The Future of Aging Therapies

Review

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Advances in understanding aging processes and their consequences are leading to the development of therapies to slow or reverse adverse changes formerly considered to be "normal" aging and processes that underlie multiple age-related conditions. Estimating the effectiveness of candidate aging therapies, whose effects on human aging may require many years to determine, is a particular challenge. Strategies for identifying candidate interventions can be developed through multiple approaches, including the screening of molecular targets and pathways in vitro and in animal models, informed as well by evidence from human genetic and epidemiologic data. A number of recently established programs and networks can serve as resources for such research. For all these research approaches, from in vitro molecular studies to clinical trials, contributions of cell and molecular biology are crucial and offer the prospect of therapeutic advances that address fundamental biological processes as well as the clinically important challenges of aging.

Introduction

Considered most broadly, the concept of aging therapies includes prevention and treatment of the variety of conditions whose rate of occurrence increases with age. Progress against many of these conditions has been ongoing for several decades. Most of this has stemmed from research on specific age-related conditions rather than on aging per se, such as clinical trials showing effective means of preventing cardiovascular disease and diabetes in older persons. It is likely that progress on these and other age-related conditions will continue. This review deals principally with therapies focused specifically on aging processes, specifically those that contribute to disease or dysfunction. The pronounced increase with age in incidence of most conditions causing morbidity and mortality, as well as long-term increases in the proportion of individuals surviving to ages when the prevalence and severity of such conditions are high, highlight the potential health benefits of better interventions to modulate such aging processes.

Aging therapies may be aimed at reversing age-related changes once they have occurred or at decelerating or preventing aging changes starting at various points in the life span. A therapy that is effective for one of these purposes may be ineffective or even

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counterproductive for the other. An intervention started before old age might slow a process that resulted in a particular pathology in late life but have adverse effects in individuals in whom that pathology is already present. This, for example, has been suggested as a possibility for effects of estrogens in regard to atherosclerosis and coronary heart disease.

Two therapeutic foci are of particular interest:

Changes with age previously considered to be "normal aging" but that have adverse consequences. Although many age-related changes are asymptomatic and are found in many or all persons, they are not necessarily benign. As understanding of adverse effects of a "normal" aging change progresses, the change may be recognized as a disease or risk factor. A familiar example is osteoporosis, which results in most cases from progressive bone loss starting in middle age. Its relation to fracture risk has been recognized for several decades, and insights into its pathophysiology have led to a variety of therapies. An analogous evolution has occurred recently regarding age-related loss of muscle mass: disability and other adverse conditions have been linked to muscle loss of sufficient severity, for which the term "sarcopenia" is now applied. Similarly, as discussed below, there is increasing evidence that an age-related increase in vascular stiffness may be a risk factor for cardiovascular disease and that therapies may be developed to enhance vascular compliance and reduce cardiovascular mortality.

The importance of identifying adverse effects of age-related changes and developing therapies that retard or reverse such changes is highlighted by the fact that, for many age-related conditions, currently known risk factors only explain a modest proportion of the risk. For example, chronologic age remains the strongest single risk factor for cardiovascular disease, presumably reflecting effects of specific age-related changes whose role has not yet been appreciated and which may be modifiable once identified. Thus, new therapies to counter aging changes whose role in disease risk is identified could, when added to existing therapies, reduce risk for age-related conditions (or delay their onset) much more than is currently possible.

Fundamental mechanisms responsible for multiple age-related pathologies. Several underlying processes, many discussed elsewhere in this issue, that may regulate multiple aging changes have been proposed and investigated. These include damage and repair of macromolecules and other tissue components; regulation of cell proliferation, differentiation, and death; control of cellular bioenergetics; and control of genomic stability. The potential health benefits of an intervention that modulates a mechanism that affects the development of multiple adverse changes with age have frequently been noted. In addition to the inherent efficiency of a single intervention with multiple benefits, this type of intervention could avoid problems of adverse effects of a therapy for one age-related condition on other age-related conditions (e.g., exacerbation of

high blood pressure by nonsteroidal anti-inflammatory drug treatment for arthritis).

Issues and Resources for Developing New Human Aging Therapies

Identification and Evaluation of Potential Therapies

Particularly for interventions whose effectiveness may depend on starting early in life, a fundamental issue for the development of aging therapies is the long time scale of human aging. Many proposed mechanisms of aging are postulated to act over the life span, so that effects of interventions that decelerate aging changes might be expected to diminish as the age when they are begun increases. This implies that testing of some human interventions may need to begin at an early age and continue for a long time. Even interventions started in late life to reverse age-related changes may take several years to show effects in humans. Thus, selection of aging therapies for testing long-term effects in humans is especially dependent on shorter-term evaluation of potential interventions. Such strategies include the following:

Identifying pathways regulating aging changes and potential molecular targets for interventions. Choices of potential therapeutic targets can be informed by evaluating genetic effects or interventions in short-lived organisms such as yeast, nematodes, fruit flies, and mice. During the past 10 years, many genetic changes as well as interventions that increase longevity in these animal models have been reported and may provide a basis for developing promising human interventions. Although genetic manipulations may not be directly applicable to humans, results of these experiments are helpful in identifying pathways for possible pharmacological intervention.

Short-lived nematodes, fruit flies, and mice have been particularly useful for identifying genes that influence longevity. Because this area has been extensively discussed by Cynthia Kenyon in this issue of *Cell* (Kenyon, 2005), we will only briefly mention the most salient results. A striking observation is that major effects on life span have been achieved by mutating genes that affect the signaling pathways related to insulin-like factors (Liang et al., 2003; Tatar et al., 2003). Significant effects have also been achieved from genetic manipulations that: (1) reduce ROS generation by mitochondrial electron transport (Feng et al., 2001; Knauf et al., 2002; Ishii et al., 2004); (2) reduce food consumption (Miskin and Masos, 1997; Lakowski and Hekimi, 1998); (3) increase expression of antioxidant genes (Larsen, 1993; Mitsui et al., 2002; Ruan et al., 2002), heat shock genes (Munoz, 2003), or sirtuins (Tissenbaum and Guarente, 2001); or (4) modify the apoptotic (Migliaccio et al., 1999; Cohen et al., 2004) or proliferative (Vellai et al., 2003) potential of cells. A limited number of potential pharmacological interventions have also been identified including antioxidants (Melov et al., 2000) and compounds that modulate protein deacetylase activity (Howitz et al., 2003; Wood et al., 2004).

Though human intervention studies based on factors identified in animal models are, for the most part, not likely to start until well into the future, in vitro studies of human cells provide another means to identify especially promising pathways and targets for human interventions. This approach has not been used extensively to date but has considerable potential. Though such studies do not directly test the effects of interventions on human aging, they do allow testing effects of genetic factors and interventions on specific functions (e.g., oxidative stress resistance or insulin sensitivity) in human cells, including tests in specific cell types, could provide clues to their in vivo effects. These in vitro approaches may be amenable to creative and high-throughput strategies. In addition, comparative studies on cells from young and old human donors may reveal age-related differences in metabolic or signaling pathways that may suggest targets for interventions to reverse or prevent such changes.

Recently, genetic epidemiologic studies of human longevity have begun to provide another means of identifying possible pathways and molecular targets. For example, such studies have identified a relationship between exceptional longevity and variants of several genes affecting lipoproteins (Schachter et al., 1994; Barzilai et al., 2003; Geesaman et al., 2003). The products of these genes may serve as targets for interventions.

New technologies are facilitating high-throughput strategies to identify potential therapeutic targets and promising interventions in very short-lived species. Using a dye-based assay for cell death, Gill et al. (2003) have developed a high-throughput assay for interventions that delay death in nematodes. Using an RNA interference screen of 5690 nematode genes, a large number of genes affecting mitochondrial function were found also to affect life span (Lee et al., 2003). Bauer et al. (2004) have developed a rapid method to identify interventions that increase longevity of fruit flies, based on the fact that mutations that change life span in fruit flies also change the timing of gene expression patterns. Because longer-lived flies express lethal reporter genes later than control flies, flies with normal aging rates can be killed off before flies with extended longevity, so these long-lived flies can be enriched in the population. This assay has also been successfully used to test pharmacological interventions as well as response to environmental conditions, showing, for example, that resveratrol and lipoic acid but not vitamin E can increase fruit fly longevity. In addition, after identification of molecular targets modulating aging changes, multiple compounds that could affect these targets can be screened. This approach has been applied to identify sirtuin activators that increase life span and decelerate aging in *S. cerevesiae* (Howitz et al., 2003), *C. elegans*, and *D. melanogaster* (Wood et al., 2004). It should be noted that NIH's Roadmap Initiatives (http://nihroadmap.nih.gov/) include efforts to establish technologies and infrastructure for high-throughput screening of molecules that impact specific metabolic pathways. These efforts will greatly facilitate aging research in this area.

Laboratory mammalian studies testing intervention effects on outcomes. Interventions for testing in mammals can be selected based on findings from the types of invertebrate studies described above, physiologic observations in mammals themselves, or epidemiologic data suggesting an effect of a particular factor in hu-

mans. Mammalian intervention studies have been conducted on the effects of interventions in abnormally short-lived strains with specific conditions or genetic defects as well as in long-lived wild-type strains. The latter type of study is widely accepted as preferable for studies to examine potential human applicability, and the following discussion is confined to such studies.

The rapid discovery rate of findings from very short-lived invertebrates, implicating potential therapeutic targets for longer-lived species, offers challenges as well as opportunities for mammalian studies: mammalian studies of effects on life span and on other responses that require observation over an appreciable percent of the life span require much more time than analogous invertebrate studies. Hence, it is likely that the fraction of candidate interventions suggested by invertebrate studies that can actually be tested in mammals will be quite limited. Expanding mammalian model testing could help to reduce this discrepancy. Strategies to help refine the selection of interventions suggested by in vitro and invertebrate studies for mammalian testing can also help. For example, it is likely that many factors with positive effects on life span operate on the same or convergent pathways (as has been found for the multiple longevity-related genes that affect the insulin-like peptide signaling pathways), and sorting these factors into a limited number of pathways will facilitate finding the most promising points on these pathways for targeting preclinical and clinical interventions. In addition, more information on functions of homologous proteins in invertebrate models and in mammalian models for therapeutic aging studies could help to distinguish those targets for which mammalian interventions are likely to have positive effects from those that affect pathways that are important in invertebrates but not in mammals.

Few mammalian life span studies have tested interventions to modulate specific processes implicated in the invertebrate studies discussed above. Antioxidant studies have not shown substantial life span effects (Golden et al., 2002), with the exception of moderate extension in rodents by nitrone free radical spin-trapping agents, though evidence suggests that this is due to inhibition of specific redox-sensitive signal transduction processes related to inflammation, rather than reduction in free radicals per se (Floyd et al., 2002). In studies of agents affecting mitochondrial function, neither α-lipoic acid (which has antioxidant effects) nor coenzyme Q10 (which increases electron transport and has antioxidant properties) affected life span in mice (Lee et al., 2004).

As information on pathways and molecular targets for interventions that may affect longevity accumulates, the number of promising interventions for mammalian testing will likely increase. Recently, NIA's Interventions Testing Program (discussed below) has initiated four mouse intervention studies testing effects on longevity of two anti-inflammatory agents (aspirin and nitroflurbiprofen), 4-hydroxy-phenyl-N-tert-butyl nitrone (a free radical scavenger), and nordihydroguariecic acid (a lipoxygenase inhibitor with structural similarities to the sirtuin activator resveratrol).

In addition to life span studies, there have been shorter-term rodent studies on interventions to reverse adverse aging changes by modulating the effects of putative aging mechanisms. There have been reports of reduction of age-related declines in cognition by compounds with antioxidant properties (reviewed in Golden et al. [2002]). There also have been rodent intervention studies of the effects of agents that modulate mitochondrial function on aging changes: acetyl-l-carnitine administration to old rats increased tissue oxygen consumption and mitochondrial membrane potential and reversed age-related declines in cognition and physical activity (Ames, 2004).

Rodent model systems have limitations for evaluating the likely effect of interventions on humans, because of the considerable differences in age-related pathologies between rodents and humans. Nonhuman primates have greater similarities, but, other than three ongoing studies of effects of caloric restriction on aging and life span (Roberts et al., 2001), they have not been used in intervention studies of aging therapies. The development of other animal models of human aging pathologies may be especially valuable for experimental therapeutic studies.

Short- or medium-term human studies of intervention effects on factors affecting risk for subsequent aging outcomes. When there is sufficient evidence to support human testing of interventions, it is necessary to decide the age of the population to be studied, the duration of administration of the intervention, and the outcomes to be measured. If the intervention is expected to reverse in older persons adverse aging changes that increase their risk of clinical outcomes, it may be possible to design a study to measure the effect of the intervention on these outcomes directly. The longer the interval needed to observe such an effect, the more challenging this task becomes. However, clinical trials with treatment periods of up to 8 years have been successfully organized, and longer trials may be possible.

If, however, the expected effect of the intervention is to decelerate aging changes, requiring that it must be initiated relatively early in life and sustained for decades before clinical effects occur, the logistical challenges of conducting a trial long enough to detect these effects are extremely formidable. Except perhaps in the case of the most promising interventions with abundant preliminary data, alternative designs to determine shorter-term effects of the intervention can serve as an intermediate step to screen potential interventions for longer-term testing. Such studies can be conducted in different age groups to determine age-related differences in response and determine, for example, whether there is a maximum age above which initiating the intervention has no effect.

These studies can examine effects on known risk factors for clinical outcomes (e.g., bone density and cholesterol), including effects on rates of age-related changes in these factors. They can also determine effects on physiologic functions affected by interventions that delay or reverse aging changes in laboratory animals. In addition, there have been attempts to identify "biomarkers of aging" that could predict very long-term consequences of aging therapies in humans, including effects on longevity (Miller, 2001; Butler et al., 2004). In mice, early life predictors of longevity have been iden-

tified (Harper et al., 2004; Warner, 2004). Though several such human biomarkers have been proposed, these have not been validated by longitudinal epidemiologic data or other evidence regarding their long-term predictive value. However, analyses of data from very long-term longitudinal studies may identify such markers.

In both human and laboratory animal intervention studies, it is particularly useful to compare the effects of the intervention on aging outcomes with its effects on its molecular targets in vivo. Such comparisons can help in refining hypotheses about aging mechanisms as well as in guiding future experimental therapeutic studies. For example, if the in vivo molecular effects of an intervention are consistent with expectations but its clinical or physiologic effects are not, this may cause reconsideration of the role that the targeted pathway actually plays in the aging outcomes being studied. In one specific example, following considerable observational evidence that oxidized low-density lipoprotein (LDL) played a significant role in progression of atherosclerosis, a human α-tocopherol intervention study found that, although α-tocopherol administration indeed markedly reduced LDL oxidation, it had no effect on atherosclerosis progression (Hodis et al., 2002).

Ascertainment of Adverse as well as Beneficial Effects of Interventions

A critical issue regarding the identification of therapeutic targets in animal models is whether longer life span associated with genetic variants or experimental manipulations is accompanied by maintenance of healthy function during this extended lifetime. Possibilities to evaluate this in invertebrates are limited by scarcity of information on their pathologies. However, the *Indy* mutation in fruit flies increases longevity "without a concomitant reduction in resting metabolic rate, flight velocity, or age-specific fecundity" (Marden et al., 2003). The *age-1* and *daf-2* mutations extend nematode longevity and slow tissue degeneration (Garigan et al., 2002; Herndon et al., 2002) and increase resistance to bacterial infection (Garsin et al., 2003; Lithgow, 2003). However, not all the effects of all factors that extend life span are beneficial. For example, although the long-lived Ames dwarf mouse has lower incidence of cancers (Ikeno et al., 2002), it also has developmental abnormalities (Carter et al., 2002) and impairments in immune function (Esquifino et al., 1991).

For interventions in human studies and mammalian models, attention to adverse effects as well as to beneficial effects on aging, is crucial. Few interventions have unmitigated benefits; it is unlikely that aging therapies will be an exception. Though human studies suggest that factors contributing to exceptional longevity also contribute to exceptional health over the life span (Hitt et al., 1999; Atzmon et al., 2004), potential adverse effects of any putative human intervention need to be explored thoroughly. Studies of effects of interventions in laboratory mammals and human cells in vitro not only can detect pathologic effects but could dissect the mechanisms responsible for them from those that produce benefits and could lead to refined interventions that slow or reverse adverse aging changes while causing minimal risk.

Interactions among Intervention Development Activities and Resources

As discussed above, the multiple approaches to developing clinical aging interventions interact with each other in several ways. This is illustrated in Figure 1. To date, such interactions have mainly been informal, depending on spontaneous collaboration and information sharing among scientists. In the future, there may be benefits from more structured ways to encourage communication and collaboration on promising lines for intervention development.

One means by which this can be done is by facilitating interactions and collaborative activities among investigators working with diverse approaches and in diverse model systems and by providing the resources relevant to their work in intervention development (see Figure 1): the NIA Longevity Assurance Genes Interactive Network, involving mainly studies in a wide range of laboratory animal models, continues to make important contributions to identifying possible molecular targets for interventions (Warner, 2003). The large number of genes influencing life span identified by this network is likely to continue to expand rapidly as high-throughput screening techniques are applied.

To promote rigorous controlled testing in mammals of interventions that appear particularly promising based on available data, the National Institute on Aging has begun an Interventions Testing Program for interventions that may hold promise for future human studies (Warner et al., 2000). In this program, candidate interventions are evaluated in terms of the likelihood of efficacy, and those selected are tested in replicate mouse studies in three laboratories using standardized diet and environmental conditions. Information about the program, including the process for proposing and selecting interventions, is available at http://www.nia.nih.gov/ResearchInformation/ScientificResources/InterventionsTestingProgram.htm.

More recently, the Longevity Consortium, involving large-scale human population studies working in collaboration with researchers in the biology of aging, has been established to identify polymorphisms affecting longevity, including variants in homologs of genes that have effects on longevity in laboratory animals (Hadley and Rossi, 2005). Additional information on the consortium is available at http://www.longevityconsortium.org/. This research should be useful in identifying or confirming targets or pathways for intervention. NIA has also begun a program to develop and screen compounds for new drug targets for age-related conditions, presently focused on neurodegenerative diseases, diabetes, congestive heart failure, and oncologic and immunologic diseases.

Identification of long-term predictors of exceptionally healthy aging, which could be used in short- and medium-term human intervention studies, could be facilitated by the recently organized NIA Longitudinal Data on Aging Working Group, comprising researchers from numerous epidemiologic studies, which is examining possibilities for enhanced uses and analyses of longitudinal data in aging research. The repor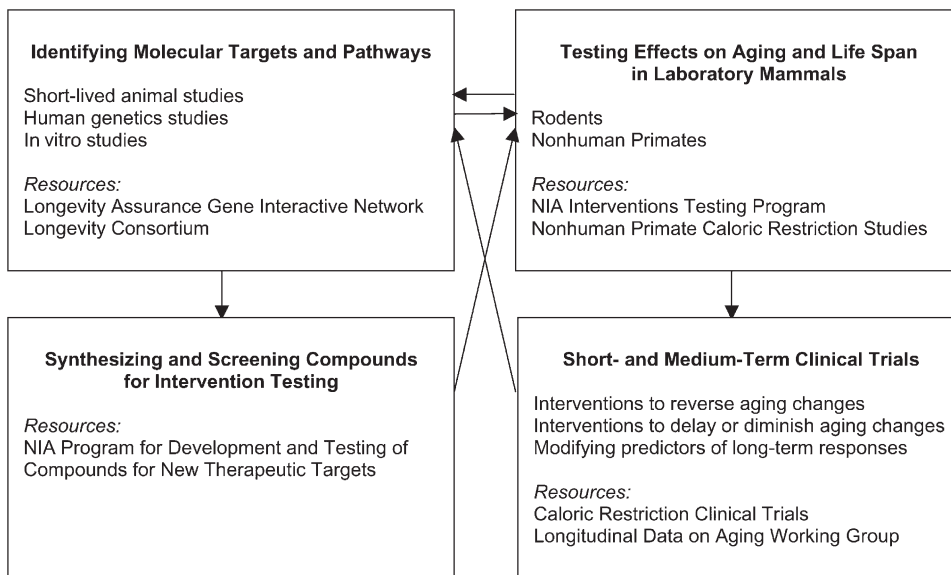t of the Working Group's first meeting is available at http://www.nia.nih.gov/ResearchInformation/ConferencesAndMeetings/.

Figure 1. Aging Therapies: Developmental Activities, Interactions, and Resources
See text for discussion.

Specific Therapies

This section discusses selected examples that illustrate the considerations discussed in preceding sections and also illustrate how development of new therapies is stimulated by different types of preliminary evidence, i.e., by biologic findings implying a cellular structure's role in aging (telomeres), by laboratory animal intervention studies (caloric restriction), and by investigations of the consequences of "normal" aging changes (vascular stiffening).

Telomere Maintenance

Critical observations by Hayflick and collaborators in the 1960s demonstrated that replicative capacity of normal somatic cells is finite and that, after a critical number of cell divisions, cells reach a state in which further division cannot occur, a state termed replicative senescence (Hayflick, 1965). This observation in turn raised the question of what biological process provides the counting mechanism that monitors cell division and underlies replicative senescence. Research over the past 15 years has provided an intriguing answer to this question in identifying the role of telomere function in regulating cell replication and senescence (reviewed by McEachern et al. [2000]). DNA replication during mitosis is incomplete and results in loss of 50–200 terminal bp per cell division. This loss of DNA occurs in telomeres, the tandem hexanucleotide repeats that constitute the ends of linear chromosomes. When cumulative loss of telomeres results in critical shortening, the "capping" function of telomeres is compromised, and recognition of this altered telomere structure results in cell senescence and/or apoptotic death. A pivotal discovery was the identification of telomerase, an RNA-dependent DNA polymerase that is capable of synthesizing terminal telomere repeats and extending the length of telomeres, thus compensating for the telomere loss that occurs with cell division. The potential implications of these findings for human aging and for clinical intervention have provoked substantial interest.

The role of telomeres in regulating the capacity for cell division, and the ability to extend cell division by intervening to manipulate telomere length and function have been well established. Cultured human cells reach replicative senescence after cell division in culture and generally express little or no detectable telomerase activity by the time that they are approaching senescence. Induction of telomerase activity in normal human somatic cells has been accomplished by transduction of telomerase genes and has been shown to result in the "immortalization" of these cells, as defined by an apparently unlimited capacity for cell division (Bodnar et al., 1998). An important question now under study is whether these immortalized cells retain their normal function or are altered in clinically relevant respects, such as loss of normal function or increased risk of malignant transformation.

What is the evidence that altered telomere length or function has consequences for multicellular organisms in vivo? Direct and compelling evidence comes from seminal experiments in which telomerase activity is eliminated by inactivation of telomerase genes. These experiments have been carried out in a number of species, but of most apparent clinical relevance are the studies carried out in knockout mice as a mammalian model (Blasco et al., 1997). During successive generations of breeding, telomeres progressively shorten, resulting in sterility due to the failure of both female and male germ cell lineages. Mice that have reached these late generations of telomere shortening also show a number of abnormalities similar to some if not all aspects of premature aging (Rudolph et al., 1999).

What, then, is the evidence that altered telomere function plays a role in normal human aging or in clinical conditions? There is considerable indication that

telomere shortening occurs in multiple cell lineages during normal human aging and that accelerated telomere shortening can occur in specific cell types and under disease-associated circumstances. These lines of evidence are consistent with but do not prove the existence of a causal relationship between telomere shortening and disease- or age-related changes in humans. In this regard, two recent findings are of particular note. In a study of men and women aged 60–97, it was observed that telomere length in peripheral blood cells is related to subsequent survival, with long telomeres associated with significantly lower risk of death (Cawthon et al., 2003). This correlation is intriguing, although it is not a direct demonstration that telomere length is a direct determinant of survival. The alternative possibility consistent with these findings is that genetic or life course events affect survival and that telomere length is affected in parallel but is an epiphenomenon rather than a causal determinant of health or survival. Perhaps the most compelling evidence for the existence of such a causal relationship is the inherited disease dyskeratosis congenita (DKC), the autosomal dominant variant of which is caused by a mutation in the telomerase RNA template gene and the X-linked variant of which is caused by mutations in the telomere-associated protein dyskerin (Mitchell et al., 1999; Vulliamy et al., 2001). DKC is marked by abnormally short telomeres, defects in epithelial and hematopoietic cell lineages, and hematopoietic malignancies. These findings suggest that telomere length or telomerase activity can affect human health.

What are the strategies by which existing information could be translated into therapeutic manipulations of telomeres? The most accessible approaches at present appear to be those in which specific human cells would be manipulated ex vivo, for example, by telomerase gene transduction to express telomerase, maintain or increase telomere length, and therefore to have extended capacity for cell division. Cells so modified could then be administered to the same individual under circumstances in which cell numbers or potential for cell division would otherwise be insufficient to serve the desired outcomes. Such approaches will require close attention to potential complications such as uncontrolled division or altered function of modified cells.

Caloric Restriction

Chronic caloric restriction (CR) ("undernutrition without malnutrition"), when started in early life or adult life, substantially extends life span in rodents as well as in multiple invertebrate species. The fact that this single intervention slows multiple age-related changes, delays the onset of cancer and multiple other age-related pathologies, and extends life span is consistent with (but does not prove) the idea that one or a few mechanisms, modulated by caloric restriction, control the rate of multiple aging changes and may be potentially controllable by other interventions as well. Chronic CR in nonhuman primates has been found to produce parallel physiologic changes to those seen in rodents; studies of its effects on nonhuman primate life span have not been completed. Despite extensive research on caloric restriction's effects on aging, we still do not understand

the mechanism(s) of its effects nor their implications for humans (Hadley et al., 2001; Roberts et al., 2001; Hursting et al., 2003; Koubova and Guarente, 2003).

There is also evidence that periodic food deprivation in mice, produced by every-other-day intermittent feeding, may induce similar physiologic effects over a period of weeks to those of caloric restriction, even when average daily intake is minimally different from ad libitum intake (Anson et al., 2003). This finding has potentially very important implications. However, a life span study in which caloric intake on an intermittent feeding regimen is documented to equal ad libitum intake and produce equivalent effects to CR's has yet to be reported.

Molecular and physiologic mechanisms proposed to explain CR's effects on life span are essentially coextensive with those proposed to regulate life span (reviewed in Masoro [2000], Koubova and Guarente [2003], Ingram et al. [2004]), including reduction of oxidative damage, increased metabolic efficiency, increased genomic stability, decreased apoptosis, increased apoptosis, lowered glucose levels, slowed glycolysis, lower insulin levels, lowered protein glycation, decreased body temperature, neuroendocrine responses, and sirtuin activation.

Exploration of potential human implications of the extension of life span by CR has taken two general directions. One derives from the search for the mechanisms mediating its effects in laboratory animals, which could be used to identify "CR mimetic" interventions that produce CR's effects in the absence of caloric restriction (Hadley et al., 2001). Since almost every mechanism proposed to modulate aging changes has been proposed to modulate CR's effects on aging, in the broadest sense, almost all studies of interventions that modulate these mechanisms could be considered CR mimetic studies. In addition to such studies discussed earlier, there have been recent CR mimetic intervention studies in rodents focused on glucose metabolism, using the glycolysis inhibitor 2-deoxyglucose and the hypoglycemic agent metformin (Ingram et al., 2004).

Another potential line of research on human implications of CR's effects on aging is controlled human trials. For reasons discussed above, medium-term studies of its effects are advisable before considering longer-term studies. NIA is supporting randomized controlled pilot trials of CR, collectively known as CALERIE (Comprehensive Assessment of Long-term Effects of Restricted Intake of Energy Intake). CALERIE will test effects of 2–3 years of caloric restriction (20%–30% reduction) in young and middle-aged nonobese persons. CR will also be compared with exercise interventions that produce weight loss to determine how their effects differ. Major goals of CALERIE are to determine whether this magnitude and duration of CR is feasible and safe, to compare human physiologic and cellular responses with effects reported in laboratory animals, and to determine its effects on disease risk factors and predictors of longevity derived from human studies. CALERIE is encouraging proposals for ancillary studies on mechanisms underlying CR's physiologic effects. Information about CALERIE, including ancillary study policies, is available at http://calerie.dcri.duke.edu/.

If findings from CALERIE indicate feasibility and

safety, a trial of a longer CR intervention may be worthwhile, particularly if CALERIE interventions have favorable effects on known disease risk factors. Prospects for long-term CR studies may be enhanced by the rapidly increasing understanding of the regulation of food intake and the possible ensuing development of better anorexiants (Flier, 2004).

Interventions against Age-Related Vascular Stiffening

One of the most salient age-associated changes in large arteries is an increase in intimal-medial thickness, which is accompanied by an increase in stiffness. A growing body of epidemiologic evidence indicates that these changes, and the ensuing increase in systolic and pulse pressure, formerly thought to be part of "normal" aging, precede and predict a higher risk for developing clinical atherosclerosis, hypertension, and myocardial infarction or stroke (Lakatta and Levy, 2003). In other words, aging blood vessels provide a milieu in which vascular diseases can flourish. These vascular changes that accompany aging in persons who do not have a diagnosis of clinical cardiovascular disease, however, largely have remained outside the bailiwick of clinical medicine and have not been the focus of preventive measures. However, if such "risky" arterial aging leads to arterial disease, it offers potential targets for treatment and prevention, involving modification of lifestyle or pharmacotherapy, to retard its rate of progression and delay or prevent clinical disease.

Cellular and molecular mechanisms that underlie arterial intimal medial thickening and stiffening with advancing age may provide targets for such interventions. These include elevated levels or activity of molecules such as matrix metalloprotease-2 (MMP2), angiotensin II (AngII), transforming growth factor β, monocyte chemoattractant protein-1 (MCP1), interstitial cell adhesion molecule-1, and NADPH oxidase. Each of these factors is a signaling target downstream of the angiotensin AT1 receptor. AngII increases MMP2 activity in the aged arterial wall and increases transcription of TGFβ and the TGFβ II receptor. Activated MMP2 activates TGFβ, leading to enhanced transcription of fibronectin and collagen genes. AngII signaling also increases the production of MCP1 and its receptor, CCR2, in arterial endothelial and vascular smooth muscle cells. Another sign of increased inflammation is enhanced NADPH oxidase activity, which also results in part from an age-associated increase in arterial AngII signaling. Excessive NADPH oxidase produces superoxide, which reacts with nitric oxide to produce the toxic species peroxynitrite, which can lead to protein nitration and "steals" nitric oxide in the process, reducing its bioavailability.

Interactions among these factors create a metabolically active environment (Lakatta, 2003; Spinetti et al., 2004; Wang et al., 2003). Some vascular smooth muscle cells (VSMCs) shift their phenotype from contractile to secretory, proliferative, and invasive and migrate into the thickened intima. Enhanced VSMC invasion within the older arterial wall is promoted by elaboration of the chemoattractant PDGFβ and its receptor, both of which are also downstream signaling effects of Ang II. VSMC invasion of matrix within the old arterial wall is facilitated both by activated MMP2 and by MCP1. Arterial

wall elastin becomes fragmented with advancing age, and excessive collagen is synthesized and becomes nonenzymatically glycated. Endothelial dysfunction and its attendant alterations in endothelial permeability and vasotonic actions occur. These same metabolic, enzymatic, cellular, and endothelial alterations appear to play a critical role in the genesis or promotion of hypertension, atherosclerosis, vascular inflammation, vascular remodeling, and oxidant stress. In other words, many of the same factors that underlie the age-associated structural and functional alterations of the arterial intima and media are also implicated in the pathogenesis of clinical arterial disease. These and other factors are the "culprits" that underlie the "risky" component of arterial aging in humans (Lakatta, 2003).

As progress is made in further elucidating the diverse molecular mechanisms that underlie the arterial alterations that accompany advancing age, novel therapies must emerge that will specifically target these pathways and retard or reverse "unsuccessful" arterial aging. Treatments targeting structural factors have begun. Nonenzymatic crosslinks between glucose (or other reducing sugars) and amino groups that generate advanced glycation end products alter long-lived proteins, e.g., collagen and elastin, and increase with advancing age, and both cause arteries and the heart to stiffen. NIA studies have demonstrated that ALT-711, a novel thiazolium agent that breaks such crosslinks, reduces arterial stiffness both in nonhuman primates (Vaitkevicius et al., 2001) and in humans (Kass et al., 2001). Chronic inhibition of angiotensin receptor signaling also substantially retards the age-associated increase in collagen content and intimal-medial thickening and stiffness in rodents (see Lakatta [2003] for review).

The substantial variability among older persons in the degree of these vascular changes also reinforces the possibility of identifying factors that modify them (Lakatta and Levy, 2003), including lifestyle factors: it is noteworthy that arterial stiffness is inversely related to physical fitness, assessed as maximum oxygen consumption, over a broad age range (Vaitkevicius et al., 1993). To date, however, clinical trial findings on the effects of physical activity on vascular stiffness have not been reported. Dietary interventions can modulate vascular properties. Food fat content has been shown to adversely affect vascular stiffness and endothelial function; diets that are reduced in sodium are associated with reduced arterial stiffening with aging, independent of their blood pressure-lowering effects (Gates et al., 2004).

While such preventive lifestyle or pharmacological strategies may be explored even now, future genetic characterization of individuals will likely allow person-specific stratification with respect to risk, efficacy, and cost effectiveness of measures to retard vascular aging in order to reduce CV functional disability and disease at older ages.

Alzheimer's Disease

Alzheimer's disease (AD) is a devastating neurodegenerative disease that afflicts older men and women, with risk increasing dramatically with age. Alzheimer's disease research has stimulated not only knowledge of the disease but also understanding of normal brain aging

and the ways in which aging of the brain both contributes to and differs from the pathology of AD. Knowledge gained from multiple avenues of research illustrates the way in which genetic, molecular, and epidemiologic information can be translated into preclinical and clinical interventions.

In the early 1900s, Alois Alzheimer described the amyloid plaques and neurofibrillary tangles characteristic of AD. Sequencing of the protein component of brain amyloid in the 1980s was followed soon thereafter by identification of the gene encoding its precursor protein, amyloid precursor protein (APP). Subsequently, it was found that mutations in the APP gene are responsible for some cases of familial early onset AD; that other cases of familial early onset AD are caused by mutations in two other genes, presenilins 1 and 2; and that mutations in all three of these genes increased the production of specific forms of amyloid. These molecular and genetic discoveries focused research on the pathways leading to amyloid formation and accumulation of plaques. The enzymes β- and γ-secretase cooperate to cleave APP and produce the peptide form, β-amyloid, that preferentially constitutes plaques in the brains of AD patients, making inhibition of these enzymes a target of current drug development strategies (history reviewed in Hardy and Selkoe [2002]). The enzyme α-secretase cleaves β-amyloid internally, potentially preventing the formation of plaques; enhancing the activity of α-secretase thus represents another target (Lichtenthaler and Haass, 2004). Yet another is degradation of amyloid (Tanzi et al., 2004) or preventing the accumulation of soluble, possibly highly toxic intermediates (Klein et al., 2001). The challenge of designing effective interventions that inhibit AD-specific pathways but do not interfere with essential normal functions is illustrated by the example of γ-secretase, which, apart from its role in β-amyloid production, is also the protease responsible for cleavage and activation of notch, a receptor that is involved in a diverse set of normal differentiative pathways (Conboy et al., 2003). This raises the possibility, being actively investigated by a number of researchers, that altered presenilin function may also lead to neurodegenerative disease by other, nonamyloidogenic pathways (Thinakaran and Parent, 2004).

The identification of mutated APP and presenilin genes that are causal for AD has also led to the development of the first transgenic mouse models of this disease, providing model systems for testing of amyloid interventions in vivo. Experimental treatments tested in these models to date include the successful reduction in amyloid plaque formation by chelation (Cherny et al., 2001) and by immunization against amyloid (Schenk et al., 1999). Clinical trials of immunization with amyloid peptide were initiated in humans but were stopped due to side effects of encephalopathy (Orgogozo et al., 2003). Researchers continue to explore different approaches to immunotherapy that would reduce amyloid load without negative side effects.

Neurofibrillary tangles are another obvious target for intervention. Research on metabolism of their protein component, tau, has led to insights into phosphorylation and other modifications of tau that favor tangle formation and dissolution of the microtubule network.

Again, genetics has played a role in highlighting the role of tau, since a number of mutations in the tau gene have been shown to cause frontotemporal dementia (FTDP-17) and related dementias (Ingram and Spillantini, 2002). The link between tau and amyloid pathology in AD is not yet clear, nor is the link between age-related accumulation of tau or amyloid and development of AD. Research on multiply transgenic mice combining abnormalities in amyloid and tau (Oddo et al., 2004) is beginning to elucidate the relationships. Interventions targeting tau phosphorylation and stabilization of microtubules are being developed.

Understanding of genetic factors in the much more common late onset form of AD was initiated by the discovery that the E4 allele of the apolipoprotein gene is a major risk factor gene for the disease (Strittmatter et al., 1993), leading to research on the mechanisms of this effect and possible therapeutic targets. No other risk factor genes have yet been unambiguously identified, although several regions of the genome have been pinpointed. The NIA's genetics initiative http://www.nia.nih.gov/ResearchInformation/ExtramuralPrograms/NeuroscienceOfAging/AlzheimersDiseaseGenetics.htm; www.ncrad.org) will make available to qualified researchers well-characterized family and case control sets presently being identified by the AD Centers (http://www.alzheimers.org/adcdir.htm) and other investigators.

Even early stages of AD are characterized by loss of synapses and neurons in entorhinal cortex and hippocampus, areas important for memory. The antecedent(s) of cell death are unknown. Possible interventions targeting cell dysfunction and death include introduction of growth factors into affected brain regions, a technique effective in nonhuman primates (Smith et al., 1999) and presently the focus of a small clinical trial in AD patients. Stimulating endogenous stem cell production of neurons may positively affect hippocampal function (Kuhn et al., 1996; Kempermann et al., 2004). Other possible targets, common to a number of neurodegenerative diseases, include boosting the pathways clearing abnormally folded and toxic proteins and developing strategies to reduce abnormal folding (Ross and Poirier, 2004). Techniques for assessing levels of thousands of individual mRNAs will reveal underlying patterns of mRNA and protein changes (Blalock et al., 2004), identifying altered pathways early in disease development.

Both AD brain pathology and epidemiological studies have implicated oxidative stress and inflammation as causal factors in AD. Diets enriched in antioxidants, such as vitamins C and E and α-lipoic acid improve learning and memory and reduce plaque load in aged canines (Milgram et al., 2004) and rats, and anti-inflammatory agents reduce plaque load in APP transgenic mice (Lim et al., 2001; Eriksen et al., 2003). Long-term prevention clinical trials for several antioxidants are ongoing. Participants in a recently suspended prevention trial of nonsteroidal anti-inflammatory drugs will continue to be followed for safety and efficacy for 2 years. Other risk factors for AD include several also related to cardiovascular disease—high blood pressure, cholesterol, and homocysteine levels. A statin is being tested in a clinical trial on patients who have AD, and another treatment trial tests efficacy of reduction in blood homo-

cysteine levels by folate/B6/B12. The effect on AD risk of lowering blood glucose levels is being tested in older diabetics. Data on most of these trials can be accessed at http://www.alzheimers.org/clintrials/search.asp.

Research on AD initially focused on late stage disease, but with better understanding and more sophisticated research tools, the focus has shifted to earlier and even preclinical stages (Grundman et al., 2004). It is likely that AD pathology is initiated many years before clinical diagnosis; by the time a person has mild cognitive impairment (MCI) with memory decline, loss of key neurons in the entorhinal cortex is profound (Price et al., 2001), and loss of neuronal function is apparent in other brain regions as well (Mufson et al., 2002). Important initiatives will therefore focus on developing prevention strategies that would slow or halt the pathology before major functional impairment. Key to early identification of persons at risk is the development of neuropsychological, clinical, and imaging techniques sensitive to characteristic early pathologies such as delayed recall memory measures, loss of hippocampal volume, and lowered metabolism in affected brain regions. Identification of suitable imaging and biological makers of progression would be critical to reducing the size, time, and cost of treatment and prevention trials. The AD Neuroimaging Initiative (ADNI) (http://www.alzheimers.org/pubs/conv11n1and2.htm#new) is a major new longitudinal study of normal controls and persons with MCI and AD to identify surrogate markers for disease onset and progression and is potentially a scaffold for ancillary studies testing specific hypotheses.

Conclusion

In recent years, cellular, molecular, and genetic studies of aging in in vitro models and in short-lived invertebrates have generated an impressive pace of discovery. These discoveries, together with outcomes of epidemiologic and clinical research, have in turn helped to identify potential therapeutic targets for testing in longer-lived mammalian species. It is important that this pace of progress continue, with application of cutting-edge basic science. It will also be critical to develop strategies that will allow selection of the most promising of candidate interventions suggested by in vitro and invertebrate studies, and by laboratory mammal and human studies, for further evaluation.

As illustrated in this review, in all aspects of the development of interventions that target aging-related processes, from in vitro to clinical studies, there are needs and opportunities for innovative molecular and cellular research strategies. Aging is a fundamental biological phenomenon with wide-ranging medical and societal effects. The discovery of effective new therapies to address it is a worthy challenge for molecular and cell biology.

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