

Telomeres and Telomerase: A Modern Fountain of Youth?

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ABSTRACT

Since ageing is a universal human feature, it is not surprising that, from the Babylonian epic of Gilgamesh to Ponce de Leon seeking the "Fountain of Youth," countless people have dreamed of finding a way to avoid ageing, to no avail. Yet the search continues. In this review, we present one of the latest candidates: the enzyme telomerase, capable of elongating the tips of chromosomes, the telomeres. Research into the causes of cellular ageing established the telomeres as the molecular clock that counts the number of times cells divide and triggers cellular senescence. Herein, we review arguments both in favor and against the use of telomerase as an anti-ageing therapy. The importance of the telomeres in cellular ageing, the low or non-existent levels of telomerase activity in human tissues, and the ability of telomerase to immortalize human cells suggest that telomerase can be used as an anti-ageing therapy. On the other hand, recent experiments in mice have raised doubts whether telomerase affects organismal ageing. Results from human cells expressing telomerase have also suggested telomerase may promote tumorigenesis. We conclude that, though telomerase may be used in regenerative medicine and to treat specific diseases, it is unlikely to become a source of anti-ageing therapies.

INTRODUCTION

AGEING is a universal human feature. It is not surprising then that since the dawn of civilization many have sought to avoid it. From the Babylonian epic of Gilgamesh, to Ponce de Leon seeking the "Fountain of Youth," countless people have dreamed of finding a way to avoid ageing, to no avail. In modern times, despite major advances in technology, there con-

tinues to be no proven way of delaying human ageing.¹ Yet the search continues. In this review, we present one of the latest candidates: the enzyme telomerase, capable of elongating the tips of chromosomes, the telomeres. We first introduce the research that led to the suggestion telomeres may be involved in ageing and later discuss whether telomerase may or not be a likely candidate for anti-ageing research.

CELLULAR AGEING REGULATION BY THE TELOMERES

In 1961, Leonard Hayflick and Paul Moorhead discovered that human cells derived from embryonic tissues can only divide a finite number of times in culture.² They noticed that cell cultures stopped dividing after an average of fifty cumulative population doublings (CPDs). This phenomenon is known as Hayflick's limit, phase III phenomenon, or, as it will be called herein, replicative senescence (RS). RS demonstrates how age changes may have a cellular origin and so understanding the mechanisms behind cellular ageing could help explain human ageing.

Hayflick and Moorhead worked with fibroblasts, a cell type found in connective tissue, but RS has been described in other cell types: keratinocytes, endothelial cells, lymphocytes, chondrocytes, etc. In addition, RS occurs in cells derived from either embryonic tissues or from adults of all ages. RS is also witnessed in cells taken from many animals, including mice, chickens, and Galapagos tortoise.³ In fact, early studies suggested a relation between the number of CPDs cells undergo in culture and the longevity of the species from which the cells were derived. For example, cells from the Galapagos tortoise, which can live over a century, divided about 110 times,⁴ while cells from the short-lived mouse divided roughly 15 times.^{5,6} In addition, cells taken from patients with progeroid syndromes—diseases resembling accelerated ageing such as Werner's syndrome (WS)—endure far less CPDs than normal cells.⁷ Exceptions exist, and certain cell lines never reach RS. As will be detailed ahead, these are said to be "immortal" and include embryonic stem cells and most cell lines derived from tumours, such as HeLa cells.³

The difference between "mortal" and "immortal" cells appears to lie in the telomeres: non-coding regions at the tips of chromosomes composed, in vertebrates, of repeated sequences of TTAGGG.⁸ Telomeres appear to form duplex loops, called t-loops, that stabilize or cap the telomeres. Initially, it was shown that telomere shortening occurs as cells divide in culture.⁹ A complex machinery maintains telomere length and structure, of which a pivotal

player is telomerase. Telomerase is a reverse-transcriptase enzyme that elongates the telomeres,¹⁰ thus counteracting the normal telomere erosion. It has two components: an RNA component¹¹ and a catalytic subunit.¹² Previously, an association between telomere shortening, telomerase, and the immortality of tumour cell lines was already apparent.¹³ Yet the definitive breakthrough came when it was shown that expression of the catalytic subunit of human telomerase (hTERT) in both retinal pigment epithelial cells and foreskin fibroblasts avoids RS.¹⁴ Human cells immortalized with hTERT divide vigorously, do not display biomarkers of senescence, and do not show signs of transformation.^{15,16} Even expression of hTERT in old cells appears to reverse the loss of function characteristic of senescent cells.¹⁷ It appears that ectopic hTERT expression is sufficient to restore telomerase activity in human cells¹⁸ and that telomere length, not hTERT expression, is the key in avoiding RS.¹⁹

All known immortal cell lines must stabilize their telomeres.²⁰ Tumour development, in particular, is dependent on telomere stabilization, normally by telomerase.²¹ In contrast, telomerase inhibition can induce senescence in cancer cells.²² Defects in telomere replication have also been shown to trigger senescence in unicellular organisms such as yeast²³ and the protozoan *Tetrahymena*.²⁴ Telomere shortening is now considered the main causal mechanism of RS and telomere length appears to be the molecular clock that counts the CPDs cells endure and triggers RS.²⁵

TELOMERASE AS AN ANTI-AGEING THERAPY

Most, not all, human somatic tissues have no detectable telomerase activity.²⁶ For example, in the bone marrow, hematopoietic cells express telomerase. Telomerase activity is higher in primitive progenitor cells and then down-regulated during proliferation and differentiation.²⁷ Other reports associate, normally low, levels of telomerase activity with human stem cells.²⁸ On the other hand, human embryonic cells and adult germ cells have been found to express hTERT.²⁹ Since normal somatic human

tissues have low or no telomerase activity, it is not surprising that telomere shortening has been reported *in vivo*.^{30–32}

hTERT expression immortalizes most, though probably not all,^{33,34} human cell types.¹⁴ Even so, the principle is that if telomerase can prevent RS, it may also prevent cellular ageing *in vivo* and serve as an anti-ageing therapy by increasing the capacity for renewal. One study found that the telomeric repair efficiency is lower in cells from an old than in cells from a young donor; and a slightly lower efficiency was also reported in WS cells.³⁵ It has been previously reported that cells from WS patients have a higher rate of telomere shortening.³⁶ In addition, a recent study found a correlation between telomere length and mortality in people over 60 years of age.³⁷ As such, telomere dysfunction may play a role in age-related debilitation.

The importance of the telomeres in RS, the low or non-existent levels of telomerase activity in human tissues, and the ability of telomerase to immortalize human cells led to the suggestion that telomerase will be used as an anti-ageing therapy.^{38,39}

RELATION BETWEEN REPLICATIVE SENESCENCE AND AGEING

It is known that cells from older donors have a slower proliferative capacity.^{3,40} This effect, known as the latent period, appears to occur because fewer cells are in the replication cycle, not because they take longer to divide.⁴¹ Therefore, changes occur with age at a cellular level. In some tissues, such as the immune system, decreased proliferative ability may play a role in age-related degeneration.⁴² Even if RS is not a faithful model of changes occurring *in vivo*,⁴³ if similar mechanisms operate to limit cell function then RS may yield insights into ageing. For instance, a small percentage of senescent cells may interfere with tissue homeostasis and function.⁴⁴

Although it is clear that human ageing has, at least in part, a cellular origin, the connection between ageing and RS is not obvious. At least *post partum*, there is no correlation between the number of CPDs cells can endure and the age of the donor.⁴⁵ Studies in centenarians failed to

find differences in the CPDs cells from centenarians could endure.⁴⁶ In addition, they raised doubts on whether telomere shortening occurs *in vivo* and whether senescence-associated genes *in vitro* are also differentially expressed *in vivo*.⁴⁷ In fact, gene expression patterns show differences between *in vitro* senescent cells and cells from old donors.⁴⁸ In addition, some types of rat cells have also been claimed as capable of evading RS.^{49,50}

The relation between a species' longevity and the CPDs its cells can endure *in vitro* may also be unrelated to ageing. Optimal culture conditions vary from species to species. For instance, O₂ partial pressure can affect cellular proliferation and recent results show that O₂ limits the replicative capacity of murine fibroblasts.⁵¹ These results show that comparisons between different species may be biased due to inter-species differences in O₂ sensitivity; instead of showing maximum cellular proliferate capacity, these results show O₂ sensitivity.⁵² In addition, since there is a positive correlation between body size and longevity,⁵³ perhaps cells taken from long-lived animals endure more CPDs because of the difference in size, not due to the difference in longevity.

TELOMERES AND ORGANISMAL AGEING

Telomerase expression has been found in lobsters and trout, two species in which ageing remains undetected.^{54,55} On the other hand, in the frog *Xenopus laevis*, an animal with a slow rate of ageing,⁵⁶ not only a great variation in telomere length exists,⁵⁷ but telomere length can diminish from parents to offspring, despite telomerase activity in germ cells, with no detectable consequences.⁵⁸ Chicken somatic tissues express telomerase,⁵⁹ but, overall, our knowledge of telomere biology is limited regarding other species.⁶⁰

No connection exists between mean telomere length and mammalian ageing. Of all studied primates, humans appear to have the shortest telomeres and the longest lifespan.⁶¹ Mice also have long telomeres and feature high telomerase activity in many organs, in contrast to humans.⁶² Interestingly, inbred mice have longer

telomeres than wild mice, suggesting telomere length does not affect organismal longevity in mice.⁶³ Therefore, telomere length and/or telomerase activity do not explain why humans age slower than other primates and mice.

Dyskeratosis congenita is an inherited disease involving skin and bone marrow failure.⁶⁴ It is caused by a mutation in the *DKC1* gene. Intriguingly, the protein encoded by *DKC1*, dyskerin, is a component of telomerase. Mutations in the RNA component of telomerase are associated with the autosomal dominant form of dyskeratosis congenita.⁶⁵ Families with this form of the disease are more severely affected in later generations, suggesting telomere shortening mechanisms are involved. Features of dyskeratosis congenita include bone marrow failure, which is the most usual cause of death, abnormal skin pigmentation, leukoplakia, and nail dystrophy.⁶⁶

As judged from the phenotype of dyskeratosis congenita and the telomerase knockout mouse (see below), telomeres are crucial in rapidly proliferating tissues but it is unclear whether telomere shortening is involved in human ageing. It is possible, however, that telomere shortening is involved in age-related deterioration. Despite having active telomerase, the telomeres of lymphocytes shorten with age.⁶⁷ A decline in telomerase activity was also found in blood mononuclear cells with age.⁶⁸ Though mean telomere length at birth does not correlate with longevity in birds, telomere shortening in erythrocytes inversely correlates with bird longevity. Telomere shortening in a variety of tissues also correlates, though to a lesser extent, with mammalian longevity.^{69,70} In fact, a correlation between erythrocyte longevity and organismal longevity was previously shown, suggesting a decrease in the number of required cell divisions in long-lived animals.⁶ It is, of course, impossible to tell whether increased telomere shortening is a cause rather than a sign of pathology and age-related debilitation.

Mice lacking telomerase were viable up to six generations. Telomeres gradually shortened leading to a number of pathologies, most notably affecting highly proliferative tissues, and cells from animals of generation four displayed aneuploidy and other chromosomal aberrations.

Knocking out telomerase in mice through deletion of its RNA component from the germline, while not preventing cancer,^{71,72} appears to increase cancer resistance^{73,74}; alternative telomere-lengthening mechanisms are likely operating to stabilize the telomeres in these cancer cells. On the other hand, telomerase overexpression in mice promoted cancer development but did not delay ageing or promote longevity.^{75,76} Of course mice and humans may feature different mechanisms of ageing, but these results show that, at least in mice, telomerase does not delay ageing.

TELOMERASE ALTERS THE NORMAL CELLULAR FUNCTIONS

Previously, experimental evidence raised questions on whether telomerase could help tumorigenesis.^{77,78} Namely, telomerase stabilizes the telomeres which promotes tumorigenesis.^{21,22,79} In addition, some reports suggest telomerase favours tumorigenesis by a telomere length-independent mechanism.⁸⁰ For example, a recent study found that hTERT expression in HDFs leads to an upregulation of epiregulin, a potent growth factor involved in tumorigenesis.⁸¹ Another recent study found that telomerase modulates the expression of growth-controlling genes to enhance cellular proliferation,⁸² and thus hTERT-immortalized cells may not be functionally equivalent to normal cells. In addition, recent results demonstrate that hTERT-immortalized cell cultures accumulate changes as they proliferate, suggesting caution in the use of such cell lines for tissue engineering.⁸³ Taken together, these results suggest that telomerase activity promotes tumorigenesis and so using hTERT for therapeutic purposes must be approached with great caution.

DISCUSSION

The connection between the telomere signalling pathways and cancer is obvious.⁸⁴ In fact, telomerase activation has been associated with skin malignancy as a result of exposure to UV.⁸⁵ Telomere shortening is most likely a tu-

mour suppressor mechanism. Telomerase-negative mice are normal up to four generations,⁷⁴ and telomerase overexpression does not alter ageing in mice.⁷⁶ On the other hand, telomerase dysfunction in humans causes dyskeratosis congenita.⁶⁵ It is clear telomere dysfunction is pivotal in RS⁸⁶ and telomerase important in cellular proliferation, but there is no evidence that the telomeres are a causal mechanism in mammalian ageing.

As with replicative potential, telomere length *in vivo* is very heterogeneous.⁸⁷ Telomere shortening *in vivo* has been reported in skin cells,³¹ blood,⁶⁸ and colon mucosa.³⁰ Other studies found weak correlations between donor age and telomere length,³² while some studies found no correlation.^{47,87,88} Moreover, long telomeres have been found in cells from centenarians.⁸⁹ Taken as a whole, these results indicate that telomere length varies widely amongst individuals and between different tissues. Although telomere shortening appears to occur in some tissues *in vivo*, there is little evidence linking telomere shortening to ageing. One hypothesis is that increased telomere shortening *in vivo* is associated with age-related pathologies because telomere shortening is a biomarker of DNA damage.⁹⁰ If so, then telomere shortening witnessed *in vivo* would be an effect rather than a cause of pathology.

As mentioned before, the relation between RS and organismal ageing is unproven. Cellular immortality just means a cell population can divide indefinitely but it does not mean that the functional capacity and differentiation of cells is preserved. In fact, many non-dividing cells are essential to the organism. Thus, whether telomere shortening plays a role in human ageing is debatable. Not only is it unproven that telomerase can be used as an anti-ageing therapy but some evidence suggests that hTERT transient expression can occur in human cell lines when necessary for regeneration,⁹¹ and there is little evidence to suggest that further hTERT expression is necessary in human tissues.^{92,93} Importantly, telomerase may alter the normal cellular functions and promote cancer. One possibility is using a transient telomerase activation in certain diseases—dyskeratosis congenita being the most obvious example—or cell lines with telomerase ex-

pression stringently controlled.⁹⁴ In regenerative medicine, telomerase expression may be necessary⁹⁵ and may be useful to treat a number of pathologies. For instance, cardiac muscle regeneration may be fostered by telomerase expression.⁹⁶

In conclusion, telomerase is a dubious candidate for Fountain of Youth: though it may be used in regenerative medicine or to treat specific diseases (e.g. dyskeratosis congenita or even WS), we think telomerase is unlikely to become a source of anti-ageing therapies.

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