A MULTIFACTORIAL GENE EXPRESSION APPROACH TO ADDRESSING BODY APPEARANCE AGING

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BACKGROUND

Aging is the result of complex multifactorial influences occurring over time between lifestyle choices, environment and genome, necessitating the study of multiple genes and their expression patterns in multiple tissues. The advent of genomic research has opened up new ways of investigating skin and body aging. From the present and continuing into the foreseeable future, vast amounts of gene expression data will need to be evaluated to produce meaningful interpretations and conclusions leading to functional interventions that mitigate age-related changes in multiple genes (1–3). Studying aging in skin is further complicated by the presence of different cell types (eg. melanocytes, keratinocytes, fibroblasts and in the subcutaneous tissue, adipocytes) which will have varying gene expression responses to different stimuli.

Here we describe the similar and differing activities of two skin care ingredients to favorably modify the genetic expression of a diverse group of genes important in body skin structure and appearance. A challenge is to distill the vast amount of gene data generated from qPCR experiments on two different cell types into manageable datasets for use in selecting actives for further investigation and product prototyping. A way for accomplishing this is proposed.

OBJECTIVE

Select an efficacious topical anti-aging ingredient for use in developing anti-aging skin care products targeting body contouring.

METHODS & MATERIALS

Two skin-active ingredient/extracts, *Laminaria digitata* Extract dissolved in water (0.5% and 1%) and Hydrolyzed Soy Protein Extract dissolved in water/glycerin (0.25%, 0.5% and 1.0%), were tested *in vitro* using two human cell systems, Human full-thickness 3D epidermal skin equivalents (FTEE, MatTek, Ashland, MA), and primary human adipocytes (Zenbio, Research Triangle Park, NC), isolated from healthy, normal subcutaneous adipose tissue obtained from elective surgery.

Full-thickness epidermal equivalent (FTEE) cultures: One hundred microliters of each test article was applied to each culture and incubated for 24 hours. Following incubation, the cultures were thoroughly washed with sterile phosphate buffered saline (PBS) to remove test materials and placed in RNAlater solution for gene expression analysis. The RNAlater tissues were incubated for 2 hours at room temperature and then stored at 4°C until processing.

Primary normal adipocytes: Cultures were allowed to acclimate 5–7 days at 37° C, 5% CO_2 in a humidified incubator prior to assay. Test articles were diluted to final assay concentration in LIP-2/3 Assay Buffer. Adipocyte growth medium was replaced with adipocyte maintenance medium (AM-1) containing the test article at the desired final concentration and incubated for 24 hours at 37° C, 5% CO_2 in a humidified incubator.

Gene set selection: A set of 376 genes (an additional 8 standard reference genes were also included) were selected from the published literature in support of the ingredient claims for body contouring purported by the ingredient manufacturer or suspected of being active in the aging process.

qPCR analysis: Custom Taqman Low Density Arrays (TLDAs) were created using Life Technologies validated gene expression assays. Each TLDA card contained 376 skin-relevant target genes, selected from published literature and grouped into four general categories, circulation/inflammation/edema, adipogenesis/lipogenesis, energy production and extracellular matrix remodeling. In addition, 4 common endogenous control genes (GUSB, HPRT, HMBS, GAPDH and 18S) were included. One gram of total RNA from each tissue sample was converted into cDNA using the High Capacity cDNA Reverse Transcription kit from Life Technologies (Foster City, CA). An Applied Biosystems 7900HT instrument was used for amplification and fluorescence detection.

Data analysis for qPCR was carried out according to the RQ analysis method using RQ Manager and StatMiner (v3.1) software programs. Expression levels were determined based on a relative quantification analysis, t-test with Benjamini and Hochberg false discovery rate correction (p value equal or less than 0.05), with a cycle threshold of less than 35.

RESULTS

The majority of genes on the custom PCR composed of 376 pre-selected genes significantly changed greater than 1.5 fold (FC) following treatment with either Hydrolyzed Soy Protein Extract or *Laminaria digitata* Extract (Figure 1). Of these changes, less than one half were in the direction expected given the claims for the ingredient or taught by the published literature. In addition, more gene expression changes were seen in the full skin thickness model than on adipocytes.

Examining only the 127 genes that increased or decreased expression as expected, the majority were found using the adipocyte culture model. Finally, it became apparent that *L. Digitata* Extract had a greater impact on overall gene expression of the adipocyte model and Hydrolyzed Soy Protein Extract had a greater impact on the full-thickness epidermal equivalent model.

Comparing the claims for each of the two ingredients to the expected gene expression changes likely for each claim gave mixed results (Table 1). Whether or not an ingredient claim could be substantiated by the expected gene expression depended on the *in vitro* model and on ingredient concentration (not shown). Some claims could not be substantiated in either *in vitro* model while some unclaimed activities could (Table 2).

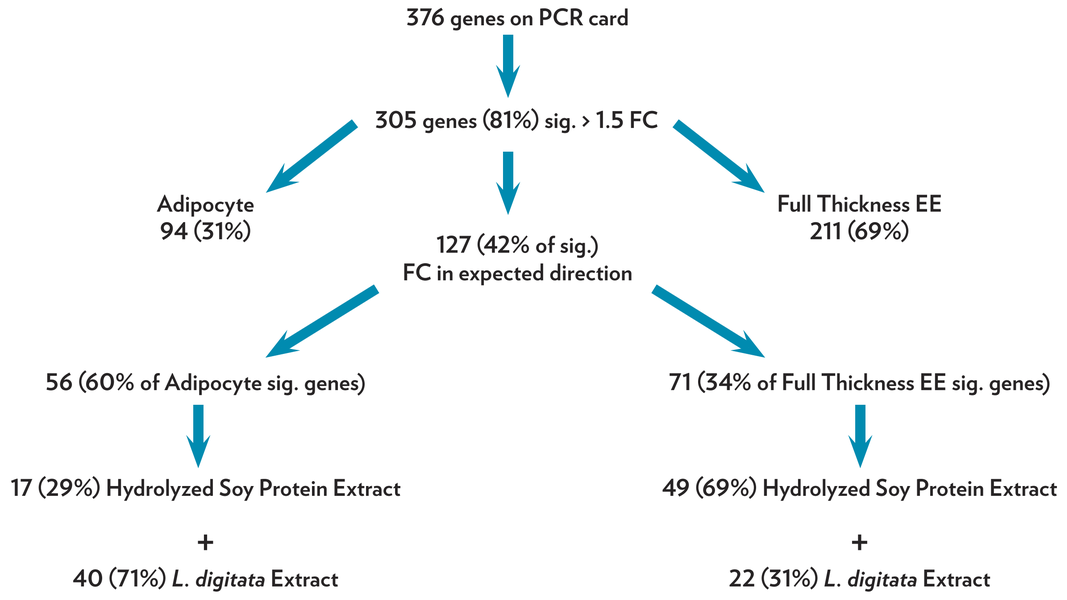

FIGURE 1. Number (percentage) of genes on the PCR card that changed by active ingredients applied on two *in vitro* model systems, full-thickness epidermal equivalent and normal primary human adipocytes.

Supplier Claims / Ingredients	Hydrolyzed Soy Protein Extract		*L. Digitata* Extract	
	Claimed?	Gene Expression Agreement: Adipocytes/Full Thickness EE	Claimed?	Gene Expression Agreement: Adipocytes/Full Thickness EE
Enhances SIRT3 expression	YES	no/no	NO	no/no
Enhances SIRT5 expression	NO	no/no	YES	no/no
Decreases cellular senescence	YES	no/no	YES	no/no
Promotes longevity	YES	yes/yes	YES	yes/no
Increases cytochrome C activity	YES	yes/no	NO	yes/no
Increases mitochondrial membrane potential	YES	yes/yes	YES	no/no
Preserves skin structure	YES	no/yes	NO	yes/yes
Increases ATP Production	YES	yes/no	YES	no/no
Preserves Apolipoprotein J (Apo-J)	NO	no/no	YES	no/no
Increases collagen type 1 synthesis	NO	no/no	YES	no/no

TABLE 1. Activity of two ingredients affecting gene expression in two *in vitro* model systems in agreement with the ingredient claims made by the supplier.

New Possible Claim	Substantiated by Gene Expression in ADP Cultures?	Gene(s)	Substantiated by Gene Expression in EFT Cultures?	Gene(s)
HYDROLYZED SOY PROTEIN EXTRACT				
Increases Hydration	Yes	Upregulation of AQP3	Yes	Upregulation of HAS3 and HAS1
Decreases Free Radical Damage	No		Yes	Upreglulation of SOD2
***L. DIGITATA* EXTRACT**				
Increases Cytochrome C Activity	Yes	Upregulation of COX1 (MT-CO1)	No	Downregulation
Preserves skin structure	Yes	Upregulation of SOD2 and COL4A3 Downregulation of ELANE	Yes	Upregulation of COL4A1, COL4A2, TIMP1 and SOD2
Increases Hydration	Yes	Upregulation of HAS3 and AQP3 Down regulation of HSPSE	Yes	Upregulation of HAS3 and HAS1
Decreases Free Radical Damage	Yes	Upregulation of SOD2	Yes	Upregulation of SOD2 and TXNRD1

TABLE 2. Additional skin-relevant claims that could be made based on gene expression analysis using two *in vitro* cell model systems.

DISCUSSION

This work was undertaken to select an efficacious topical anti-aging ingredient for use in developing anti-aging skin care products targeting body contouring. Both skin cells and subcutaneous adipocytes were studied using different ingredients to assess effects on gene expression. Since aging is multifactorial, when focusing on body contouring it is important to study multiple genes involved in skin structure and appearance as well as lipid metabolism. However, performing whole-genome expression analyses are time consuming and costly. Further, analyzing large data sets containing multiple gene expressions is complex and remains an emerging field, bioinformatics. To focus our study and simplify the process we constructed a PCR card for gene expression based on the claims for the ingredients and the published literature and examined the response of two *in vitro* cell systems to two candidate active ingredients.

In our analyses of the data, our first step was to study only those genes that had a greater than 1.5 fold change in expression. We then further subdivided based on a comparison with expected expression changes as reported in the literature and also with claimed activities for the ingredient. Finally, we looked at the data in relation to the *in vitro* model used. Some ingredient claims were substantiated, others were not. In many cases, we found novel actions additional to those reported by the supplier and previously not documented in the scientific literature.

CONCLUSION

Since aging is multifactorial, we believe that the construction of an effective anti-aging product necessitates an approach targeting more than one gene, therefore using multiple actives to obtain best efficacy. In this work, each ingredient elicited some common and many unique genetic expression changes suggesting that a comprehensive anti-aging product be composed of more than one ingredient to affect a breadth of necessary changes in the expression of groups of genes involved in aging. The availability of modern genomic techniques allows the investigation of multiple genes in a single experiment, therefore making this approach of targeting the expression of multiple genes by several ingredients useful in the development of functional skin care products.

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