

THE BASIS OF AGELOC SCIENCE

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INTRODUCTION: AGELOC SCIENCE

With the Nu Skin Anti-Aging Scientific Advisory Board, Nu Skin brings together a world-class team of scientists who have individually conducted pioneering research in their respective fields of anti-aging research for over 30 years. Collectively, they have published over 300 scientific papers, and their insights have changed the understanding of aging. Their work details the genetic basis of aging while demonstrating that biological aging is no longer considered an inexorable or inevitable process.

Building on these insights, Nu Skin and our collaborators are focusing on identifying the ultimate sources of aging called “age related super markers” or arSuperMarkers. Nu Skin’s first arSuperMarker, arNOX, is an age-related ECTO-NOX protein found on the cell surface that is capable of superoxide generation.

Nu Skin next identified groups of genes that play a central role in the aging process, key arSuperMarkers called Youth Gene Clusters (YGCs). Using proprietary ageLOC science and ingredients, we have found ways to reset these YGCs to reflect a more youthful gene expression profile.

ageLOC science is not dependent on any single discovery or scientific paper; instead our Nu Skin research and development scientists have incorporated key learnings from leading genetic researchers and anti-aging specialists (see Appendix for background). Our knowledge and understanding of key learnings are then applied in the development of proprietary, innovative, safe, and effective products. ageLOC science focuses on the ultimate sources of aging.

We believe that over the next five to 10 years as the ageLOC scientific approach matures, its full impact will be realized in many ways, including both topical and ingestible applications. The ageLOC scientific approach provides Nu Skin with the technology to further enhance our tradition of innovation. It’s an exciting time for Nu Skin science!

The following collection of scientific publications, which includes the work of Nu Skin scientists, Nu Skin Anti-Aging Scientific Advisory Board members, and academic partners, represents the foundational work from which we have gained key insights into the aging process. We believe that ongoing research is needed and will further enhance ageLOC science into the future.

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

MOL CELL BIOCHEM. 2003 DEC;254(1-2):101-9.

AN AGING-RELATED CELL SURFACE NADH OXIDASE (ARNOX) GENERATES SUPEROXIDE AND IS INHIBITED BY COENZYME Q

Morré DM, Guo F, Morré DJ.

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This report describes a novel ECTO-NOX protein with an oscillating activity having a period length of ca. 26 min encountered with buffy coat fractions and sera of aged individuals (70-100 years) that generates superoxide as measured by the reduction of ferricytochrome c. The oscillating, age-related reduction of ferricytochrome c is sensitive to superoxide dismutase, is inhibited by coenzyme Q and is reduced or absent from sera of younger individuals (20-40 years). An oscillating activity with a regular period length is a defining characteristic of ECTO-NOX proteins (a group of cell surface oxidases with enzymatic activities that oscillate). The period length of ca. 26 min is longer than the period length of 24 min for the usual constitutive (CNOX) ECTO-NOX proteins of the cell surface and sera which neither generate superoxide nor reduce ferricytochrome c. The aging-related ECTO-NOX protein (arNOX) provides a mechanism to transmit cell surface oxidative changes to surrounding cells and circulating lipoproteins potentially important to atherogenesis. Additionally, the findings provide a rational basis for the use of dietary coenzyme Q to retard aging-related arterial lesions.

PMID: 14674687 [PubMed - indexed for MEDLINE]

BIOFACTORS. 2003;18(1-4):33-43.

SPECIFICITY OF COENZYME Q INHIBITION OF AN AGING-RELATED CELL SURFACE NADH OXIDASE (ECTO-NOX) THAT GENERATES SUPEROXIDE

Morré DM, Morré DJ.

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Our laboratories have described a novel class of ectoproteins at the cell surface with both NADH or hydroquinone oxidase (NOX) and protein disulfide-thiol interchange activities (ECTO-NOX proteins). The two activities exhibited by these proteins alternate to generate characteristic patterns of oscillations where the period length is independent of temperature. The period length for the constitutive ECTO-NOX is 24 min. Here we describe a distinctive age-related ECTO-NOX (arNOX) whose activity is blocked by coenzyme Q10. arNOX occurs exclusively in aged cells and tissues. The period length of the oscillations is 26 min. Rather than reducing $1/2 O_2$ to H_2O , electrons are transferred to O_2 to form superoxide. Superoxide formation was demonstrated by superoxide dismutase-sensitive reduction of ferricytochrome c and by reduction of a superoxide-specific tetrazolium salt. Quinone inhibition was given by coenzymes Q8, 9 and Q10 but not by Q0, Q2, Q4, Q6 or 7. The arNOX provides a mechanism to propagate reactive oxygen species generated at the cell surface to surrounding cells and circulating lipoproteins of importance to atherogenesis. Inhibition of arNOX by dietary coenzyme Q10 provides a rational basis for dietary coenzyme 10 use to retard aging-related arterial lesions.

PMID: 14695918 [PubMed - indexed for MEDLINE]

BIOFACTORS. 2009;34(3):237-44.

AGE RELATED ENOX PROTEIN (ARNOX) ACTIVITY CORRELATED WITH OXIDATIVE SKIN DAMAGE IN THE ELDERLY

Morré DM, Meadows C, Hostetler B, Weston N, Kern D, Draelos Z, Morré DJ.

Department of Foods and Nutrition, Purdue University, West Lafayette, IN 47907-2059, USA.

ENOX proteins with an oscillatory pattern of production of superoxide (measured by ferricytochrome c reduction) and with a period length of 26 min increase linearity with age beginning at about 30 y to a maximum of about age 60. The proteins are shed and appear in serum, saliva and urine. Enhanced arNOX activity correlates with age and with oxidative changes contributing to skin aging. Topical cosmetic preparations containing substances that block arNOX activity are under evaluation to reduce visible symptoms of skin aging.

PMID: 19734125 [PubMed - in process]

J COSMET DERMATOL. 2009 JUN;8(2):77-82.

A NEW SOURCE OF AGING

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There has been a considerable increase in understanding how skin ages, along with significant progress toward the correction and prevention of the visible signs of aging. However, there are still many unknown factors regarding why we age - and why we all seem to age differently. An area of high interest is the biological or intrinsic processes that affect our appearance over time. This article describes a recent discovery of a membrane bound enzyme proven to be present in skin and increases its activity as biological age increases. The enzyme is located on the external surface of both fibroblast and keratinocytes, and generates free radicals. Therefore, as we age there appears to be a biological mechanism that further increases the production of free radicals. Additionally, there appears to be a relationship between activity of the enzyme and appearance. Data showed that subjects who look younger than their biological age had lower enzyme activity and conversely, subjects who looked older than their biological age had higher enzyme activity. Free radicals are believed to be a major contributing factor in the production of fine lines and wrinkles by destroying the collagen and elastin network keeping skin supple and firm.

PMID: 19527329 [PubMed - indexed for MEDLINE]

REJUVENATION RESEARCH—IN PRESS JAN 2010.

CONTROLLING REACTIVE OXYGEN SPECIES AT THEIR SOURCE TO REDUCE SKIN AGING

Kern D, Morre DM, Morre DJ, Draelos, Z.

Nu Skin Enterprises, Provo, Utah. Department of Foods and Nutrition, Purdue University, West Lafayette, IN, USA.

Activity of an age-related, superoxide-forming cell surface oxidase (arNOX) comparing dermis, epidermis, serum and saliva from female and male subjects ages 28 to 72 y measured spectrophotometrically using reduction of ferricytochrome c correlated with oxidative skin damage as estimated from autofluorescence of skin using an AGE-Reader (DiagnOptics B.V., Netherlands). By reducing arNOX activity in skin with arNOX-inhibitory ingredients (NuSkin's ageLOC technology) skin appearance is improved through decreased protein cross linking and an accelerated increase in collagen.

COSMETICS AND TOILETRIES. 2009;124: 48-52.

THE ARNOX ENZYME: IMPLICATIONS FOR INTRINSIC AGING

Knaggs HE.

Nu Skin Enterprises, Provo, Utah.

This article describes a membrane-bound enzyme found in skin whose activity increases as biological age increases. The enzyme located on external surface of fibroblasts and keratinocytes generates free radicals. The present work identifies the biological mechanism of the enzyme and its relationship to the appearance of aging in skin

BIOFACTORS. 2008;32(1-4):221-30.

SUPPLEMENTATION WITH COQ10 LOWERS AGE-RELATED (AR) NOX LEVELS IN HEALTHY SUBJECTS

Morré DM, Morré DJ, Rehmus W, Kern D.

Department of Foods and Nutrition, Purdue University, West Lafayette, IN, USA.

Our work has identified an aging-related ECTO-NOX activity (arNOX), a hydroquinone oxidase which is cell surface located and generates superoxide. This activity increases with increasing age beginning >30 y. Because of its cell surface location and ability to generate superoxide, the arNOX proteins may serve to propagate an aging cascade both to adjacent cells and to oxidize circulating lipoproteins as significant factors determining atherogenic risk. The generation of superoxide by arNOX proteins is inhibited by Coenzyme Q10 as one basis for an anti-aging benefit of CoQ10 supplementation in human subjects. In a preliminary pilot study, 25 female subjects between 45 and 55 y of age were recruited at Stanford University from the Palo Alto, CA area. Informed consent was obtained. Ten of the subjects received Coenzyme Q10 supplementation of 180 (3 x 60 mg) per day for 28 days. Serum, saliva and perspiration levels of arNOX were determined at 7, 14 and 28 days of CoQ10 supplementation and compared to the initial baseline value. Activity correlated with subject age up to a maximum between age 50 and 55 years of age for saliva and perspiration as well and then declined. With all three sources, the arNOX activity extrapolated to zero at about age 30. Response to Coenzyme Q10 also

increased with age being least between ages 45 and 50 and greatest between ages 60 and 65. With all three biofluids, arNOX activity was reduced between 25 and 30% by a 3 x 60 mg daily dose Coenzyme Q10 supplementation. Inhibition was the result of Coenzyme Q10 presence.

PMID: 19096119 [PubMed - indexed for MEDLINE]

PRESENTED AT THE SOCIETY OF COSMETIC CHEMISTS TECHNOLOGY SHOWCASE, DEC. 2009 **NEW SKIN BENEFITS IDENTIFIED FOR WHITE WILLOW BARK EXTRACT**

Remona Gopaul, Anna Langerveld, Jan Lephart, Helen Knaggs

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White Willow Bark is well-known for its anti-inflammatory benefits; however, there is limited research on its skin aging benefits. In this study, we used high throughput, gene expression technologies to evaluate the effects of an extract from White Willow Bark, salicin, on cultured human equivalent skin. Affymetrix microarray analysis was conducted using a 3' expression array (HG U133 plus 2.0), followed by validation with quantitative real time PCR. Data analysis showed that 24 and 48 hr exposure to salicin produced statistically significant changes in genes involved in multiple biological functions. Specifically, salicin significantly increased expression of the mitochondrial protective enzyme, manganese superoxide dismutase (MnSOD/SOD2) and one of its transcriptional activators, Forkhead Box O1 (FOXO1). SOD2 is one of the skin's major protectors, and plays an integral role in protecting cells from skin aging damages caused by environmental stress factors. This study indicates that salicin is capable of reducing the appearance of skin aging caused by extrinsic factors through the induction of many different groups of genes, which we are terming "**functional gene clusters.**"

SECTION 2: PRIOR ANTI-AGING RESEARCH CONDUCTED BY NU SKIN PARTNERS

CURR OPIN BIOTECHNOL. 2007 AUG;18(4):355-9. EPUB 2007 AUG 2. **SYSTEMS BIOLOGY OF AGING IN FOUR SPECIES**

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 mouse, 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.

PMID: 17681777 [PubMed - indexed for MEDLINE]

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

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**

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 human and mouse. The motif most strongly associated with aging was that of the transcription factor NF-kappaB. Inducible genetic blockade of NF-kappaB for 2 wk 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.

PMID: 18055696 [PubMed - indexed for MEDLINE]

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

COMMON AGING PATHWAYS IN WORMS, FLIES, MICE AND HUMANS

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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.

PMID: 17449826 [PubMed - indexed for MEDLINE]

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

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

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.

PMID: 16789832 [PubMed - indexed for MEDLINE]

PLOS BIOL. 2004 DEC;2(12):E427. EPUB 2004 NOV 30.

A TRANSCRIPTIONAL PROFILE OF AGING IN THE HUMAN KIDNEY

Rodwell GE, Sonu R, Zahn JM, Lund J, Wilhelmy J, Wang L, Xiao W, Mindrinos M, Crane E, Segal E, Myers BD, Brooks JD, Davis RW, Higgins J, Owen AB, Kim SK.

Division of Nephrology, Stanford University Medical Center Stanford, California, USA.

In this study, we found 985 genes that change expression in the cortex and the medulla of the kidney with age. Some of the genes whose transcripts increase in abundance with age are known to be specifically expressed in immune cells, suggesting that immune surveillance or inflammation increases with age. The age-regulated genes show a similar aging profile in the cortex and the medulla, suggesting a common underlying mechanism for aging. Expression profiles of these age-regulated genes mark not only age, but also the relative health and physiology of the kidney in older individuals. Finally, the set of aging-regulated kidney genes suggests specific mechanisms and pathways that may play a role in kidney degeneration with age.

PMID: 15562319 [PubMed - indexed for MEDLINE]

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

REVERSAL OF AGING BY NFKAPPAB BLOCKADE

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

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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.

PMID: 18256548 [PubMed - indexed for MEDLINE]

APPENDIX: BACKGROUND RESEARCH CONDUCTED BY INDEPENDENT PARTIES

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

INITIAL SEQUENCING AND ANALYSIS OF THE HUMAN GENOME

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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.

PMID: 11237011 [PubMed - indexed for MEDLINE]

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

THE SEQUENCE OF THE HUMAN GENOME

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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.

PMID: 11181995 [PubMed - indexed for MEDLINE]

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

EXPRESSION PROFILING USING AFFYMETRIX GENECHIP MICROARRAYS

Auer H, Newsom DL, Kornacker K.

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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.

PMID: 19212713 [PubMed - indexed for MEDLINE]

BMC BIOINFORMATICS. 2008 JUN 17;9:284.

METHODS FOR EVALUATING GENE EXPRESSION FROM AFFYMETRIX MICROARRAY DATASETS

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BACKGROUND: 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. **RESULTS:** 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. CONCLUSION: 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.

PMID: 18559105 [PubMed - indexed for MEDLINE]

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

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

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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.

PMID: 12063017 [PubMed - indexed for MEDLINE]

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

QUANTITATIVE REAL-TIME RT-PCR: A PERSPECTIVE

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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.

PMID: 15956331 [PubMed - indexed for MEDLINE]

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

EXPRESSION PROFILING OF AGING IN THE HUMAN SKIN

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

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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 2135 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.

PMID: 16530368 [PubMed - indexed for MEDLINE]

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

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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.

Journal of Investigative Dermatology Symposium Proceedings (2008) 13, 15-19; doi:10.1038/jidsymp.2008.3

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

UNVEILING THE MOLECULAR BASIS OF INTRINSIC SKIN AGING

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

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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.

PMID: 18492207 [PubMed - as supplied by publisher]

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

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

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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. 5330 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 5105 tags analyzed from a preauricular serial analysis of gene expression library. Of the total of 4742 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 (5257 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.

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

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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 H₂O₂, 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 H₂O₂-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 H₂O₂ treatment. Both MudPIT and cDNA microarray analyses indicate that H₂O₂ 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.

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RETINOID-RESPONSIVE TRANSCRIPTIONAL CHANGES IN EPIDERMAL KERATINOCYTES

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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.

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**MOISTURIZERS CHANGE THE MRNA EXPRESSION OF
ENZYMES SYNTHESIZING SKIN BARRIER LIPIDS**

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In a previous study, 7-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.

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GENOMIC-DRIVEN INSIGHTS INTO CHANGES IN AGING SKIN

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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.