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The Future of Aging Interventions

Current Status of Efforts to Measure and Modulate the Biological Rate of Aging

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Biomarkers of aging would be highly desirable, but so far, a definitive panel of biomarkers to predict mortality risk has not been obtained, even though many traits that vary with age have been identified. This lack hinders the search for interventions that may retard the rate of aging in mammals. The recent discovery and characterization of many longevity genes in animal model systems, such as nematodes, fruit flies, and mice, are providing new targets for research by providing insight into mechanisms of longevity regulation in these model systems. It is hoped that this will ultimately lead to interventions to delay the development of age-related pathology in humans.

BIOMARKERS are measurements or other observations made on biological material that provide information about the physiological status of that material. As such, they may indicate the relative risk to an individual of developing any given disease or of death due to any of a variety of causes. Although chronological age is extensively used by the life insurance industry, it is a poor indicator of the overall health status of an individual. Thus, biomarkers of aging have long been sought by the gerontological research community in its attempt to identify what causes the adverse phenotypes that accompany aging, and what can be done to prevent, reverse, or at least retard the development of these phenotypes.

National Institute on Aging's Biomarkers Initiative

Fifteen years ago, Baker and Sprott laid out the case for identifying biomarkers of aging that could predict functional capability better than does chronological age (1). They also suggested that such biomarkers should fulfill the following criteria:

1. Reflect some basic biological process of aging (rather than diseases),
2. Have high reproducibility in cross-species comparisons,
3. Change independently of passage of time,
4. Be obtainable through nonlethal means, and
5. Be measurable during a relatively short time interval compared to the life span of the animal.

The critical question is whether there are any parameters that can be reproducibly measured that will reflect a physiological process more dependent on aging factors than on disease factors, although there exists controversy about how to unequivocally distinguish between the two.

With the intent to "assess putative biomarkers of aging in a species where there is a well-defined genetic background, with a longitudinal and cross-sectional protocol, and with extensive pathological examinations," the National Institute on Aging (NIA) began a program in 1988 to identify such biomarkers of aging, and funded the program for 10 years. The program used genetically homogeneous strains of mice and rats, and produced many interesting papers, but a definitive panel of biomarkers to be used for assessing the physiological age of individuals within a population was not achieved. Although the results of this program have not been comprehensively summarized in the literature, a series of seven papers was published in the November and December 1999 issues of the *Journal of Gerontology: Biological Sciences* (volume 54A, pages B464–B566). Of particular interest is the summary of age-related pathology observed in rats and mice used in the study, and how caloric restriction alters it (2,3), as well as extensive characterization of growth and survival characteristics of the strains used (4). Dr. Sprott's own published assessment of the 10-year program was that, "although progress is being made in developing biomarkers of aging, it is too early to constitute a definitive panel of biomarkers for either animal models or humans" (5).

Use of Genetically Heterogeneous Mouse Stocks

Although this early failure to establish a definitive panel of aging biomarkers does not preclude the eventual identification of true biomarkers of aging, it does suggest that new approaches are needed. Richard Miller (6,7) has embarked on such a quest, but using genetically heterogeneous stocks of mice generated in the laboratory for this purpose (8). Such mice provide greater genetic diversity than do inbred mouse strains for identifying loci associated

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with aging and longevity regulation (9). For this program, Dr. Miller suggested a slightly different list of requirements for a biomarker of aging.

1. It should predict the outcome of a wide range of age-sensitive tests in multiple physiological domains,
2. It should predict remaining longevity at an age when 90% of the population is still alive, and
3. Its measurement should not alter either life expectancy or the outcome of subsequent tests of other age-sensitive traits.

Miller reported that four T-cell subset measurements conducted on 18-month-old mice vary with age and correlate with longevity (7,10); these include CD4, CD4 memory, CD4 naïve, and CD4 cells with P-glycoprotein. In a separate article, Miller and Chrisp (11) show that an index made up of all of these T-cell subsets does better than any one of them alone in predicting life spans, even as early as 8 months of age. Miller and colleagues (12) have also shown that body size measured early in adult life varies negatively with longevity. Serum levels of thyroxine, insulin-like growth factor-I (IGF-I), and leptin can also be used to predict longevity, but only in a sex-specific manner (13).

However robust any of these correlations are, they are statistical correlations, and it is difficult to see at this time how any test done on an individual, especially in an outbred population, could be used to predict the remaining longevity of that individual, although that objective may eventually be better realized for humans if a well-validated panel of such age-sensitive traits can be developed. This difficulty is at least partially due to the tremendous cross-sectional variability among individuals within an outbred population for most parameters that can be measured noninvasively. Whereas the effect of the environment and behavior on biological parameters has long been recognized, it has only recently become clear just how widely our individual human genomes can vary without causing obvious pathology (14), as single nucleotide polymorphisms (SNPs) occur at least as frequently as once every 1900 bases in human DNA (15). The identification of SNPs in DNA that affect our risk for developing Alzheimer's disease, diabetes, or vascular disease (16–18), as well as affecting longevity (19), represent only the tip of the huge iceberg of SNPs that could affect fundamental biological processes and thus have slight but unknown effects on the aging pattern of each individual. Thus, such polymorphisms may impact on biomarker measurements in unknown ways and diminish their predictive value for individuals.

Longevity Regulation in Animal Models

At the time the Biomarkers Program was started, very little was known about what biological factors might affect longevity, but the past decade has seen remarkable progress in identifying biological factors involved in regulation of life span, both mean and maximum (20,21). This has been accomplished largely through the use of model organisms with short life spans and well-developed genetic systems, particularly nematodes and fruit flies, and fortunately these results have been translatable into the mouse in most cases. This may provide new insights into biomarkers if Edward Masoro (22) is correct in arguing that "predicators of the maximum life span of a species have a high probability of being valid biomarkers of aging." In 1988, caloric restriction was the only known intervention to retard aging in mice, but it is now known that a variety of factors that influence the activity of the insulin-signaling pathway, or the various stress response systems, chromatin structure, or energy metabolism, also affect longevity.

It is not clear exactly how longevity regulation relates to organismal aging per se. A dramatically shortened life span produced by obvious pathology, such as seen in mice lacking mitochondrial manganese superoxide dismutase activity, is clearly not equivalent to rapid aging (23). In contrast, it is hard to imagine that the longevity of a population could be increased substantially without slowing some aspect of aging in a fundamental way. Flurkey and colleagues (24) recently attempted to relate life span extension to known aging-related changes. They showed that the increased longevity observed in Snell dwarf mice unable to produce growth hormone correlates negatively with collagen cross-linking and age-related changes in immune system status. From this result, they concluded that "a single gene can control maximum lifespan and the timing of both cellular and extracellular senescence in a mammal." However, those correlations do not establish causal relationships among these parameters.

Other examples of delayed aging phenotypes that accompany life span extension in mice include the delayed occurrence and reduced incidence of fatal cancers (25), and memory retention and delayed age-related decline in locomotor activity in Ames dwarf mice, which also fail to produce growth hormone (26). Delayed physiological aging has also been observed in long-lived nematodes; Herndon and colleagues (27) demonstrated that the progressive muscle degeneration observed during normal aging is delayed in the long-lived *Caenorhabditis elegans age-1* mutant.

Stress resistance and age are generally negatively correlated in most species, so it is of interest that many long-lived mouse, fruit fly, and nematode mutants are more stress-resistant than is the wild-type organism (28–30). Cells taken from long-lived dwarf mice are also stress resistant (31). These results strongly support the idea that life span extension does reflect at least some fundamental processes involved in aging, and encourage us to believe that information on longevity regulation may provide a whole new range of targets for future biomarker research. Possible candidates identified in this way include the IGF-I receptor protein (28,32,33), phosphastidylinositol-3′-kinase (34), histone/p53 deacetylases (35), dicarboxylic acid transporter proteins (36), p53 (37), transcription factors (38,39), heat shock proteins (38), and a variety of other stress response and repair proteins (30,40–42).

Gene Expression Microarray Analysis

Another recent development that may contribute to biomarker research is the use of gene expression microarrays. The NIA provided administrative supplements to funded grants in 2000 and 2001 to stimulate the use of gene expression microarray technology in aging research, and this is

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beginning to pay off in documenting age-related changes in gene expression. Comprehensive studies of gene expression at different ages and in different tissues (43,44), in response to caloric restriction (45,46), and in pathological states such as Hutchinson-Gilford syndrome (47) have been carried out, and may help to identify gene expression patterns and signatures that will prove useful in estimating the physiological age of an individual.

Another technology that may prove useful is the developing area called proteomics, which has shown some early promise in predicting risk for specific cancers (48). The development of high-resolution, noninvasive imaging methods, for example, magnetic resonance imaging, positron emission tomography, and so forth, for assessing anatomical and physiological status of animal models during aging may also provide biomarkers of aging that will prove useful in human studies.

Human Biomarkers of Aging

The difficulty in identifying useful biomarkers of aging in mice and rats raises the question of whether this will be even more difficult in people. There have been a few attempts to do this. For example, Richard Hochschild (49) selected 12 candidate biomarkers of sensory and physical function, and these were then combined with a variety of behavioral factors to predict rates of common functional declines with age. Cawthon and colleagues (50) found that human survival correlates with telomere length in DNA extracted from blood cells, suggesting that "telomere shortening contributes to mortality in many age-related diseases." In this study, mortality was mainly associated with higher incidence of infectious diseases and heart disease. Aviv and colleagues (51) have also suggested that telomere length measured in somatic cells early in life may be predictive for successful aging in humans, even though telomere length among species does not correlate with species longevity. Roth and colleagues (52) found that three biological parameters that correlated with increased longevity in humans in the Baltimore Longitudinal Study of Aging, that is, lower body temperature, circulating insulin, and higher circulating DHEAS (dehydroepiandrosterone sulfate), were also modified in monkeys subjected to caloric restriction. However, biological parameters have not yet been combined in a panel to predict longevity in either monkeys or humans.

Testing Interventions to Retard, Prevent, or Reverse Adverse Phenotypes Associated With Aging

One of the main objectives of developing biomarkers of aging would be their value in testing dietary and pharmacological interventions to retard aging. The anti-aging industry has promoted a variety of compounds to slow down aging, including growth hormone and other hormones whose levels decline with increasing age, antioxidants, and a variety of other dietary supplements [for example, see (53)]. Hormone replacement therapy is an obvious strategy, as it seems reasonable to assume that the hormone levels associated with youth are likely to be beneficial compared with the lower levels found in older people. However, this ignores the possibility that the declining hormone levels may represent a protective mechanism against the development of late-life pathologies such as cancer, diabetes, and cardiovascular disease. Also, while most gerontologists accept that oxidative stress is probably a factor in aging, oxygen free radicals also play important signaling roles in normal metabolism and are involved in responses to infection. Thus, the choice of interventions and how they are administered may require more knowledge than is currently available.

The work with model organisms on longevity regulation described above may thus be informative. These results suggest that the insulin-signaling pathway, stress response systems, and interventions that in some way mimic caloric restriction and/or affect energy metabolism are promising targets for intervention to retard aging and should be critically evaluated (see above under Longevity Regulation in Animal Models). Other possibilities in mammals include the ability to resist infections and reduce inflammation, both of which are thought to impact negatively on the health and longevity of older animals.

The only intervention currently known to work reliably across many species to retard aging, improve overall health, and increase longevity is caloric restriction (54), but the mechanism by which it does so remains obscure. Although the literature is replete with reports of aging interventions in mice, many of these studies are compromised by one or more design flaws, for example, too few animals per cohort, failure to control for possible caloric restriction, use of wrong animal model, poor housing conditions, no accompanying pathology assessment, and so forth. Therefore, the NIA initiated a program in 2003 to rigorously test pharmacological and dietary agents that may extend the longevity of mice (55). Besides survival and pathology assessment, biochemical, physiological, and functional tests to measure what are known to be "age-sensitive traits" will be used to judge the efficacy and safety of the compounds being tested. What tests will actually be used remains undecided because of the lack of validated biomarkers of aging, and will depend on the intervention being tested, but if the program is successful, it may actually shed some light on promising biomarkers of aging. To increase the probability of finding and validating useful interventions, testing will take place in three distinct sites. The entire gerontological community is welcome to nominate compounds to be tested, and information about nominating interventions for testing can be obtained by accessing http://www.nia.nih.gov/research/ITPsponsorApplicationForm.htm.

Summary

Although there are many known age-sensitive traits, a panel of biomarkers of aging that predicts remaining longevity of either rodents or primates does not currently exist. A large number of possible new targets for biomarker research is emerging through research on the genetic regulation of longevity in invertebrate model organisms and mice, and the use of new technology such as gene expression microarray analysis. A panel of validated biomarkers would be very useful for testing aging interventions, so

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biomarker development remains a priority in gerontological research.

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