THE GENETICS OF YOUTH

TABLE OF CONTENTS

INTRODUCTION: THE GENETICS OF YOUTH

SECTION 1: SKIN CARE RESEARCH ON AGELOC CONDUCTED BY NU SKIN AND/OR ITS PARTNERS

SECTION 2: NUTRITIONAL RESEARCH ON AGELOC CONDUCTED BY NU SKIN AND/OR ITS PARTNERS

SECTION 3: PRIOR ANTI-AGING RESEARCH CONDUCTED BY NU SKIN PARTNERS

APPENDIX: BACKGROUND RESEARCH CONDUCTED BY INDEPENDENT PARTIES

INTRODUCTION: THE GENETICS OF YOUTH

With our in-house scientists and Nu Skin Anti-Aging Scientific Advisory Board (SAB), Nu Skin brings together a world-class team of scientists whose anti-aging research and insights have changed the understanding of how and why we age. Our SAB scientists have individually conducted pioneering anti-aging research in their respective fields for over 30 years and have published over 300 scientific papers on the genetic basis of aging. Their extensive work, combined with that of our in-house scientists, demonstrates that biological aging should no longer be considered an inevitable process.

Building on these insights, Nu Skin and our collaborators are focusing on identifying the ultimate sources of aging with proprietary ageLOC® science. Nu Skin has identified groups of genes that play a central role in the aging process, called Youth Gene Clusters (YGCs).

ageLOC science is not dependent on any single discovery or scientific paper; instead, our Nu Skin research and development scientists have incorporated key discoveries from leading genetic researchers and anti-aging specialists (see Appendix for background). Our knowledge and understanding of these discoveries are then applied in the development of proprietary, innovative, safe, and effective products. We believe that over the next five to 10 years as ageLOC science matures, its full impact will be realized in many ways as we expand our ageLOC product innovations, which include both topical and ingestible applications. ageLOC science provides Nu Skin with the ingenuity to further enhance our tradition of innovation. It's an exciting time for Nu Skin!

The following collection of scientific publications, which includes the work of Nu Skin scientists, SAB members, and academic partners, represents the foundational work from which we have gained key insights into the aging process, as well as more recent work that substantiates our ageLOC approach and our ability to identify, target, and reset YGCs. We believe that ongoing research will further enhance ageLOC science into the future.

INFLUENCING THE EXPRESSION OF GENES ASSOCIATED WITH SKIN INFLAMMATION BY COMBINING NARCISSUS TAZETTA BULB EXTRACT AND SCHIZANDRA CHINENSIS FRUIT EXTRACT—AN IN VITRO ANALYSIS

Presented at: Society of Cosmetic Chemists Annual Scientific Seminar, New York, NY December 2011.

Authors: R Gopaul, HE Knaggs, DG Kern

Narcissus Tazetta Bulb Extract, a bulb extract from the daffodil plant family has been used topically to delay cellular proliferation. Schizandra Chinensis Fruit Extract, a red berry fruit extract belonging to the magnolia plant family, has been used for decades in Chinese medicine to promote general well being and vitality when taken orally. Recent studies involving these extracts in finished cosmetic formulations have led to the hypothesis that when combined, Narcissus Tazetta Bulb Extract and Schizandra Chinensis Fruit Extract may be able to reduce skin inflammation. This research investigates the in vitro effect of a proprietary blend of these extracts on genes related to skin inflammation when applied topically. A combination of Narcissus Tazetta Bulb Extract and Schizandra Chinensis Fruit Extract was applied to human equivalent skin cultures containing normal human epidermal keratinocytes and fibroblasts. RNA extracted after 24 hours of culture incubation from both treated and untreated culture samples were used to conduct RT-PCR experiments. Results showed that the combination of these extracts reduced the expression of genes associated with skin inflammation while increasing the expression of genes related to anti-inflammation. These include the down-regulation of CASP8, LTBR4, PTAFR, HRH1 and SLC6A4 and the up-regulation of IL1RN, MT1H, MT2A and VEGFA. The findings from this study suggest a possible role of a combination of Narcissus Tazetta Bulb Extract and Schizandra Chinensis Fruit Extract on skin inflammation when applied topically.

SO WHY DON'T I LOOK YOUNGER?

Presented at: the International Federation of Societies of Cosmetic Chemists, Bangkok, Thailand December 14-15, 2011.

Authors: DG Kern, A Chang, SM Wood, R Gopaul, J Lephart, HE Knaggs

The Asian population is often considered to age better than Western counterparts. We studied a Western cohort of subjects in California and a Japanese cohort recruited in Tokyo, recording their chronological ages as well as their 'apparent age'. The apparent age of all subjects was assessed based on how old they looked as a result of dermatology clinicians grading key facial aging parameters such as pigmentation and age spots. In the U. S. populations, we found spread in the error of age assessment (the numerical difference between apparent age and chronological age) of 16 years, with the average at +/- 7 years. Data from a follow-up study with a Japanese cohort showed a similar pattern, but exhibited a smaller range in the average error in age assessment. In the Japanese population, subjects' appearance, on average, ranged from 6.2 years older than their chronological age to 4.5 years younger than their chronological age. Several biological parameters were measured and a correlation with apparent age was found:

those subjects who appeared older for their chronological age had elevated levels of PGF2 isoprostanes, known to be *in vivo* biomarkers of oxidative stress. Conversely, subjects who appeared younger than their biological age had correspondingly lower levels of these markers. In a third study, oral supplementation targeting the antioxidant defense network using normal and oxidatively stressed (smoking) subjects showed an improvement in skin aging attributes among oxidatively stressed subjects receiving supplementation. Overall, these studies demonstrate a link between how old someone appears and the endogenous level of oxidation. Additionally, we found that nutritional supplementation of subjects with high levels of oxidative stress (smoking) resulted in an improvement in skin aging attributes.

DERMATOLOGICAL SURGERY. HTTP://ONLINELIBRARY.WILEY.COM/DOI/10.1111/J.1524-4725.2011.02235.X/ABSTRACT DECEMBER 5, 2011

AN EXPLORATORY STUDY TO DETERMINE THE ASSOCIATION BETWEEN ASSESSED FACIAL SKIN AGING AND PLASMA ISOPROSTANE LEVELS IN MIDDLE-AGED JAPANESE WOMEN

Authors: Anne Lynn S. Chang MD, Bharathi Lingala, Tiffany C. Chang, Dale G. Kern MS, Steve M. Wood PhD, Hidekazu Toyoda MS, Helen E. Knaggs PhD

One of the central mechanisms of aging is hypothesized to be oxidative stress. Quantification of oxidative stress in human organ systems has been difficult. One of the best methods is using plasma isoprostane levels, which have been shown to reflect oxidative stress in multiple nondermatologic organ systems. The goal of the study was to determine whether severity of aging of human skin is associated with plasma isoprostane levels, specifically prostaglandin F2a (PGF2a) and 8-iso-PGF2a, while controlling for covariates such as body mass index, ultraviolet light exposure, diet, medication, supplement use, and stress levels. Facial skin aging assessments performed by four blinded dermatologists were correlated with plasma isoprostane levels in 46 healthy, nonsmoking Japanese women aged 45 to 60. Individuals whose assessed skin age exceeded chronological age had mean plasma isoprostane levels of PGF2a and 8-iso-PGF2a that were higher than those whose skin age was assessed to be less than chronological age ($p = .001$ and .001, respectively). These results remained statistically significant when adjusted for confounding variables (8-iso-PGF2a, $p = .02$; PGF2a, $p = .03$). Plasma isoprostanes as markers of accelerated aging of the skin merit further study.

RESTRUCTURING OF THE ECM IN HUMAN SKIN BY EQUOL: A PLANT AND SOY-DERIVED ISOFLAVENOID

Presented at: The 4th International Conference and Exhibition for Nutraceuticals and Functional Foods, Sapporo, Japan, November 2011

Authors: R Gopaul, HE Knaggs, ED Lephart

Equol, an isoflavenoid, has affinity for estrogen receptor subtypes and acts as a selective androgen modulator (binding 5 alpha-DHT). This study investigated the effects of equol on the expression of human skin genes and proteins. Equol (@0.3 and 1.2%) in qPCR experiments examined human skin gene expression. Equol, 5 alpha-DHT and 17 beta-estradiol (10 nM) were studied in human monolayer

fibroblasts cultures (hMFC) and in 3-dimensional organotypic cultures for protein expression via fluorescent activated cell sorting (FACS). In qPCR experiments: equol significantly influenced skin extracellular matrix (ECM), antioxidant and anti-aging genes. In hMFCs, equol significantly increased collagen type I. FACS analysis showed equol and 17 beta-estradiol significantly stimulated collagen type I & II and elastin, while matrix metallproteinases were significantly decreased, and while tamoxifen blocked these influences. These findings suggest that equol has the potential to be used topically for treatment of skin aging, by enhancing ECM components in human skin.

A MULTIFACTORIAL APPROACH TO ADDRESSING BODY APPEARANCE AGING

Presented at: The European Society for Dermatological Research (ESDR) Sep 2011, J. Invest Dermatol 131. S. 54 (2011).

Authors: HE Knaggs,[1] DG Kern,[1] R Gopaul,[1] A Langerveld[2]

[1]Nu Skin Enterprises, Inc., Provo, Utah, USA
[2]Genemarkers LLC, Portage, Michigan, USA

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. Among the most promising routes are methodologies that seek to understand the interactions of multiple genes during aging. Aging is the result of complex multifactorial influences occurring over time between lifestyle choices, environment and genome, necessitating the study of multiple genes and expression patterns in multiple tissues. Here we describe the ability of several skin care ingredients to favorably modify the genetic expression of a diverse group of genes important in body skin structure and appearance. Three skin-active ingredients were tested on human full–thickness 3D epidermal skin equivalents and primary human adipocytes. Gene expression was measured by quantitative PCR using custom TaqMan Low Density Arrays (TLDA). Compared to each other, each ingredient exhibited some common and many unique genetic expression changes, suggesting that comprehensive anti-aging products be composed of more than one ingredient to affect the necessary changes in expression of groups of genes involved in aging.

ASSOCIATION OF SKIN AGING SEVERITY WITH BLOOD ISOPROSTANE LEVELS IN HEALTHY MIDDLE-AGED JAPANESE WOMEN

Presented at: The Society for Investigative Dermatology (SID) May 2011, J. Invest Dermatol 131. S. 85 (2011).

Authors: T Chang, B Lingala, D Kern, S Wood, H Toyoda, H Knaggs, A Chang

One of the central mechanisms of aging is hypothesized to be oxidative stress. To date, quantification of oxidative stress in human organ systems has been difficult. One of the best methods currently available is blood isoprostane levels, which not only reflect systemic oxidative damage, but are also associat-

ed with oxidative stress in multiple non-dermatologic organ systems. We explored the connection between human skin aging and oxidative stress as measured through blood isoprostane levels. The purpose was to determine whether human skin aging severity associates with blood isoprostanes levels, specifically PGF2a, 8-iso-PGF2a, and 15R-8-iso-PGF2a, while controlling for covariates such as sun exposure, diet, medication or supplement use. We designed a prospective cohort study using a community setting in the Tokyo, Japan metropolitan area with participants that included 36 healthy, nonsmoking middle-aged Japanese women aged 45-60 years with body mass index (BMI) within one standard deviation of the mean, out of an original 70 volunteers seeking participation. The main outcome measure(s) included statistically significant increase in PGF2a, 8-iso-PGF2a, and 15R-8-iso-PGF2a isoprostane levels in participants with increased skin aging. Our results showed that mean blood isoprostane levels of PGF2a and 8-isoPGF2a was significantly increased in the group of subjects whose skin age was assessed to exceed chronological age, with p-values <0.01 for both. When these mean values were adjusted for age, BMI, education level, UV exposure, work stress, diet, medication and supplement use, the p-values for these 2 isoprostanes remained <0.01 for both. In conclusion, this study represents the first report in medical literature that increased skin aging phenotype may be reflected in systemic levels of PGF2a and 8-isoPGF2a isoprostanes.

J. INVEST DERMATOL 131. S. 57 (2011)

A MULTIFACTORIAL GENE EXPRESSION APPROACH TO UNDERSTANDING SKIN AGING

Authors: HE Knaggs, DG Kern, M Bartlett, SM Wood, A Mastaloudis, SB Ferguson, A Langerveld

The advent of genomic research has opened up new ways of investigating skin aging as well as overall 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. Among the most promising routes are methodologies that seek to understand the interactions of multiple genes during aging. Aging is the result of complex multifactorial influences occurring over time between lifestyle choices, environmental and genome, necessitating the study of multiple genes and expression patterns in multiple tissues. Additionally, tissues age at different rates and their capacity to repair varies. By scanning the entire genome of multiple key tissues using microarrays, we have identified multiple genetic expression patterns that occur with aging. Further, comparing these genetic expression changes across many individuals has enabled the identification of changes that are common and have strong correlation with aging or youthfulness. This permits the designation of a key set of genetic markers that can be used to screen for active ingredients affecting these genetic markers. Using data from muscle and skin, we describe our approach to discover multiple markers of skin aging and their use in screening for candidate aging response modulators, and we demonstrate the efficacy of modulation in the gene expression of what we call skin youth gene clusters.

COSMETICS & TOILETRIES (FEBRUARY 2011):112-117

A REVIEW OF GENOMIC TECHNIQUES IN COSMETIC TESTING

Authors: R. Gopaul, H. Knaggs, R. Wickett

Genomics assists product developers in understanding the expression of specific genes and their relationship to particular skin attributes. This article reviews commonly used testing techniques, such as DNA microarray, RT-PCR, SAGE, northern blot and RNA sequencing, and describes their application in testing the effects of cosmetic ingredients and products on skin.

EXPLORATORY STUDY TO ASSESS ASSOCIATION OF SKIN AGING WITH PLASMA ISOPROSTANE LEVELS IN HEALTHY MIDDLE-AGED JAPANESE WOMEN

Presented at: The American Academy of Dermatology's 69th Annual Meeting, New Orleans, LA, February 2011

Authors: AL Chang, TC Chang, B Lingala, DG Kern, SM Wood, H Toyoda, HE Knaggs

One of the central mechanisms of aging is hypothesized to be oxidative stress. To date, quantification of oxidative stress in human organ systems has been difficult. Exploratory studies determine if human skin aging severity associates with plasma isoprostane levels, specifically PGF2a, 8-iso-PGF2a, while controlling for covariates. Plasma isoprostanes merit further study as markers of oxidative stress in the skin and possible biomarkers of skin aging.

LINKS TO SKIN: GENES AND AGING

Presented at: In-Cosmetics Asia; Bangkok, Thailand, November 2010

Author: HE Knaggs

The advent of the Human Genome project, the development of better methodology for probing genetic material, and the improvement in accessibility of these methods has collectively resulted in the generation of a large amount of scientific data describing individual genes, as well as families of genes. Application of these methods to skin tissue has been no exception, with much of the available data coming from a variety of different techniques as methods have evolved and newer methods have become available, thus making it difficult to compare across studies and determine consistent findings. Additionally, the complexity of the skin tissue containing multiple cell types and many different proposed mechanisms for aging make it hard for clear data interpretation.

This presentation will review and summarize current genetic findings associated with skin aging. Review of these papers indicates that there is a substantial body of work published on changes in skin in both intrinsic and extrinsic aging. Data has been collected from different sources, including human skin biopsies, tissue culture of aged and young fibroblasts, and keratinocytes. One way authors are dealing with the overwhelming amount of data generated is by attributing these genes to families of genes or gene

'themes' defined by the Genetic Ontology (GO) library of gene pathways, then looking for changes in pathways that can be related to the different mechanisms of skin aging. Other groups are focused on understanding the effect of anti-aging actives (eg. retinoic acid) or clinical interventions (laser therapies, microdermabrasion), which are known to improve aged skin appearance, and could act as a positive control to understand and develop new ways to treat aging. Clearly, the challenge facing the Personal Care industry is to be able to use this abundance of scientific data and interpret it into meaningful data and marketing concepts to deliver a consumer-compelling story.

JOURNAL OF COSMETIC SCIENCE 9(3) 2010; 260-263

INNOVATION IN THE PERSONAL CARE INDUSTRY

Author: HE Knaggs

When considering opportunities to develop novel, eye-catching, and consumer-relevant personal care (PC) products, it is important to understand and reflect on how science has changed over the last two decades and how this has generated a new body of data from which to draw ideas and technologies. This article outlines some advances in scientific technologies and new ways of thinking in science, which lead to new insights into skin biology. How these innovations may impact and be leveraged into the development of new product in PC is also discussed. For example, fundamental discoveries in skin biology and advancement of scientific methodologies are enabling step changes in technology in PC. Two examples of areas where we have seen much advancement are discussed. This article is based on and summarizes a presentation given at the HBA in Sep 2009 as part of a session entitled "Emerging Technologies and New Opportunities in Anti-aging in PC."

AGING AND GENES-THE LINK WITH SKIN

Presented at: The New York Society of Cosmetic Chemists, March 2010

Author: HE Knaggs

The advent of the Human Genome project, the development of better methodology for probing genetic material, and the improvement in accessibility of these methods has collectively resulted in the generation of a large amount of scientific data describing individual genes, as well as families of genes. Application of these methods to skin tissue has been no exception, with much of the available data coming from a variety of different techniques as methods have evolved and newer methods have become available, thus making it difficult to compare across studies and determine consistent findings. Additionally, the complexity of the skin tissue containing multiple cell types and many different proposed mechanisms for aging make it hard for clear data interpretation.

This presentation will review and summarize current genetic findings associated with skin aging. Review of these papers indicates that there is a substantial body of work published on changes in skin in both intrinsic and extrinsic aging. Data has been collected from different sources, including human skin biopsies, tissue culture of aged and young fibroblasts, and keratinocytes. One way authors are dealing with the overwhelming amount of data generated is by attributing these genes to families of genes or gene 'themes' defined by the Genetic Ontology (GO) library of gene pathways, then looking for changes in

pathways that can be related to the different mechanisms of skin aging. Other groups are focused on understanding the effect of anti-aging actives (eg. retinoic acid) or clinical interventions (laser therapies, microdermabrasion), which are known to improve aged skin appearance, and could act as a positive control to understand and develop new ways to treat aging. Clearly, the challenge facing the Personal Care industry is to be able to use this abundance of scientific data and interpret it into meaningful data and marketing concepts to deliver a consumer-compelling story.

TRANSCRIPTIONAL BIOMARKERS OF AGE AND THEIR MODULATION BY DIETARY INTERVENTIONS

Presented at: International Congress on Controversies in Longevity, Health and Aging (COLONGY) Barcelona, Spain. June 26, 2010.

Authors: Barger JL[1], Wood SM[2], Weindruch R[1], Prolla TA[1]

[1] LifeGen Technologies, Madison WI (USA)
[2] Pharmanex R&D, Provo UT (USA)

Studies using whole-genome transcriptional profiling have identified thousands of genes that are changed in expression with age. However, many of these age-related changes are not universal, but instead are specific to the genetic background of the organism being studied. Thus, there is great interest in identifying robust biomarkers of age across multiple experimental models that are applicable to human aging. We used gene expression profiling to identify transcripts that were consistently changed in expression with age (5 vs. 28–30 month old) in seven mouse strains. This analysis was performed in four tissues (heart, cerebral cortex, gastrocnemius and adipose tissue) and RT-qPCR was used to confirm a panel of 10–20 genes in each tissue. Interestingly, we found minimal overlap across the four tissues studied, suggesting that aging at the individual gene level is tissue-specific. We then assessed whether the age-related change in these biomarkers was effected by caloric restriction (CR), the only intervention known to extend lifespan by slowing the aging process. Depending on the tissue studied, CR opposed 3–24% of the overall aging change. Finally, we assessed the ability of dietary ingredients to attenuate age-related changes in these biomarkers. An extract of pomegranate was the most effective compound tested, opposing 32–65% of the overall aging change depending on the tissue studied. In summary, we have identified robust, tissue-specific panels of the transcriptional biomarkers that are relevant to human aging. We are currently using these biomarkers in a large-scale screen of compounds to determine if they have efficacy in preventing aging at the transcriptional level.

ANTIOXIDANT AND LIFESPAN-EXTENSION ACTIVITIES OF CORDYCEPS SINENSIS CS-4 IN OXIDATIVE STRESS AND AGING MODELS

Presented at: Oxygen Club of California 2010 World Congress at Santa Barbara, CA. March 17–20, 2010.

Authors: Jia-Shi Zhu,[1,2,3] Yan Zhang,[2] Jieying Yang,[2] Ningzhi Tan,[2] Angela Mastaloudis,[1] and Chunsheng Zhao[2]

[1]Pharmanex Research Institute, Provo, UT 84601

[2]Pharmanex Beijing Clinical Pharmacology Center: 2 Xinkang Road, Beijing 100088, China

[3]School of Pharmacy, Shihezi University, Shihezi City, Xinjiang 832000, China

Cordyceps sinensis has traditionally been used in China as a strategy to combat aging. We have reported

the effects of *Cordyceps sinensis* Cs-4 (Cs-4), a mycelia fermentation product of C. sinensis, in glucose-lipid-energy metabolism, anti-fatigue, and endurance enhancement. In this study, we examined the effect of Cs-4 on lifespan extension and antioxidant status in mice. For the lifespan extension study, 250 mice 12 months of age (both sexes) received either vehicle or Cs-4 (0.5, 1.0 or 1.5g/kg diet). Caloric intake was adjusted to match the levels for controls twice per week. Compared to controls, the Cs-4 dosage group's 75% survival time was extended 94-108 days, 50% survival time extended 10-66 days, 25% survival time extended 29–44 days, and 12.5% survival time extended 7–50 days (86 weeks so far; treatment continues). The Kaplan-Meier Survivor analysis revealed the extended lifespan and the reduced risks of death by Cs-4. The antioxidant activity was tested in mice (6 months old) that received 60 days of vehicle or Cs-4 (0.5, 1.0 or 1.5 g/kg diet) and a single dose of 11Gy 60Co gamma-radiation on day 60. Compared to controls, Cs-4 prevented the depletion of plasma total thiol-groups, GSH and GSH-peroxidase, liver CAT, SOD, and GSH-reductase ($p<0.05$) caused by radiation exposure. Cs-4 also prevented the radiation-induced increases in liver protein carbonyls and 8-OHdG ($p<0.05$) observed in the control group. In conclusion, Cs-4 supplementation significantly improves the body's antioxidant capacity and extends the lifespan in mice, supporting the traditional anti-aging uses of Cs-4 in humans.

TRANSCRIPTIONAL BIOMARKERS OF MITOCHONDRIAL AGING AND MODULATION BY CORDYCEPS SINENSIS CS-4

Presented at: Biology of Aging, Determinants of Health-Span: From Cells to Humans. Gordon Research Conferences. Les Diablerets, Switzerland. August 22-27, 2010.

Authors: SM Wood[1], JL Barger[2], TA Prolla[2], R Weindruch[2], A Mastaloudis[1], SB Ferguson[1].

[1] Pharmanex R&D, Provo UT (USA)
[2] LifeGen Technologies, Madison WI (USA)

One of the earliest manifestations of human aging is a decline in energy, which begins as early as 30 years of age. The source of this decline is multi-factorial, yet changes in mitochondria (ie. function and number) have been implicated as an integral component of the age-associated decline in humans. Therefore, we set out to identify mitochondrial nuclear encoded genes that consistently change in expression with aging. *Cordyceps sinensis* Cs-4 (Cs-4) is a natural ingredient that has been shown to have anti-aging properties and positive effects on energy, including maximal oxygen consumption (VO2max). Therefore, we examined whether age-related gene expression changes could be opposed by Cs-4. Mice (C57Bl/6), aged 5 (n = 5; young control (YC)) and 22 (n = 10; old) months of age were fed an AIN 93M diet. The old animals were divided into two groups and fed either the diet alone (old control (OC)) or supplemented with Cs-4 (300 mg/kg body weight)(old supplemented (OS)), for three months. Tissues were collected from skeletal muscle (gastrocnemius) and brain (cerebral cortex); gene expression was analyzed using microarrays. Gene expression profiling was used to identify mitochondrial-related transcripts that consistently changed with age in brain and muscle. Gene ontology terms were used and Parametric Analysis of Gene set Enrichment (PAGE) performed to determine effects of age (YC vs. OC) and supplementation with Cs-4 (OC vs. OS). We identified 393 out of 1241 mitochondria-related nuclear encoded transcripts in the muscle (220) and brain (173) that changed in expression with age. Cs-4 opposed the age-related changes in 48 of the genes ($p<0.05$). In addition, Cs-4 opposed the effects of aging in several gene ontology pathways. We identified mitochondrial-related nuclear encod-

ed genes, which changed consistently in expression with age. Using this methodology, we found that Cs-4 opposed many of these changes in aging brain and muscle. Ongoing studies are utilizing this technique to investigate the effects of a variety of natural ingredients in brain, muscle and other tissues.

A NUTRITIONAL STRATEGY TO OPPOSE THE GENETIC EXPRESSION OF AGING AND LOSS OF VITALITY

Presented at: 1st World Congress on Targeting Mitochondria, Strategies, Innovations & Clinical Applications. Berlin, Germany; November 18-19, 2010.

Authors: Wood SM[1]; Ferguson SB[1], Barger JL[2], Prolla TA[2], Weindruch R[2], Bartlett M[1].

1 Nu Skin Center for Anti-Aging Research, Provo 84601, UT.
2 LifeGen Technologies, LLC, Madison 53706, WI

One of the earliest manifestations of human aging is a decline in vitality. Age-related mitochondrial dysfunction yields bioenergetic defects within the cell that influence physical and mental vitality. The purpose of this study was to identify and target tissue-specific transcriptional biomarkers (Super Markers of aging) and functional youth gene clusters associated with the mitochondria (mtYGCs) in mouse brain (cerebral cortex) and skeletal muscle (gastrocnemius) tissues. Furthermore, we screened natural ingredients using Super Markers of aging and mtYGCs to identify candidates for a nutritional formula to oppose age-related gene expression changes that influence vitality.

Methods: *Study 1. Super Markers of Aging:* Seven strains of mice (six inbred and one F1 hybrid strain: 129sv, BALB/c, CBA, DBA, C57bl/6, C3H, and B6C3F1) aged 5 mo (young;Y) and 25 mo (old;O) were compared for consistent age-related changes in gene expression in select tissues including the brain and skeletal muscle. Once age-related gene expression changes common to all seven strains (Super Markers of aging) were identified, several natural ingredients were screened for activity that opposed aging effects.

Study 2. mtYGC Identification: Brain and skeletal muscle tissues were collected from mice (C57Bl/6) aged 5 mo (young control;YC) and 25 mo (old control;OC) of age and gene expression profiles were compared. Nuclear mitochondrial-associated genes whose expression changed with age (OC vs. YC, $p<0.05$) were grouped and defined as an mtYGC. Old supplemented (OS) mice were fed *Cordyceps sinensis* Cs-4 (Cs-4) at 300 mg/kg body weight from age 22–25 mo. Tissues from OS were compared to OC for gene expression differences and whether changes were towards a more youthful (YC) gene expression pattern.

Results: *Study 1.* Super Markers of aging were identified by their consistency of change in all strains of mice and included 10–15 genes per tissue. Pomegranate fruit (PFE) was one of the most potent natural ingredients that opposed changes in the Super Markers of aging muscle and brain tissue, respectively (32–65%; $p<0.05$).

Study 2. Of the 20,687 gene transcripts measured, 1,241 were classified with the mitochondria in some fashion (structural, enzymatic, etc). Of these genes, 172 changed in expression with aging in brain and 220 in muscle tissues (OC vs. YC). Cs-4 strongly opposed ($p<0.05$) the age-related changes in mtYGCs in both tissues.

We identified Super Markers of aging as well as mtYGCs whose expression changed with age. We then screened ingredients that opposed these changes and identified PFE and Cs-4 as potent mitochondrial anti-aging ingredients. The gene expression changes we observed following supplementation provide mechanistic evidence of anti-aging effects noted by other researchers. Studies designed to elucidate the functional benefits (i.e. vitality) of resetting mtYGC, as well as a novel formula containing PFE and Cs-4, are ongoing.

TARGETING AGE-RELATED GENE EXPRESSION IMPROVES MENTAL AND PHYSICAL VITALITY

Presented at: 1st World Congress on Targeting Mitochondria, Strategies, Innovations & Clinical Applications. Berlin, Germany; November 18-19, 2010.

Authors: Ferguson SB[1], Tan NZ[2], Dong YZ[2], Lu JH[2], Fisk NA[1], Wood SM[1], Zhu JS[1,2], Bartlett M[1].

[1] Nu Skin Center for Anti-Aging Research, Provo 84601, UT.
[2] Pharmanex Beijing Clinical Pharmacology Center, Beijing 100088 China.

Vitality loss is a universal complication of the aging process. Age-related mitochondrial dysfunction yields bioenergetic defects within the cell, having profound effects on physical and mental vitality. We previously identified functional groups of genes, or gene clusters, associated with mitochondrial aging, and transcriptional biomarkers of aging in multiple tissues. Using these as targets, we performed large scale screening of natural products and identified two ingredients in particular that were able to restore the transcriptional profile of these genes to a more youthful pattern. For example, a unique pomegranate fruit extract (PFE) opposed 32–65% of the overall aging changes depending on the tissue studied. *Cordyceps sinensis* Cs-4 (Cs-4), a natural ingredient, was also shown to significantly restore the expression pattern of mitochondrial gene clusters to a more youthful level.

Published studies have established that Panax Ginseng Root Extract (PG) improves mitochondrial function by increasing key transcriptional factors (AMPK, PGC-1 alpha, Nrf2). These findings, coupled with our own transcriptional profiling experiments on Cs-4 and PFE, have led us to our present objective: to better understand the functional benefits of targeting age-related gene expression changes, via dietary supplementation with combinations of Cs-4, PFE, and PG.

Female ICR mice (8 and 18 months of age) were fed either control or a blend of Cs-4+PG +/- PFE; 400 or 800 mg/kg) (n=15). Treadmill exercise to exhaustion was examined at Week five, and swim to exhaustion tested at Week seven. After two weeks of Cs-4+PG+PFE supplementation, the following parameters were tested: plasma lactate, muscle and liver glycogen, muscle mitochondria enzyme activities, and muscle superoxide. In a separate human cognitive study, male and female human subjects aged 28–50 were randomized into four independent arms (n=10/group): 1. Positive-control: 200mg Phosphatidylserine DHA, 300mg Bacopan, 30mg Vinpocetine; 2. Vitality blend: 2270 mg/day (Cs-4+PG+PFE); 3. Placebo: mono-crystalline cellulose, caramel color; 4. No-supplementation. Subjects underwent cognitive testing (designed by MyBrainTrainer.com) twice a week for 12 weeks. Parameters measured: three-choice reaction time, short term memory, executive function, information processing, pattern recognition, and working memory. The six scores were averaged to generate a Composite Cognition Index (CCI).

In mice, Cs-4+PG treatment increased treadmill time to exhaustion by 63% ($p<0.01$), increased activities of mitochondrial complexes I+III and II+III ($p<0.05$), and decreased blood lactate by 25% ($p<0.01$). Treatment with Cs-4+PG+PFE increased swim time to exhaustion by 22% ($p=0.02$), spared glycogen in muscle ($p=0.003$), and lowered muscle superoxide production by 32% ($p=0.005$). In humans, treatment with Cs-4+PG+PFE (vitality blend) improved overall cognitive function by 20% (as measured by the CCI), and improved reaction time by 110 ms (14%) vs. placebo ($p<0.05$).

We report improvements in treadmill performance, swimming performance, and glycogen sparing in mice. Considerable enhancement in cognitive performance was achieved in a blinded human clinical study. These results demonstrate that targeting the genetic basis of the aging process (changes in gene expression), can yield powerful anti-aging benefits. This work indicates that a blend of Cs-4, PG, and PFE can alleviate some of the physical and mental symptoms of age-related vitality loss.

ROLE OF MITOCHONDRIA IN AGE-RELATED HEARING LOSS AND ITS PREVENTION BY CALORIC RESTRICTION

Presented at: International Symposium on Aging and Anti-Aging; Tokyo, Japan. Sept 24, 2010.

Author: Tomas A. Prolla, Ph.D.

Professor, Departments of Genetics and Medical Genetics,
University of Wisconsin-Madison

Age-related hearing loss (AHL, also known as presbycusis) is a universal feature of mammalian aging and is the most common sensory disorder in the elderly population, affecting 50% of individuals over 60 years of age. AHL is a complex disorder, but it is widely accepted that AHL is generally caused by degeneration of the inner ear (cochlea). AHL is associated with age-dependent loss of sensory hair cells, which function as mechanosensory transducers, and spiral ganglion neurons, which relay information from the hair cells to the CNS. Because these cells do not regenerate in mammals, cochlear cell loss eventually leads to AHL.

We have recently demonstrated that AHL can be prevented in mice by deletion of Bak, a mitochondrial pro-apoptotic protein. Bak-mediated cell death of cochlear cells is induced by oxidative stress, and prevented by overexpression of a mitochondrial-targeted catalase, or dietary antioxidants that target mitochondria.
We have also shown that caloric restriction can prevent AHL, and that prevention is associated with the induction of mitochondrial transcripts, and reduction in the expression of genes involved in the mitochondrial apoptotic pathway. Induction of the mitochondrial sirtuin sirt3 appears to be essential for the positive effects of CR, since sirt3 gene deletion prevents the beneficial effects of CR. These findings suggest novel pathways for intervention in aging and AHL through the development of CR mimetic compounds.

A PCR-BASED SCREEN TO IDENTIFY NATURAL COMPOUNDS WITH THE ABILITY TO INFLUENCE NRF2-MEDIATED TRANSCRIPTIONAL INDUCTION OF DETOXIFICATION/ANTIOXIDANT GENES

Presented at: Gordon Research Conference : Oxidative Stress and Disease. Emerging Research Areas in the Study of Oxidative Stress and Disease. March 13–18, 2011, Ventura, CA.

Authors: A Mastaloudis,[1] JL Barger,[2] TA Prolla,[2,3] R Weindruch,[2,3] SB Ferguson,[1] SM Wood[1]

[1]Nu Skin Enterprises, Provo, Utah, U.S.A.;

[2]LifeGen Technologies, Madison, Wisconsin, U.S.A.;

[3]University of Wisconsin, Madison, Wisconsin, U.S.A.

It is widely accepted that oxidative injury and inflammation are intimately involved in the aging process and the development of age-related conditions. To date, most anti-aging strategies have focused solely on the delivery of exogenous antioxidants to combat the negative effects of aging. A promising new strategy is to identify nutrients and plant extracts that can directly target intrinsic cytoprotective mechanisms, including: 1) up-regulation of genes involved in the detoxification of xenobiotic and xenobiotic metabolites, 2) up-regulation of genes involved in the synthesis and regulation of intrinsic antioxidants and antioxidant enzymes, and 3) modulation of genes involved in the regulation of inflammation. Therefore, the purpose of this study was to evaluate natural compounds for the ability to modulate a representative panel of genes from key age-related pathways: the Nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE)/Phase II detoxification and inflammatory pathways. Nrf2 is a transcription factor that regulates the basal and inducible expression of a large battery of genes encoding for cytoprotective factors, including those that defend against electrophilic stressors and oxidative insults. We selected a panel of eight genes representative of the Nrf2 and inflammatory pathways based on a review of the literature. Mice (C57Bl/6), aged 8 weeks were fed an AIN 93M diet without (n=8) or with one of 12 compounds (n=8/group) for 14 weeks. In a second, exploratory study, we compared the effects of a subset of compounds in young mice (8 weeks) and old mice (14 months) with and without supplementation for 16 months. The compounds included a variety of nutrients and plant extracts. Tissues were collected and gene expression of the eight-gene panel was analyzed by RT-qPCR. Finally, nutrients and plant extracts were ranked based on the number of Nrf2 genes up-regulated, number of inflammation-related genes down-regulated, and the robustness of the changes in gene expression. In summary, we have identified a robust panel of genes representative of the Nrf2 and inflammatory pathways that can be used as a rapid screening tool to evaluate the effects of specific nutrients on cellular detoxification, antioxidant status, and inflammatory balance. This technique affords an opportunity to define the optimal blend of ingredients that can oppose gene expression changes in these key pathways that directly relate to human aging.

TRANSCRIPTIONAL BIOMARKERS OF MITOCHONDRIAL AGING AND MODULATION BY CORDYCEPS SINENSIS CS-4

Presented at: Biology of Aging, Determinants of Health-Span: From Cells to Humans. Gordon Research Conferences. Les Diablerets, Switzerland. August 22–27, 2010.

Authors: SM Wood,[1] JL Barger,[2] TA Prolla,[2] R Weindruch,[2] A Mastaloudis,[1] SB Ferguson[1]

[1]Nu Skin Enterprises, Provo, Utah, U.S.A.

[2]LifeGen Technologies, Madison, Wisconsin, U.S.A.

One of the earliest manifestations of human aging is a decline in energy, which begins as early as 30 years of age. The source of this decline is multi-factorial, yet changes in mitochondria (function, size, and number) have been implicated as an integral component of the age-associated decline in humans. Therefore, we set out to identify mitochondrial-related nuclear genes that consistently change in expression with aging. *Cordyceps sinensis* Cs-4 (Cs-4) is a natural ingredient that has been shown to have anti-aging properties and positive effects on energy, including maximal oxygen consumption (VO2max). Therefore, we examined whether age-related gene expression changes could be opposed by Cs-4.

Mice (C57Bl/6), aged 5 (n=5; young control-YC) and 22–25 (n=10; old-O) months of age were fed an AIN 93M diet. The old group was divided and fed the diet alone (old control-OC) or supplemented (old supplemented-OS) with Cs-4 (30 mg/kg body weight) for three months. Tissues were collected from skeletal muscle (gastrocnemius) and brain (cerebral cortex); gene expression was analyzed by microarrays. Gene expression profiling was used to identify mitochondrial-related transcripts that consistently changed with age in brain and muscle. Gene ontology terms were used and Parametric Analysis of Gene set Enrichment (PAGE) performed to determine effects of age (YC vs. OC) and supplementation with Cs-4 (OC vs OS).

We identified 393 out of 1,241 mitochondria-related nuclear transcripts in the muscle and brain that changed in expression with age. Cs-4 opposed the age-related changes in 52 of the genes ($P<0.05$). In addition, Cs-4 opposed the effects of aging in several gene ontology pathways.

We identified mitochondrial-related nuclear genes that consistently change in expression with age. Using this methodology, we found that Cs-4 opposed many of these changes in aging brain and muscle. Ongoing studies are utilizing this technique to investigate the effects of a variety of natural ingredients in brain, muscle, and other tissues.

A UNIQUE BLEND OF NATURAL COMPOUNDS WITH THE ABILITY TO OPPOSE AGE-RELATED CHANGES IN GENE EXPRESSION RELATED TO DYSREGULATION OF METABOLISM, CELLULAR DETOXIFICATION, ANTIOXIDANT PROTECTION AND INFLAMMATORY BALANCE.

Authors: Mastaloudis A[1]; Barger JL[2]; Prolla TA[2, 3]; Weindruch R[2, 3]; Bartlett, M[1]; Wood SM[1]

[1]Nu Skin Enterprises, Provo, Utah, USA;

[2]LifeGen Technologies, Madison, Wisconsin, USA;

[3]University of Wisconsin, Madison, Wisconsin, USA.

Aging is a complex multi-factorial process that remains poorly understood. Changes in cellular metabolism, energy production, nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE)/Phase II detoxification and inflammatory balance are consistently observed, even in 'healthy' aging models. One hallmark of the aging process is the dysregulation of gene expression associated with these pathways, as well as the inflammation and accumulation of damaging metabolic byproducts resulting from alterations in gene expression. To date, the few anti-aging strategies designed to target gene expression have focused solely on single genes and/or individual tissues. A promising new strategy is to first identify multiple genes in diverse tissues that change in expression with aging and then to select nutrients and plant extracts that can directly oppose age-related changes in gene expression and in doing so, combat the negative effects of aging. Therefore, the purpose of this study was to test a blend of natural ingredients in middle-aged mice for the ability to oppose age-related changes in gene expression of several genes in multiple tissues.

Mice (CBA/J), aged 16 months were fed an AIN 93M diet without (middle-age controls (MAC); n = 8) or with the Formula (middle-age supplemented (MAS); n = 8) for three months. The supplement consisted of a blend of natural compounds: Cordyceps sinensis and extracts of pomegranate seed, Panax ginseng, broccoli seed, blood orange, and grape seed. Full gene expression profiling was performed using Affymetrix Mouse Genome arrays in liver and gastrocnemius muscle tissues. Gene expression profiles were compared in order to identify changes in gene expression in response to the supplement.

We have identified a unique blend of natural compounds with the ability to positively influence gene expression related to dysregulation of metabolism, cellular detoxification, antioxidant protection, and inflammatory balance typically associated with aging. These effects, elicited by a mid-life nutritional intervention, likely have positive implications for healthy human aging or 'youthspan.'

GENES DEV. 2007 DEC 15;21(24):3244-57. EPUB 2007 NOV 30.

MOTIF MODULE MAP REVEALS ENFORCEMENT OF AGING BY CONTINUAL NF-KAPPAB ACTIVITY

Authors: Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, Chang HY.

Program in Epithelial Biology and Cancer Biology Program, Stanford University School of Medicine, Stanford, California 94305, USA.

Aging is characterized by specific alterations in gene expression, but their underlying mechanisms and functional consequences are not well understood. Here we develop a systematic approach to identify combinatorial cis-regulatory motifs that drive age-dependent gene expression across different tissues and organisms. Integrated analysis of 365 microarrays spanning nine tissue types predicted fourteen motifs as major regulators of age-dependent gene expression in humans and mice. The motif most strongly associated with aging was that of the transcription factor NF-kappaB. Inducible genetic blockade of NF-kappaB for two weeks in the epidermis of chronologically aged mice reverted the tissue characteristics and global gene expression programs to those of young mice. Age-specific NF-kappaB blockade and orthogonal cell cycle interventions revealed that NF-kappaB controls cell cycle exit and gene expression signature of aging in parallel but not sequential pathways. These results identify a conserved network of regulatory pathways underlying mammalian aging and show that NF-kappaB is continually required to enforce many features of aging in a tissue-specific manner.

CELL CYCLE. 2008 MAR 1;7(5):556-9. EPUB 2007 DEC 26.

REVERSAL OF AGING BY NFKAPPAB BLOCKADE

Authors: Adler AS, Kawahara TL, Segal E, Chang HY.

Program in Epithelial Biology and Cancer Biology Program, Stanford University School of Medicine, Stanford, California 94305, USA.

Genetic studies in model organisms such as yeast, worms, flies, and mice leading to lifespan extension suggest that longevity is subject to regulation. In addition, various system-wide interventions in old animals can reverse features of aging. To better understand these processes, much effort has been put into the study of aging on a molecular level. In particular, genome-wide microarray analysis of differently aged individual organisms or tissues has been used to track the global expression changes that occur during normal aging. Although these studies consistently implicate specific pathways in aging processes, there is little conservation between the individual genes that change. To circumvent this problem, we have recently developed a novel computational approach to discover transcription factors that may be responsible for driving global expression changes with age. We identified the transcription factor NFkappaB as a candidate activator of aging-related transcriptional changes in multiple human and mouse tissues. Genetic blockade of NFkappaB in the skin of chronologically aged mice reversed the global gene expression program and tissue characteristics to those of young mice, demonstrating for the first time that disruption of a single gene is sufficient to reverse features of aging, at least for the short-term.

APPENDIX: BACKGROUND RESEARCH CONDUCTED BY INDEPENDENT PARTIES—SKIN CARE

NATURE. 2001 FEB 15;409(6822):860-921.

INITIAL SEQUENCING AND ANALYSIS OF THE HUMAN GENOME

Authors: Lander ES, et al

Whitehead Institute for Biomedical Research, Center for Genome Research, Cambridge, Massachusetts 02142, USA. lander@genome.wi.mit.edu

The human genome holds an extraordinary trove of information about human development, physiology, medicine, and evolution. Here we report the results of an international collaboration to produce and make freely available a draft sequence of the human genome. We also present an initial analysis of the data, describing some of the insights that can be gleaned from the sequence.

SCIENCE. 2001 FEB 16;291(5507):1304-51.

THE SEQUENCE OF THE HUMAN GENOME

Authors: Venter JC, et al

Celera Genomics, 45 West Gude Drive, Rockville, MD 20850, USA. humangenome@celera.com

A 2.91-billion base pair (bp) consensus sequence of the euchromatic portion of the human genome was generated by the whole-genome shotgun sequencing method. The 14.8-billion bp DNA sequence was generated over nine months from 27,271,853 high-quality sequence reads (5.11-fold coverage of the genome) from both ends of plasmid clones made from the DNA of five individuals. Two assembly strategies—a whole genome assembly and a regional chromosome assembly—were used, each combining sequence data from Celera and the publicly funded genome effort. The public data were shredded into 550-bp segments to create a 2.9-fold coverage of those genome regions that had been sequenced, without including biases inherent in the cloning and assembly procedure used by the publicly funded group. This brought the effective coverage in the assemblies to eightfold, reducing the number and size of gaps in the final assembly over what would be obtained with 5.11-fold coverage. The two assembly strategies yielded very similar results that largely agree with independent mapping data. The assemblies effectively cover the euchromatic regions of the human chromosomes. More than 90% of the genome is in scaffold assemblies of 100,000 bp or more, and 25% of the genome is in scaffolds of 10 million bp or larger. Analysis of the genome sequence revealed 26,588 protein-encoding transcripts for which there was strong corroborating evidence and an additional approximately 12,000 computationally derived genes with mouse matches or other weak supporting evidence. Although gene-dense clusters are obvious, almost half the genes are dispersed in low G+C sequence separated by large tracts of apparently noncoding sequence. Only 1.1% of the genome is spanned by exons, whereas 24% is in introns, with 75% of the genome being intergenic DNA. Duplications of segmental blocks, ranging in size up to chromosomal lengths, are abundant throughout the genome and reveal a complex evolutionary history. Comparative genomic analysis indicates vertebrate expansions of genes associated with neuronal function, with tissue-specific developmental regulation, and with the hemostasis and immune systems. DNA sequence comparisons between the consensus sequence and publicly funded genome data provided locations of 2.1 million single-nucleotide polymorphisms (SNPs). A random pair of human haploid genomes differed at a rate of 1 bp per 1250 on average, but there was marked heterogeneity in the level of polymorphism across the genome. Less than 1% of all SNPs resulted in variation in proteins, but the task of determining which SNPs have functional consequences remains an open challenge.

METHODS MOLECULAR BIOLOGY. 2009, 509: 35-46.

EXPRESSION PROFILING USING AFFYMETRIX GENECHIP MICROARRAYS

Authors: Auer H, Newsom DL, Kornacker K.

Columbus Children's Research Institute, Columbus, OH, USA.

The approximately 25,000 genes in mammalian genomes can be transcribed at different levels. Measurements of gene expression for ten thousands of genes in parallel give the most comprehensive picture of steady-state levels of transcripts and is used in basic and applied research. Microarrays are the most frequently used technology for genome-wide expression profiling; from the various available microarray platforms, Affymetrix GeneChips are most frequently used for expression profiling and over 3,000 scientific publications describe results of this technology. In medical research, expression profiling by microarrays holds great promises for better understanding of diseases, identification of new therapeutic targets, and subclassification of diseases to identify individualized treatment strategies.

BMC BIOINFORMATICS. 2008 JUN 17;9:284.

METHODS FOR EVALUATING GENE EXPRESSION FROM AFFYMETRIX MICROARRAY DATASETS

Authors: Jiang N, Leach LJ, Hu X, Potokina E, Jia T, Druka A, Waugh R, Kearsey MJ, Luo ZW.

School of Biosciences, The University of Birmingham, Edgbaston Birmingham B15 2TT, England, UK. nxj677@bham.ac.uk

Affymetrix high density oligonucleotide expression arrays are widely used across all fields of biological research for measuring genome-wide gene expression. An important step in processing oligonucleotide microarray data is to produce a single value for the gene expression level of an RNA transcript using one of a growing number of statistical methods. The challenge for the researcher is to decide on the most appropriate method to use to address a specific biological question with a given dataset. Although several research efforts have focused on assessing performance of a few methods in evaluating gene expression from RNA hybridization experiments with different datasets, the relative merits of the methods currently available in the literature for evaluating genome-wide gene expression from Affymetrix microarray data collected from real biological experiments remain actively debated. The present study reports a comprehensive survey of the performance of all seven commonly used methods in evaluating genome-wide gene expression from a well-designed experiment using Affymetrix microarrays. The experiment profiled eight genetically divergent barley cultivars each with three biological replicates. The dataset so obtained confers a balanced and idealized structure for the present analysis. The methods were evaluated on their sensitivity for detecting differentially expressed genes, reproducibility of expression values across replicates, and consistency in calling differentially expressed genes. The number of genes detected as differentially expressed among methods differed by a factor of two or more at a given false discovery rate (FDR) level. Moreover, we propose the use of genes containing single feature polymorphisms (SFPs) as an empirical test for comparison among methods for the ability to detect true differential gene expression on the basis that SFPs largely correspond to cis-acting expression regulators. The PDNN method demonstrated superiority over all other methods in every comparison, whilst the default Affymetrix MAS5.0 method was clearly inferior. A comprehensive assessment of seven commonly used data extraction methods based on an extensive barley Affymetrix gene expression dataset has shown that the PDNN method has superior performance for the detection of differentially expressed genes.

EXP HEMATOL. 2002 JUN;30(6):503-12.

GENE QUANTIFICATION USING REAL-TIME QUANTIFICATION PCR: AN EMERGING TECHNOLOGY HITS THE MAINSTREAM

Author: Ginzinger DG.

Genome Analysis Core Facility, Comprehensive Cancer Center, School of Medicine, University of California at San Francisco, 94143-0808, USA. dginzing@cc.ucsf.edu

The recent flood of reports using real-time Q-PCR testifies to the transformation of this technology from an experimental tool into the scientific mainstream. Many of the applications of real-time Q-PCR include measuring mRNA expression levels, DNA copy number, transgene copy number and expression analysis, allelic discrimination, and measuring viral titers. The range of applications of real-time Q-PCR is immense and has been fueled in part by the proliferation of lower-cost instrumentation and reagents. Successful application of real-time Q-PCR is not trivial. However, this review will help guide the reader through the variables that can limit the usefulness of this technology. Careful consideration of the assay design, template preparation, and analytical methods are essential for accurate gene quantification.

J MOL ENDOCRINOL.2005 JUN;34(3):597-601.

QUANTITATIVE REAL-TIME RT-PCR: A PERSPECTIVE

Authors: Bustin SA, Benes V, Nolan T, Pfaffl MW.

Institute of Cellular and Molecular Science, Barts and the London, Queen Mary's School of Medicine and Dentistry, University of London, London, UK. s.a.bustin@qmul.ac.uk

The real-time reverse transcription polymerase chain reaction (RT-PCR) uses fluorescent reporter molecules to monitor the production of amplification products during each cycle of the PCR reaction. This combines the nucleic acid amplification and detection steps into one homogeneous assay and obviates the need for gel electrophoresis to detect amplification products. Use of appropriate chemistries and data analysis eliminates the need for Southern blotting or DNA sequencing for amplicon identification. Its simplicity, specificity and sensitivity, together with its potential for high throughput and the ongoing introduction of new chemistries, more reliable instrumentation and improved protocols, has made real-time RT-PCR the benchmark technology for the detection and/or comparison of RNA levels.

EXP GERONTOL. 2006 APR;41(4):387-97. EPUB 2006 MAR 10

EXPRESSION PROFILING OF AGING IN THE HUMAN SKIN

Authors: Lener T, Moll PR, Rinnerthaler M, Bauer J, Aberger F, Richter K.

Department of Cell Biology, University of Salzburg, Hellbrunnerstrasse 34, A-5020 Salzburg, Austria.

During the last years it was shown that the aging process is controlled by specific genes in a large number of organisms (C. elegans, Drosophila, mouse or humans). To investigate genes involved in the natural aging process of the human skin we applied cDNA microarray analysis of naturally aged human foreskin samples. For the array experiments a non-redundant set of 2,135 pre-selected EST clones was used. These arrays were used to probe the patterns of gene expression in naturally aged human skin of five young (3-4 years of age) and five old (68-72 years of age) healthy persons. We found that in total 105 genes change their expression over 1.7-fold

during the aging process in the human skin. Of these 43 genes were shown to be down-regulated in contrast to 62 up-regulated genes. Expression of regulated genes was confirmed by real-time PCR. These results suggest that the aging process in the human skin is connected with the deregulation of various cellular processes, like cell cycle control, cytoskeletal changes, inflammatory response, signaling and metabolism.

J INVEST DERMATOL (2008) 13, 15–19

SKIN IMMUNE SYSTEMS AND INFLAMMATION: PROTECTOR OF THE SKIN OR PROMOTER OF AGING?

Authors: Mary F. Bennett, Michael K. Robinson, Elma D. Baron and Kevin D. Cooper

Department of Dermatology and Skin Diseases Research Center, University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, Ohio.

The immune system may either have a protective role against sunburn and skin cancer or, conversely, promote solar damage. The skin is poised to react to infections and injury, such as sunburn, with rapidly acting mechanisms (innate immunity) that precede the development of acquired immunity and serve as an immediate defense system. Some of these mechanisms, including activation of defensins and complement, modify subsequent acquired immunity. An array of induced immune-regulatory and pro-inflammatory mediators is evident, at the gene expression level, from the microarray analysis of both intrinsically aged and photoaged skin. Thus, inflammatory mechanisms may accentuate the effect of UV radiation to amplify direct damaging effects on molecules and cells, including DNA, proteins, and lipids, which cause immunosuppression, cancer, and photoaging. A greater understanding of the cutaneous immune system's response to photo–skin interactions is essential to comprehensively protect the skin from adverse solar effects. Sunscreen product protection measured only as reduction in redness (current "sun" protection factor) may no longer be sufficient, as it is becoming clear that protection against UV-induced immune changes is of equal if not of greater importance. Greater knowledge of these processes will also enable the development of improved strategies to repair photodamaged skin.

INT J COSMET SCI. 2005 OCT;27(5):263-269

UNVEILING THE MOLECULAR BASIS OF INTRINSIC SKIN AGING

Authors: Holtkötter O, Schlotmann K, Hofheinz H, Olbrisch RR, Petersohn D.

Henkel KGaA, Henkelstraße 67, 40 191 Düsseldorf, Germany.

The process of skin aging is a combination of an extrinsic and intrinsic aspect, and knowing the molecular changes underlying both is a prerequisite to being able to effectively counter it. However, despite its importance for a deeper understanding of skin aging as a whole, the process of intrinsic skin aging in particular has barely been investigated. In this study, the molecular changes of intrinsic skin aging were analyzed by applying 'Serial Analysis of Gene Expression' (SAGE(TM)) to skin biopsies of young and aged donors. The analysis resulted in several hundred differentially expressed genes with varying statistical significance. Of these, several genes were identified that either have never been described in skin aging before (e.g. APP) or have no identified function, e.g. EST sequences. This is the first time that intrinsic skin aging has been analyzed in such a comprehensive manner, offering a new and partially unexpected set of target genes that have to be analyzed in more detail in terms of their contribution to the skin aging process.

J INVEST DERMATOL. 2002 JUL;119(1):3-13

A SERIAL ANALYSIS OF GENE EXPRESSION IN SUN-DAMAGED HUMAN SKIN

Authors: Urschitz J, Iobst S, Urban Z, Granda C, Souza KA, Lupp C, Schilling K, Scott I, Csiszar K, Boyd CD.

The Pacific Biomedical Research Center, University of Hawaii, 1993 East-West Road, Honolulu, HI 96822, U.S.A.

To study the phenotypic changes in human skin associated with repeated sun exposure at the transcription level, we have undertaken a comparative serial analysis of gene expression of sun-damaged preauricular skin and sun-protected postauricular skin as well as sun-protected epidermis. Serial analyses of gene expression libraries, containing multiple mRNA-derived tag recombinants, were made to poly(A+)RNA isolated from human postauricular skin and preauricular skin, as well as epidermal nick biopsy samples. 5,330 mRNA-derived cDNA tags from the postauricular serial analysis of gene expression library were sequenced and these tag sequences were compared to cDNA sequences identified from 5,105 tags analyzed from a preauricular serial analysis of gene expression library. Of the total of 4,742 different tags represented in both libraries we found 34 tags with at least a 4-fold difference of tag abundance between the libraries. Among the mRNAs with altered steady-state(1) levels in sun-damaged skin, we detected those encoding keratin 1, macrophage inhibitory factor, and calmodulin-like skin protein. In addition, a comparison of cDNA sequences identified in the serial analysis of gene expression libraries obtained from the epidermal biopsy samples (5,257 cDNA tags) and from both full-thickness skin samples indicated that many genes with altered steady-state transcript levels upon sun exposure were expressed in epidermal keratinocytes. These results suggest a major role for the epidermis in the pathomechanism of largely dermal changes in chronically sun-exposed skin.

BIOGERONTOLOGY. 2009 APR;10(2):125-51. EPUB 2008 JUL 25

GENOMIC AND PROTEOMIC PROFILING OF OXIDATIVE STRESS RESPONSE IN HUMAN DIPLOID FIBROBLASTS

Authors: Xie L, Pandey R, Xu B, Tsaprailis G, Chen QM.

Department of Pharmacology, College of Medicine, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ 85724, USA.

A number of lines of evidence suggest that senescence of normal human diploid fibroblasts (HDFs) in culture is relevant to the process of aging in vivo. Using normal human skin diploid fibroblasts, we examine the changes in genes and proteins following treatment with a mild dose of H_2O_2, which induces premature senescence. Multidimensional Protein Identification Technology (MudPIT) in combination with mass spectrometry analyses of whole cell lysates from HDFs detected 65 proteins in control group, 48 proteins in H_2O_2-treated cells and 109 proteins common in both groups. In contrast, cDNA microarray analyses show 173 genes up-regulated and 179 genes down-regulated upon H_2O_2 treatment. Both MudPIT and cDNA microarray analyses indicate that H_2O_2 treatment caused elevated levels of thioredoxin reductase 1. Semi-quantitative RT-PCR and Western-blot were able to verify the finding. Out of a large number of genes or proteins detected, only a small fraction shows the overlap between the outcomes of microarray versus proteomics. The low overlap suggests the importance of considering proteins instead of transcripts when investigating the gene expression profile altered by oxidative stress.

J CELL PHYSIOL. 2009 AUG;220(2):427-39

RETINOID-RESPONSIVE TRANSCRIPTIONAL CHANGES IN EPIDERMAL KERATINOCYTES

Authors: Lee DD, Stojadinovic O, Krzyzanowska A, Vouthounis C, Blumenberg M, Tomic-Canic M.

New York University School of Medicine, Department of Dermatology, Biochemistry and The Cancer Institute, New York, NY, USA.

Retinoids (RA) have been used as therapeutic agents for numerous skin diseases, from psoriasis to acne and wrinkles. While RA is known to inhibit keratinocyte differentiation, the molecular effects of RA in epidermis have not been comprehensively defined. To identify the transcriptional targets of RA in primary human epidermal keratinocytes, we compared the transcriptional profiles of cells grown in the presence or absence of all-trans retinoic acid for 1, 4, 24, 48, and 72 h, using large DNA microarrays. As expected, RA suppresses the protein markers of cornification; however the genes responsible for biosynthesis of epidermal lipids, long-chain fatty acids, cholesterol, and sphingolipids, are also suppressed. Importantly, the pathways of RA synthesis, esterification and metabolism are activated by RA; therefore, RA regulates its own bioavailability. Unexpectedly, RA regulates many genes associated with the cell cycle and programmed cell death. This led us to reveal novel effects of RA on keratinocyte proliferation and apoptosis. The response to RA is very fast: 315 genes were regulated already after 1 h. More than one-third of RA-regulated genes function in signal transduction and regulation of transcription. Using in silico analysis, we identified a set of over-represented transcription factor binding sites in the RA-regulated genes. Many psoriasis-related genes are regulated by RA, some induced, others suppressed. These results comprehensively document the transcriptional changes caused by RA in keratinocytes, add new insights into the molecular mechanism influenced by RA in the epidermis and demonstrate the hypothesis-generating power of DNA microarray analysis.

ARCH DERMATOL RES. 2009 SEP;301(8):587-94. EPUB 2009 MAY 23

MOISTURIZERS CHANGE THE MRNA EXPRESSION OF ENZYMES SYNTHESIZING SKIN BARRIER LIPIDS

Authors: Buraczewska I, Berne B, Lindberg M, Lodén M, Törmä H.

Department of Medical Sciences, Dermatology and Venereology, University Hospital, 751 85 Uppsala, Sweden.

In a previous study, seven-week treatment of normal human skin with two test moisturizers, Complex cream and Hydrocarbon cream, was shown to affect mRNA expression of certain genes involved in keratinocyte differentiation. Moreover, the treatment altered transepidermal water loss (TEWL) in opposite directions. In the present study, the mRNA expression of genes important for formation of barrier lipids, i.e., cholesterol, free fatty acids and ceramides, was examined. Treatment with Hydrocarbon cream, which increased TEWL, also elevated the gene expression of GBA, SPTLC2, SMPD1, ALOX12B, ALOXE3, and HMGCS1. In addition, the expression of PPARG was decreased. On the other hand, Complex cream, which decreased TEWL, induced only the expression of PPARG, although not confirmed at the protein level. Furthermore, in the untreated skin, a correlation between the mRNA expression of PPARG and ACACB, and TEWL was found, suggesting that these genes are important for the skin barrier homeostasis. The observed changes further demonstrate that long-term treatment with certain moisturizers may induce dysfunctional skin barrier, and as a consequence several signaling pathways are altered.

JOURNAL OF DRUGS IN DERMATOLOGY. (JULY 2009) VOL 8 ISSUE 7(SUPPL) S8

GENOMIC-DRIVEN INSIGHTS INTO CHANGES IN AGING SKIN

Authors: Michael K. Robinson PhD, Robert L. Binder PhD, Professor Christopher E.M. Griffiths

Like all tissues, the skin ages due to the passage of time (chronologic aging). However, skin is also exposed to external insults such as sunlight. Aging due to chronic sun exposure (photoaging) is characterized clinically by wrinkling, dyspigmentation and other changes. Chronologic and photoaging of skin have been distinguished at the structural, cellular and molecular levels. However, many underlying mechanisms remain a mystery. Recent sequencing of the human genome and development of genome-wide microarray platforms now permit analysis of skin aging at the level of gene expression. Analysis of gene expression differences between young and old sun-protected and sun-exposed skin showed that photoaging produces many similar (but more severe) changes in gene expression versus chronologic aging. However, some changes are unique to one form of aging or the other. Bioinformatics tools also enable an integrated analysis of gene expression themes and pathways, which may provide new insights into the mechanisms of skin aging and possible interventions.

CURR OPIN BIOTECHNOL. 2007 AUG;18(4):355-9. EPUB 2007 AUG 2.

SYSTEMS BIOLOGY OF AGING IN FOUR SPECIES

Authors: Zahn JM, Kim SK.

Department of Developmental Biology, Stanford University Medical Center, Stanford, CA 94305, USA.

Using DNA microarrays to generate transcriptional profiles of the aging process is a powerful tool for identifying biomarkers of aging. In *Caenorhabditis elegans*, a number of whole-genome profiling studies identified genes that change expression levels with age. High-throughput RNAi screens in worms determined a number of genes that modulate lifespan when silenced. Transcriptional profiling of the fly head identified a molecular pathway, the 'response to light' gene set, that increases expression with age and could be directly related to the tendency for a reduction in light levels to extend fly's lifespan. In mice, comparing the gene expression profiles of several drugs to the gene expression profile of caloric restriction identified metformin as a drug whose action could potentially mimic caloric restriction *in vivo*. Finally, genes in the mitochondrial electron transport chain group decrease expression with age in the human, mouse, fly, and worm.

NOTE: Full text can be viewed here: http://cmgm.stanford.edu/~kimlab/COBIOT460.pdf

J EXP BIOL. 2007 MAY;210(PT 9):1607-12.

COMMON AGING PATHWAYS IN WORMS, FLIES, MICE AND HUMANS

Author: Kim SK.

Department of Developmental Biology and Genetics, Stanford University Medical Center, Stanford, CA 94305-5329, USA. kim@cmgm.stanford.edu

Development of functional genomics tools has made it possible to define the aging process by performing genome-wide scans for transcriptional differences between the young and the old. Global screens for age regulation have been performed for worms and flies, as well as many tissues in mice and humans. Recent work has begun to analyze the similarities and differences in transcriptional changes in aging among different species. Most age-related expression changes are specific for a given species, but genes in one pathway (the electron transport chain pathway) show common age regulation in species from worms to humans. Evolutionary theories of aging provide a basis to understand how age regulation of a genetic pathway might be preserved between distantly related species.

ZPLOS GENET. 2006 JUL;2(7):E115. EPUB 2006 JUN 9.

TRANSCRIPTIONAL PROFILING OF AGING IN HUMAN MUSCLE REVEALS A COMMON AGING SIGNATURE

Authors: Zahn JM, Sonu R, Vogel H, Crane E, Mazan-Mamczarz K, Rabkin R, Davis RW, Becker KG, Owen AB, Kim SK.

Department of Developmental Biology, Stanford University Medical Center, Stanford, California, USA.

We analyzed expression of 81 normal muscle samples from humans of varying ages, and have identified a molecular profile for aging consisting of 250 age-regulated genes. This molecular profile correlates not only with chronological age, but also with a measure of physiological age. We compared the transcriptional profile of muscle aging to previous transcriptional profiles of aging in the kidney and the brain, and found a common signature for aging in these diverse human tissues. The common aging signature consists of six genetic pathways; four pathways increase expression with age (genes in the extracellular matrix, genes involved in cell growth, genes encoding factors involved in complement activation, and genes encoding components of the cytosolic ribosome), while two pathways decrease expression with age (genes involved in chloride transport and genes encoding subunits of the mitochondrial electron transport chain). We also compared transcriptional profiles of aging in humans to those of the mouse and fly, and found that the electron transport chain pathway decreases expression with age in all three organisms, suggesting that this may be a public marker for aging across species.