

COUNTERACTING AGING PHENOMENA BY NEW PURE TETRAPEPTIDES WITH TARGETED EFFICACY

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Summary

In the skin, proteoglycans are present both in the epidermis and the dermis. In the latter layer, a group of small proteoglycans (< 60 kDa) plays an important role in fibrillogenesis, growth factors modulation and dermal homeostasis. The epidermis also synthesizes several small proteoglycans, involved in keratinocyte functionality.

The knowledge about the alteration of the synthesis and the structure of such small proteoglycans during skin aging used to be rather limited in the past. In fundamental studies, Laboratoires Sérobiologiques could recently identify two small proteoglycans whose influence in skin proper functioning is significant, and whose synthesis decreases with aging. Lumican is located in the dermis, where it is involved in the formation of the collagen fibers and consequently in the skin firmness and thickness. Syndecan-1 occurs preferentially in the supra-basal layers of the epidermis; it is jointly implicated in the epidermal cohesion.

The dermo-epidermal junction assures the connection between dermis and epidermis. With certain anchor molecules like collagen XVII, hemidesmosomes attach the basal cells to the basal membrane.

The stimulation of the synthesis of lumican in dermal cells, of syndecan-1 in epidermal cells and of collagen XVII in the basal layer counteracts the aging process on three different levels in the skin. In order to identify ingredients with a corresponding activity, skin specific DNA-arrays were used, allowing to selectively analyze the expression of genes highly implicated in the skin physiology. Two efficient acetylated tetrapeptides (respectively AcTP1 and AcTP2) were selected.

The efficacy of both peptides was further demonstrated on cell cultures, skin models and finally in clinical studies.

Riassunto

I proteoglicani sono presenti sia a livello dell'epidermide che del derma. Nel derma un gruppo di proteoglicani a basso peso molecolare (p.m) (<60k Da) gioca un ruolo importante nella fibrillo genesi, nella modulazione dei fattori di crescita e nell'omeostasi di questo strato cellulare. Comunque, anche l'epidermide sintetizza diversi piccoli proteoglicani, coinvolti nella funzionalità dei cheratinociti.

Negli anni passati l'alterazione che si verifica durante l'invecchiamento nella sintesi di strutture quali i proteoglicani a basso p.m era poco conosciuta. Recentemente i laboratori della Seròbiologique hanno identificato due piccoli proteoglicani che influenzano in modo significativo le funzioni cutanee, la cui sintesi viene a ridursi durante l'invecchiamento.

Così il proteoglicano denominato Lumican è localizzato nel derma dove è coinvolto nella formazione delle fibre di collagene e quindi nello spessore e nella compattezza della cute.

Il proteoglicano Syntecam-1 si riscontra normalmente negli strati soprabasali dell'epidermide ed è implicato nella coesione delle cellule epidermiche.

La giunzione dermo-epidermica rappresenta il punto di congiunzione tra epidermide e derma attraverso l'attività di legame svolta da alcune molecole quali il collagene XVII e gli emodesmosomi.

La stimolazione esercitata rispettivamente da Lumican a livello delle cellule dermiche e del collagene XVII esercita un effetto positivo sul processo dell'invecchiamento cutaneo a tre diversi livelli.

Per verificare l'effettiva attività svolta dagli ingredienti in studio, ne è stata verificata l'efficacia con una serie di DNA specifici analizzando selettivamente l'espressione dei geni implicati a livello fisiologico. Sono stati così selezionati due specifici tetrapeptidi acetilati (AcTP1 e AcTP2). L'efficacia di entrambi i peptidi è stata dimostrata su culture cellulari, su modelli di cute ed infine a livello clinico.

INTRODUCTION

Cosmeceuticals or so-called 'doctor' brands have recently enjoyed increasing appeal to consumers because they offer anti-ageing benefits without the need to undergo invasive procedures. To achieve this, a growing number of active ingredients is being incorporated into skincare products.

This trend holds out the possibility of sustainable growth for cosmeceutical products but also poses challenges to suppliers of active ingredients. Cosmetics manufacturers are seeking ever more effective and innovative cosmetic ingredients, such as enzymes, amino acids and peptides, with identified and specific mechanisms of action and demonstrable benefits that perfectly fit into the growing cosmeceutical trend.

The phenomenon of skin ageing and the identification of its biological pathways remain a constant focus of many research programmes. Laboratoires Sérobiologiques (LS), the active ingredient business of Cognis Care Chemicals, has recently undertaken a fundamental research study targeting one specific family of molecules, small proteoglycans.

Small proteoglycans are characterised by glycosaminoglycan chains fixed to a linear core protein by covalent bonds. They are present both in the epidermis and the dermis and act as biomechanical supports, tissue organisers and biological filters, thereby playing an important role in skin homeostasis. However, knowledge of their synthesis and structure during skin ageing has always been rather limited.

Our research programme has identified two specific proteoglycans – lumican1 and syndecan-1 - whose synthesis counteracts the ageing process in the skin and has been demonstrated to decrease with ageing.

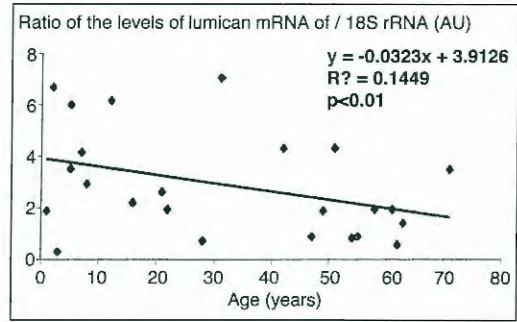


Fig. 1 Lumican during skin aging – Variation in dermal fibroblasts with age (Northern-blot).

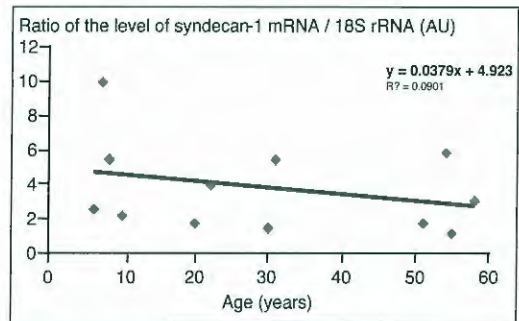


Fig. 2 Syndecan-1 during skin aging – Variation in keratinocytes with age (Northern-blot).

Lumican is located in the dermis, where it is involved in the formation of collagen fibres and consequently in skin resistance to traction. Syndecan-1 occurs preferentially in the supra-basal layers of the epidermis and is involved in epidermal cohesion.

In order to identify ingredients with corresponding activity, skin-specific DNA arrays² were used. This allowed us to screen selectively potential actives that would influence the expression of genes coding for the two identified proteoglycans but also growth factors and structural proteins involved in skin structure and homeostasis.

Based on these findings, LS has developed two new pure tetrapeptides, **ACTP1** (INCI name: Glycerin (and) Acetyl Tetrapeptide-9) and **ACTP2** (INCI name: Glycerin (and) Acetyl

Tetrapeptide-11), which are designed to fight the skin ageing process. The efficacy of both was further demonstrated on cell cultures, skin models and finally in clinical studies.

At the dermis level: to optimize collagen fibers functionality

ACTP1 has been singled out for its unique biological anti-age action, which is targeted at increased collagen network functionality via lumican synthesis. DNA array tests² made it possible to evaluate ACTP1's influence on the gene-

ral gene expression profile of dermal fibroblasts including lumican and the structural protein COL1A1 coding for collagen I.

Stimulation of lumican synthesis by aged human dermal fibroblasts showed that ACTP1 achieved increases of 66% and 115% over a control in the expression index, as measured in terms of the number of stained pixels by fluorescence intensity of the green channel in arbitrary unit, when used at 0.74 and 2.2 $\mu\text{g/ml}$ respectively. IL-4 at 0.1 $\mu\text{g/ml}$ scored a 73.6% increase over the control

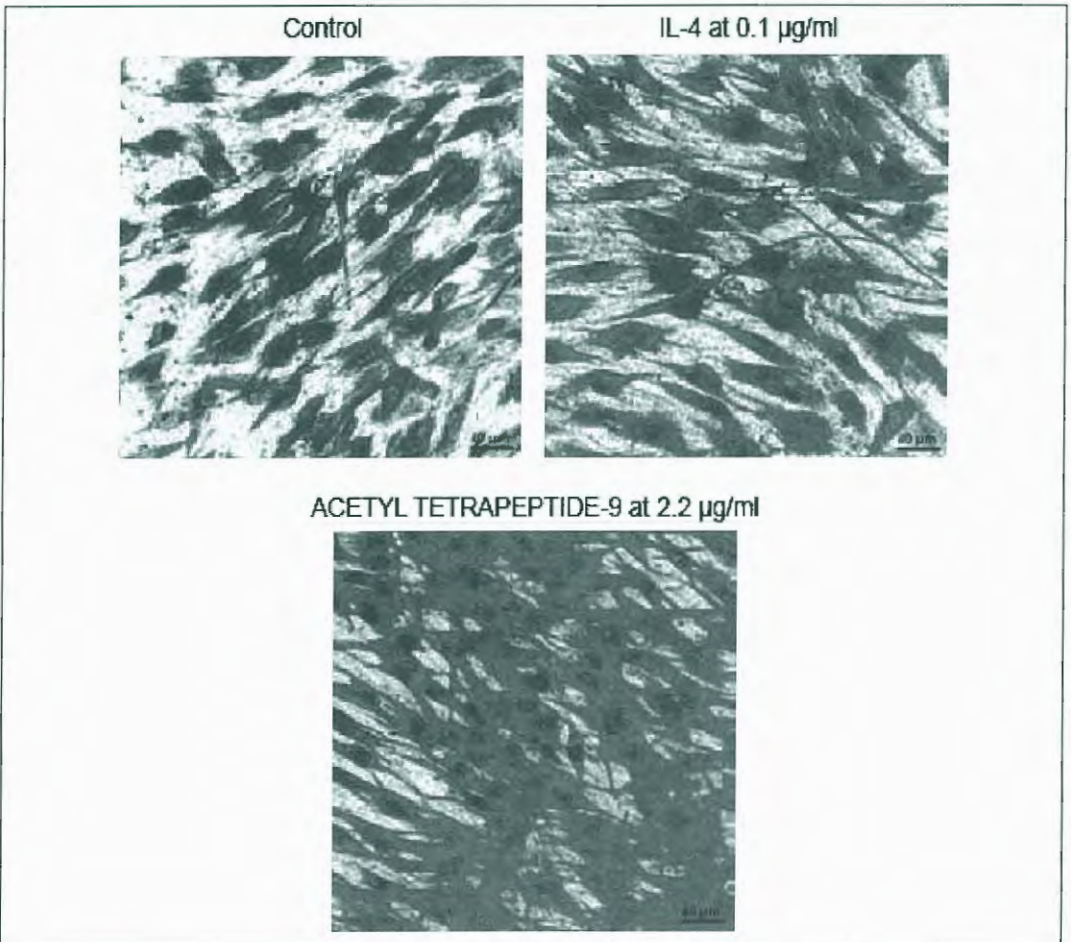


Fig. 2 Stimulation of lumican synthesis by aged human dermal Fibroblasts: vizualisation (lumican in green).

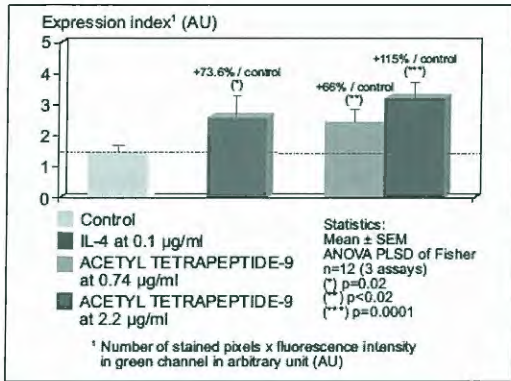


Fig. 3 Stimulation of lumican synthesis by aged human dermal Fibroblasts: quantification.

Likewise, in the expression of COL1A1 gene, 2.2 µg/ml ACTP1 showed a 22% increase over the control, while TGF-β1 (the reference substance)³ at 10 ng/ml showed a 125% increase. In the stimulation of collagen I synthesis, ACTP1 showed increases of 49% and 112% at 2.2 and 7.4 µg/ml respectively; TGF-β1 at 3 ng/ml showed a 65% increase. For proteins in the same test, the increases were 4%, 5% and 20% respectively.

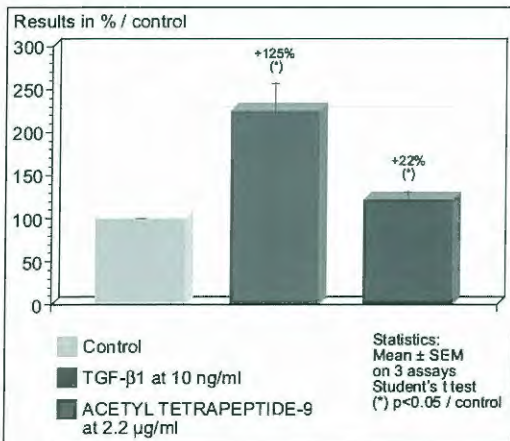


Fig. 4 Stimulation of the expression of collagen I COL1A1 gene by human dermal fibroblasts.

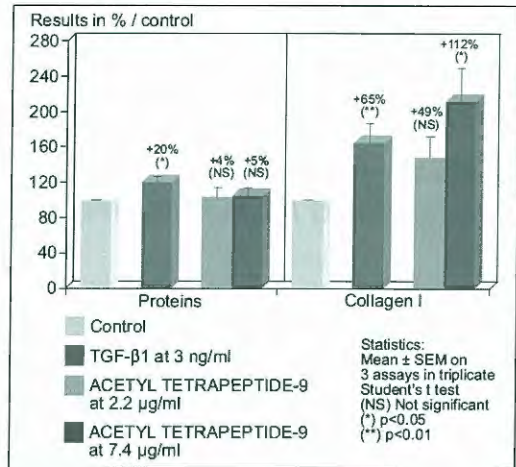


Fig. 5 Stimulation of collagen I synthesis by human dermal fibroblasts using DOT-BLOT technique⁴.

Thus, lumican is directly involved in the synthesis of the collagen fibers (fibrillogenesis) and consequently in the organisation of the collagen bundles, resulting in tighter and firmer skin. Several *in vitro* tests have demonstrated ACTP1's capacity to boost the synthesis of lumican and collagen I.

ACTP1's efficacy and tolerability were demonstrated in a clinical study with 17 female volunteers aged between 45 and 55. After four months of treatment, it induced an increase in skin thickness (evaluated on the forearm by echography/ultrasound^{5,6} using the 2D DermaScan C[®] equipment with a 20MHz probe) and an improvement of skin firmness (the evaluation of variations in the extensibility and elasticity of superficial cutaneous layers was performed using a SEM 575 CUTOMETER^{®7}).

By acting both on the structure and mechanical properties of skin, it confirmed its anti-ageing efficacy, leading to a firmer and plumper skin. With ACTP1, we are moving to the next generation of anti-ageing products. Beyond collagen synthesis, we also ensure that newly synthesized fibers are fully functional.

Based on its mechanism of action, this active

ingredient is also suitable for other applications related to firmer skin, and it is thus distinctly possible that ACTP1 may improve stretch marks as well.

At epidermis level: to restore the radiance and firmness of mature skin

With age, the epidermis loses its resistance and radiance, mainly due to reduced cohesion of the epidermal cells. As a result, skin becomes dry and slack and is easily damaged with even the lightest friction or shock. To prevent this, manufacturers of personal care products must design products to meet the specific needs of mature skin.

ACTP2 specifically targets the two constituents

of the epidermis that are primarily responsible for its cohesion: syndecan-1 and collagen XVII. The latter is a protein that occurs in the hemidesmosomes, a microscopic, rivet-like structure that affects the strength of the bond between the epidermis and the dermo-epidermal junction⁸.

The overall effects of ACTP2 were established by DNA array² technology. This test made it possible to evaluate ACTP2's influence on the general gene expression profile^{9,10} of human primary epidermal keratinocytes.

Several in vitro tests on keratinocytes demonstrated ACTP2's ability to boost the synthesis of syndecan-1, reinforcing epidermal cohesion, and the production of collagen XVII protein, leading to an improvement of the adhesion between the epidermis the and dermo-epidermal junction.

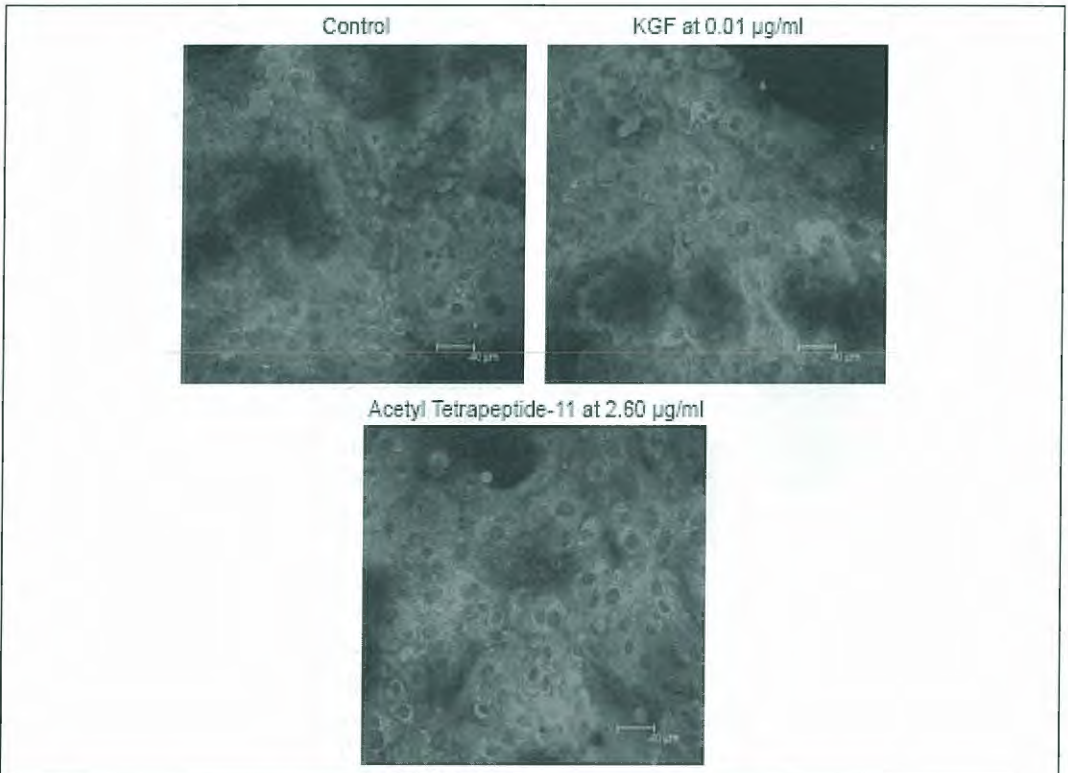


Fig. 6 Stimulation of syndecan-1 synthesis by human keratinocytes: visualization at 5 days (syndecan-1 in green).

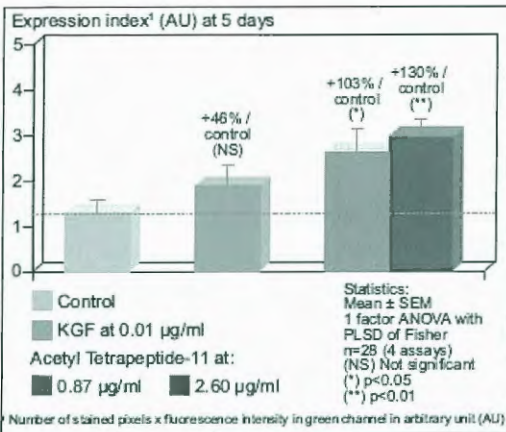


Fig. 7 Stimulation of syndecan-1 synthesis by human keratinocytes: quantification.

Over five days, ACTP2 achieved increases of 103% and 130% in the synthesis of human keratinocytes by comparison with a control, as measured by the number of stained pixels by fluorescence intensity of the green channel in arbitrary unit, when used at 0.87 and 2.6 µg/ml respectively. KGF (the reference substance¹¹) at 0.01 µg/ml, meanwhile, scored a 66% increase over the control.

In the expression of the collagen XVII COL17A1 gene over three days, meanwhile, ACTP2 achieved increases of 13% and 18% over a control when used 0.87 and 2.6 µg/ml respectively.

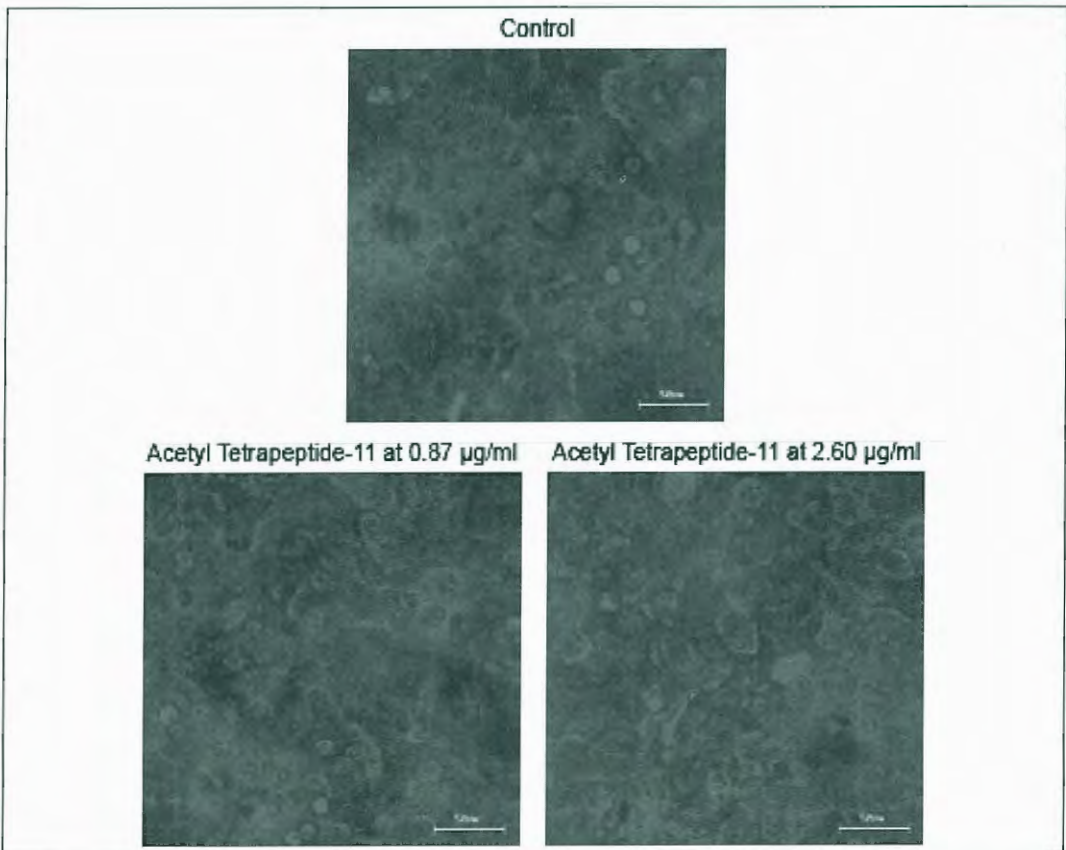


Fig. 8 Stimulation of the synthesis of collagen XVII protein by human epidermal keratinocytes: visualization at 3 days.

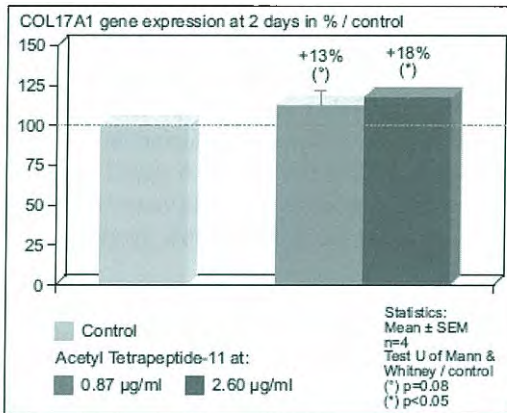


Fig. 9 Stimulation of the expression of collagen XVII COL17A1 gene by human epidermal keratinocytes: visualization at 3 days.

The efficacy and tolerability of ACTP2 was proven in a clinical test on 19 female volunteers aged between 60 and 70. After a two-month course of treatment, the biomechanical firmness and elasticity of the superficial epidermal layers had increased significantly and the skin texture was refined, so that the subjects looked visibly younger.

For a comprehensive anti-age approach, the combination of ACTP2, specific for the epidermis, with ACTP1, which specifically targets aging of the dermis is an optimal choice of ingredients for various easy-to-use products in the growing market for cosmeceutical skin care products.

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