

P1618 Reduction in Gene Expression Related to Inflammation by Skin Barrier Improving Agent, Niacinamide

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INTRODUCTION

Skin photo-aging is associated with chronic inflammation. However, it has not been clear whether inflammation is a driving factor in skin aging or an epiphenomenon (1). Recent genome-wide analysis of gene expression changes in photo-aged skin (2) revealed that inflammation is a central theme in the process and is directly related to other key processes, such as protease activity, oxido-reductase activity, and extracellular matrix. A known skin benefit agent, niacinamide, has been shown to improve the skin barrier (3) and has been reported to reduce inflammation in vitro and in some clinical conditions (4,5). In this study, a new theme analysis approach was used to assess the subtle effects of skin application of a niacinamide-containing formulation by analysis of biomarkers at the gene expression level.

OBJECTIVE

Assess gene expression changes due to repeated topical application of a niacinamide-containing formulation in human subjects:

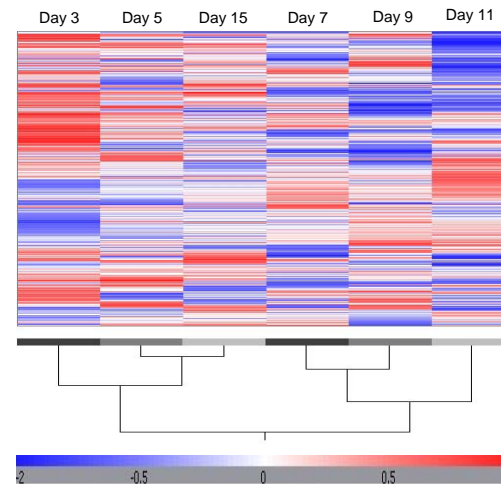
- Identify increased or decreased gene expression changes
- Identify functional themes among affected biomarker genes
- Determine if known skin benefits from repeat topical application of a niacinamide-containing cosmetic formulation may be expressed as a reduction in inflammatory biomarkers

METHODS

Individual study subjects (females 35-55 years of age) were treated with 3.5% niacinamide-containing or vehicle control lotion formulations on the volar forearm twice daily for up to 15 days. Full-thickness skin biopsies were taken from the treatment sites over the 15 day period. Total RNA was extracted from the biopsies and labeled for Affymetrix GeneChip® analysis. The synthesized target cRNA was hybridized to Affymetrix HG U133A microarrays. Statistically analyzed data were filtered by significance ($p < .05$, niacinamide vs. vehicle treatments) to identify genes showing an increase or decrease in expression levels, a standard bioinformatics approach. The number of significant changes was within the range expected by chance, suggesting subtle skin effects. Therefore, biological themes were examined using a different, new type of analysis in which over-represented gene ontology terms were identified from those biomarker genes affected by application of the niacinamide formulation.

RESULTS

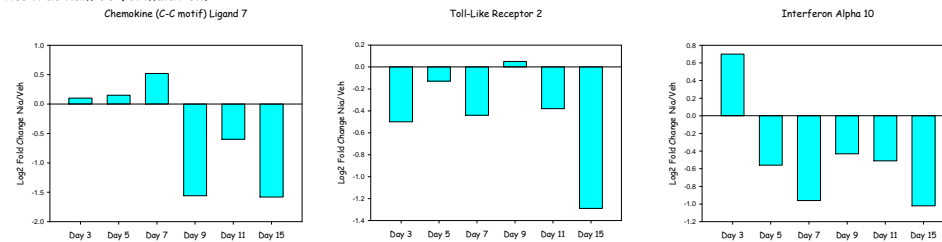
Figure below shows patterns (row averaged and Log2 normalized) of probe sets with levels of expression up (red) or down (blue) after application of a niacinamide-containing formulation vs. vehicle, across 3-15 days of application. The expression clustering pattern indicated that there was not a visually consistent pattern of changes across the days of treatment, using this standard method of depicting treatment- and time-dependent effects. This is not unexpected for a short term application of a cosmetic formulation across different individuals.



An alternative method for examining early functional gene expression differences was through theme analysis. Biological themes emerging from the Day 11 and Day 15 niacinamide formulation vs. vehicle were identified by statistical analysis of over-represented gene ontology terms associated with the differentially expressed biomarker genes vs. all genes expressed in the study. Increasing significance for each listed term is indicated by yellow → orange color. Separate listings are provided for all genes (All), genes showing increased expression (Up), and genes showing decreased expression (Down). Themes marked by asterisk (*) were among those most significantly expressed in an opposite direction in photo-aged skin (1).

Biological Process	All	Up	Down
defense response	32	10	22
inflammatory response	22	8	14
acute inflammatory response	7	2	5
response to wounding	33	11	22
regulation of leukocyte mediated cytotoxicity	2	0	2
regulation of epidermal growth factor receptor activity	2	2	0
regulation of epithelial cell proliferation	4	0	4
cellular lipid metabolic process	36	20	16
NAD biosynthetic process	2	0	2
Molecular Function			
cytokine activity	12	2	10
oxidoreductase activity	38	28	10
Cellular Component			
fibril	2	2	0
microfibril	2	2	0

Examples of individual inflammation-theme biomarker genes decreased in expression (particularly at day 15) after application of the niacinamide formulation.



SUMMARY

Theme analysis of biomarker genes significantly increased or decreased in expression after 11-15 days of application of a niacinamide formulation provided a novel means to detect functional biological improvements in skin condition:

- General decrease in the defense and inflammatory response themes, including cytokine activity, suggesting a decrease in inflammatory state.
- Increase in themes related to oxido-reductase activity and lipid metabolism. The first suggests a possible anti-oxidant benefit and the latter appears relevant to skin barrier improvement.
- Directional changes in gene expression in many themes are opposite to those seen in photo-aged skin (2), suggesting an improvement in the condition of aged skin.

CONCLUSION

Theme analysis is a sensitive new approach to examine the rebalancing of biomarkers of chronic inflammation in photoaged skin after short term application of a niacinamide-containing formulation.

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