Skin Science: The Use of Genomics Tools in Skin Care

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What is “Skin Science?”

- The use of “hard science” techniques and principles in guiding cosmeceutical product development
  - Genomics - analysis of genes
  - Proteomics – analysis of proteins
  - Histological techniques – visual (microscopic) analysis of skin cells

- Based on the fact that changes in the skin’s appearance (wrinkles, fine lines, dullness, age spots) result from changes in basic biological and physiological processes
  - Cellular aging (cell cycle, growth factors, aging factors – sirtuins)
  - Anti-oxidant production/removal
  - Inflammation
  - DNA repair
  - Extracellular matrix (i.e., collagens, elastins)
The Biological Basis of Skin Characteristics

**Physical Characteristics**
Fine lines, wrinkles, age spots, dullness

**Changes in Biological/Physiological Processes**
Inflammation, cell cycle/regeneration, oxidant formation/anti-oxidant production, aging molecules, extracellular matrix integrity

**Changes in gene expression and protein expression**
(sirtuins, collagens, keratins, growth factors, metalloproteinases, interleukins)
Changes in Gene Expression Are Biomarkers

- Easily measurable (using high tech genomics methods)
- Indicative of biological events that underlie skin’s appearance

For cosmeceuticals:
- Faster and less subjective than clinical testing
- Identify underlying biological mechanisms
  - For example, multiple different biological pathways involved in inflammation and anti-aging in skin
Genomics: History and Current Applications

• Field was borne from the human genome project
  ▫ identified and sequenced 30,000 human genes

• Produced high throughput technologies that measure/analyze:
  ▫ Gene expression (which genes are turned on or off in a given condition)
  ▫ Gene sequence (determines if the DNA code is altered)
    - SNP’s – single nucleotide polymorphisms

• Technologies are being used in all areas of human health (personalized medicine), animal health, environmental and agricultural industries

• Just beginning to emerge in the cosmeceutical industry
Benefits of Genomics to the Cosmeceutical Industry

• Improve product formulations to achieve desired effects

• Validate product efficacy at biological level
  ▫ Faster and less subjective than clinical studies

• Cost-savings
  ▫ May find that a small amount of expensive ingredient produces same effect as higher concentration

• Ensure product safety

• Each of these things will *improve marketability*
Current Uses in Skin Care Industry

Most common:
- Determine how specific ingredients or products regulate gene expression
- Create “custom” products based on an individual’s gene sequence

Additional uses:
- Compare different skin conditions to understand how they differ at the molecular level (i.e., aging vs young skin, hyperpigmentation vs normal pigmentation)
- Identify biomarkers of efficacy for quicker analysis of product safety and efficacy
  - p53 a gene that regulates DNA repair was recently identified as a biomarker for testing sunscreen efficacy
  - Authors suggest this is a better measure than SPF
Gene Expression is Based on the Central Dogma of Biology

- All cells in a person’s body have the same DNA or “genes”
- When the gene is activated it is turned into RNA
- Different cell types are produced by activation of unique sets of genes
- Specific RNAs assemble specific proteins such as collagen and keratin in skin cells
- Aging, disease and other conditions will influence the regulation of specific genes
- Gene expression technologies measure the amount of RNA in a given cell or tissue
Genomics Technologies: Affymetrix Microarrays (Gene Chips)

- Measure up to 30,000 genes at a time
- Chips available for over 30 organisms
- Ideal for discovery
  - What does my product/ingredient do?
  - Identifying novel mechanisms of action
  - Comparing biological activities of different products
Taqman Real Time PCR (Polymerase Chain Reaction)

- Taqman Low Density Array (TLDA)
- Less expensive than microarrays; very sensitive method – highly quantifiable
- Can measure up to 384 genes at a time
- Arrays are custom-designed with your genes of interest
- More focused experimental approach
Genomics Study for *Elina* Organic Skin Care

- Used quantitative real time PCR to understand how *Elina Ambra-Lift* acts at the biological level; which genes were turned “on or off” by the product
  - we tested the final product rather than individual ingredients

- For the study, *Elina Ambra-Lift* was applied to a 3D *in vitro* skin culture model that contains both epidermal and dermal skin cell layers
  - The test product was applied for 48 hrs.
  - The control cultures received no cream

- Expression of 91 custom-selected genes that regulate biological functions that underlie skin cell aging and anti-aging processes were measured (plus 5 control genes)

- Assessed skin cell integrity using a standard histological stain (hematoxylin & eosin)
**In Vitro 3D Skin Culture System**

- Using a 3D human *in vitro* skin model (EpiDerm FT; purchased from MatTek)

- 3D model contains epidermis (keratinocytes, stratified corneum layer) and dermis (viable fibroblasts)

- *Model allows application and testing of final cosmetic product, rather than single ingredients*
Elina Ambra-Lift Regulated Expression of Specific Genes Important for Maintaining Healthy Skin

- 48 hr exposure to Elina Ambra-Lift produced statistically significant changes in 47 out of 91 genes tested
  ▫ T-tests were performed to determine statistical significance
  ▫ All of the 47 genes were statistically significant at p<0.05 or less

- 30 genes were downregulated; their levels were decreased after exposure to Ambra-Lift

- 17 genes were upregulated; their levels were increased after exposure to Ambra-Lift

- Detailed results can be obtained at www.elinaskincare.com
Elina *Ambra-Lift* Increases Expression of Anti-Aging and Anti-Wrinkle Genes

- **Sirtuin 1 (SIRT1)** is a well characterized anti-aging gene; *Ambra-Lift* increased expression

- **Collagen 7A1 (COL7A1)** is considered a “biochemical marker of wrinkles,” whereas decreased expression is associated with wrinkles; *Ambra-Lift* increased expression

- **Collagen 4A1 and 4A2** are important for maintaining skin firmness; *Ambra-Lift* increased expression

* Statistically significant at p<0.02; ** statistically significant at p<0.06
Elina Ambra-Lift Induces Anti-Oxidant and Anti-Inflammatory Gene Expression

- *Elina Ambra-Lift* increased expression of 2 important anti-oxidant genes which help protect cells from damage (metallothionein 2, superoxide dismutase 2)

- *Elina Ambra-Lift* increased expression of the interleukin 1 receptor antagonist (IL1RN), which decreases activity of a powerful inflammatory molecule, interleukin 1 beta (IL-1β)

- These results demonstrate that *Elina Ambra-Lift* activates genes involved in protective cellular mechanisms
Summary: Elina *Ambra-Lift* Activates Genes that Promote Anti-Aging and Anti-Wrinkle Effects

- The use of genomics to validate the biological effects is an innovative approach

- The experimental study design allowed for testing of the final product rather than single ingredients

- *Elina Ambra-Lift*, an organic product, naturally induced expression of genes that regulate anti-wrinkle and anti-aging effects in the skin
  - Collagens
  - Anti-Oxidants
  - *Sirtuin-1*

- Elina will use these data to increase her presence from a localized, midwest market to a more prominent, national product leader
Future Studies/Capabilities

- Test additional products

- Experimental design can be modified to test other conditions or endpoints. For example:
  - Protection from UV exposure or other types of damage
  - Repair from damage caused by UV exposure or other conditions
Summary and Market Projection

- Genomics testing methods will play a more prominent role in cosmeceutical product development and testing

- This will be driven by:
  - Changes in Federal guidelines for more stringent regulations on marketing claims and safety guidelines
  - Consumer awareness and their demand for products with proven efficacy
  - Competition; keeping up with companies/products that are using “hard science” approaches in their product development