column indicates the expression of one gene of the 8 genes and each row represents one condition. In the figure 2, we observed that young fibroblasts cluster together in the same branch as the old donor cells treated with retinol. Cells treated with retinol and young cells are clustered in two branches next to each other. The older donors are far apart with high transcriptional noise. Thus, this signature appears to highlight retinol's ability to mitigate the effect of age and bring physiologically aged cells closer to young cells.

Figure 2. Visualization of gene expression profiles for the different conditions. The qPCR expression values are shown for each of the 8 signature genes. Expression values are normalized to housekeeping gene expression. Values are shown in CT units: higher values mean lower gene expression. Cells from two aged donors (Old1 and Old2) were treated with Retinol (R) and Astragaloside IV (AS4), for comparison with untreated (NT), and untreated younger donors (Young1 and Young2). Hierarchical clustering was then performed using the distances between gene expression profiles.

An average of the significance of gene expression across all the genes and the samples is used to create a score. This score represents a numerical value quantifying the alignment of a specific condition to a "youthful" biological profile (young donors). The further a condition is from young donors and retinols, the more the score decreases. Therefore, higher signature scores indicate greater anti-aging effects, as they reflect closer alignment with the youthful signature (young donors).

This score is crucial to identify treatments with the strongest retinol-like effect i.e. products with promising anti-aging potential.

Figure 3 shows Retinol signature score for aged, retinol treated-aged cells and young cells. We showed that fibroblast cells treated with retinol scored as well as young cells.

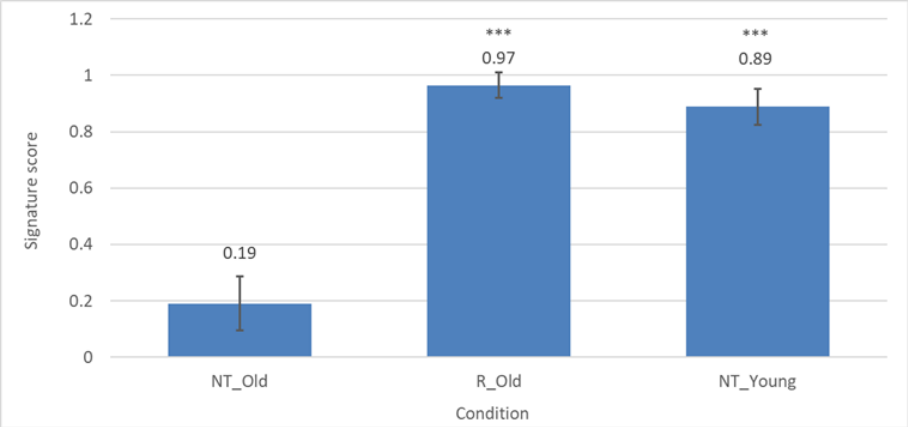

Figure 3. Retinol signature score. The gene expression profile of each sample was analyzed using the age signature model. Resulting scores were averaged for each condition. The error bars represent the standard error of the mean. Student t-test were performed against the untreated old condition (*p-value <0.05, **p-value < 0.005, ***p-value < 0.001).

2.2. Anti-aging signature validation

After developing the anti-aging model, we tested its capabilities by evaluating a novel metabiotic ingredient - produced through a multi-fermentation process, has garnered attention for its potential role in promoting longevity and combating signs of aging - and a known anti-aging ingredient - Astragaloside IV. To do this, 63 and 62-aged fibroblasts were treated or not with Astragaloside IV and GLYULT1. We assessed the expression levels of the 8 genes of the retinol like-signature and define the signature score for each ingredient. They are also compared to aged, young donors and aged donors treated with retinol.

In Figure 4, the methodical application of the signature score analysis is presented, specifically evaluating the anti-aging potential of Astragaloside and GLYULT1. Aged cells treated with Astragaloside and GLYULT1 have a much better score than aged control donors. This suggests the anti-aging potential of these ingredients.

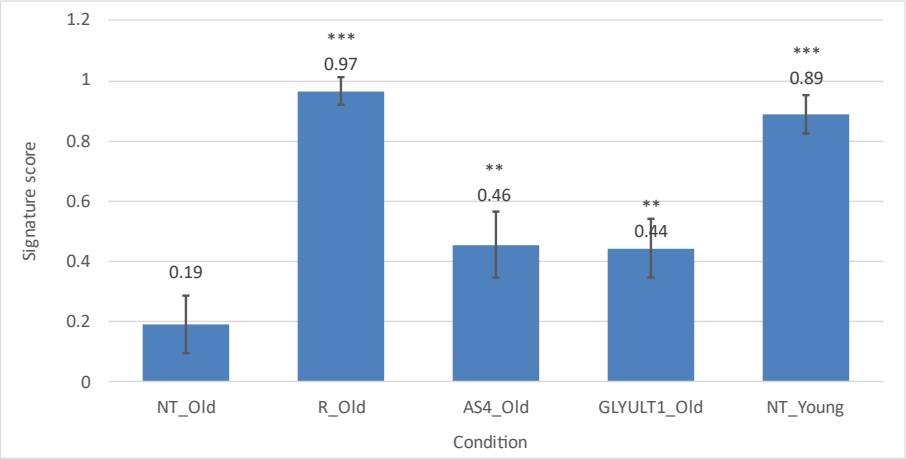

Figure 4. Signature score. The gene expression profile of each sample was analyzed using the age signature model. Resulting scores were averaged for each condition. The error bars represent the standard error of the mean. Student t-test were performed against the untreated old condition (*p-value <0.05, **p-value < 0.005, ***p-value < 0.001).

4. Discussion

Skin aging is a natural process resulting from several factors, including cellular aging. Over time, skin cells undergo significant changes that affect their ability to divide, regenerate, and function optimally. **[2]** This phenomenon, known as cellular senescence, is largely responsible for the visible signs of skin aging. **[11]** Additionally, cellular renewal slows down, leading to an accumulation of dead cells on the surface and a dull complexion. Skin cells also become less efficient at repairing damage caused by environmental factors such as UV rays, pollution, and oxidative stress. **[11]** In parallel, cellular aging is often accompanied by a reduction of autophagy, which is the process of cleaning and recycling damaged cellular components. **[12]** Consequently, skin aging leads to visible alterations in skin appearance, such as wrinkles, dark spots, and dryness. **[11]** These changes can greatly impact our self-esteem.

Developing active ingredients that combat the signs of aging can be beneficial in addressing both the physical and psychological effects, thereby enhancing our quality of life. By targeting the underlying causes of cellular aging, these products can help maintain younger, firmer, and more radiant skin, while delaying the appearance of wrinkles and age spots. we evaluated the potential of retinol, a vitamin A derivative, widely regarded as a key ingredient in skincare due to its extensive anti-aging properties.**[4]** The active ingredient GLYULT1 provided by Sethic Innovation Labo, was tested in this study by RT-qPCR to assess which pathways were regulated, and by aging signature. We confirmed the efficacy of the active compound by assessing the potential of Astragaloside IV, a plant featured in the Chinese Pharmacopoeia and renowned for its anti-aging properties through telomerase activation and oxidative stress reduction.**[8,9]** The analysis presented in this study provides a comprehensive evaluation of the anti-aging potential of retinol, Astragaloside IV, and GLYULT1.

Firstly, the anti-aging signature was built using results from retinol-treated old and young donors, with a Lasso regression analysis performed to identify the genes with the strongest predictive potential. 8 markers demonstrated high predictive capability and were selected for use in the downstream model. The anti-aging model showed great results where the young donor groups, and the retinol-treated old donor groups are closely aligned.

Then we confirmed this signature using Astragaloside IV and evaluated the potential of a new metabiotic ingredient. This metabiotic product, produced by a multi-fermentation process, has garnered significant attention for its potential role in promoting longevity and combating aging. GLYULT1-treated groups cluster alongside Astragaloside IV-treated groups, both indicating a more modest effect compared to retinol. Moreover, the signature score analysis introduced, a metric derived from the 8-gene anti-aging signature, quantifies how closely a condition aligns with a youthful biological profile. The results validate the aging signature, showing retinol's strong anti-aging effect with scores clustering closely to the young donor group. Astragaloside IV demonstrates notable, albeit less pronounced, efficacy, while GLYULT1 exhibits a comparable response to Astragaloside IV. These findings highlighted that GLYULT1 has a potential as good as Astragaloside IV, an anti-aging molecule widely described in the literature.

5. Conclusion

Through this new anti-aging signature A.I retinol based, we studied the anti-aging potential of a new compound which our study demonstrated a promising anti-aging effect. Additionally, by leveraging biomarkers of aging, we achieved a comprehensive perspective on skin aging, encompassing donor characteristics and gene expression profiles. This approach significantly expands our understanding of retinol alternatives and complementary solutions.
By leveraging biomarkers of aging, our comprehensive AI-driven strategy provides an unparalleled understanding of skin aging, encompassing donor characteristics, gene expression profiles, and more. This holistic approach is instrumental in advancing our exploration of retinol alternatives and complements, paving the way for innovative solutions.

6. References

[1] Fore J. A review of skin and the effects of aging on skin structure and function. *Ostomy Wound Manage*. 2006;52(9):24-37.
[2] Farage MA, Miller KW, Elsner P, Maibach HI. Intrinsic and extrinsic factors in skin ageing: a review. *Int J Cosmet Sci*. 2008;30(2):87-95.
[3] Shin SH, Lee YH, Rho NK, Park KY. Skin aging from mechanisms to interventions: focusing on dermal aging. *Front Physiol*. 2023;14:1195272. Published 2023 May 10.
[4] Quan T. Human Skin Aging and the Anti-Aging Properties of Retinol. *Biomolecules*. 2023;13(11):1614. Published 2023 Nov 4.

[5] Shao Y, He T, Fisher GJ, Voorhees JJ, Quan T. Molecular basis of retinol anti-ageing properties in naturally aged human skin in vivo. *Int J Cosmet Sci*. 2017;39(1):56-65.
[6] SCCS., S. C. REVISION of the scientific Opinion (SCCS/1576/16) on vitamin A.
2022 Oct.
[7] López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. Hallmarks of aging: An expanding universe. *Cell*. 2023;186(2):243-278.
[8] Lin R, Chen H, Callow D, et al. Multifaceted effects of astragaloside IV on promotion of random pattern skin flap survival in rats. *Am J Transl Res*. 2017;9(9):4161-4172. Published 2017 Sep 15.
[9] Chen B, Li R, Yan N, et al. Astragaloside IV controls collagen reduction in photoaging skin by improving transforming growth factor-β/Smad signaling suppression and inhibiting matrix metalloproteinase-1. *Mol Med Rep*. 2015;11(5):3344-3348.
[10] C. Nikopoulou, S. Parekh, P. Tessarz, Ageing and sources of transcriptional heterogeneity, Biol. Chem., 400 (2019), pp. 867-878
[10] Chin T, Lee XE, Ng PY, Lee Y, Dreesen O. The role of cellular senescence in skin aging and age-related skin pathologies. *Front Physiol*. 2023;14:1297637. Published 2023 Nov 22.
[11] Murase D, Kusaka-Kikushima A, Hachiya A, Fullenkamp R, Stepp A, Imai A, Ueno M, Kawabata K, Takahashi Y, Hase T, et al. Autophagy Declines with Premature Skin Aging resulting in Dynamic Alterations in Skin Pigmentation and Epidermal Differentiation. *International Journal of Molecular Sciences*. 2020; 21(16):5708.