RETINOL+ EMULSION 0.3 GENE ACTIVATION STUDY

STUDY OBJECTIVE

Retinol+ Emulsion 0.3 was evaluated for its epigenetic ability to influence gene expression in the skin.

STUDY DESIGN

Differential gene expression was evaluated 48 hours after an application protocol designed to mimic typical at-home product use. Gene sequencing was performed. Differential gene expression analysis and biological pathway analyses were performed using several bioinformatics software programs.

This laboratory method[1,2,3,4,5] incorporates ribonucleic acid (RNA) sequencing using next-generation sequencing (NGS) to quantify messenger RNA (mRNA). This allows the ability to look at changes in global gene expression patterns over time. This technology offers advantages of higher specificity, sensitivity, and a broader range over previous laboratory technologies such as gene microarrays. This allows for millions of small stretches of genes to be mapped and tracked back to the human genome. Statistical analysis is used to evaluate differentially sequenced genes (DEGs) and their significance with respect to the genome.

Multiple combinations of genes are involved in different biologic processes throughout the skin. These were also statistically evaluated. To be classed as showing significant changes, values were required to reach a statistical significance of $p \geq 0.05$ and a log2 fold change value of 0.06.

Quadruplicate testing was performed and Control tissues to which normal saline (0.9% saline) was applied were also included in the analysis.

SIGNIFICANCE OF STUDY

Operational data about gene functions "turned on" or "turned off" explains the many ways a skin product may work to affect cells and tissues downstream from the genes. In any biologic process, genes become activated as the first step and can be a very sensitive measurement of product actions. All other physiologic events then occur in response to direction given by the genes. Genes do not operate in isolation but are part of the entire holistic milieu of skin. Skincare products may initiate epigenetic messages in the skin. Gene function is heavily influenced by epigenetic messages[6], such as those evaluated here.

The practical use of the specific laboratory methods used herein combined with bioinformatics platforms allowed for a broader and more specific evaluation of a topical product's actions on skin than has been previously possible. This yields important and more comprehensive information about the effectiveness and usefulness of a product.

RESULTS AND CONCLUSIONS

Differential gene expression for Retinol+ Emulsion 0.3 compared to Control was demonstrated for 3,233 genes out of approximately 20,000. These were found to message significantly in the following biological processes, genes, and gene groupings:

Upregulated processes included:

- Wound healing and skin repair
- Apoptotic processes consistent with biological programmed cell death of already devitalized cells, leading to creation of improved aging and lessening the creation of the Senescent Associated Secretory Phenotype (SASP)[7,8]
- Extracellular matrix disassembly and skin homeostasis
- Antioxidant activity and response to endoplasmic reticulum (ER) stress
- Dermal epidermal junction (DEJ) maintenance and integrity
- Upregulated cell-cell adhesion
- Cysteine and methionine metabolism, incorporated in skin tone, elasticity, and texture
- Keratolytic events related to inflammation
- Skin aging via the galactose-mediated pathway

Downregulated processes included:

- Inflammatory pathways via arachidonic acid metabolism
- Inflammatory pathways via histidine metabolism
- Biologic processes having a role in epithelial and skin barrier development, including keratinization, cornification, skin barrier establishment, intermediate filament organization, fatty acid metabolism, ceramide synthesis

Retinol+ Emulsion 0.3 demonstrated, upon complete skin gene activation analysis using bioinformatics software, a wide range of epigenetic messaging. These messages included activities involving keratolytic pathways, such as ceramide synthesis, fatty acid metabolism, keratinization, and cornification. There was some upregulation of inflammatory pathways. Since Retinol+ Emulsion 0.3 contains Retinol and was designed to be keratolytic, these activities are expected. The pro-inflammatory regulation was also countered by other effects, including strong antioxidant activity, response to stress, and downregulation of the arachidonic pathway which is one of the strongest stimuli for inflammation. Multiple other regenerative processes were also upregulated.

Retinol+ Emulsion 0.3 may be described as a product that meets the formulating goals of being keratolytic and providing benefits associated with a Retinol-containing product while, at the same time, offering strong regenerative and healing abilities. Even though it is a keratolytic Retinol product, Retinol+ Emulsion 0.3 is, on balance, anti-inflammatory.

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DISCLOSURES

Study performed by Genemarkers, LLC.

Study type: Gene Activation

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