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IN THE SPOTLIGHT

Are Short Telomeres Predictive of Advanced Cancer?

Jerry W. Shay

Summary: The combination of variable telomere length in cancer cells and shorter telomere length in cancer-associated stromal cells strongly correlates with progression to prostate cancer metastasis and cancer death. The implication is that telomere length measurements have potential not only as prognostic indicators of prostate cancer outcomes but also as risk stratification enrichment biomarkers for individualized therapeutic interventions. *Cancer Discov; 3(10); 1096–8. ©2013 AACR.*

See related article by Heaphy et al., p. 1130 (6).

Telomeres are the repetitive TTAGGG DNA ends of human linear chromosomes. Telomeres do not encode genes but have a specialized structure including shelterin proteins to hide/cap/protect DNA ends from exonuclease activity and DNA damage recognition. Telomeres progressively shorten throughout life, including in stem cells and highly proliferative transit-amplifying cells, due to failure of lagging DNA strand synthesis to be completed to the very end, often referred to as the end replication problem. In addition, oxidative damage responses may accelerate the loss of telomeres. Almost all *in situ* preneoplastic lesions (sometimes referred to as indolent lesions of epithelial origin) have critically shortened telomeres, which may be an initial protective mechanism limiting the maximum number of divisions human cells can undergo. Because a large number of genetic and epigenetic alterations are required for a normal cell to become malignant, limiting the number of cellular divisions in human cells results in a preneoplastic proliferative growth arrest state referred to as senescence. Replicative senescence may have evolved as an initial potent anticancer molecular mechanism (1). Premalignant cells expressing viral oncoproteins can bypass senescence, move into an extension of cell growth phase, and finally enter a state termed "crisis," or what we now know as terminal telomere shortening. In crisis, telomeres are so short that end-to-end fusions occur, followed by bridge–breakage–fusion cycles, and only rarely in humans does a cell engage a mechanism to escape from crisis. The relationship of shortened telomeres in the preneoplastic cells in crisis compared with the contribution of short telomeres in the cancer-associated stromal cell compartment, including inflammatory cells, is much less clearly understood.

In approximately 85% to 90% of all carcinomas, the molecular mechanism to bypass the crisis is through activation of the gene *TERT*, or telomerase reverse transcriptase (2). The mechanisms of activation of telomerase are still controversial but include mutations in the *TERT* promoter, engagement of *TERT* alternative splicing, *TERT* gene amplification, and epigenetic changes. Another intriguing possibility is that the human *TERT* gene may autoregulate itself, as it is located very close to the telomere end of chromosome 5. In many large long-lived species, *TERT* is also close to a telomere, but in small short-lived species such as mice, *Tert* is not located near a telomere. Interestingly, telomerase is more promiscuous in mice, and inbred strains of mice have very long telomeres compared with humans, but the reasons for this are not understood. One could speculate that the *TERT* gene being located near a telomere in long-lived species may have been selected for over evolutionary time to regulate telomerase and thus the maximal telomere length (3). Telomerase is active during early human fetal development, then becomes silenced in most tissues. Thus, when telomeres reach a certain length (~15–20 kb) during human development, chromatin modifications involving telomere position effects may silence the *TERT* gene (3). As part of cancer progression, as telomeres shorten, the chromatin silencing effects may become relaxed, making a permissive environment for telomerase reactivation. This is consistent with the observation that almost 70% of all cancers are in the segment of the population that is 65 years and older.

Mice with the *Tert* gene develop short telomeres and phenocopy many of the hallmarks of human aging after several generations. In humans with rare disorders of telomere maintenance (called telomeropathies), there is an early onset of diseases such as bone marrow failure, idiopathic pulmonary fibrosis, and dyskeratosis congenita (a disease showing age-associated tissue dysfunctions and a modest increase in cancer in highly proliferative tissues). These diseases suggest that short telomeres in combination with additional genetic and epigenetic alterations contribute to malignant cell transformation. There is no convincing evidence that shortened telomeres without other alterations lead to genomic instability or cancer. In a large population study, a statistically significant inverse relationship between telomere length and both cancer incidence and mortality has been reported (4). In addition to short telomeres correlating with poor prognosis (4), short telomeres in both the epithelial and stromal cell compartments have been reported to activate a senescence-associated secretory pathway, making the

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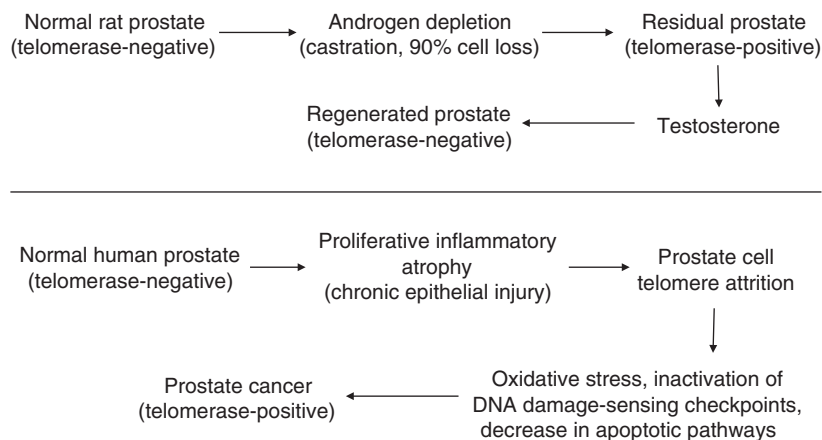
Figure 1. Evidence for a stem cell population in the rat prostate (top, based on data from ref. 8). Evidence for the role of localized proliferative inflammatory atrophy in the human prostate (bottom, based on data from ref. 11).

microenvironment more permissive for cancer progression (5). Senescent cells may also promote inflammation, which is a common feature of all major age-related diseases including cancer, and proliferation in the setting of chronic inflammation predisposes to cancer.

Thus, we come to the present study by Alan Meeker's laboratory in this issue of *Cancer Discovery* (6). Heaphy and colleagues (6) showed that the combination of variable telomere length in cancer cells and shorter telomere length in cancer-associated stromal cells strongly correlates with progression to metastasis and cancer death. The study included approximately 600 men of an average age of 65 years at diagnosis who were enrolled in the Health Professionals Follow-up Study. These men had had a prostatectomy with pathologically organ-confined disease (Gleason sum of 7, clinically localized disease). The mean follow-up times were approximately 13 years for lethal prostate cancer. The cumulative incidences for biochemical recurrence were 33%, for lethal prostate cancer 19%, for prostate cancer death 17%, and for non–prostate cancer death 56%. The results showed that men with more variable telomere length in prostate cancer cells and shorter cancer-adjacent stromal cell telomere length had eight times the risk of progressing to lethal prostate cancer and 14 times the risk of dying of their prostate cancer. Men with the less variable/longer combinations of telomere length were much less likely than expected to die of their prostate cancer, and their time to advanced disease was much longer. Importantly, these associations were independent of currently used prognostic indicators in prostate cancer. This provocative study suggests the translation potential of telomere length measurements as prognostic indicators and potentially as risk stratification biomarkers for individualized therapeutic interventions. The telomere biomarker adds to the predictive capability of the currently used prognostic indicators and may also result in less-aggressive therapy for those men at low risk of recurrence of disease.

Previous studies on prostate cancer and telomeres/telomerase biology have been consistent (7, 8). Using high-resolution *in situ* methods, extensive telomere shortening has been observed in cancer cells compared with normal epithelial cells in the vast majority of prostate cancers and in high-grade prostatic intraepithelial neoplasias (PIN). In normal prostate tissue or benign prostatic hyperplasia

(BPH), there is very little or no detectable telomerase activity (8). In more than 60% of microdissected high-grade PIN lesions, there is detectable telomerase activity. Ninety percent of all prostate carcinomas have telomerase activity and 98% of patients with a Gleason's score >7 have telomerase activity (8). There is mounting evidence that telomerase may be present in some rare human prostate stem cells (9, 10). In one study (Fig. 1, top), the rat prostate had no detectable telomerase activity. After androgen depletion via castration, there was 90% cell loss from the prostate. In the residual prostate, there was some detectable telomerase activity. Then testosterone was added, the prostate regenerated, and telomerase activity was silent again. This study (8) suggests that turnover of stem cells may lead to progressive telomere shortening.

Another mechanism to increase turnover in the noncancerous human prostate is termed focal prostatic atrophy (11), which is associated with chronic inflammation resulting in increased proliferation, cell turnover, and ultimately shortened telomeres (Fig. 1, bottom). Inflammation is frequently present in BPH biopsies and in radical prostatectomy specimens. Inflammatory infiltrates are often observed in areas of prostatic atrophy that are characterized by increased proliferation (11). Chronic inflammation may result in increased oxidative stress, leading to increased cell proliferation and to telomere attrition. The end result is inactivation of DNA damage checkpoint pathways, accumulation of driver genetic and epigenetic oncolytic changes, and emerging prostate cancer cells expressing telomerase.

One way to turn these observations into translational opportunities is to use telomerase inhibitors in selective cohorts of men with the highest risk factor for recurrence of disease (12). Following prostatectomy, the small pool of residual cancer cells with greatly shortened telomeres may be removed by a period of antitelomerase treatment. The telomerase enzyme seems like a perfect cancer target as it is only expressed in a small subset of proliferative stem cells and cancers. Telomerase expression is essential for the proliferation of most advanced cancer cells, but the enzyme is inactive in the vast majority of normal human tissues (2). This suggests that inhibiting the telomere maintenance enzyme should, in theory, be a relatively safe and effective way to drive cancer cells back into crisis before or after treating with cytolytic

agents. The results of the first antitelomerase clinical trials have just been completed (Geron Corp.; Geron.com). Subgroup analyses from a phase II advanced non–small cell lung cancer maintenance trial, following standard induction chemotherapy, showed that imetelstat (a telomerase competitive inhibitor) was most effective in patients whose lung tumors at baseline had the shortest telomeres. Although it did not reach statistical significance, the early analysis suggested a modest trend of efficacy in favor of the imetelstat arm. In a retrospective measurement of tumor telomere length, the analyses suggested that patients whose tumors had short telomeres at baseline experienced an increase in progression-free survival when treated with imetelstat in comparison with patients in the control arm. The treatment effect was not observed in imetelstat-treated patients whose tumors had medium-to-long telomeres.

Overall, these new results (6) could be interpreted to indicate that patients with prostate cancer having short/variable telomeres in their tumor cells and short telomeres in their associated stromal cells at the time of diagnosis may be an enrollment enrichment biomarker for more aggressive treatments. Future studies should address the use of short or variable telomere length as a prognostic tool at the time of biopsy and in risk stratification to individualize treatment and surveillance strategies. Tissue-based markers are urgently needed to improve treatment and surveillance decision-making in men with prostate cancer, and this study is an important advance.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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