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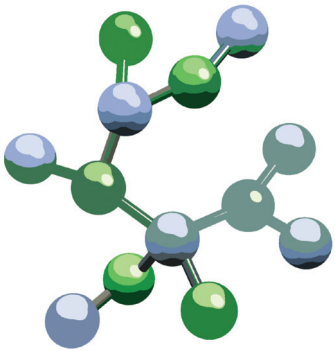


NUTRITIONAL and GENETIC Strategies for LONGEVITY

By Mark R. Bartlett, PhD

Abstract

A genomic understanding of aging is paving the way to identify interventions that can have significant impact on the aging process. The polymorphic nature of aging indicates that any anti-aging strategy has to start with a better understanding of genes that affect tissue viability.

Our anti-aging approach has always centered on the foundation of good macro and micro-nutrition, including the consumption of plentiful plant-based antioxidants and phytonutrients. However recent advances, especially with the mapping of the human genome and the subsequent development of DNA microarrays provide (a) an opportunity to explore the mechanisms of aging and (b) the tools to begin addressing aging at its most fundamental level. We believe that if we are to widen the gap between chronological and biological age we must better understand the role of gene expression in aging and how dietary ingredients interact with gene expression in a positive way.

Introduction

Aging is not an episodic process; rather, it is the consequence of a continuum of cumulative damage occurring at the molecular, cellular and tissue levels. The rate of aging rests on factors, internal and external, that can either positively or negatively influence the balance between tissue preservation or repair, and damage. Attenuation of aging is entirely dependent on mitigating such molecular damage by augmenting protection and compensatory repair mechanisms or slowing the degenerative processes. In a practical sense we've all probably witnessed clear discrepancies between chronological and biological age in certain individuals. And while it has been proposed that genetic factors contribute to the phenomenon of people looking old, or young, for their years, most of us intuitively suspect that there are some environmental components over which we wield a certain amount of control. Therefore, if we are to widen the gap between chronological and biological age, we must understand the various mechanisms involved in aging and devise effective strategies that turn these mechanisms in favor of tissue protection or repair and regeneration. The question we are asking is: what are the lifestyle factors and nutritional components that may assist us in taking control of our own aging process so that we can age healthily and reduce age-related morbidity?

Macro and Micro Nutrition

A major factor in healthy aging involves what, and how much, we eat. There is ample evidence that poor nutrition, which includes overeating and poor nutrient density, is linked with an increased risk for many degenerative diseases including heart disease, diabetes and cancer. It is now also becoming clear that even marginal micronutrient deficiencies over time lead to accelerated aging.[1] Deficiencies in several different micronutrients including folic acid, vitamin D and Magnesium lead to DNA damage and accelerate age-related mitochondrial dysfunction; which in turn leads to further oxidative damage to DNA, RNA, proteins and membrane lipids leading to functional decline in mitochondria, cells, tissues and organs. Since multiple studies and extensive government-commissioned surveys point to the widespread nature of inadequate dietary intakes of fruits and vegetables (and therefore vitamins and minerals)[2] it seems prudent that all individuals either improve their diets or supplement their diets with a multivitamin mineral supplement to ensure that there are no shortfalls in essential nutrients.

FIGURE 1: LGT identified biomarkers of age across seven strains of mice (5 months vs. 28-30 months old) so that only the most conserved relevant patterns of age-related gene expression markers were considered. RTqPCR was used to confirm a panel of 10-20 genes in each tissue.

Free-radical Biology and Antioxidants

A leading hypothesis of aging is based on the free radical theory of aging by Harman[3] who argued that oxygen-free radicals produced during normal cellular respiration cause cumulative damage to molecules which progressively leads to loss of functionality of the organism. Since Harman's theories were first proposed, a huge body of literature has emerged providing evidence that free radicals and oxidative stress are involved in many disease states, especially age-related degenerative disease. Although oxidative stress may be a significant factor associated with aging, it is clearly not the only contributor and recently evidence is emerging to support the concept that vitamins, minerals, and phytonutrients not only fight free radicals, but they exert perhaps even more powerful anti-aging effects through a non-antioxidant role. Phytonutrients, many of which are antioxidants, also influence the expression or activity of factors involved in aging including, for example, sirtuins, AMPK, NFKB and PGC-1 alpha to name a few.[4-6] Thus it is becoming increasingly clear that the phytonutrients we thought were merely antioxidants are also capable of modulating gene expression.

Gene Expression Science

It is clear that a nutraceutical approach to anti-aging must take into account the polymorphic nature of aging, and that the crosstalk among multiple genes plays a more important role than the action of a single gene in mediating the survival of an organism. Since the development of DNA microarrays that allow scientists to measure the work output of all of the genes in a single experiment, it is now possible to rapidly explore the differences in the expression of multiple genes between two or more biological conditions in a single experiment. Our research and development team at Nu Skin became intrigued with the possibility of measuring the aging process objectively at the genetic expression level after reading some of the exceptional work published by Weindruch, Prolla and colleagues (LifeGen Technologies, LLC) (LGT) wherein a powerful technique of differential expression analysis was being used to conduct genome-wide searches for consistent changes in gene expression patterns that occur during the aging process.[7, 8]

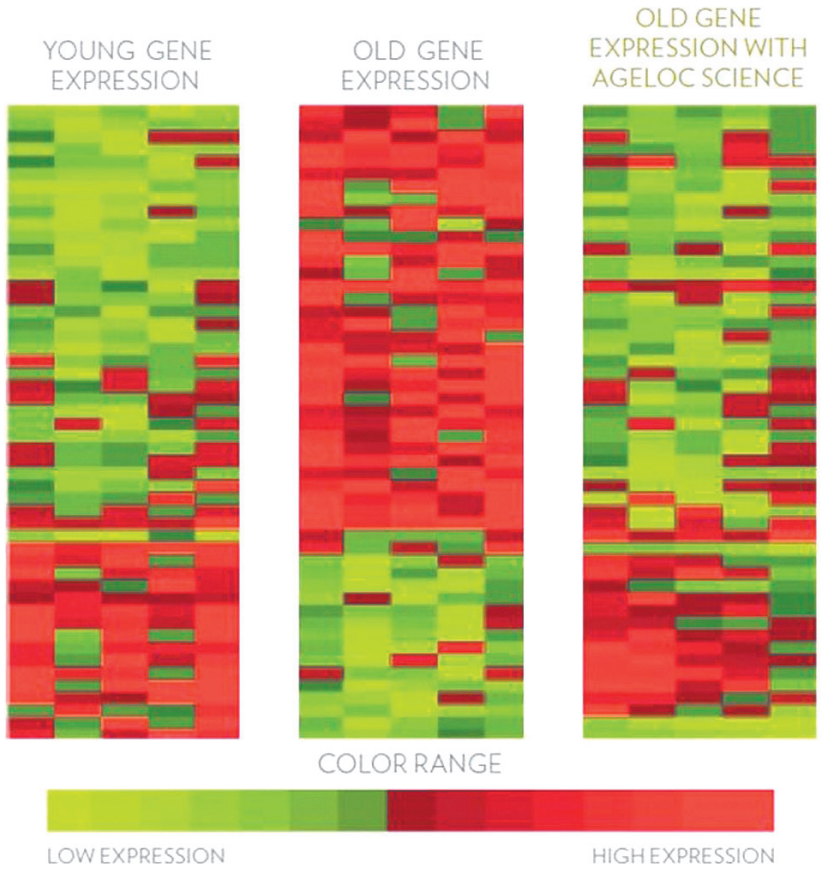
Studies using whole-genome transcriptional profiling typically identify thousands of genes that are changed in expression with age. Since many of these age-related changes are not universal, but rather are specific to the genetic background of the organism being studied, LGT identified biomarkers of age across seven strains of mice (5 months vs. 28-30 months old) so that only the most conserved relevant patterns of age-related gene expression markers were considered. Moreover, these analyses were performed in

three tissues (heart, cerebral cortex and gastrocnemius) and real-time quantitative PCR was used to confirm a panel of 10-20 genes in each tissue. Data generated from such a model are not only of a higher standard of rigor, but they are more likely to be applicable to human aging as well. Using this approach statistically robust patterns, or signatures, of youthful and older gene expression have emerged which enabled us to essentially measure aging at the genetic level. The possibility now existed to screen for ingredients or formulations for their ability to retard the aging process. This is the procedure that Pharmanex has adopted in collaboration with LGT to target aging at the source, gene expression, in an approach that we call ageLOC science.

Microarrays, Databases and Bioinformatics as a Guide to Product Development

In our first screening experiment certain ingredients emerged for their abilities to reset gene expression to that of a more youthful pattern. A particular preparation of pomegranate, for example, was the most effective compound tested, opposing 32-65% of the overall aging change depending on the tissue studied. Other ingredients and formulations also emerged as having potent effects on gene expression that attenuated age-associated patterns of expression. The results of our first round of screening provided an important insight into ingredients that influence gene expression in a positive way and served as an important foundation to further product development.

In addition to helping identify individual gene expression signatures associated with aging, DNA microarray technology in conjunction with gene databases and bioinformatics can also be used to identify the expression levels of groups of genes that work together to serve a particular metabolic pathway. We added the use of such pathway analyses to our repertoire of microarray-related gene tools to help further guide our product development in formulating anti-aging products by applying it to the concept of age-related vitality loss. One of the earliest manifestations of human aging is a decline in vitality. Mitochondrial dysfunction associated with aging yields



Transcriptional Biomarkers of Mitochondrial Aging and Modulation by Cordyceps Sinensis Cs-4. Gordon Research Conference, Biology of Aging, Determinants of Health-Span: From Cells to Humans, August 22-27, 2010. Les Diablerets Conference Center, Les Diablerets, Switzerland.

FIGURE 2: This heat map illustrates gene expression of three groups from a pre-clinical test with one of the ageLOC Vitality ingredients: young (column 1), old (column 2), and old with ageLOC science (column 3). Each row represents one of 52 genes comprising the mitochondrial Youth Gene Cluster (mtYGC). Columns 1 and 2 show that each of the 52 genes became more or less active during the aging process. In column 3, the YGC activity pattern of the old with ageLOC science group has been reset to a gene expression pattern similar to the young group in column 1.

bioenergetic defects within the cell[9] that exert profound effects on physical and mental vitality. Our goal was to identify and target functional gene clusters associated with mitochondrial aging.

In our attempt to identify these gene pathways we found that of 20,687 gene transcripts measured by the Affymetrix Mouse Genome array, 1241 were associated with the mitochondria by pathway ontology (using a gene ontology database). After our murine feeding studies and microarray screening we found that 172 of these genes changed in expression during aging in cerebral cortex tissue. In gastrocnemius tissue 220 genes changed which age. Cs-4 opposed the age-related changes in 52 of these genes ($P<0.05$). In addition, Cs-4 opposed the effects of aging in several gene ontology pathways. In essence we were able to identify mitochondrial-related nuclear encoded genes which changed consistently in expression with age, or mitochondrial youth gene clusters (YGC). A number of natural compounds were screened for their ability to reset the expression profile of these genes to a more youthful level. One ingredient, Cordyceps sinensis Cs-4 (Cs-4)[10] was shown to markedly attenuate these age-related gene expression changes in the mitochondria, suggesting its potential use as a therapeutic intervention of age related vitality loss. Ongoing studies are utilizing this technique to investigate the effects of a variety of natural ingredients in brain, muscle and other tissues, but the sum of such explorations so far, into the ability of certain natural products to

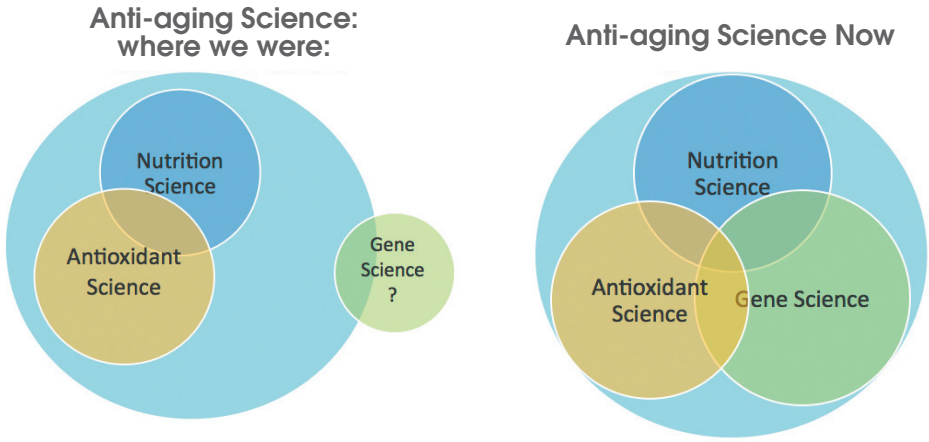


FIGURE 3: Mainstream anti-aging approaches for years have centered on the foundation of good macro and micro-nutrition and antioxidants. However it is becoming increasingly clear that some of the phytonutrients we thought were merely antioxidants are also capable of modulating gene expression and play a role in aging.

influence gene expression in a positive way, has provided strong guidance to our product development process targeted at attenuation of the aging process.

Functional Studies

The techniques that we have used for studying gene expression have not disappointed us in their promise as tools explore the mechanisms of aging and drive us towards meaningful product development strategies. We see great promise in the ability of certain nutraceutical ingredients and formulations to have a marked effect on gene expression to oppose age-related changes. The next logical step is to support these gene expression data with functional studies. Indeed, the ingredients that we selected for gene expression screening were based on promising functional studies that had already been performed. However, to close the circle, we have followed up these promising gene expression data with further functional, safety and efficacy studies in both animals and humans. Some of these studies are already completed and have provided positive correlation and confirmation of the gene expression data; other studies are still underway.

Conclusion

Since aging can be considered as a function of how genes respond to diet and environmental perturbations through gene expression while maintaining their primary function to survive, we chose to exploit a gene expression approach to screen several nutraceutical ingredients and formulations for their effects on retarding the aging process. We called this approach ageLOC science. Our first foray into this approach involved targeting age-related vitality loss through an exploration of the gene expression changes involved in mitochondrial aging. We identified tissue-specific functional YGCs, or signatures of gene expression changes associated with mitochondrial aging and screened for ingredients that restored the more youthful pattern of gene expression. Functional studies have confirmed the promise offered by the gene expression study results. It is our opinion that while a foundation sound of nutrition and a positive lifestyle are key to healthy aging and compression of morbidity, there is much to be gleaned from an understanding of gene expression as it relates to the aging process as we pursue the goal dying young - as late in life as possible. ◆

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Dr. Bartlett has degrees in Biochemistry and Organic Chemistry from the Australian National University and a Ph.D. in Immunology and Cell Biology from the John Curtin School of Medical Research in Canberra, Australia. In Australia he conducted research on cardiovascular disease with an emphasis on the role of reactive oxygen species and free radicals. He also studied the role of blood platelets in heart disease, and helped publish the first scientific report of a biochemical link between cigarette smoking and atherosclerosis.

Later Dr. Bartlett became interested in autoimmune inflammatory diseases and examined a number of plant-derived substances for their ability to inhibit graft rejection, inhibit cancer metastasis – or spreading – as well as natural products that were able to inhibit autoimmune disease.

Before joining Pharmanex Dr. Bartlett was a visiting scientist at the National Institutes of Health, National Cancer Institute in Bethesda, MD where, at the National Cancer Institute, he investigated the interaction of T-cells with the blood vessel wall, and the role of various adhesion molecules that are used by these cells to communicate with one another. He is currently the Vice President of Global Research and Development for Pharmanex.