

Telomerase and the Aging Cell

Implications for Human Health

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Recent research has shown that inserting a gene for the protein component of telomerase into senescent human cells reextends their telomeres to lengths typical of young cells, and the cells then display all the other identifiable characteristics of young, healthy cells. This advance not only suggests that telomeres are the central timing mechanism for cellular aging, but also demonstrates that such a mechanism can be reset, extending the replicative life span of such cells and resulting in markers of gene expression typical of “younger” (ie, early passage) cells without the hallmarks of malignant transformation. It is now possible to explore the fundamental cellular mechanisms underlying human aging, clarifying the role played by replicative senescence. By implication, we may soon be able to determine the extent to which the major causes of death and disability in aging populations in developed countries—cancer, atherosclerosis, osteoarthritis, macular degeneration, and Alzheimer dementia—are attributable to such fundamental mechanisms. If they are amenable to prevention or treatment by alteration of cellular senescence, the clinical implications have few historic precedents.

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But the lengthening of the thread of life itself, and the postponement for a time of that death which gradually steals on by natural dissolution and the decay of age, is a subject which no physician has handled in proportion to its dignity.

Francis Bacon,
The Advancement of Learning

THERE IS A natural inclination to believe that scientific and medical advances will be accretional: gradual, predictable additions to our current body of knowledge and practice. Although this is an appropriate view of the overwhelming bulk of day-to-day science and medicine, history also shows unpredictable and innovative transitions in our paradigms and, subsequently, in clinical practice. The most notable of such shifts has been germ theory (with the consequent development of immunizations, antibiotics, and sterile surgical technique). The same is likely to be true of genetics, although, as yet, the clinical benefits remain largely theoretical.

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An equally remarkable potential—in regard to cancer, aging, and age-related disease—is highlighted in the startling articles by Bodnar et al¹ and Vaziri and Benchimol,² which demonstrate that human cell senescence can be reversed (and the “Hayflick limit” extended) by transfection with a gene for the catalytic component of telomerase. The publication of these results makes it appropriate to review the field and outline the prospects for clinical medicine.

BEYOND THE HAYFLICK LIMIT

In 1961, Hayflick and Moorehead³ showed that normal somatic cells have a limited replicative potential, roughly 50 in young skin fibroblast cells. Prior to achieving this maximum, they slow their rate of division (to such an extent that they probably rarely reach their replicative limit in vivo) and manifest identifiable and predictable morphologic changes characteristic of “senescent cells.”⁴ Since that time, we have defined patterns of predictable changes in genetic expression (“senescence-associated gene expression”) that accompany the replicative block in a variety of cell types.⁵⁻⁹

In the early 1970s, Olovnikov¹⁰ and Watson¹¹ independently pointed out that DNA polymerase primers overlay and “mask” a portion of the terminal chromosome from DNA polymerase; a por-

tion of the telomere is not replicated and therefore shortens with each cell division. During the 1980s, molecular biologists began to address this hypothesis, and by 1990, research showed that telomeres are indeed shorter in senescent cells than in younger cells.^{12,13} Since then, results have accumulated¹⁴⁻¹⁶ that (not without exception¹⁷) imply, but did not prove, that telomere shortening is the clock that results in the shift to a senescent pattern of gene expression and ultimately cell senescence and the Hayflick limit.

This barrier to unlimited cell replication, the Hayflick limit, has been reliably reproduced¹⁸ and has few (and identifiable) exceptions in multicellular organisms.¹⁹ These exceptions include cancer cells,²⁰⁻²² the germ cell lineage,²³⁻²⁷ and certain stem cell lines. These latter cells (including hematopoietic stem cells,²⁴ gastrointestinal crypt cells, hair follicles,²⁵ and perhaps liver cells²⁶) also demonstrate replicative senescence, but do so with a much extended cellular life span (a greater number of divisions) compared with other somatic cell types. This extension correlates with transient expression of telomerase^{30,31}—a reverse transcriptase comprising (at least) an RNA template³² and a catalytic protein component³³—that allows them to slow the rate at which the erosion of telomere bases occurs. Telomerase expression also occurs in the germ cell line and primordial stem cells,^{34,35} whose potential clinical applications include the ability to grow tissues and organs without being limited by replicative senescence, as has been the case until now.

The acceptance of cellular senescence raised 2 immediate and critical questions: (1) what is the cellular “clock” that timed cell replicative senescence, and (2) what is the relationship between cell senescence and aging in the organism? Bluntly, does replicative senescence tell us anything clinically useful about the causes of (and therefore potential treatments for) diseases such as cancer, atherosclerosis, arthritis, and dementia?

After 35 years of research, Bodnar et al¹ have emphatically answered the first

of these 2 questions: the telomere is the clock of replicative senescence and it can be reset. The work has been independently confirmed by Vaziri and Benchimol,² as well as by others.³⁶ Telomerase acts by adding DNA bases to telomeres. In addition to its RNA template that is present in cells normally (and which acts as a “die” for the TTAGGG sequence), it contains a catalytic protein component that is not found in normal, aging, somatic cells. In the first published work, Bodnar et al¹ transfected a gene for the catalytic component into such cells and showed that this resulted in extended telomeres. More importantly, however, was that this extended the replicative life span of cells and gave them a pattern of gene expression typical of young cells. By the time results were submitted, treated cells already showed 40% more population doublings and showed no evidence of slowing their rate of cell division. Although this result was strongly implied by initial work on cell hybrids in which telomere lengthening correlated with extended cell life span,³⁷ it is the article by Bodnar et al that effectively establishes that telomeres are the clock of replicative aging. Telomeres not only shorten with cell aging, but relengthening the telomere appears to reset gene expression (as measured by expression of β -galactosidase, an established biomarker of aging in these cells³⁸), cell morphology, and the replicative life span. Furthermore, and despite the appropriate concern that this should raise, there is no evidence yet that telomerase expression per se causes malignant transformation.

Remarkable as these implications are, it is far more important in allowing us to begin answering the second question, viz, what does replicative senescence have to do with cancer and other age-related diseases and therefore with their treatment? It is here that the historical transition may be in the offing. The potential is therapeutic modification of the cellular mechanisms underlying age-related diseases to an as-yet-unparalleled and effective degree. These possibilities include effective cancer therapy and potentially, but more distantly, the effective prevention and treatment of atherosclerosis, osteoarthritis, immune senescence, dermatologic aging, macular degeneration, and Alzheimer dementia at a fundamental cellular level. While such possibilities have been discussed previously and speculatively,³⁹ we now have the tools to begin testing them.

IMPLICATIONS FOR UNDERSTANDING DISEASE

The potential impact of altering aging and age-related diseases at the genetic

and cellular level is enormous, both medically and economically.⁴⁰ Although some hormonal therapies, such as estrogen replacement,^{41,42} perhaps growth hormone,⁴³ and possibly dehydroepiandrosterone (DHEA),⁴⁴⁻⁴⁶ have evidence of efficacy, their clinical role is as yet poorly defined and their indications are limited. Estrogen replacement, for example, lowers the atherosclerotic death rate in women (and may delay or ameliorate Alzheimer dementia⁴⁷), but is not appropriate in men, may increase the risk of breast cancer^{48,49} or endometrial cancer,^{50,51} and does not prevent aging overall. With even less to offer and more caveats, human growth hormone,⁵² DHEA,⁵³ and the like may be found to play a clinical role in some aging-related diseases or in the quality of life,⁵⁴ but there is no evidence that these hormones (or any others) underlie fundamental aging processes.

Nor should we expect hormones to play such a role. While it is true that hormones show age-related changes,⁵⁵ such models implicitly assume that “wear and tear” of the secreting organ assumes the role of a “clock.”⁵⁶ The more appropriate and intriguing model is that suggested by the correlation between replicative senescence and life span between and within species¹⁵ (as well as in progeric syndromes such as Hutchinson-Gilford disease and Werner syndrome⁵⁷).

With regard to cell senescence, there is a growing literature supporting a central role for cell aging in organismal aging. The “limited model”—that telomeres play a pivotal role in replicative senescence—has been addressed in recent reviews^{7,58} and receives significant support in the results of Bodnar et al¹ and Vaziri and Benchimol.² The “general model”—that cell aging underlies the general process of aging in the organism—however, remains more speculative. This more general model relies not only on changes in dividing cells (which approach their replicative limits), but on nondividing cells (which depend on cells that *do* divide) and whose dysfunction ultimately results in clinical disease.

The limited model is relatively simple, although much is still unknown about the mechanisms involved. As cells divide, telomeres shorten, resulting both in senescence-associated gene expression^{38,59,60} and in inhibition of cell replication⁶¹ via the cell cycle braking system.⁶² Telomere loss is detected as genetic damage⁶³ and invokes the p53 cascade, the failure of which is implicated in oncogenesis.⁶⁴ The linkage for the first arm of this mechanism—senescence-associated gene expression—is under debate, but current data suggest that it results from changes in the heterochromatin,⁶⁵ conformational changes in

the chromosome itself,⁶⁶ directly through a checkpoint arrest,⁶¹ or from an interaction of these mechanisms. In cells that do not show replicative senescence—cancer cells, germ line cells, and some stem cells—telomerase maintains telomere lengths, and, according to this model, thereby prevents cell senescence. Both Bodnar and her colleagues¹ (including Vaziri and Benchimol²) have provided substantial proof for this model by showing 2 critical results. Lengthening telomeres results in cells that have longer replicative limits and are “younger” (not senescent) as assessed by their patterns of gene expression, physiologic markers, and morphology. Perhaps more importantly, considering the permissive role (necessary but not sufficient) that telomerase expression appears to play in malignancy, telomerase expression and telomere lengthening per se give no evidence—so far—of altering normal cell cycle control, chromosome complement, or cell morphology, or of resulting in any other markers of malignant transformation.

Furthermore, and as a result of their work, the general model now becomes testable. This model goes further, suggesting that replicative senescence itself times and underlies organismal senescence. Replicative senescence is not solely responsible for clinical aging: there are too many data that environmental factors (eg, tobacco use, oxidative damage, ultraviolet exposure) and genetics (eg, diabetes, hypertension, hypercholesterolemia) have major roles in many age-related diseases (such as atherosclerosis). Nevertheless, cell aging probably plays a central role in the timing and course of such diseases through a number of mechanisms. This senescence-associated gene expression model of aging does not suggest that older tissues have a preponderance of (or even necessarily any) cells that have reached their replicative limits; rather it suggests that a sufficient number of senescing cells have an altered pattern of gene expression and that this alteration in cell function is both directly and indirectly responsible for the cascade of processes (such as atherogenesis) that we encounter clinically as “aging” in organs and tissues.

Perhaps the best example (in terms of currently available supporting data) of this is dermatologic aging. Many of the changes that occur in the dermis with age—loss of collagen, increased collagenase, and decreased elastin⁶⁷—are paralleled by the changes in senescent dermal fibroblasts *in vitro*.⁶⁰ Although we lack a comprehensive understanding of how skin aging occurs, the model that senescing cells may contribute to this process is now potentially testable, per-

haps by directly modulating telomere length. We might now, for example, attempt to delay senescence in vivo (in dermal fibroblasts) by using telomerase analogs, transfection, or activation of gene expression.

Skin aging is not merely cosmetic, having measurable clinical morbidity, but there is greater clinical interest in the effects of aging in other tissues (and cell types), for example, joints (eg, chondrocytes), the central nervous system (eg, glia and secondarily the nondividing neurons that depend on them), and the immune system (eg, lymphocytes). Current technical prowess is not up to these goals yet, but already allows us to extend telomeres in vitro, which may suffice to permit clonal expansion of human cells for vaccine production or to grow skin cells for burn patients. Similarly we can now test the ex vivo clinical potential in human immunodeficiency virus disease and marrow transplants, in which replicative senescence may be reset using currently available transfection techniques. Recent work by Shay,⁶⁸ Moore,⁶⁹ and others,⁷⁰ for example, suggests that telomere shortening may play a role in limiting the clonal expansion of bone marrow for transplants. This is precisely the sort of clinical possibility that current technology is most capable of testing, with the potential benefit of more successful bone marrow transplants and better patient survival.

Aging vessels—specifically atherosclerosis and its clinical outcomes (largely myocardial damage and stroke)—are a major cause of death and morbidity in developed countries. Here too, the senescence-associated gene expression model offers to clarify and extend our basic model of atherogenesis and suggests an alternative therapeutic approach. Cellular senescence has been observed in arterial endothelial cells,⁷¹ which are themselves implicated in triggering atherosclerosis.⁷² In those portions of the vascular tree under high stress (shear stress due to hypertension or vessel bifurcation, for example), cells show shorter telomeres, attributed in this model to greater cell division and cell replacement in these areas. Such older endothelial cells have an altered pattern of trophic factor production, part of the cascade of atherogenesis. Dividing cells (vascular endothelial cells) can cause age-related pathology in nondividing cells (myocardial cells): many cells do not divide, yet—in this general model—are critically dependent on cells that do. Once again, the importance of the new research is not that it proves or extends this model of vascular aging, but rather that it opens the door to testing the model if we can extend telomeres in vivo in vascular endo-

thelial cells by transfection or gene activation. Such techniques (in vivo) still lie beyond our grasp, yet are tantalizingly close and possess provocative implications: might we prevent (or to an extent even reverse) atherosclerotic lesions and thus favorably alter their clinical outcomes?

MALIGNANT TRANSFORMATION

The other major cause of death in developed nations is cancer. Malignant transformation requires that cancer cells evade replicative senescence, making aging and cancer “the double-edged sword of replicative senescence.”⁷³ This raises the concern that in lengthening telomeres to reverse replicative senescence, we might increase the risk of cancer. Such a concern arises not because telomerase expression causes cancer, but rather because it allows transformed cells to continue dividing and therefore to attain clinical significance. As predicted by this model, telomerase expression is a superb marker of malignancy,^{22,29} but is not itself a cause of cancer, a conclusion that begs testing, as in Bodnar et al¹ and studies like it. In fact, the absence of telomerase in most normal somatic cells may play a protective role, forcing the cancer cells in many would-be tumors to stop dividing prior to becoming clinically significant. Ironically then, our growing knowledge of replicative senescence also implies a novel potential therapy forcing cancer cells to senesce by inhibition of telomerase activity.²⁰ Here again, Bodnar et al provide a first step, showing that—so far and as predicted—telomerase activity per se does not show any evidence of causing malignant transformation in this study. This initial study is supported and confirmed by a more extensive recent study,⁷⁴ which again finds no evidence of malignancy: telomerase-transformed cells appear to be otherwise normal cells. Equally, these results show that we are growing more adept at using the tools of telomere biology and are thereby moving slowly closer to trials of telomerase inhibitors to treat cancer. Such inhibitors would be used to reinstate a replicative limit in cancer cells, with the clinical aim of braking further tumor growth and forcing such cells to senesce.

IMPLICATIONS FOR AGING

The possibility of directly undercutting the cellular mechanisms causing age-related diseases by altering the mechanisms of cellular senescence suggests a more distant and unprecedented prospect: we might extend not only the mean life span (as with every clinical advance), but potentially the maximum life span as well. The correlation between

species life span and the replicative life span of cells from such species raises this (perhaps soon testable) possibility. Such a potential increase in life span, while unintentional, would have a pervasive impact on our culture.

Any prevention, postponement, or reversal of replicative senescence may carry implications for aging and age-related diseases, and although this work is extraordinary, it is by no means conclusive. Telomere biology and replicative senescence still abound with uncertainties and superficially contradictory data, as does any field at its inception.⁷⁵ For example, there are multiple ways of inducing replicative senescence or senescence-like states (oxidative damage, DNA damage, ultraviolet exposure, the *ras* oncogene,⁷⁶ etc) independent of telomere shortening,^{77,78} and there are pathways for extending telomeres without telomerase activity.⁷⁹ Additionally, although senescence-associated gene expression and growth arrest serve to define senescence, there is a great deal still to understand, even when we restrict the discussion to a single cell type within a single species. The telomerase knockout mouse¹⁷ (in which cells do not express the gene for telomerase and the offspring appear normal for several generations) clearly suggests that telomere function differs between species, but does not directly contradict the contribution of senescence-associated gene expression to aging,⁸⁰⁻⁸² nor does it undermine the significance of the work by Bodnar et al¹ or Vaziri and Benchimol.²

The importance of this work does not lie merely in our nascent ability to alter cell senescence. Nor are the implications that we may simply be able to test an increasingly complete and elegant theory of aging. Rather the importance, ultimately, lies in its potential to treat human disease, to alleviate patient suffering, and—raising the possibility “in proportion to its dignity”—that we may alter “the thread of life itself.”

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