Increased telomerase activity and comprehensive lifestyle changes: a pilot study

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Summary

Background **Telomeres are protective DNA–protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere shortness in human beings is emerging as a prognostic marker of disease risk, progression, and premature mortality in many types of cancer, including breast, prostate, colorectal, bladder, head and neck, lung, and renal cell. Telomere shortening is counteracted by the cellular enzyme telomerase. Lifestyle factors known to promote cancer and cardiovascular disease might also adversely affect telomerase function. However, previous studies have not addressed whether improvements in nutrition and lifestyle are associated with increases in telomerase activity. We aimed to assess whether 3 months of intensive lifestyle changes increased telomerase activity in peripheral blood mononuclear cells (PBMC).**

Methods **30 men with biopsy-diagnosed low-risk prostate cancer were asked to make comprehensive lifestyle changes. The primary endpoint was telomerase enzymatic activity per viable cell, measured at baseline and after 3 months. 24 patients had sufficient PBMCs needed for longitudinal analysis. This study is registered on the ClinicalTrials.gov website, number NCT00739791.**

Findings **PBMC telomerase activity expressed as natural logarithms increased from 2·00 (SD 0·44) to 2·22 (SD 0·49; p=0·031). Raw values of telomerase increased from 8·05 (SD 3·50) standard arbitrary units to 10·38 (SD 6·01) standard arbitrary units. The increases in telomerase activity were significantly associated with decreases in low-density lipoprotein (LDL) cholesterol (r=–0·36, p=0·041) and decreases in psychological distress (r=–0·35, p=0·047).**

Interpretation **Comprehensive lifestyle changes significantly increase telomerase activity and consequently telomere maintenance capacity in human immune-system cells. Given this finding and the pilot nature of this study, we report these increases in telomerase activity as a significant association rather than inferring causation. Larger randomised controlled trials are warranted to confirm the findings of this study.**

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Introduction

Telomeres are protective DNA–protein complexes at the end of linear chromosomes that promote chromosomal stability. Telomere maintenance is required for the complete replication of DNA, protecting chromosomes from nuclease degradation, from end-to-end fusion, and from cellular senescence.[1] Telomere length and rate of telomere shortening are indicators of mitotic cell age, because telomeres shorten during normal cell divisions. Telomere shortening is counteracted by the cellular enzyme telomerase.

Telomere shortness in humans is emerging as a prognostic marker of disease risk, progression, and premature mortality. The aspect of cellular ageing that is conferred by diminished telomere maintenance seems to be an important precursor to the development of many types of cancer.[2,3] Shortened telomeres predict poor clinical outcomes, including increased risk of metastasis in patients with breast cancer,[4] increased risk of bladder, head and neck, lung, and renal-cell cancers,[5] worse progression and prognosis of patients with colorectal cancer,[6] prostate-cancer recurrence in patients undergoing radical prostatectomy,[7] and decreased survival in patients with coronary heart disease and infectious disease.[8]

Although telomere length predicts clinical outcomes and mortality, cells with shortened telomeres can remain genetically stable if the telomere maintenance system, which includes telomerase, is fully functioning.[9] Telomerase adds telomeric repeat sequences to the chromosomal DNA ends, preserving not only telomere length, but also healthy cell function and long-term immune function.[10]

Telomerase is expressed at low concentrations in peripheral-blood mononuclear cells (PBMCs).[11] Until now, few studies have studied telomerase activity in these cells because of the high detection threshold. In previous studies, we adapted the standard telomerase enzymatic activity assay[12] to quantify the low activity of telomerase in normal unstimulated human PBMCs.[13] In these studies, and in the current study, telomerase activity was measured as telomerase enzymatic activity per viable cell in PBMC samples.

Decreased telomere maintenance capacity has also been linked to increased risk of cardiovascular disease, independent of chronological age. In a study of healthy women, we reported that telomerase activity in PBMCs, although not telomere length, was inversely associated with six major cardiovascular disease risk factors.[14] These findings suggested that telomerase activity might be a more direct and potentially earlier predictor than telomere length of long-term cellular viability or genomic stability (or both) and disease processes.

Lifestyle factors known to promote cancer and cardiovascular disease might also adversely affect telomerase and, eventually, telomere length. For example, increases in obesity and insulin resistance over 10–13 years are associated with decreases in telomere length.[15] However, previous studies have not addressed whether improvements in nutrition and lifestyle are associated with increased telomerase activity.

In previous randomised controlled trials, we reported that interventional comprehensive lifestyle changes (improved nutrition, moderate exercise, stress management techniques, and increased social support) beneficially affected the progression of both coronary heart disease[16,17] and early-stage prostate cancer.[18] In the Gene Expression Modulation by Intervention with Nutrition and Lifestyle (GEMINAL) study,[19] a prospective single-arm pilot clinical intervention study in men with indolent low-risk prostate cancers, we reported that such comprehensive lifestyle changes were associated with modulations of gene expression profiles in healthy prostate tissue. Two-class paired analysis of global gene expression by use of significance analysis of microarrays detected 48 upregulated and 453 downregulated transcripts after 3 months of lifestyle intervention. Pathway analysis identified substantial beneficial modulation of biological processes that have crucial roles in tumorigenesis, including protein metabolism and modification, intracellular protein traffic, and protein phosphorylation (all $p<0\cdot05$). Because these patients chose active surveillance for reasons unrelated to both the GEMINAL study and the current study, it was possible to assess the association between changes in lifestyle and changes in telomerase without confounding interventions such as radical prostatectomy, radiation, or chemotherapy.

In the current study, we aimed to assess the hypothesis that in the same cohort of patients in the GEMINAL study, this behavioural intervention might be associated with increased PBMC telomerase activity after 3 months.

Methods

Patients

Men with low-risk prostate cancer willing to make comprehensive lifestyle changes gave written informed consent under a protocol approved by the University of California San Francisco Institutional Review Board. These patients chose active surveillance rather than conventional treatments for prostate cancer for reasons unrelated to this study (eg, advice from their physician, concerns about side-effects of treatment). Eligibility criteria included: pathology-confirmed prostate cancer, prostate-specific antigen (PSA) concentration 10 ng/mL or lower (or <15 ng/mL if there was documented benign prostatic hyperplasia or prostatitis) at the time of screening, Gleason score of 6 or lower, stage T1 or T2a tumour (according to the Tumour, Nodes, Metastases staging system), 33% or less of biopsy cores positive for the presence of adenocarcinoma, and 50% or less of the length of a tumour-core positive for the presence of adenocarcinoma.

A detailed description of patient recruitment has been reported elsewhere.[19] Standard clinical methods were used for waist circumference, weight, height, blood pressure, serum lipids, C-reactive protein, and PSA.

Lifestyle intervention

A 3-month comprehensive lifestyle modification was comprised of a 3-day intensive residential retreat, followed by an outpatient phase where participants met with staff for 4 hours per week and had weekly telephone contact with a study nurse. Lifestyle modifications included a low-fat (10% of calories from fat), whole foods, plant-based diet high in fruits, vegetables, unrefined grains, legumes, and low in refined carbohydrates; moderate aerobic exercise (walking 30 min/day, 6 days/week); stress management (gentle yoga-based stretching, breathing, meditation, imagery, and progressive relaxation techniques 60 min/day, 6 days/week), and a 1-h group support session once per week.[20] The diet was supplemented with soy (one daily serving of tofu plus 58 g of a fortified soy protein powdered beverage), fish oil (3 g daily), vitamin E (100 IU daily), selenium (200 µg daily), and vitamin C (2 g daily).[21,22] Participants were provided with all of their food during the intervention period. A registered dietician, exercise physiologist, clinical psychologist, nurse, and stress management instructor were available for education and counselling. Adherence was assessed with self-reported questionnaires which were used to compute a mean adherence score.[16–18] The decision to use a 3-month duration was arbitrary and was based on the resources available.

Measurement of telomerase activity

Changes in telomerase activity were measured in PBMC samples by comparing telomerase activity per viable cell in PBMC samples at baseline and at 3 months. PBMCs were separated from serum and red blood cells by