# EPIGENETIC MODULATION OF SKIN EQUIVALENTS BY COSMETIC INGREDIENTS

Jin Namkoong[1], Dale G. Kern[1], Remona Gopaul[1], Elisabeth A. Lehigh[2], Anna J. Langerveld[2], and Helen E. Knaggs[1]

[1]Center for Anti-Aging Research, Nu Skin Enterprises, Inc., Provo, UT, United States
[2]Genemarkers, Kalamazoo, MI, United States

## ABSTRACT

Numerous topical ingredients are available for formulating cosmetic products. A variety of biological assessment methods aids in the selection of cosmetic ingredients for finished products. Differences in the source or manufacturing process used to obtain the ingredient and the blending of ingredients impact biological efficacy. Modulation of gene expression is commonly used to evaluate biological activities. Epigenetic changes play a significant role in how genes are expressed and translated into functional proteins. Epigenetics represents the heritable changes without altering DNA sequences; it is critical in biological functions, since epigenetics allows tissue-specific changes in gene expression. Examples of epigenetic modifications are DNA methylation, histone modification, and regulatory RNAs. Cosmetic ingredients are evaluated for epigenomics, utilizing the DNA methylation arrays for more global assessments or individual genes/targets by looking at the specific promoter regions for histone modification or methylation patterns. High throughput screening methods can also be used to measure expression of microRNAs, small non-coding regulatory RNAs that play a significant role in gene expression. A qPCR-based screening method was used to examine microRNAs with established roles in skin biology. This method was used to investigate how combinations of topical ingredients modulate gene and microRNA expression in full-thickness skin equivalent models. Results showed changes in several mRNAs gene expression, with some correlated changes in microRNAs, such as SIRT1 stimulation through the inhibition of miR-9. Additional correlations between epigenetic modulation and mRNA gene expression that influence skin aging are being investigated.

## INTRODUCTION

A finished cosmetic product requires multiple ingredients. In order to formulate safe and effective cosmetic products, it is important to understand how ingredients are interacting with each other as well as with the skin. Utilizing skin equivalents, topical ingredients can be readily evaluated for different biological assessments. Gene expression analysis by PCR is one of the most readily used biological assessments for topical ingredients. In more recent years, additional attention has been made on how gene expression is regulated, such as looking at epigenomics. Epigenetics represents the heritable changes without altering DNA sequences. Examples of epigenetic modifications are DNA methylation, histone modification, and regulatory RNAs. In order to expedite research, high-throughput methods were developed for epigenome. Currently, methylation arrays and microRNA arrays are broadly used in research, including evaluating topical ingredients.

microRNAs are small non-coding RNAs that are found in the genome. They were isolated from various locations within the genome, like introns, within exons, or intergenic regions. They are transcribed by RNA polymerase II, and processed by Drosha and Dicer to form mature microRNAs that target different genes. In the human genome, there are close to 2000 microRNAs identified so far (1), though the number is growing rapidly. With growing interests in microRNAs, target gene identification and validation are an important part of the research focus, since regulation by microRNAs does not require the perfect pairing (2). microRNAs will guide Argonaute (AGO) to target sites and that will lead to the target silencing by mRNA cleavage, degradation or inhibition of translation (2). Interestingly, microRNAs can also stimulate target gene expression under certain conditions (2).

MicroRNAs play a key role in skin health; ablation of miRNAs from the epidermis by deletion Dicer or dgcr8, resulted in a barrier defect and hair follicle abnormalities (3). One of the most widely studied skin microRNA is miR-203, which was identified to promote epidermal differentiation through p63 (4). In order to evaluate microRNAs specific to skin, microRNAs known to be involved in skin functions were assessed in a Taqman PCR array format. In this study, topical ingredients were evaluated for gene and microRNA expression using human skin equivalents.

## OBJECTIVE

To investigate microRNAs and gene expression modulation by cosmetic ingredients using human skin equivalents.

## METHODS

In order to evaluate different cosmetic ingredients on genetic and epigenetic modulations, full-thickness human skin equivalents were obtained from MatTek (Ashland, MA). These human skin equivalents were made from normal human fibroblasts and normal human keratinocytes. After equilibrating the skin equivalents according to the manufacturer's protocol, they were dosed with different concentrations of cosmetic actives in six replicates. Extract 1 is *Oryza sativa* extract; Blend 2 is a mixture of *Narcissus tazetta* bulb extract and *Schizandra chinensis* fruit extract; and Blend 3 is a mixture of Extract 1 and Blend 2. After a 24-hour incubation period, the skin equivalents were harvested.

For qPCR analysis, Maxwell 16 LEV Simply RNA Tissue Kits (Promega, Madison, WI) were used to isolate RNA, followed by determination of RNA concentration and quality using a NanoDrop 2000 (Thermo Scientific, Waltham, MA). cDNA was synthesized using High Capacity DNA Synthesis Kits (Life Technologies, Grand Island, NY). 384 validated Taqman gene expression assays with endogenous control genes were put together in custom OpenArray (Life Technologies) formats and analyzed using the QuantStudio 12K Flex instrument (Life Technologies). Data was processed using RealTime StatMiner software v 4.2 for statistical analysis.

For microRNA analysis, RNA was isolated using miRNeasy Mini Kits (Qiagen, Valencia, CA), followed by determination of RNA concentration and quality using a NanoDrop 2000. cDNA was synthesized using Taqman microRNA Reverse Transcription kits and preamplified for 12 cycles using a Taqman Custom microRNA Preamplification Primer Pool and Taqman PreAmplification Master Mix (Life Technologies). Epigenetic modulation was examined using Genemarkers' microRNA Skin Panel, which used validated Taqman gene expression assays in the QuantStudio 12K Flex instrument (Life Technologies). Data was processed using RealTime StatMiner software v 4.2 for statistical analysis.

## RESULTS

Three topical ingredients were selected to be evaluated using human skin equivalents. Extract 1 (*Oryza sativa* extract) was applied alone, whereas *Narcissus tazetta* bulb extract and *Schizandra chinensis* fruit extract (Blend 2) were applied together as a blend. Blend 3 is the mixture of all three ingredients (*Oryza sativa* extract, *Narcissus tazetta* bulb extract, and *Schizandra chinensis* fruit extract). For gene expression, only Extract 1 and Blend 3 results are shown (Figure 1). Multiple antioxidant genes, growth factors, ECM, and barrier-associated genes were modulated by these ingredients.

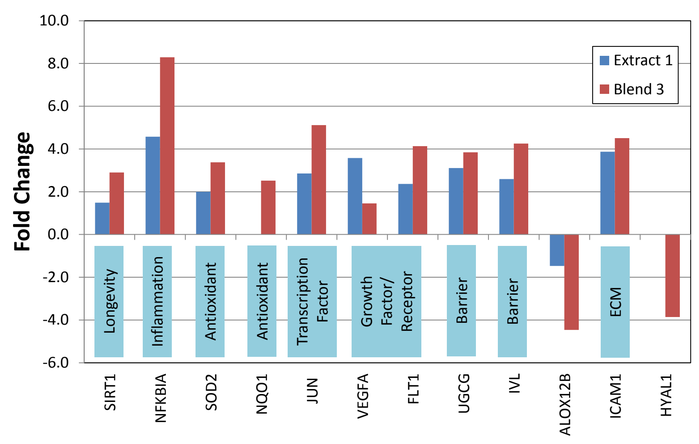


Figure 1. Extract 1 or Blend 3 was examined for gene expression using human skin equivalents in six replicates. 384 validated Taqman gene expression assays were examined based on relevance to the skin function. Unpaired t-tests were performed using StatMiner and all data shown are statistically significant from the untreated (P≤0.05). Selected gene expression data are shown on the graph.




Figure 2. Extract 1, Blend 2, or Blend 3 was examined for microRNA expression using human skin equivalents in six replicates. 105 validated targets with 7 endogenous controls were examined in an OpenArray format. Unpaired t-tests were performed using StatMiner and all data shown are statistically significant from the untreated (P≤0.05). Only selected microRNAs are shown on the graph.

In addition to gene expression analysis, microRNAs were analyzed with the same ingredients. A selection of microRNAs shown to be involved in skin functions is shown (Figure 2). miR-146a is an important inflammatory regulator, whose expression is partially controlled by NF-κB. As a feedback mechanism, miR-146a also inhibits the upstream of NF-κB signaling pathways (5). miR-22 was identified to be upregulated when human keratinocytes were exposed to UV irradiation, to stimulate cell survival affecting PTEN expression (6).

Different microRNAs are shown to target SIRT1, a well-known longevity gene, which controls cellular metabolism and stress response. miR-9 regulates SIRT1 expression. miR-9 is downregulated, whereas SIRT1 mRNA expression is upregulated in this study. However, there are also other microRNAs, such as miR-22, which also targets SIRT1 and is upregulated in this study. It would be intriguing to identify how and which microRNAs would take precedent over another.

The regulation of gene expression by microRNAs is more complicated than initially thought. An example is the interaction between miR-155 and c-Jun. In the microRNA skin panel study, miR-155 is upregulated by 1.5 fold. A previously reported study identified c-Jun to be a target of miR-155 (6). miR-155 mimic decreased c-Jun protein levels and miR-155 inhibitor increased c-Jun protein levels (6). However, in our study, c-Jun expression was actually stimulated. It is interesting to note that miR-155 affects c-Jun protein levels post-transcriptionally and does not affect c-Jun mRNA expression (6).

The environment influences microRNA expression as well. There were several published studies examining environmental exposure *in utero* modulating microRNAs (7). Comparing microRNAs from placentas procured during delivery presented downregulation of miR-146a, miR-16, and miR-21, when the mother had a history of smoking during pregnancy (7). In this study, all three microRNAs were upregulated.

Many studies examining different microRNAs from skin are examining microRNAs in association with skin cancers or skin diseases. It is also important to understand how microRNAs keep the skin healthy, to prevent skin aging.

## CONCLUSIONS

- Different topical ingredients affect gene and microRNA expression. These were measured efficiently using PCR arrays.
- Some gene expression modulation was correlated to microRNA expression changes.

## REFERENCES

1. miRBase: the microRNA database www.mirbase.org
2. Nature Reviews: Genetics (2012) 13: 271-282
3. Journal of Investigative Dermatology (2011) 131: 20-29
4. Nature (2008) 452: 225-229
5. Journal of Investigative Dermatology (2014) 134: 1931-1940
6. Current Drug Targets (2013) 14: 1128-1134
7. Current Opinion in Pediatrics (2014) 26: 243-251