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How the genomic analysis could help the Development of the cosmetic formulation

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1. Introduction

The skin, as the body's outermost organ, serves as a complex and dynamic interface with the external environment. It plays essential roles in protection, thermoregulation, hydration, and immune surveillance [1, 2]. These functions are supported by a multilayered structure, including the epidermis, dermis and hypodermis, which relies on the coordinated activity of keratinocytes, fibroblasts and adipocytes to maintain tissue homeostasis [3]. However, with age, the skin undergoes a progressive decline in regenerative capacity, elasticity, and hydration, driven by both intrinsic genetic programming and extrinsic factors such as ultraviolet (UV) exposure, pollution, and oxidative stress [4, 5].

Aging skin is marked by alterations in the extracellular matrix (ECM), including reduced levels of hyaluronic acid and collagen, as well as by dysregulation of cellular signaling pathways that control differentiation, proliferation, and inflammation [6]. At the molecular level, transcriptomic studies have revealed widespread changes in gene expression patterns with age, affecting genes involved in tissue remodeling, barrier function, and immune responses [7, 8]. These changes not only reflect the skin's diminished capacity to regenerate, but also its altered responsiveness to cosmetic or therapeutic interventions.
In this context, the cosmetic industry increasingly relies on high-throughput genomic technologies such as DNA microarrays or RNA sequencing to investigate the biological effects of active ingredients and complex formulations [9]. These approaches allow for comprehensive profiling of cellular responses, helping to identify molecular signatures linked to hydration, anti-aging, barrier reinforcement, or inflammation modulation. Such mechanistic insights are crucial for developing next-generation cosmetic products that are both effective and biologically targeted.

Among the most promising cosmetic strategies are formulations combining different types of hyaluronic acid (HA), a key molecule for hydration and tissue volume maintenance. In this study we test a complex formulation composed by different ingredients. CRISTALHYAL E-PERFECTION is a high molecular weight (HA), with bioactive compounds capable of

stimulating ECM synthesis, modulating inflammation or promoting the hypodermis structure [10]. PrimalHyal™ Ultrafiller is a HA derivative designed to penetrate twice deeper into the skin than standard HA and is resistant to hyaluronidases thanks to its full acetylation, for a longer lifetime in the skin. Likewise, rhamnose-based compounds have emerged for their soothing and anti-inflammatory properties [11], though the molecular mechanisms behind these effects remain underexplored. However, the Si-Rhamnose, a derivative of organic silicon and rhamnose sugar, has shown strong anti-wrinkle, lifting, anti-aging effect, optimizing skin repair processes. Finally, the LYSO-BOOST (from Terra Cosmetics) is a lysophospholipid (LPL)-based compound. LPL have been shown to restore skin barrier integrity by modulating lipid composition and enhancing keratinocyte cohesion. It has been shown to improve skin hydration and elasticity while reducing transepidermal water loss.

Here, we investigated the transcriptomic impact of a novel cosmetic complex composed of hyaluronic acid derivatives (CRISTALHYAL E-PERFECTION, PRIMALHYAL ULTRAFILLER) and bioactive ingredients (LYSO-BOOST TC, SIRHAMNOSE). Using ex vivo human skin explants and microarray analysis, we aimed to characterize the evolution in gene expression induced by a cosmetic formulation and evaluate the relevance of transcriptomic tools in guiding the development of next-generation dermocosmetic products.

2. Materials and Methods

2.1 Skin explants preparation

Human abdominal skin explants were obtained from healthy female donors aged between 35 and 60 years, undergoing elective abdominoplasty. Written informed consent was obtained in accordance with the Declaration of Helsinki and French Public Health Code (Article L.1243-4) from the patients who allowed the anonymous utilization of their surgical waste for the ex-vivo studies there is no need the ethics committee approval for the use of surgical waste in Europe. For this study, 21 skin explants were prepared in total from a 43-year-old Caucasian woman (Fitzpatrick phototype II), by carefully removing subcutaneous fat under sterile conditions and punching dermal discs of 11 ± 1 mm in diameter. Explants were immediately transferred to the specific BIO-EC's Explants Medium (BEM) (laboratory own culture medium) and maintained in a humidified atmosphere at 37°C with 5% CO_2 throughout the experiment.

2.2 Test products and study groups

The study included four groups: untreated controls collected at baseline (Day 0, n=3), untreated controls at Day 3 and Day 8 (n=3 per time point), excipient controls (DMSO-treated, n=3 per time point), and treated samples receiving the cosmetic complex (n=3 per time point). The tested formula consisted of 1% CRISTALHYAL E-PERFECTION, 0.2% PRIMALHYAL ULTRAFILLER, 1.25% LYSO-BOOST TC, and 5% SIRHAMNOSE dissolved in DMSO. A topical dose of 2 µL/cm² (approximately 2 mg/cm²) was applied topically on the surface of each explant on days 0, 1, 2, 3, 6, and 7. Culture medium (BIO-EC's Explants Medium, BEM) was renewed on days 2, 3, and 6 to maintain explant viability.

2.3 RNA extraction and quality control

On Day 3 and Day 8, explants were collected and immediately frozen at -80°Cfor molecular analysis. Total RNA was extracted from each sample using the ReliaPrep™ RNA Tissue

Miniprep System (Promega) after tissue homogenization in TRIzol reagent. Genomic DNA was removed by DNase I treatment. RNA concentration and purity were assessed by spectrophotometry (BioDrop), with A260/A280 ratios above 1.8 and A260/A230 ratios above 1.6. RNA integrity was confirmed by microcapillary electrophoresis (Agilent 2100 Bioanalyzer), and only samples with RNA quality indicator (RQI) values above 7.0 were used. RNA yields ranged from 2.8 to 6.8 µg per sample.

2.4 Gene expression analysis

Transcriptome profiling was carried out using Agilent SurePrint G3 Human Gene Expression v3 microarrays (8×60K format, Design ID: 072363). For each sample, 100 ng of total RNA was used to synthesize and label cRNA with Cy3 using the One-Color Low Input Quick Amp Labeling Kit (Agilent Technologies). The labeled complementary RNA (cRNA) was hybridized to the arrays according to the manufacturer's protocol.

Raw data were background-corrected using the NormExp method and normalized by quantile normalization using the limma package in R. Differential gene expression was analyzed by two-way ANOVA taking into account the treatment and the time as variables. Genes were considered significantly expressed if they met the criteria of adjusted *p*-value < 0.05 and absolute $\log_2$ fold change > 1.5.

To gain functional insights, differentially expressed genes were analyzed for enrichment in Gene Ontology (GO) terms, biological pathways (Kyoto Encyclopedia of Genes and Genomes -KEGG), and protein-protein interaction networks using the Metascape platform. This allowed for the identification of key biological processes and molecular functions modulated by the cosmetic complex.

3. Results

3.1 Global transcriptomic modulation induced by the cosmetic complex

After microarrays qualitative validation and normalization of the data, the selection of modulated genes was performed by calculating, for each gene, the ratio corresponding to the normalized intensity of treated with the cosmetic complex compared to those of control treated by excipient samples. Based on this method, modulated genes were selected when the ratio was ≥ 1.5 (upregulated) or ≤ 0.65 (downregulated) at Day 3 (D3) and at Day 8 (D8). A total of 122 genes were significantly modulated by the cosmetic complex at D3 (p-value < 0.05), including 65 upregulated and 57 downregulated transcripts. The number of differentially expressed genes decreased at D8 by 13% when 105 genes were significantly modulated by the complex including 40 upregulated and 65 downregulated transcripts.

As shown on the Venn diagrams (Figure 1), only 8 genes were commonly modulated by study complex, both at D3 and D8. This was consistent with the Main Component Analysis indicating that the time has a great influence on the results and distinct number of genes was modulated by the cosmetic complex at D3 or at D8.

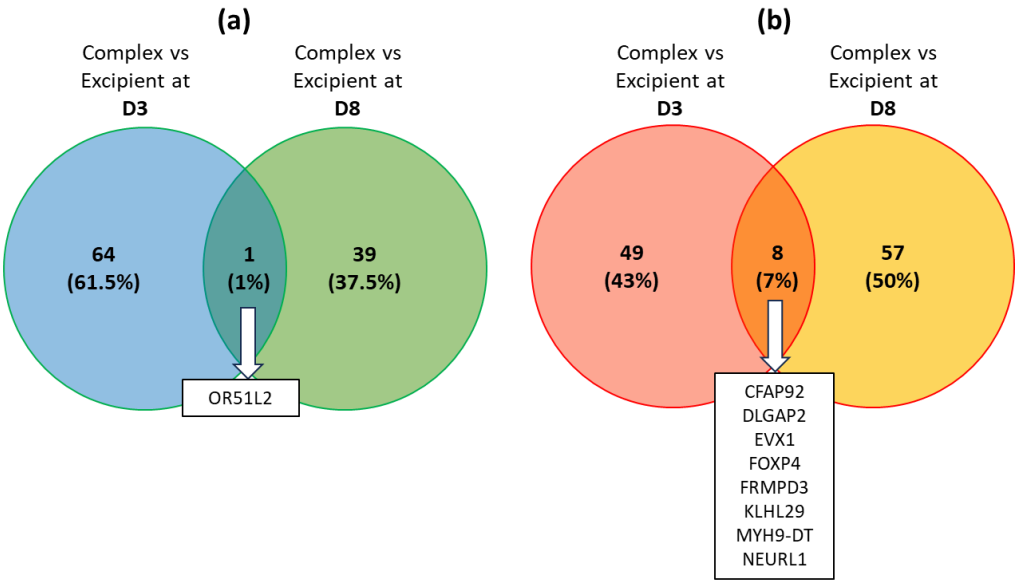

Figure 1. Venn diagrams representing the modulated genes between D3 and D8. The number of genes that were induced by study complex were represented in panel (a), and repressed genes are represented in panel (b). The commonly modulated genes by complex treatment at D3 and D8 are listed in the white boxes.

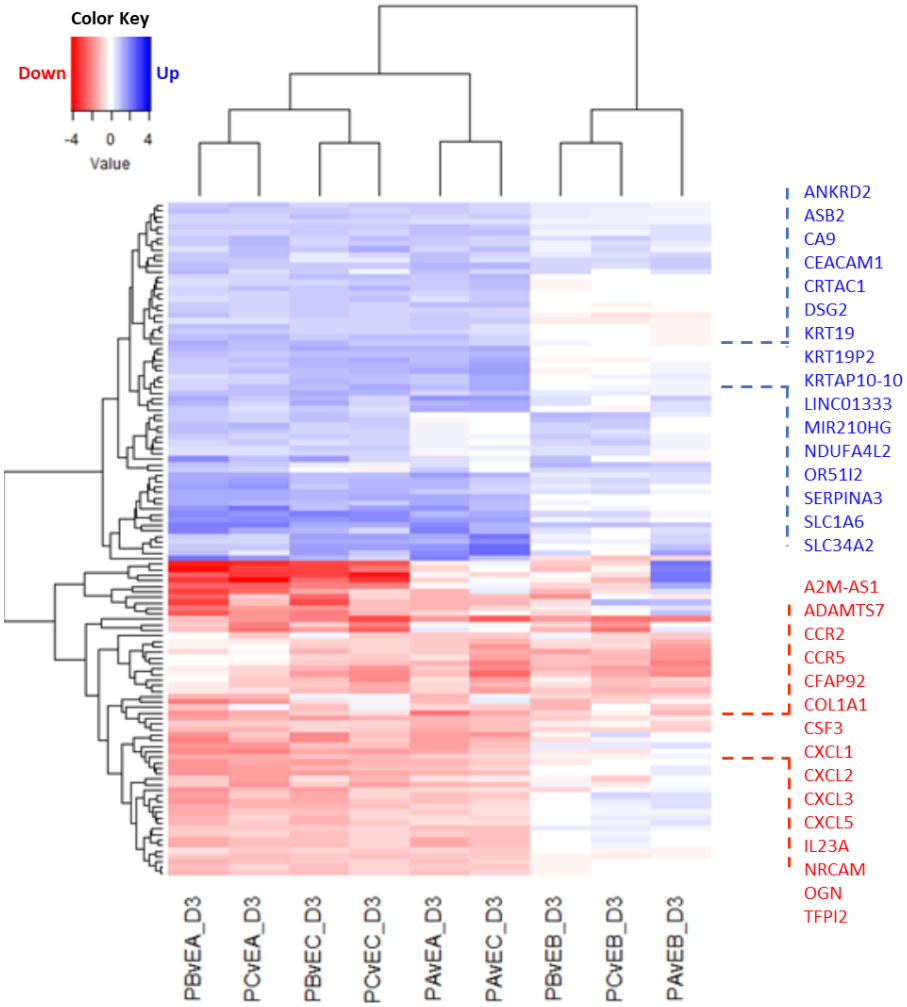

Figure 2. Heatmap of modulated genes at D3 after the cosmetic complex treatment. Heatmap was performed on the modulated genes by the treatment vs excipient at D3 (122 genes) based on ratio approach and Log^2(ratio) values were considered to plot the heatmap.

3.2 Induction of Regenerative and Differentiation Programs

In order to identify the biological processes associated with a modulated gene list, an enrichment analysis by Metascape was performed and focused on Day 3 (D3). Based on the enrichment results (Table 1), the biological activities identified in response to the cosmetic complex at D3 can be potentially related to differentiation process of keratinocytes/regeneration of skin based on the identified terms as “keratinization process”, “inorganic cation transmembrane transport”, “vitamin D receptor pathway” and “homeostasis”.

Table 1. Representative biological process associated with the upregulated genes list at D3 identified by Metascape analysis. For each of the biological terms associated with the selected genes in response to treatments, the $-log^{10}$(P) values were calculated and classified from the highest to the lowest score. Only scores greater than or equal to 2 were considered significant.

Category of Data base	Biological process	Number of genes	$-Log^{10}$(P)	Gene Name
Reactome Gene Sets	Keratinization	6	5.33	DSG2, KRT16, KRT19, PI3, KRTAP10-10, LIPN
GO Biological Processes	regulation of sprouting angiogenesis	3	3.59	CEACAM1, S100A1, DLL1
GO Biological Processes	homophilic cell adhesion via plasma membrane adhesion molecules	4	3.44	CEACAM1, CEACAM5, DSG2, PCDHGC4
GO Biological Processes	digestive system process	3	3.28	SERPINA3, KCNN4, MUC2
WikiPathways	Vitamin D receptor pathway	4	3.25	CEACAM1, CA9, KRT16, SLC34A2
Reactome Gene Sets	Transport of inorganic cations/anions and amino acids/oligopeptides	3	2.86	SLC1A6, SLC9A6, SLC34A2
Reactome Gene Sets	Hemostasis	6	2.81	A1BG, SERPINA, CEACAM1, CECAM5, RASGRP2, KLC2
GO Biological Processes	inorganic cation transmembrane transport	6	2.79	KCNN4, CACNA1H, PLCH2, SLC9A6, SLC34A2, NIPAL3
GO Biological Processes	muscle organ development	4	2.44	CACNA1H, ANKRD2, DLL1, ASB2

GO Biological Processes	regulation of heart contraction	3	2.09	DSG2, S100A1, CACNA1H

Among above mentioned upregulated genes, some have important functional roles in cellular activity and repair process. Here are some examples:

- KRT19 (keratin 19), is a keratinization associated genes, specifically expressed in the periderm, the transient superficial layer that envelopes the developing epidermis is also considered as a differentiation gene marker.
- KRT16 and KRT6 keratin pair functions to maintain collective cell migration and integrity by increasing cell-cell and cell-matrix contact. Their main functions include improving the cytoskeletal structure, wound healing and stress response and improving the barrier function. The tested complex increased significantly this pair.
- Similarly, the upregulated LIPN gene is highly expressed in granular keratinocytes in the epidermis. It encodes the lipase and plays a role in the differentiation of keratinocytes.
- DSG2 is a calcium-binding transmembrane glycoprotein component of desmosomes, cell-cell junctions between epithelial, myocardial, and other cell types.
- S100A1 is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells and involved in the regulation of several cellular processes such as cell cycle progression and differentiation.
- As several keratins, upregulation of PI3/elafin known to contribute also to the cornifyed cell envelope assembly.
- CEACAM1 (CEA Cell Adhesion Molecule 1) was found to be a cell-cell adhesion molecule detected on leukocytes, epithelia, and endothelia. Multiple cellular activities have been attributed to the encoded protein, including roles in the differentiation and arrangement of tissue three-dimensional structure, angiogenesis, apoptosis, tumor suppression, metastasis, and the modulation of innate and adaptive immune responses.
- The cell surface pH regulatory enzyme, CA9 (carbonic anhydrase IX), catalyzes the reversible hydration of carbon dioxide to acidify the cellular microenvironment and provides an extracellular pH control mechanism. In normal skin, the highest expression of CA9, whose inhibition can contribute to increase cellular pH, was detected in hair follicles, sebaceous glands, and basal parts of epidermis.

3.3 Downregulation of Inflammatory and Catabolic Pathways

In parallel with the induction of regenerative genes, enrichment analysis by Metascape was performed on downregulated genes by the cosmetic complex treatment (Table 2)

Table 2. Representative biological process associated with the down-regulated genes list at D3 identified by Metascape analysis. For each of the biological terms associated with the selected genes in response to treatments, the $-\log^{10}(P)$ values were calculated and classified from the highest to the lowest score. Only scores greater than or equal to 2 were considered significant.

Category of Data base	Biological process	Number of genes	$-Log^{10}(P)$	Gene Name
Reactome Gene Sets	Chemokine receptors bind chemokines	6	9.32	CCR5, CXCL1, CXCL2, CXCL3,

				CXCL5, CCR2
WikiPathways	Burn wound healing	3	3.63	COL1A1, ELN, CXCL1
GO Biological Processes	Regulation of T cell mediated immunity	3	3.28	ARG1, IL23A, CCR2
GO Biological Processes	Second-messenger-mediated signaling	3	2.19	CCR5, GUCA2B, CCR2
Reactome Gene Sets	Neutrophil degranulation	4	2.16	A1BG, ARG1, CXCL, FRMPD3
GO Biological Processes	Biological process involved in symbiotic interaction	3	2.06	CCR5, IFI27, SPRR2A

Down-regulation of genes including CCR5, CXCL1, CXCL2, CXCL3, CXCL5, CCR2, ARG1 and IL23a suggest anti-inflammatory properties of the tested formulation according to the identified terms such as “chemokine receptors binding and “regulation of T cell mediated immunity”.
Similarly, silencing of COL1A1 (Collagen I), a major ECM (extracellular matrix molecules) component can suppress self-renewal and promote differentiation process in various epithelial cells. ELN (elastin) was also found to be downregulated. In addition to its structural and mechanical roles, ELN has inherent cell signaling properties that promote a diverse range of cellular responses including chemotaxis, cell attachment, proliferation, and differentiation.

3.4 Temporal Dynamics of the Transcriptomic Response
The temporal analysis of gene expression revealed a dynamic modulation pattern over time. At Day 3 (D3), 122 genes were differentially expressed (65 up-regulated and 57 down-regulated), whereas at Day 8, 105 genes were affected (40 up-regulated, 65 down-regulated). Interestingly, only eight genes were consistently regulated across both time points, indicating a substantial shift in the transcriptomic landscape between the early and late phases of treatment. This suggests that the initial response to cosmetic complex involves a burst of regenerative and anti-inflammatory activity, followed by a transition toward stabilization and tissue remodeling. At Day 8, we found that newly regulated genes were primarily associated with cellular signaling and transport. Enrichment analysis identified significant representation of SLC-mediated transport (p=0.02) and cell fate commitment pathways (p=0.02), suggesting an ongoing tissue reprogramming and sustained cellular adaptation. The shift in gene expression patterns also suggests a resolution phase, in which the tissue re-establishes homeostasis after the initial activation of repair mechanisms. These results highlight the time-dependent nature of the cosmetic complex effect and support a biphasic model of action: an early activation phase that drives tissue repair, followed by a modulatory phase aimed at long-term maintenance of skin integrity.

4. Discussion

High-throughput transcriptomic analysis has become a cornerstone in functional dermatological research, enabling the exploration of complex biological responses to environmental factors and active compounds at a genome-wide scale. In the context of cosmetic science, this technology offers a unique approach to evaluate the molecular efficacy of formulations and to uncover the underlying mechanisms. In the present study, we used transcriptomic profiling via

DNA microarrays to identify biologically relevant signaling pathways modulated by a hyaluronic acid-based cosmetic complex, highlighting its potential for rational product development and targeted claims. By analyzing human skin explants at two distinct time points (Day 3 and Day 8), we were able to capture both the immediate and delayed molecular effects of the cosmetic formulation. This temporal dimension revealed a biphasic response: an early phase characterized by the activation of genes involved in keratinocyte differentiation and barrier repair, followed by a second phase marked by the regulation of genes related to cellular transport and tissue homeostasis. Our findings are consistent with previous studies demonstrating the dynamic nature of the skin transcriptome following topical intervention. Previous studies reported time-dependent transcriptional shifts in response to environmental stressors, with early activation of stress response genes giving way to repair-associated pathways within days [12, 13]. Similarly, a study by Lee et al. on retinoid-based formulations demonstrated a rapid induction of epidermal differentiation genes followed by homeostatic adjustments, mirroring the kinetics observed in our dataset [14]. Among up-regulated genes at Day 3, KRT19, DSG2, S100A1, and LIPN genes suggest an enhancement of epidermal maturation and barrier reinforcement by the cosmetic complex treatment. Enrichment of pathways such as keratinization and cornified envelope formation supports the hypothesis that the cosmetic complex promotes physiological regeneration rather than mere superficial hydration.

A particularly interesting aspect of our findings is the concurrent down-regulation of pro-inflammatory and catabolic mediators. Genes such as CXCL1, CCR5, and IL23A commonly associated with acute inflammation and immune cell recruitment, were significantly repressed following treatment. Moreover, the observed decrease in COL1A1 and ELN expression suggests a loss of cells adhesion to the ECM components which can potentially reflect a remodeling phase rather than a loss of function.

The methodology used in this study integrating microarray analysis and enrichment through platforms such as Metascape, has proven effective in identifying both individual gene changes and broader functional shifts. While RNA sequencing (RNA-seq) is often viewed as the gold standard in transcriptomics due to its greater sensitivity and dynamic range, microarrays remain a valid and cost-effective option for studies focusing on annotated transcripts, particularly in human skin where many targets are well-characterized.

Nonetheless, it is important to acknowledge the limitations of transcriptomic data as a standalone endpoint. Changes in mRNA levels do not always correlate directly with protein expression or functional outcomes, particularly for low-abundance transcripts or genes regulated post-transcriptionally. Additional studies incorporating proteomic or metabolomic profiling, as well as functional assays (such as histology or immunostaining), would provide a more comprehensive understanding of the complex’s biological activity.

5. Conclusion

In conclusion, this study illustrates the value of transcriptomic analysis as a strategic tool in cosmetic development. By enabling the identification of key signaling pathways involved in skin regeneration and inflammation, such approaches support the development of scientifically proven products with measurable biological effects. The cosmetic complex investigated here demonstrates a dual activity: promoting epidermal and dermal repair while attenuating inflammation, consistent with the goals of next-generation dermo-cosmetic formulations.

These findings encourage the continued integration of omics technologies in cosmetic research pipelines, not only to validate efficacy claims but also to guide the rational design of bioactive ingredients and synergistic formulations.

6. References

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