

SPERMIDINE MAINTAINS TELOMERE LENGTH AND DELAYS AGING

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Abstract

Spermidine, a natural polyamine, has been noticed for its anti-aging properties. Supplementation of this drug prolongs lifespan and diminishes the incidence of age-related pathology. In the human population, spermidine levels decrease as aging progresses, and a potential link between diminished endogenous spermidine levels and age-related decline has been studied. At the cellular level, autophagy is the prime mode of action of spermidine known to decline with the progress of aging, similarly contributing to the accretion of impaired macromolecules and organelles through aging. Epidemiological statistics support the concept, suggesting that elevated uptake of polyamine delays aging. Here, we overview the effect of autophagy on cellular processes and age-associated diseases, emphasizing the importance of these events to the hallmarks of aging.

There are numerous factors like shortening telomere, oxidative stress, mitochondrial damage, and impaired intracellular calcium signaling, which are influenced by the aging process. We hypothesize that spermidine supplements in the diet increase the telomere length. The proposed hypothesis also brings to light the differentially regulated genes involved in telomere maintenance and aging after spermidine treatment. Knowing the role of spermidine in telomere maintenance would help us understand the molecular mechanism of spermidine's effect on aging.

Keywords: Spermidine, Aging, Diet, Hypothesis

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INTRODUCTION

Aging is the natural process in all living organisms defined as weakening the cellular function due to the damage augmentation over time [1, 2]. Three principles govern aging at the organism level: the repair of oxidative damage, balanced distribution of resources, and stochastic (aging results from random damage accumulation) events. On the molecular level, some mechanisms show modulation of cellular or organismal longevity. These factors interact with each other in a complex manner. Several possible factors responsible

for aging and their interactions suggest possible medication against aging. Polyamines, particularly spermidine, have also arisen as potential candidates, and this report will lend support to the participation of spermidine in aging.

Spermidine, spermine, putrescine and cadaverine are common polyamines. Being polycations, they interact readily with negatively-charged molecules, comprising DNA, RNA, and lipids. This property to interact with various molecules is the basis for polyamines' role in

different cellular processes like DNA stability, cell growth, division, and death [3-5]. Studies have suggested that polyamine levels decline with age [6]. However, only recently, the effect of polyamines, specifically spermidine, on aging has been studied. Recent studies show the levels of spermidine in human elderly in the range 60 to 80 years to be less than in humans below 50 years, but humans above than 90 show levels comparable to the population below 50. This study indicates that the maintenance of spermidine levels retards aging [7]. Intriguingly, spermidine's exogenous supplementation has multiple impacts on aging and age-related pathologies in various model systems along with mice. Diet with spermidine extends lifespan across species, promotes cardio, and neuroprotection [8-10], leads to stimulation of antineoplastic immune response [11], and can avoid immune-senescence through the stimulation of memory T-cell formation [12]. Many of these antiaging properties have been associated with spermidine's potency to exhibit proteostasis on the activation of cytoprotective macroautophagy [13, 14]. A recent report showed that endogenous spermidine's genetic modulation affects growth and development in *Dictyostelium discoideum* [15]. Spermidine levels can be augmented in model organisms and humans when supplemented in food or water [8]; therefore, the impact of polyamines on aging is comparatively feasible to study.

The influence of spermidine and spermine on the delay of brain aging is exhibited principally by the mechanism of autophagy in senescence-accelerated mouse-8 (SAMP8 mice) [16]. Autophagy is the core cellular mechanism, where destruction and re-use of unwanted or damaged molecules or entire organelles occur. Impaired autophagy has been related to a plethora of age-related diseases. This observation is shown in yeast, worms, flies, and mouse hepatocytes. Reports in mice suggest that the deletion or depletion of crucial autophagy genes in myocardial or cancer cells reduces spermidine's beneficial effects on cardiovascular disease and cancer, respectively [11, 17]. Impaired autophagy in yeast cells, worms, and flies failed to prolong lifespan when fed with spermidine, illustrating the vital role of autophagy on spermidine's extension of lifespan [8, 18, 19]. Therefore, the approach is to use the antiaging effect of spermidine on various hallmarks of aging.

Regardless of the conventionally believed theory that aging is a multifaceted progression, different ideas have arisen to describe it as only predominant age-related change. According to the widely accepted "stochastic theory," aging is the consequence of accretion of random

damage and loss of a biological system's repairing ability. On the contrary, other theories present aging as a highly regulated process governed by the genetic code, telomere length, the number of divisions that a somatic cell can undergo (the "Hayflick limit"), and Spatio-temporal modulation of gene expression [20, 21]. Moreover, we know that telomere length is one of the potent biomarkers for the detection of aging. Here, the focus lies on spermidine's effect (an antiaging drug) on the telomere length.

In eukaryotes, telomeres contain DNA repeats capping the ends of chromosomes, shortening on each mitotic cycle in all cells devoid of active telomerase because of the end replication problem. This shortening is common in somatic cells, whereas in germline and stem cells, telomerase remains active, ensuring telomere length maintenance. Significantly, the reactivation of telomerase correlates with cancer. The repetitive telomeric cap reduction in normal cells eventually halted at a DNA damage checkpoint, prohibiting further cell division. In each cell cycle, the telomere length gets shortened, thus making the chromosomal end increasingly vulnerable to other DNA active processes. This event is designated as telomere attrition, one of the important hallmarks of aging [22]. Reports suggest that stem cell function and self-renewal are restricted as aging is prolonged by tumor-suppressor mechanisms [23, 24]. Reports in telomerase-deficient mice show that telomere shortening is responsible for age-related diseases and longevity. Premature aging phenotypes are observed in low proliferating heart and brain and high-proliferating compartments: bone marrow, gut, skin, and testis [25-28]. Telomere shortening with age has been investigated in several mouse stem cells in various tissues of high or low tissue proliferation rates [29, 30].

Compared with age-matched controls, the telomere length acts as a diagnostic biomarker for physiological aging and assessing severity and disease risk. With a gene therapy approach, adult and old mice treated with a virus to express telomerase reverse transcriptase (TERT) show lifespan extension up to 24% without the prospect of the risk of cancer [31]. A study in aged individuals shows mitochondria involvement in modulating biological aging when an increase in DNAm-PhenoAge as a biomarker and leukocyte telomere length was observed [32].

Remarkably, studies in mice show the synergistic effect of TERT treatment with dietary restriction in increasing longevity, highlighting the array of pathways that initiate natural aging. Taji et al. have reported that the overexpression of Beclin1 in HeLa cells led to a decrease

in telomerase activity on autophagy induction [33]. This is the basis of the hypothesis that autophagy is the key player in tumor suppression by modulating the telomerase activity in somatic cells. The molecular and cellular mechanisms through which spermidine delays aging have been elucidated to some extent but not thoroughly studied. After considering all the studies reporting the potential effect of spermidine on aging, we propose that spermidine treatment increases human cells' telomere length. We have also proposed a method to determine how spermidine affects the expression of genes involved in telomere maintenance and autophagy.

HYPOTHESIS

It is evident from the above discussion that autophagy has been known to date as the principal mode of spermidine action on aging. Autophagy causes a selective clearance of organelles that regulate cellular homeostasis in the model system from yeast to humans. Damaged organelles are removed, which relieves the cell of potentially toxic byproducts and allows organelle constituents' salvage for bioenergetics. Moreover, it is reported that spermidine levels decline in aging. Telomere length is one of the fundamental hallmarks of aging and cancer. Telomere length gets shortened with each cycle of replication, which eventually results in aging. Here we suggest that spermidine treatment enhances the telomere length and improves the healthspan of human cells. Telomerase is a specialized reverse transcriptase that maintains the telomere length by actively dividing cells, viz. stem cells and cancer cells. We also propose to analyze the telomerase expression and its activity following spermidine treatment. The proposed hypothesis would also discover the differentially regulated genes involved in telomere maintenance and aging after spermidine treatment. Future studies should consider the relations of dietary spermidine with other types of cancer, particularly in the necessity of polyamines for cancer growth. The spermidine-rich diet could increase tumor growth in human patients and open new questions, requiring comprehensive investigation before such diets can be recommended.

VALIDATION OF THE HYPOTHESIS

There are several steps to take for the validation of the hypothesis.

Optimization of concentration of spermidine

Different spermidine concentrations would be administered for regular time intervals to deduce the time and dose-dependent effect of spermidine.

Identification of different cell lines: To see the effect of spermidine on telomere length, we need different cell lines

Two primary non-cancerous cell lines are required where there is no maintenance of telomere length either through telomerase or alternative telomere lengthening (ALT) pathway. HEK293 and MCF-10A are two excellent human cell lines derived from normal kidney and breast epithelial cells.

Two cancerous cell lines, such as Hela and MCF-7, which are cervical and breast cancer cell lines, respectively, have an endogenous expression of telomerase. They would be used to see the effect of spermidine treatment on telomerase activity and its expression. Hela and MCF-7 will also be used to see the telomere length change following hTERT (the catalytic and rate-limiting component of telomerase) knockdown and spermidine treatment.

Two ALT cell lines are required to maintain telomere length by homologous recombination-based ALT mechanism and not by telomerase. U2OS and VA13 are osteosarcoma and lung fibroblast cell lines, respectively, in which the ALT mechanism maintains telomere length.

HYPOTHESIS TESTING

Quantitative real-time PCR is performed to measure telomere length after spermidine treatment:

1. To see the effect of spermidine on the telomere length of two primary non-cancerous cell lines, HEK293 and MCF-10A cells will be treated with different concentration of spermidine, and after 24, 48, and 96 hrs treatment Q-RT PCR will be performed by using the already published protocol [31]. This experiment will tell the difference in telomere length of these two different cell lines before and after spermidine treatment at a different time interval, which eventually will indicate the spermidine effect on normal non-cancerous cells aging (Fig 1).
2. Another Q-RT PCR will be done after hTERT knockdown in Hela and MCF-7 cells. First, we will check the telomere length after hTERT knockdown and then after spermidine treatment at a different time interval. This experiment will tell spermidine's effect on the telomere length of hTERT knockdown cancer cells (Fig 1).
3. Next, Q-RT PCR will be done in U2OS and VA13 cells after inhibiting the ALT pathway by knocking-down the TERRA (telomeric repeat-

containing RNA) molecule, which is a hallmark of the ALT pathway, and followed by spermidine treatment. This experiment will again tell the effect of spermidine in ALT cells in which telomere length is maintained by a homologous recombination-based pathway and not by telomerase (Fig 1).

4. After spermidine treatment in two primary non-cancerous cell lines, HEK293 and MCF-10A-To know the differentially expressed genes involved in different signaling pathways of aging and autophagy, global expression profile after spermidine treatment RNA-seq can be done after spermidine treatment in HEK293 and MCF-10A cells (Fig 2). This experiment will tell about the differentially expressed genes similarly affected in both the cell lines by spermidine treatment and involved in the aging process. It would be good to see the differential expression of genes involved in telomere maintenance and protection, including the shelterin complex's genes.

EFFECT OF SPERMIDINE ON TELOMERASE EXPRESSION AND ACTIVITY

Telomerase is a specialized enzyme expressed in 90% of the cancer cell and maintains a certain telomere length, and provides immortality to the cells. We also propose to see the effect of spermidine telomerase expression and activity. For telomerase expression, we can do western blot and real-time PCR, and for telomerase activity, we would suggest doing a TRAP (telomere repeat amplification protocol) assay. Because there are reports showing spermidine also promotes tumor progression, so it would be good to see its effect on telomerase expression and activity. We expect that spermidine treatment would lead to telomerase expression's upregulation and enhance its activity (Fig 1).

IMPLICATIONS OF THE HYPOTHESIS

Our hypothesis will significantly impact future research in the aging and telomere field. The previous study on the role of Spermidine on aging has advanced this field, and our study can add more to it. Testing the role of Spermidine in telomere maintenance will motivate the researcher to validate Spermidine's role in aging and telomere maintenance and test in the clinical trial as well.

CONCLUSION

Spermidine is a crucial molecule involved in aging. Its role in the different aging pathways is already being established. Telomere length is a hallmark of cancer, and it gets shortened with human age. Till now, there is no study done to know the effect of spermidine on telomere length. This study would propose the effect of spermidine on the telomere length of human non-cancerous and cancer cells. We hypothesize that telomere length will increase after spermidine treatment. Spermidine is involved in many cell signaling pathways, and to explore more pathways affected by spermidine treatment, RNA-seq should be done after spermidine treatment in HEK293 and MCF-10A cells (Fig 2). This will suggest a few more pathways that can be further explored to determine the exact molecular mechanism involved in telomere maintenance affected by spermidine.

ABBREVIATIONS

SAMP8 mice- senescence accelerated mouse-8.
TERT- Telomerase reverse transcriptase.
ALT- Alternative telomere lengthening (ALT).
TERRA- Telomeric repeat-containing RNA.
TRAP- Telomere repeat amplification protocol.

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AUTHOR CONTRIBUTIONS

RJ hypothesized the concept. PS and RJ both drafted the manuscript. Both authors read and approved the final manuscript.

CONFLICTS OF INTEREST

Both authors have completed the ICMJE Disclosure Form (<http://www.icmje.org/disclosure-of-interest/>; available on request from the corresponding author). Both authors declare that there are no potential conflicts of interest.

DISCLAIMER

No part of the final manuscript is copied or published elsewhere in whole or in part.

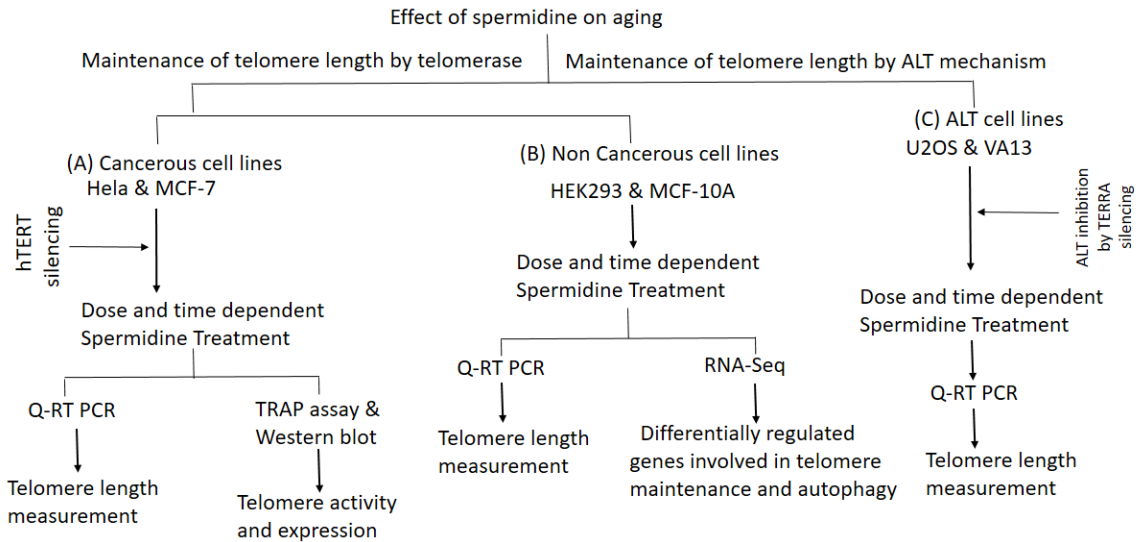


Fig.1. Impact of spermidine on telomere length on aging and cancer. Picture showing three different microenvironments after the exogenous modulation of spermidine levels in the cancerous and non-cancerous cells on the telomerase activity. In aging, lack of telomerase activity in cells leads to telomere shortening. (A) Effect of hTERT gene transduction and spermidine supplementation on telomere length in cancerous cell lines. (B) Analysis of telomere length and expression of differentially regulated genes involved in telomere maintenance and autophagy after spermidine supplementation in non-cancerous cell lines. (C) Measurement of telomere length on spermidine supplementation in ALT cell lines.

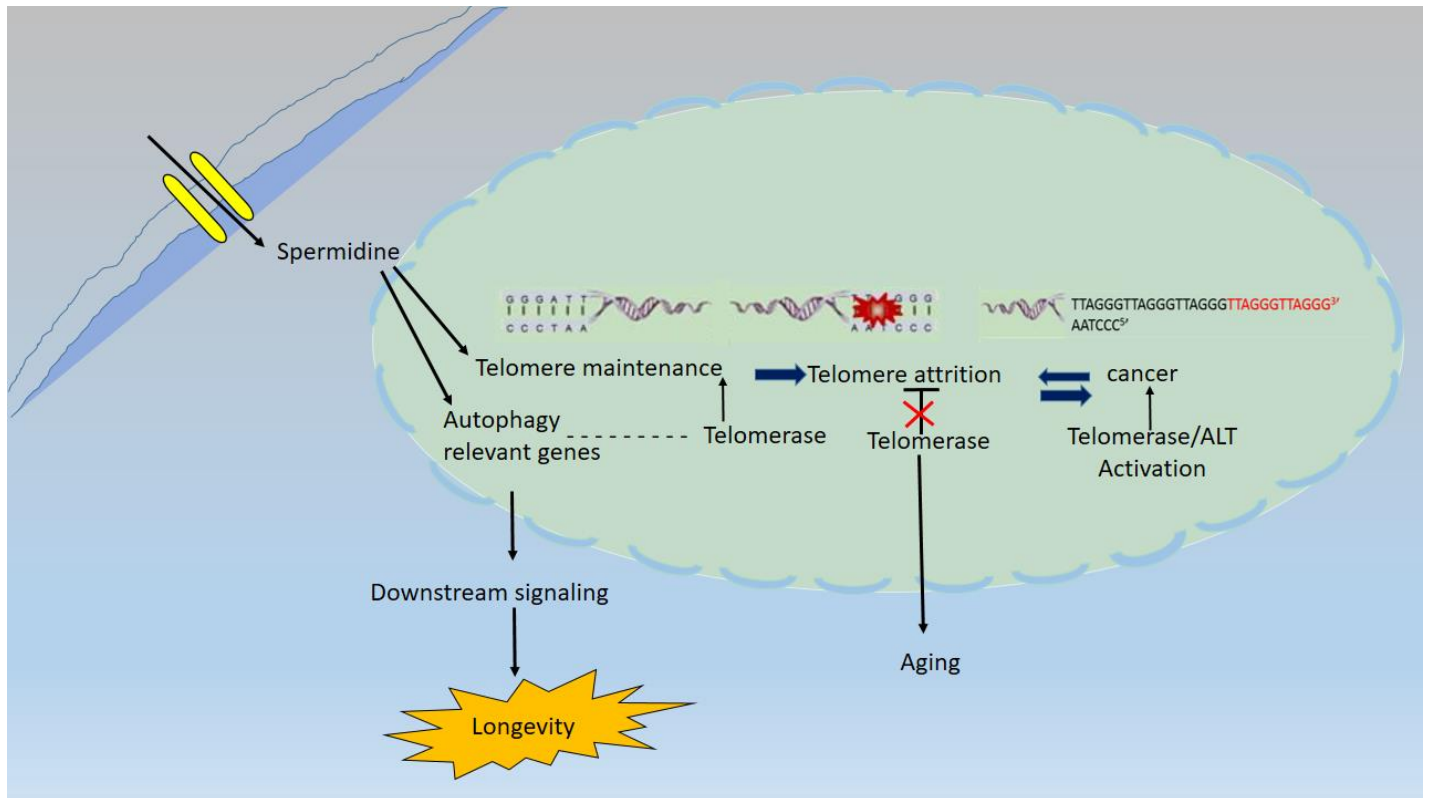


Fig. 2. A schematic representation of experimental strategy and expected outcome considering if spermidine is positively regulating the expression of telomerase.

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СПЕРМИДИН ПОДДЕРЖИВАЕТ ДЛИНУ ТЕЛОМЕРА И ЗАДЕРЖИВАЕТ СТАРЕНИЕ**Резюме**

Спермидин, природный полиамин, известен своими антивозрастными свойствами. Прием этого препарата продлевает продолжительность жизни и снижает частоту возрастных патологий. У взрослого человека уровень спермидина снижается по мере старения, была изучена связь между снижением уровня эндогенного спермидина и возрастными изменениями. На клеточном уровне аутофагия является основным механизмом действия спермидина, который, как известно, снижается по мере старения, способствуя при этом накоплению поврежденных макромолекул и органелл. Эпидемиологическая статистика поддерживает эту концепцию, предполагая, что повышенное потребление полиамина замедляет старение. В статье авторы рассматривают влияние аутофагии на клеточные процессы и возрастные заболевания, подчеркивая важность этих факторов для процесса старения. Существует множество факторов, на которые влияет процесс старения, к ним относятся сокращение теломер, окислительный стресс, повреждение митохондрий и нарушение внутриклеточной кальциевой передачи сигналов. Авторы предполагают, что добавки спермидина в рацион питания увеличивают длину теломер. Предлагаемая гипотеза также выявляет дифференциально регулируемые гены, участвующие в поддержании теломер и старении после лечения спермидином. Изучение роли спермидина в поддержании теломер поможет понять молекулярный механизм воздействия спермидина на старение.

Ключевые слова: спермидин, старение, диета, гипотеза

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