

Review Article

Up-Regulating Telomerase and Tumor Suppressors: Focusing on Anti-Aging Interventions at the Population Level

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ABSTRACT: Most human populations are undergoing a demographic transition regarding their age structure. This transition is reflected in chronic non-communicable diseases featuring among the main contributors to burden of disease. Considering that the aging process is a major risk factor for such conditions, understanding the mechanisms underlying aging and age-related diseases is critical to develop strategies to impact human health at population and/or individual-levels. Two different aspects of aging process (namely, telomere shortening and DNA damage accumulation) were shown to interact in positively impacting mice median survival. However, strategies aimed at translating such knowledge into actual human health benefits have not yet been discussed. In this manuscript, we present potential exposures that are suited for population-level interventions, and contextualize the roles of population (based on behavioral exposures) and individual-level (based on small-molecule administration) anti-aging interventions in different levels of disease prevention. We suggest that exposures such as moderate wine consumption, reducing calorie intake and active lifestyle are potentially useful for primordial and primary prevention, while small-molecules that activate telomerase and/or tumor suppression responses are more suited for secondary and tertiary prevention (although important for primary prevention in specific population subgroups). We also indicate the need of studying the impacts, on aging and age-related diseases, of different combinations of these exposures in well-conducted randomized controlled trials, and propose Mendelian randomization as a valuable alternative to gather information in human populations regarding the effects of potential anti-aging interventions.

Key words: Aging, Telomerase, Tumor suppression, Population-level interventions, Levels of disease prevention

Age-related impairments and diseases are currently among the most prevalent causes of morbidity and mortality worldwide. Conditions such as cardiovascular disease, cancer and diabetes account for a significant portion of the burden of disease in the majority of countries, since the age structure of human populations has changed and continues to change towards an increase in the proportion of the old-aged group (as a consequence of increases in life expectancy and decreases in fertility rates) [1]. Although not being the only risk factor for such conditions, the aging process itself is a fundamental and the most predictable cause of impairments that tend to

occur in the elderly. Therefore, interventions that target the underlying mechanisms of the physiological aging process are promising for treatment and (at least partial) prevention of age-related diseases.

There are several mechanisms evidenced to cause or favor aging, which have recently being integrated as contributing causes of the multifactorial process called aging. In this regard, the proposed aging hallmarks are mechanisms that primarily act at the molecular or cellular levels, resulting in aging at the organism level by cumulative effects of each individual factor as well as by a rather complex interplay between two or more of these

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[2]. In spite of recognizing the complexity and multifactorial nature of the aging process as it is currently understood, this manuscript will focus on telomere biology, and, more specifically, the interplay between telomere length and tumor suppression responses regarding adult stem cells (ASCs) function. This is justified by the fact that telomere biology is among the best established and mostly studied aging mechanisms, which importance can be observed in the fact that the 2009 Nobel Prize in Medicine and Physiology was awarded to the discoverers of telomerase [3]. In addition, two more aging hallmarks, as well as their interplay, are considered: DNA damage accumulation (here discussed with regards to tumor suppression responses) and ASC exhaustion; thus, at least a portion of the complex interaction between different causal factors of aging is addressed.

Telomeres, tumor suppression and ASCs in aging and cancer: an overview

Telomeres are guanine-rich (5' TTAGGG 3') DNA repeats *in tandem* located at the ends of eukaryotic chromosomes in association with a protein complex called shelterin [4]. Telomere integrity is essential to prevent chromosome ends from being recognized as double-strand DNA breaks and from being fused together [5]. Due to the incapacity of the cellular machinery to replicate the very ends of linear chromosomes (i.e., the end replication problem) [6] and to other phenomena such as oxidative stress [7], telomeres are shortened after each cell division. In some cell types – including embryonic stem cells, germline stem cells and ASCs – the activity of a ribonucleoprotein complex called telomerase counteracts telomere shortening by elongating these structures through *de novo* reverse transcription [8, 9]. Telomerase is mainly composed of two subunits: the telomerase reverse transcriptase (encoded by *TERT* – EntrezGene ID: 7015), of which expression is the main rate-limiting factor for telomerase activity (since the other component is found at varying levels in several tissues). This subunit catalyzes the reaction based on an RNA template called telomerase RNA component (encoded by *TERC* – EntrezGene ID: 7012).

Telomere dysfunction (caused by progressive telomere shortening or telomere uncapping) triggers tumor suppression responses (apoptosis and/or senescence), thus limiting cell viability. In ASCs, telomerase levels are sufficient only to delay telomere shortening [10], resulting in ASCs eventually reaching a critical telomere length state with time. Considering the fundamental roles of ASCs in maintenance of organism homeostasis by promoting tissue self-renewal, telomere shortening plays a major role in organism aging and age-

related diseases by limiting ASCs viability. Indeed, an elegant study using transgenic mice showed that removing senescent [i.e., p16(Ink4a)-positive] cells can extend health span not only by preventing or delaying tissue dysfunction, but also by alleviating already established age-related impairments [11].

Many age-related conditions were evidenced to have telomere dysfunction as an important causal factor. Indeed, a collection of telomere-related disorders recently termed “telomere syndromes” was proposed as important for understanding age-related diseases [12]. In this regard, it is important to note that telomere biology also plays critical roles in cancer. The genomic instability caused by telomere dysfunction highly predisposes the accumulation of mutations and, consequently, acquiring tumorigenic characteristics [13]. Moreover, telomerase activity is the main immortalization mechanism of tumors (being present in approximately 85%-90% of human cancers) [14, 15], thus being a highly prevalent cancer biomarker. In fact, both telomere dysfunction and telomerase activity are regarded as two cancer hallmarks [16]. Therefore, the exposed illustrates the interplay between telomere biology and tumor suppression in age-related impairments (especially regarding tissue self-renewal failure) and cancer, with the capacity of the tumor suppression machinery to sense critically shortened telomeres playing a crucial role in the “balance” between these two age-related conditions with regards to telomere dysfunction. Such interplay was discussed in more detail elsewhere in the context of dyskeratosis congenita (a telomere syndrome) [17].

Telomere length and tumor suppression: potential anti-aging effects

One of the earliest notions that chromosome ends were somehow related to the capacity of cells to divide *in vitro* was formalized in a theory of *in vitro* aging published more than 30 years ago [18]. Among the earliest solid experimental evidences for a role of telomeres in aging, *in vitro* studies of human fibroblasts can be cited, which showed that telomere shortening occurs during serial passages [19] and that initial telomere length predicts capacity to replicate *in vitro* [20]. It is important to note that, by that time, the roles of tumor suppression in inducing telomere shortening-dependent senescence (which was shown to depend on DNA damage checkpoint proteins, such as p53 and RB – encoded by *TP53* and *RB1*; EntrezGene ID: 7157 and 5925, respectively) were already being recognized [21]. Subsequently, it was shown that introducing *TERT* expression reconstitutes telomerase activity and extends the lifespan (concomitantly with longer telomeres, vigorous cell

division and reduced senescence) of normal human cells *in vitro* [22-24].

Although several studies of different natures could be cited, two landmark studies in mice provided invaluable evidence relevant to this discussion. One of them used an elegant genetic engineering strategy that allowed inducible reactivation of *TERT* in late-generation telomerase-null mice (i.e., telomere-deficient animals) to show that telomerase can reverse age-related tissue degeneration in several organs (e.g., testes, spleen and intestines), notably including the neural tissue (which is considered a low-turnover tissue) [25]. The other study combined constitutive *TERT* expression in epithelial tissues (i.e., under the regulation of the *KRT5* – EntrezGene ID: 3852 – promoter) and increased expression (under their normal regulation) of tumor suppression genes [namely, *CDKN2A* (EntrezGene ID: 1029) – encoding the proteins p16 and Arf – and *TP53*], resulting in long-lived cancer-resistant mice called SUPER-M. These mice were used to assess the longevity effects of telomerase in a cancer-resistant background (which is important given the cancer risk associated with constitutive telomerase activity). In both overall and cancer-free mice, a clear effect of telomerase in extending median survival was observed [26]. Furthermore, it is

important to consider that the increased expression of tumor suppression genes in mice had been already shown to be protective against aging [27], which is in accordance to the notion that tumor suppressors restrain ASCs proliferation (thus delaying their exhaustion) and have been evidenced to reduce or prevent the accumulation of ASCs with DNA damage [28].

In addition to the fact that the SUPER-M mice study represented a major contribution for the understanding of telomerase and tumor suppression in aging and cancer, a more detailed observation of the median survival results (as presented in a subsequent publication [29]) reveals that telomerase and tumor suppression are effect modifiers of each other in both overall and cancer-free animals (Table 1). Indeed, such effect modification could already be detected by comparing median survival differences of Tg*TERT* - Sp53 with SUPER-M - cancer-resistant mice, with the last showing a much greater difference (and, then, a stronger effect of telomerase) than the first. However, based on table 1, it can also be noted that the same occurs for tumor suppression (i.e., it has a much greater effect on median survival when occurs concomitantly with constitutive telomerase activity in the epithelia).

Table 1. Median survival differences for overall and cancer-free mice of the SUPER-M mice study.

Median survival comparison	Median survival increase	
	Overall	Cancer-free
Tg <i>TERT</i> - Sp53	9.0%	18.0%
Cancer-resistant - Sp53*	11.3%	8.7%
SUPER-M - Sp53	40.2%	50.0%
SUPER-M - Tg <i>TERT</i> *	28.6%	27.1%
SUPER-M - Cancer-resistant	26.0%	38.0%

Tg*TERT*: Mice constitutively expressing *TERT* under the *KRT5* promoter. Sp53: Control group, characterized by increased *TP53* expression only. *Median survival differences (for both overall and cancer-free mice) calculated based on the available results for the other groups.

Importantly, the values in table 1 also indicate [although the median survival in the SUPER-M mice (comparing with Sp53 mice) was greater for cancer-free animals] that adding Tg*TERT* in a cancer-resistant background has similar if not slightly higher effects on overall median survival. Such observation allows the

speculation that, if combined with cancer resistance, increases in telomerase activity (at the very least) do not increase cancer risk, and might even have modest anti-cancer effects. On the other hand, the observed differences in median survival of overall and cancer-free animals when comparing SUPER-M and Sp53 mice could

simply indicate that such an increase in lifetime implicates in cancer being an important cause of death even in a background of telomerase and tumor suppression up-regulation. Such rationale is in accordance to the multifactorial nature of cancer (with many hallmarks other than telomere dysfunction and DNA damage/mutation accumulation) [16] and to the notion that tumorigenic telomerase activation is commonly regarded as a relatively late event in carcinogenesis, possibly occurring a consequence of the genomic instability caused by, for example, telomere dysfunction [13].

Population-level interventions based on telomerase and/or tumor suppression up-regulation

The literature exposed so far (although insufficient as a comprehensive review) provides solid evidence for the potential of up-regulating telomerase and tumor suppressors to reduce the risk and/or ameliorate the gravity of age-related diseases, especially with regards to tissue/organ degeneration (which has loss of tissue self-renewal capacity due to ASC exhaustion as a key causal factor). In humans, implementing such interventions is a non-trivial challenge, involving several individual and population (including biological/medical, socioeconomic and political) factors. In spite of this complexity, it is evident that certain interventions that would be highly useful in the clinical setting (e.g., for treatment purposes) would not even be feasible for reducing the risk of age-related diseases in an entire group of people at the population level (and vice-versa). It is hardly arguable that, at the population level, interventions aiming at preventing or reducing disease risk are preferable over therapeutic approaches. Moreover, preventive strategies are normally cheaper and easier to implement by health planning and policies. The most common and illustrative examples are interventions related to dietary or exercising habits. Since these are, in essence, behaviors, they can be influenced at the population level by mass-targeted strategies, such as educational campaigns, economic policies (e.g., making healthy food products more accessible) and infra-structure initiatives (e.g., bike paths).

Resveratrol

Regarding telomerase activity, there are examples of both dietary and exercise-related interventions with great potential to be applied at the population level. Perhaps one of the most popular examples is resveratrol, although definitive results are yet to be obtained. There is, however, evidence for a benefic role of this phenol in human health by delaying aging. Taking atherosclerosis as an example, *TERT* expression was shown to be activated by resveratrol

treatment in a dose-dependent manner in endothelial progenitor cells *in vitro*, which was accompanied by an inhibition of *in vitro* endothelial progenitor cells senescence onset [30]. Furthermore, there is evidence that resveratrol treatment can immortalize p53-deficient cells, thus reinforcing the importance of effective tumor suppression responses in counteracting potentially tumorigenic consequences of telomerase up-regulation [31]. The relevance of these findings is highlighted by the evidence for causal roles of telomere shortening in atherosclerosis [32] and that *TERT* up-regulation has been discussed as a potential strategy to promote angiogenesis for tissue engineering applications [33].

The translation of the potential benefits of resveratrol into actual health gains for the population would depend on, for example, incorporating such substance in diet. In this regard, red wine and oral administration of resveratrol in normal rats have been shown to preserve vascular function by delaying its aging, although no differences in lifespan were observed [34]. Importantly, a reduction in p53 levels was also observed, which raises a concern regarding cancer risk associated with resveratrol. In this regard, a study using cancer cell lines reported that resveratrol treatment activates the p53 pathway [35]. Such controversy can be speculated to be attributable to the markedly different physiological states of the biological systems where the experiments were performed. It is plausible to hypothesize that, while in cancer cell lines resveratrol activates the p53 pathway in response to a cellular environment with high levels of stress and genomic instability, in normal rat cells there would be no such “triggering signaling” for the p53 pathway. Therefore, p53 levels in normal rat cells could be diminished as a rather indirect consequence of the healthier cellular environment state induced by resveratrol administration (such hypothesis, although plausible, requires experimental confirmation). Nevertheless, resveratrol is a potential candidate for delaying the onset of age-related diseases at the population level (possibly through wine consumption) in the context of telomerase and tumor suppression up-regulation. In fact, it has been shown that resveratrol increases lifespan and promotes physiological benefits for middle-aged mice on a high-calorie diet [36], thus possibly being a benefic intervention even for individuals with bad dietary habits and/or obese.

Caloric/dietary restriction

Calorie intake is a crucial aspect of diet, with important implications for different human health states, including increasing the risk for several age-related diseases. Interestingly, caloric restriction (sometimes referred to as dietary restriction, which consists of reducing total calorie intake while maintaining adequate nutrition) has been

evidenced to increase lifespan and reduce the risk of age-related diseases (including cancer) [37]. Differently than resveratrol, the benefits of caloric restriction for healthier aging have been exhaustively investigated and demonstrated in different species and contexts, including normal and disease states (as reviewed elsewhere [38]). However, it is important to note that this intervention is not universally acknowledged as effective in humans [39]. Indeed, the effects of caloric restriction have been evidenced to depend on aspects such as genetic background [40] and the phase of life when it occurs [41-44].

Considering the evidence linking caloric restriction with aging, it is somehow intuitive to investigate the roles of telomere biology in such relationship. However, evidence in this context is relative scarce. A negative association between telomere activity (and cell proliferation) and caloric restriction has been reported in a study on hepatic cells of mice pre-treated with aflatoxin B₁ [45], which is known to induce DNA damage and considered an important risk factor for hepatocellular carcinoma [46]. In a more recent study on normal and immortalized fetal lung fibroblasts, glucose restriction has been shown to inhibit growth, induce apoptosis and decrease *TERT* expression in the last, but to extend lifespan and up-regulate *TERT* in the former, and that such differential impacts on *TERT* expression were partially mediated by epigenetic mechanisms [47]. This study, then, provided evidence that glucose (and possibly caloric) restriction may have both anti-cancer (when occurring in cancer or precancerous cells) and anti-aging effects (in normal cells), with different consequences regarding telomerase regulation depending on the cellular and pathological context. In the same year, another study (in mice) has found that adult-onset, short-term caloric restriction reduces the number of senescent cells in the small intestinal epithelium and liver in association with improved telomere maintenance in the absence of telomerase up-regulation [48]. Evidence for a role of caloric restriction in up-regulating telomerase activity by increasing the levels of *TERT* expression have been recently reported in mice, in addition to a synergic role between telomerase and caloric restriction in mice health span and longevity [49].

The studies discussed above are in accordance with the theory that caloric restriction protects against DNA damage by restraining the metabolism [50, 51], thus extending lifespan by reducing DNA damage accumulation (which is a hallmark of aging [2]). Considering that DNA damage accumulation is also a cancer hallmark [16], caloric restriction is suggested to partially mimic a cancer-protective environment and, therefore, would be expected to interact with telomere maintenance in regulating aging and age-related

impairments given the findings of the SUPER-M mice study [26] and another pieces of evidence (as reviewed elsewhere [17]). Such expectation has been, indeed, observed experimentally by subjecting transgenic mice over-expressing *TERT* to caloric restriction [49]. Furthermore, both rapamycin and metformin (two substances that have been suggested to act as caloric restriction mimetics [38]) have been evidenced to up-regulate tumor suppression responses (as reviewed elsewhere [52]). Thus, the notion that caloric restriction has both anti-cancer and anti-aging properties may be explained by the up-regulation of tumor suppression responses by this intervention (in addition to the recent evidence for a role in increasing *TERT* expression), thus either inducing senescence and/or apoptosis in cancer cells or extending health span in normal cells.

Physical activity

The awareness of the benefits of physical activity for human health is widespread not only in the media and general culture, but also in the scientific literature. The public health potential of promoting physical activity is hardly arguable, since physical inactivity has been shown to be an important risk factor for several chronic diseases and to reduce life expectancy worldwide [53]. Importantly, physical inactivity prevalence tends to increase with age, probably as a consequence of conditions such as sarcopenia (which is, in turn, ameliorated by physical activity) [54-56].

The well-established benefits of physical activity for lifespan and health (especially regarding chronic diseases) raise the possibility of a role for telomere biology. In a study of protective cardiac effects of physical activity, it was shown that both short and long-term voluntary exercise increase cardiac *TERT* expression and telomerase activity in mice, which was evidenced to be a mediator of such benefits [57]. More recently, long-term effects of exercise (over a 1-year period) on mice (8 weeks-old at baseline) on telomere dynamics have been evaluated. Although exercise was observed to reduce telomere shortening in liver and cardiac tissues, opposite effects were found in skeletal muscle. Notably, telomerase activity was up-regulated by exercise in skeletal muscle, but did not change in neither liver nor cardiac tissues [58]. Importantly, a recent randomized controlled trial of qigong exercise (4-month intervention in 64 patients with chronic fatigue or chronic fatigue syndrome) showed increased telomerase activity in peripheral blood cells [59], thus providing important evidence for a causal role of physical activity in telomere biology. Regarding tumor suppression responses, although there is some evidence for an up-regulation effect associated with physical activity, the literature is scarce. Acute exercise was shown to activate AMPK (encoded by *PRKAA1* and *PRKAB1*;

EntrezGene ID: 5562 and 5564, respectively) and p38 MAPK (encoded by *MAPK14*; EntrezGene ID: 1432) phosphorylation in skeletal muscle biopsies from young males (n=6) [60], suggesting that p53 activation could also be occurring [61, 62]. Indeed, a more recent study in 10 active men compared two different physical activity

protocols and obtained similar results, with increased phosphorylation of AMPK, p39 MAPK and p53 in skeletal muscle biopsies [63].

Levels of prevention of age-related diseases			
Primordial	Primary	Secondary	Tertiary
Population-level interventions: economic and social policies to stimulate low-calorie diets, physical activity and moderate wine consumption (only if proved to promote telomerase activity and especially if such promotion does not depend on alcohol) to promote healthy aging.	Population-level interventions: active efforts to reduce calorie intake and stimulate active lifestyle and moderate wine consumption in a given population, considering its particularities (e.g., stimulating wine consumption would not be advisable in a population where alcohol abuse and/or dependence is prevalent), in order to reduce age-related diseases incidence. Individual-level interventions: acute anti-aging effect (e.g., in ex-heavy smokers); reduce age-related diseases incidence in physically-disabled individuals, elderly population and individuals with predisposition to premature aging.	Population-level interventions: used in combination with therapeutic strategies to improve the treatment and reduce disability resulting from an age-related disease. Individual-level intervention: treat early-stage age-related diseases to reduce disease prevalence, disability resulting from the disease and individual and/or public costs with treatment of disease in more advanced stages.	Individual-level intervention: promote rapid and strong anti-aging effects on individuals with either cancer (focusing, at first, on enhancing tumor suppression responses) or stem cell exhaustion-related diseases (combining telomerase and tumor suppression responses up-regulation) by the administration of small-molecules.

Figure 1. Summary of anti-aging interventions in different levels of disease prevention. For each level of age-related diseases prevention (gray boxes), ways of exploring telomerase and/or tumor suppression responses up-regulation at population and/or individual level are described. While in primordial and primary levels there is more applicability for population-level interventions, individual-level interventions receive a greater importance in secondary and tertiary levels.

Final remarks and conclusions

In this manuscript, three commonly studied interventions with potential to be translated into population-level anti-aging strategies were pointed and briefly discussed. It is important to note that proper evaluation of the existing evidence regarding anti-aging interventions that impact telomere lengthening and/or tumor suppression would require an extensive systematic review. The aim of the present manuscript, however, was to present the concept

of transposing the knowledge obtained from the SUPER-M mice study into actual public health benefits based on scientific literature. It is also worth noting that the manuscript focused on interventions suited for population-level strategies, although interventions more suited for individual-level application are also important, especially in the clinical setting. Examples of such interventions would be telomerase activators (e.g., TA-65® [64-66], food supplement 20070721-GX [67], GRN510 [68], astragaloside IV and cycloastragenol [69,

70]) and MDM2 (a p53 inhibitor, encoded by *MDM2*; EntrezGene ID: 4193) inhibitors [71-74] to improve telomere maintenance and up-regulate tumor suppression responses, respectively.

Indeed, individual-level interventions aimed at up-regulating telomerase and tumor suppression responses would have several applications. The most obvious would be to treat age-related diseases, such as cancer or impairments caused by ASC exhaustion. This application can be easily extended to prevention of further development of diseases diagnosed in early stage, thus evidencing the potential of such interventions to both secondary and tertiary levels of disease prevention [75]. Additionally, individual-level interventions would be applicable at the primary level of disease prevention in the context of the “high-risk-individual strategy” in two distinct manners. One would be to promote an acute anti-aging effect (to be followed by population-level strategies) to counteract either the side effects of therapeutic approaches that the individual had been submitted to (e.g., chemotherapy, which has been evidenced to induce both telomere shortening and DNA damage [76, 77]) or the negative effects of behaviors such as heavy smoking (which is considered to accelerate telomere shortening [78, 79]). The other application would be to reduce disease risk in susceptible individuals (e.g., carriers of highly penetrant disease-associated genetic profiles).

Individual-level interventions would also be useful in population sub-groups where the population-level interventions available would be not feasible and/or harmful/controversial. For example, stimulating physical activity is less effective (or even not effective) for individuals with premature physical disability or congenital defects that imposes major difficulties for doing regular physical activity (especially in individuals of low socioeconomic position). Another example is caloric restriction in the elderly due to the risk of favoring muscle mass loss [38]. A summary of the applications of population-level and individual-level interventions aimed at up-regulating telomerase and tumor suppression responses is provided in figure 1.

In spite of the important applications of individual-level anti-aging interventions, public health strategies benefit more from population-level interventions. In this manuscript, we suggest that resveratrol (mainly through wine consumption), caloric restriction and physical activity are exposures with a potential anti-aging effect regarding telomere maintenance and tumor suppression responses. In order to obtain further evidence in human populations, an interesting strategy would be to employ Mendelian randomization using genetic markers in genes involved in pathways related to those interventions (e.g., *MTOR* SNPs as caloric restriction proxies and *ALDH2*

SNPs – in populations where wine is the most consumed alcoholic beverage – as wine consumption proxies) as instrumental variables as an attempt to gather robust causal evidence (assuming the requirements of Mendelian randomization are met) [80]. Nevertheless, more definitive assessments depend on well-conducted randomized controlled trials. In this regard, it would be particularly important to have the groups randomized to receive different combinations of each strategy in order to understand how they act together in impacting aging and age-related diseases. Finally, given the multifactorial nature of aging, interventions that target factors other than telomere maintenance and tumor suppression responses are important to be considered and studied in order to elaborate the most appropriate population and individual-level anti-aging interventions.

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Disclosure statement

The authors declare no competing interests.

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