Telomere Shortening Is an Early Somatic DNA Alteration in Human Prostate Tumorigenesis

Alan K. Meeker, Jessica L. Hicks, Elizabeth A. Platz, et al.

Cancer Res 2002;62:6405-6409.

[CANCER RESEARCH 62, 6405–6409, November 15, 2002]

Advances in Brief

Telomere Shortening Is an Early Somatic DNA Alteration in Human Prostate Tumorigenesis[1]

Alan K. Meeker, Jessica L. Hicks, Elizabeth A. Platz, Gerrun E. March, Christina J. Bennett, Michael J. Delannoy, and Angelo M. De Marzo[2]

Brady Urological Institute [A. K. M., E. A. P, A. M. D. M.], Department of Pathology [A. K. M., J. L. H., G. E. M., A. M. D. M.], Department of Oncology [E. A. P., A. M. D. M.], and Microscope Facility [M. J. D.], Johns Hopkins University School of Medicine, and The Johns Hopkins University Bloomberg School of Public Health [E. A. P], Baltimore, Maryland 21231

Abstract

Chromosomal instability appears to be key to the pathogenesis of malignant transformation in human cancers, yet the precise molecular mechanisms underlying chromosomal rearrangements remain largely unknown. Telomeres stabilize and protect the ends of chromosomes, but shorten because of cell division and/or oxidative damage. Critically short telomeres, in the setting of abrogated DNA damage checkpoints, have been shown to cause chromosomal instability *in vitro* and in animal models, leading to an increased cancer incidence as a result of chromosome fusions, subsequent breakage, and rearrangement. We present results from a quantitative, high-resolution, *in situ* method for telomere length assessment used to test the hypothesis that telomere shortening is an early contributor to human tumorigenesis. High-grade prostatic intraepithelial neoplasia (HGPIN) is a putative preinvasive precursor of prostatic adenocarcinoma, the most common noncutaneous malignancy in Western men. The telomere lengths of epithelial cells within HGPIN lesions were strikingly shorter than those of adjacent normal appearing epithelial cells in 93% (28 of 30) of lesions examined. This shortening is similar to what has been shown in fully invasive prostate adenocarcinomas. Interestingly, telomere shortening was restricted to the luminal epithelial cells of HGPIN and was not present in the underlying basal epithelial cells; this provides strong evidence that basal cells are most likely not the direct targets of neoplastic transformation. These findings reveal that telomere shortening is a defining somatic DNA alteration characterizing HGPIN. The implications of this are that the earliest phase of human prostate carcinogenesis may proceed as a consequence of chromosomal instability mediated by shortened, dysfunctional telomeres.

Introduction

The majority of human malignancies develop from an accumulation of a number of somatic genomic alterations in key regulatory genes (1, 2). The accrual of many such changes is widely assumed to result from an underlying genetic instability (3). The vast majority of epithelial neoplasms appear to develop from morphologically defined intraepithelial precursor lesions (4). Although not extensively studied, examinations of intraepithelial precursor lesions, including those of prostate cancer, have shown at least some evidence of genetic instability (5–7). Two major types of genetic instability have been identified in human malignancies (8). The first involves simple DNA base changes that occur because of defects in DNA repair processes such as mismatch repair and nucleotide excision repair (9, 10). The second type of instability, which is present in the majority of human carcinomas, is characterized by grossly abnormal karyotypes, featuring both structural and numerical chromosome abnormalities (11, 12). Whereas a number of genes are known to be involved in chromosomal stability (13), only a small subset of human cancers have thus far been found to harbor mutations in such genes (14), and therefore, the molecular mechanisms responsible for most of these chromosomal changes remain unknown.

As demonstrated more than 50 years ago (15), telomere dysfunction can be a major mechanism for the generation of chromosomal instability. Telomeres are composed of a tandemly repeated DNA sequence (TTAGGG in vertebrates) and specific binding proteins located at the ends of eukaryotic chromosomes (16). These structures stabilize chromosome ends and prevent them from being recognized by the cell as DNA double-strand breaks. Telomeres are dynamic entities subject to shortening because of the end replication problem (17), loss of telomere-binding proteins (18), or oxidative stress (19, 20). Conversely, telomeres may be elongated via recombination or, as seen in the vast majority of human cancers, by action of the enzyme telomerase (21).

In human cells, the tumor-suppressor proteins p53 and pRB are key components of a telomere length monitoring system that responds to short telomeres by initiating either replicative senescence or apoptosis (22, 23). Inactivation of these checkpoints allows critical shortening, such that the telomeres are no longer able to perform their protective capping function, resulting in chromosome end-to-end fusions, producing dicentric and ring chromosomes that often break or mis-segregate during mitosis (24). The resulting broken ends can undergo additional rounds of recombination resulting in complex chromosomal rearrangements and aneusomies (15, 25, 26). It has been postulated that such dysfunctional telomeres may promote neoplastic transformation by contributing to genetic instability, therefore playing a causal role in tumorigenesis (27–29). Results from the telomerase template RNA knockout mouse model, in which late generation animals possessing critically short telomeres exhibit abnormal karyotypes and an increased cancer incidence, support this concept (26). Additional support comes from the work of Artandi *et al.* (30), in which crossing telomerase knockout mice with p53+/− mice results in a shift in the spectrum of tumors normally seen in the p53-defective background (primarily lymphomas and sarcomas) to one dominated by carcinomas displaying the types of karyotypic abnormalities, such as nonreciprocal translocations, that are often observed in human epithelial cancers.

In humans, several studies have shown that tumor telomeres are often shorter than those of adjacent normal tissue (31–35). In addition, anaphase bridges, likely because of dysfunctional telomeres, have been noted in some human tumor cells *in vitro* (36, 37), as well as *in situ* in early stage colon cancers (38). If critical telomere shortening does, in fact, contribute to the genetic instability thought to underlie human epithelial neoplasia, then it should presumably occur during or before the invasive phase of carcinogenesis. Unfortunately, studies of telomere length in preinvasive lesions have been hampered by the technical requirement for

Received 4/29/02; accepted 10/3/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by USPHS grants NIH/National Cancer Institute K08CA78588, DK07552, and National Cancer Institute Specialized Programs of Research Excellence P50CA58236.

[2] To whom requests for reprints should be addressed, at Department of Pathology, Division of Genitourinary Pathology, Bunting/Blaustein Cancer Research Building, Room 153, 1650 Orleans Street, Baltimore, MD 21231-1000. Phone: (410) 614-5686; Fax: (410) 502-5158; E-mail: ademarz@jhmi.edu.

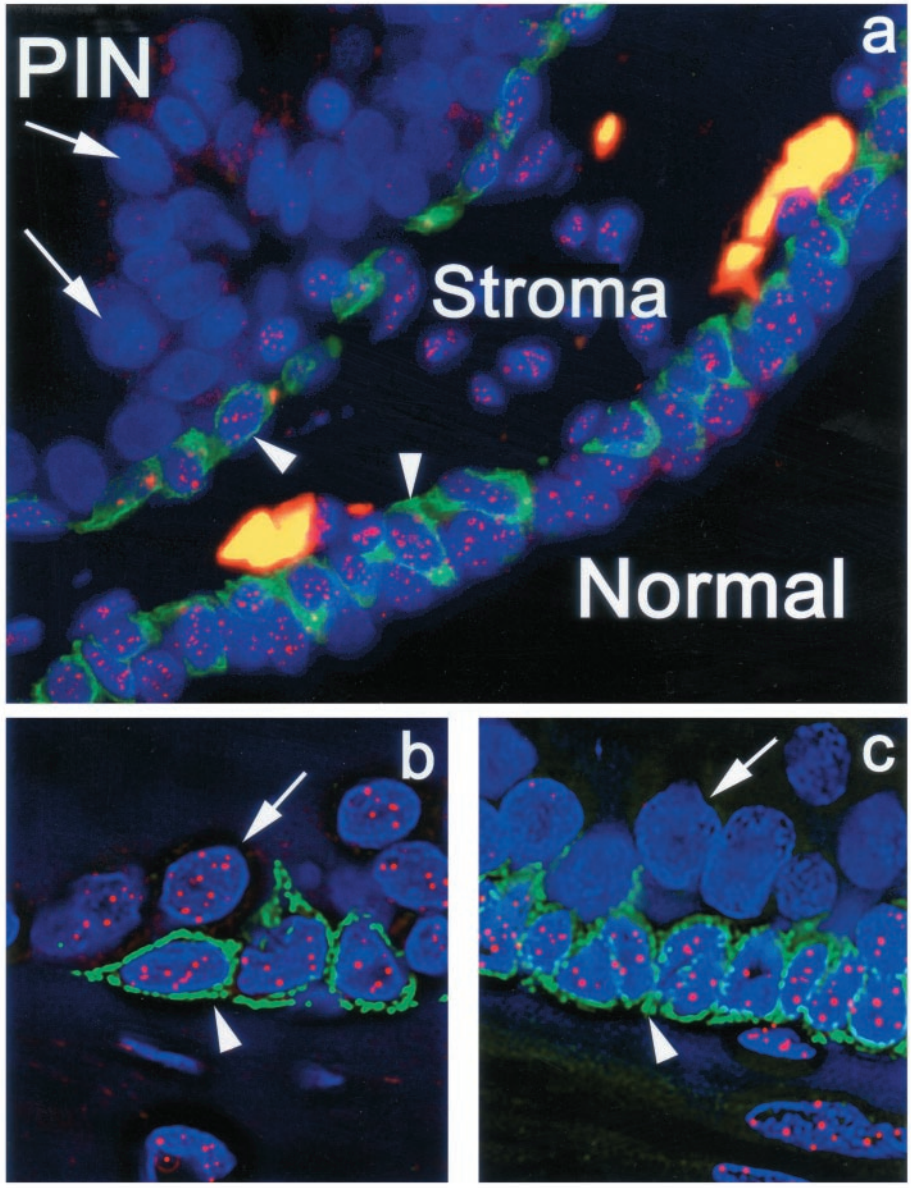

Fig. 1. Short telomeres in the luminal epithelial cells of PIN lesions. Representative images of normal and PIN human prostate glands stained for telomeres (*Red*; Cy3-labeled antitelomeric probe), basal cell-specific cytokeratins (*Green*; antibody 34bE12), and DNA (*Blue*; DAPI). *a*, PIN lesion adjacent to normal gland with intervening stroma. PIN luminal epithelial cells are indicated by *arrows*, underlying basal cells by *arrowheads*. *b*, fluorescence deconvolution image of normal prostate epithelium displaying equal telomere signals in the luminal epithelial cells (*arrow*) and basal cells (*arrowhead*). *c*, fluorescence deconvolution image of a PIN lesion displaying reduced telomeric intensities in the luminal cells (*arrow*) compared with the underlying basal cells (*arrowhead*).

fairly large (~100,000 cells) unfixed tissue samples and by the cell type heterogeneity typical of such samples (39).

In this study, we used a recently validated fluorescent *in situ* method for telomere length assessment (40) to test the hypothesis that telomere shortening is an early contributor to human tumorigenesis. To this end, we probed telomere lengths in HGPIN,[3] the putative preinvasive precursor to adenocarcinomas of the prostate. Because the method provides single cell resolution, we were able to directly compare the telomere lengths of epithelial cells within PIN lesions to those of adjacent normal prostatic epithelial cells. We found clear evidence of significant telomere shortening in PIN epithelial cells in the vast majority of lesions examined. In addition, the simultaneous application of telomere fluorescence *in situ* hybridization and immunofluorescence allowed us to compare telomeres between basal and secretory epithelial cell subtypes within the same high-grade PIN lesion to provide definitive evidence regarding precisely which cell type exhibits this somatic molecular alteration.

[3] The abbreviations used are: HGPIN, high-grade prostatic intraepithelial neoplasia; PIN, prostatic intraepithelial neoplasia; DAPI, 4′,6-diamidino-2-phenylindole.

Materials and Methods

Tissue Samples. Human tissues were obtained from the Department of Surgical Pathology at the Johns Hopkins University School of Medicine. Radical prostatectomy specimens ($n = 6$) from patients that underwent surgery for localized adenocarcinoma of the prostate were obtained fresh from the operating room. Patient ages ranged from 47 to 67 years. Radical prostatectomy Gleason scores ranged from 6 to 8. Specimens were immediately inked and processed by vigorous injection of formalin, followed by microwave treatment. Prostates were then sectioned, and tissues were additionally fixed for 1–4 h and then subjected to standard tissue processing and paraffin embedding. Twenty formalin-fixed needle biopsy core specimens from separate patients with histopathological diagnoses of PIN without frank carcinoma were also obtained.

Telomere Fluorescence *in Situ* Hybridization and Immunofluorescence. The protocol for combined staining of telomeric DNA (FISH probe) and immunostaining was performed without protease digestion as described previously (40). Briefly, deparaffinized slides underwent heat-induced antigen retrieval followed by hybridization with a Cy3-labeled, telomere-specific peptide nucleic acid probe, and were then processed for indirect immunofluorescence using an anticytokeratin primary antibody (34BE12; Enzo Diagnostics, Farmingdale, NY), followed by

Table 1 *Telomere length comparisons: normal luminal epithelial cells versus HGPIN luminal epithelial cells*

Patient no.	Lesion no.	Gleason score	Age	Stage	Normal luminal			PIN luminal			Normal: PIN Ratio	*P*
					Mean	SD	*n*	Mean	SD	*n*		
1	1	6	67	T2N0MX	345	80	20	41	30	20	8.4	<0.0001
1	2	6	67	T2N0MX	345	80	20	47	15	20	7.3	<0.0001
2	3	8	59	T3AN0MX	628	325	13	278	220	14	2.3	0.0029
2	4	8	59	T3AN0MX	628	325	13	111	31	7	5.7	0.0006
2	5	8	59	T3AN0MX	628	325	13	159	29	5	4.0	0.006
3	6	6	53	T2N0MX	435	159	12	151	85	14	2.9	<0.0001
3	7	6	53	T2N0MX	189	78	12	169	58	15	1.1	0.45
4	8	6	57	T2N0MX	162	69	14	116	39	9	1.4	0.08
5	9	6	47	T2N0MX	239	61	17	110	66	17	2.2	<0.001
6	10	8	57	T3AN0MX	118	53	7	25	23	11	4.7	0.0001
6	11	8	57	T3AN0MX	118	53	7	48	37	6	2.5	0.021

application of rabbit IgG fraction Alexa Fluor 488 (Molecular Probes) and counterstaining with DAPI (Sigma Chemical Co., St. Louis, MO).

Microscopy and Image Analysis. Normal glands and PIN lesions were identified on H&E-stained adjacent tissue sections before fluorescence microscopy. The staining of telomeres revealed widely and apparently randomly distributed nuclear signals in all of the cases examined, with no evidence of either peripheral or other nuclear sublocalization, in keeping with the results of previous studies (41–43). The dispersed nature of telomeric signals was additionally verified by optical sectioning followed by deconvolution and 3-dimensional image reconstruction.

Telomere signals were quantitated by a method validated recently in which the sum of pixel intensities in the Cy3 channel for a given cell nucleus is normalized to the DAPI signal. DAPI staining provides a robust measure of DNA content, being largely insensitive to cell type, proliferation status, and degree of chromatin condensation (44, 45). To rule out the possibility that DNA denaturation (in the presence of formamide) and renaturation that occurs during the staining protocol affects the quantitative binding of DAPI to DNA, control experiments were conducted. The comparison of the DAPI signal on adjacent tissue sections from several different cases using a tissue microarray either with or without subjecting the slides to the fluorescent *in situ* method for telomere length assessment staining procedure showed no consistent effects on the quantification of the DAPI signal.

Slides were imaged with a Zeiss Axioskop epifluorescence microscope equipped with appropriate fluorescence filter sets (Carl Zeiss Inc, Thornwood, NY and Omega Optical, Brattleboro, VT). During fluorescence microscopy, basal cells were differentiated from stromal cells and luminal epithelial cells by positive staining with the basal cell-specific anticytokeratin antibody 34bE12. Fluorescent images were captured with a cooled CCD camera (Micro MAX digital camera; Princeton Instruments, Trenton, NJ), and the digitized fluorescent telomere signals were quantified using a semiautomated algorithm written with the image analysis software package IPLabs (Scanalytics, Inc., Fairfax, VA), as described previously (40). An average of 12 representative nuclei per cell subtype (stromal, basal epithelial, luminal epithelial, and cancer) were quantified. Rare nuclei that were obviously undergoing mitosis were excluded from our analysis. For each patient sample, comparisons of the mean ratios of telomeric signal to DAPI between the various cell types were done using the paired *t* test with STATA 6.0 for Microsoft Windows (Stata Corp., College Station, TX). Using this technique for telomere quantification, telomere signals were shown previously to be linearly proportional to average telomere length, as assessed by Southern blotting, in a variety of cell lines representing different cell types, ploidies, and proliferation rates (40).

To obtain high-quality images, and to verify the distributed nature of the telomere signals within the nuclei of prostatic tissue sections, images were also obtained using an Applied Precision Deconvolution System with an Olympus IX 70 epifluorescence microscope (Olympus America, Melville, NY) with a ×100 Plan Apo objective lens (1.35 numerical aperature) and appropriate fluorescence filter sets. Raw data slices were captured with a Roper CH350 1K × 1K, 16-bit, CCD camera (Roper Scientific, Trenton, NJ), using 0.2 μM z-steps and processed by conservative deconvolution iterations using the appropriate optical transform function, as determined by the lens point spread function with the Softworx software package (version 2.50; Applied Precision, Issaquah, WA).

Results

Microscopic examination of prostate tissue sections hybridized with a telomere-specific fluorescent peptide nucleic acid probe showed markedly reduced telomere signals in HGPIN lesions when compared with either the surrounding stroma, or to the stroma and epithelia of adjacent normal-appearing glands ($n = 11$ HGPIN lesions from 6 separate radical prostatectomy specimens; Fig. 1). Quantification of the telomere signals confirmed statistically significant telomere shortening in luminal cells in 9 of the 11 PIN lesions (82%) when compared with adjacent normal-appearing prostate luminal epithelial cells (Table 1). Normal luminal epithelial cells had, on average, 3.9-fold (range: 1.1–8.4) more telomeric fluorescence than did the PIN luminal cells. This implies that the telomeres of PIN lesions are, on average, ~4-fold shorter than their normal counterparts. Unfortunately, no definitive statements can be made regarding the precise lengths of the telomeric tracts within these cells, as absolute internal standards for telomeric length do not exist in our tissue sections. Two of the six specimens contained foci of invasive cancer, which also exhibited shortened telomeres, the magnitude of which was similar to that seen previously (40) and in the corresponding PIN lesions (data not shown). Stromal cells generally exhibited telomere lengths similar to, or slightly longer than, adjacent benign normal-appearing prostatic epithelium and also displayed a greater degree of heterogeneity (Fig. 2).

Strikingly, the telomere shortening in PIN lesions was restricted to the luminal epithelial cells and did not appear to occur within the basal cell compartment (Fig. 1). When compared with the underlying basal cell

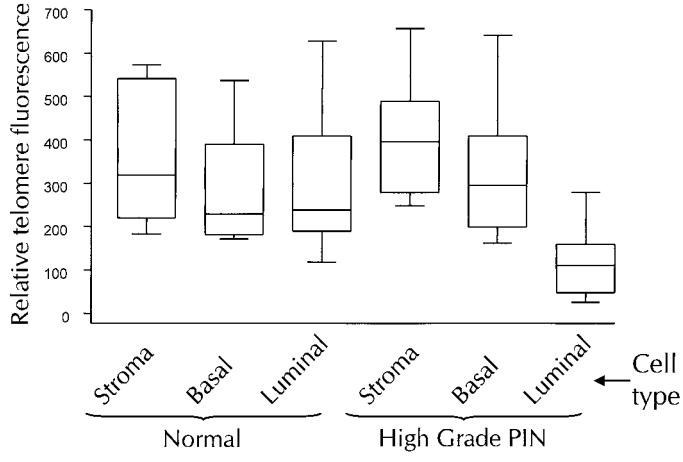

Fig. 2. Mean normalized telomeric fluorescent intensities by cell type for 11 HGPIN lesions ($n = 6$ patients) and adjacent normal tissues. Data representing the means of DAPI-normalized telomere fluorescence signals from individual cells, grouped according to cell type, are displayed in *box plot* format. *Boxed regions* enclose the 25th through 75th percentiles. *Vertical lines* indicate medians. *Whiskers* include the 5th to 95th percentiles.

Table 2 *Telomere length comparisons: HGPIN luminal epithelial cells versus underlying PIN basal cells*

Patient no.	Lesion no.	PIN basal			PIN luminal			Basal: luminal ratio	*P*
		Mean	SD	*n*	Mean	SD	*n*		
1	1	162	33	13	41	30	20	4.0	<0.0001
1	2	168	69	10	47	15	20	3.6	<0.0001
2	3	641	293	16	278	220	14	2.3	0.0007
2	4	430	103	9	111	31	7	3.9	<0.0001
2	5	409	130	7	159	29	5	2.6	0.0019
3	6	297	80	17	151	85	14	2.0	<0.0001
3	7	394	107	7	169	58	15	2.3	<0.0001
4	8	237	75	12	116	39	9	2.0	0.0003
5	9	221	58	15	110	66	17	2.0	<0.0001
6	10	295	56	11	25	23	11	11.8	<0.0001
6	11	199	128	5	48	37	6	4.2	0.021

layer within the same PIN lesions, telomere lengths of the PIN luminal cells were statistically significantly shorter than the basal cells in all of the PIN lesions examined ($n = 11$; Table 2). PIN basal cells had, on average, 3.7-fold (range, 2.0–11.8) more telomeric fluorescence than did the PIN luminal cells. By contrast, in normal-appearing epithelium, the telomere lengths of the luminal and underlying basal cells were essentially equal (data not shown).

To determine whether telomere shortening occurs in HGPIN lesions from patients without evidence of concurrent prostatic carcinoma, 20 samples consisting of diagnostic prostate needle biopsy specimens, with histopathologic diagnoses of HGPIN without associated cancer, were also examined. Visual inspection revealed that, in every case, the telomere fluorescent signals within the PIN luminal compartments were markedly diminished as compared with either the underlying basal cells or the adjacent normal epithelium.

Discussion

The origins of chromosomal instability in the majority of human epithelial cancers and their intraepithelial precursor lesions are unknown. In this study, we find direct evidence for telomere shortening in the epithelial cells of a human cancer precursor lesion, consistent with the hypothesis that telomere shortening may play an early role in human tumorigenesis. Specifically, the epithelial cells in 28 of 30 HGPIN lesions (93%) show marked telomere shortening, similar in magnitude to what is observed for invasive carcinoma (40). In addition, we find that basal cells within HGPIN do not show this somatic DNA alteration, and, therefore, are most likely not the direct targets of neoplastic change in the prostate.

Prostate epithelium contains two major cell types, basal and secretory. The vast majority of prostate cancer cells and the atypical cells within HGPIN lesions possess a phenotype that is most consistent with secretory luminal cells. For example, HGPIN cells express abundant androgen receptor, PSA, prostate specific acid phosphatase, high levels of cytokeratins 8 and 18, and generally lack cytokeratins 5 and 14, as well as p63 (46). Because basal cells are thought to give rise to secretory cells, they have been postulated to be prostatic stem cells. In most organs where epithelial neoplasms predominate, stem cells are widely thought to be the cells that undergo neoplastic transformation (47). However, there is no definitive molecular evidence that shows whether basal cells or secretory type cells are the ultimate targets of neoplastic transformation in the prostate. The data presented here suggest that measurable somatic telomere shortening does not occur to a large degree in basal cells.

Because the overwhelming majority of normal prostate luminal cells are not proliferative and are thought to be terminally differentiated, the current finding of telomere shortening only in the luminal compartment supports the concept that proliferative cells possessing a phenotype intermediate between basal and luminal cells are the targets for neoplastic transformation in the prostate (46, 48, 49). Other indirect evidence suggests that basal cells are not the target for transformation. For example, normal prostate basal cells express proteins, such as glutathione *S*-transferase π, thought to function in the defense against electrophilic carcinogens. The *GSTP1* gene is inactivated by biallelic methylation in ~90% of prostate cancers and at least 70% of HGPIN (50–52). Because basal cells in PIN lesions maintain GSTP1 expression, it can be inferred that, unlike prostate cancer cells, these cells do not undergo somatic biallelic methylation of the *GSTP1* gene, the product of which may therefore function to protect the genome.

While established invasive prostatic adenocarcinomas contain telomerase activity in at least 85% of cases, telomerase activity has been detected in only 16% of HGPIN cases (53). Because PIN lesions are highly proliferative compared with adjacent normal tissue (54, 55), our results suggest that PIN cells undergo a number of cell divisions without telomerase (Fig. 3). This proliferation in cells lacking a telomere maintenance mechanism, possibly in conjunction with immune-mediated oxidant stress (49), would promote telomere shortening, eventually generating one or more dysfunctional telomeres. We propose that telomeres are subsequently stabilized in the subset of PIN lesions that activate telomerase, and that it is these PIN lesions, theoretically capable of unlimited cell division, that may eventually progress to fully invasive adenocarcinoma. This is similar to what is thought to occur during human colorectal carcinoma progression (38). It will be of interest to know how widespread a phenomenon early telomere shortening is in other cancers. To this end, studies applying this method to archival specimens containing the recently identified precursor lesions of pancreatic cancer, pancreatic intraepithelial neoplasia, showed marked telomere shortening in the vast majority of specimens (56).

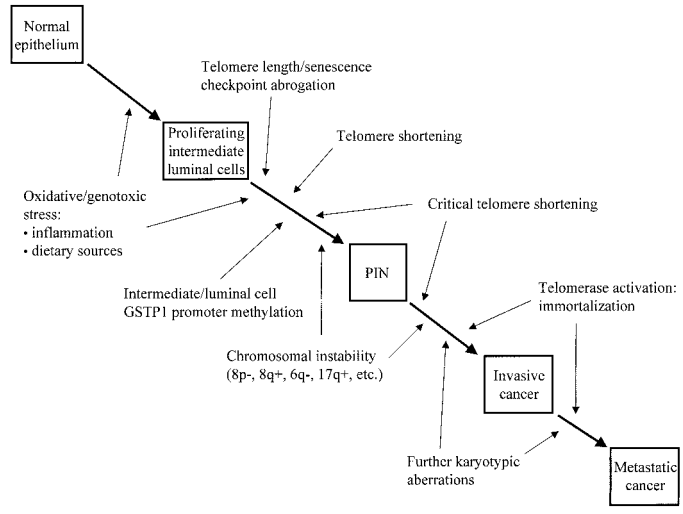

Fig. 3. Model for involvement of telomere shortening in human prostate tumorigenesis. Telomere shortening occurs early, at or before the PIN stage, and is proposed to contribute to chromosomal instability, hence neoplastic transformation.

Acknowledgments

We thank Dr. Donald S. Coffey, William B. Isaacs, and William G. Nelson for helpful comments and critical reading of the manuscript. We thank Christina Bennett, Helen Fedor, and Marcella Southerland for diligent efforts in tissue harvesting and organization. We also thank James Morgan for computer programming assistance for telomere data processing.

References

1. Knudson, A. G. Genetics of human cancer. Annu. Rev. Genet., *20:* 231–251, 1986.
2. Fearon, E. R., and Vogelstein, B. A genetic model for colorectal tumorigenesis. Cell, *61:* 759–767, 1990.
3. Loeb, L. A. Mutator phenotype may be required for multistage carcinogenesis. Cancer Res., *51:* 3075–3079, 1991.
4. O'Shaughnessy, J. A., Kelloff, G. J., Gordon, G. B., Dannenberg, A. J., Hong, W. K., Fabian, C. J., Sigman, C. C., Bertagnolli, M. M., Stratton, S. P., Lam, S., Nelson, W. G., Meyskens, F. L., Alberts, D. S., Follen, M., Rustgi, A. K., Papadimitrakopoulou, V., Scardino, P. T., Gazdar, A. F., Wattenberg, L. W., Sporn, M. B., Sakr, W. A., Lippman, S. M., and Von Hoff, D. D. Treatment and prevention of intraepithelial neoplasia: an important target for accelerated new agent development. Clin. Cancer Res., *8:* 314–346, 2002.
5. Shih, I. M., Zhou, W., Goodman, S. N., Lengauer, C., Kinzler, K. W., and Vogelstein, B. Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res., *61:* 818–822, 2001.
6. Qian, J., Jenkins, R. B., and Bostwick, D. G. Genetic and chromosomal alterations in prostatic intraepithelial neoplasia and carcinoma detected by fluorescence *in situ* hybridization. Eur. Urol., *35:* 479–483, 1999.
7. Al-Maghrabi, J., Vorobyova, L., Chapman, W., Jewett, M., Zielenska, M., and Squire, J. A. p53 Alteration and chromosomal instability in prostatic high-grade intraepithelial neoplasia and concurrent carcinoma: analysis by immunohistochemistry, interphase *in situ* hybridization, and sequencing of laser-captured microdissected specimens. Mod. Pathol., *14:* 1252–1262, 2001.
8. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instabilities in human cancers. Nature (Lond.), *396:* 643–649, 1998.
9. Schmutte, C., and Fishel, R. Genomic instability: first step to carcinogenesis. Anticancer Res. *19:* 4665–4696, 1999.
10. Eshleman, J. R., and Markowitz, S. D. Microsatellite instability in inherited and sporadic neoplasms. Curr. Opin. Oncol., *7:* 83–89, 1995.
11. Mittelamn, F. Catalog of Chromosome Aberrations in Cancer, 6 edition. New York: Alan R. Liss, 1998.
12. Sandberg, A. A., Chromosomes in human neoplasia. Curr. Probl. Cancer. *8:* 1–52, 1983.
13. Hartwell, L. H., and Kastan, M. B. Cell cycle control and cancer. Science (Wash. DC), *266:* 1821–1828, 1994.
14. Jallepalli, P. V., and Lengauer, C. Chromosome segregation and cancer: cutting through the mystery. Nature Rev. Cancer, *1:* 109–117, 2001.
15. McClintock, B. The stability of broken ends of chromosomes in Zea mays. Genetics, *26:* 234–282, 1941.
16. Blackburn, E. H. Telomeres. Trends Biochem. Sci., *16:* 378–381, 1991.
17. Olovnikov, A. M. Principle of marginotomy in template synthesis of polynucleotides. Dokl. Akad. Nauk., *201:* 1496–1499, 1971.
18. Smogorzewska, A., van Steensel, B., Bianchi, A., Oelmann, S., Schaefer, M. R., Schnapp, G., and de Lange, T. Control of human telomere length by TRF1 and TRF2. Mol. Cell. Biol. *20:* 1659–1668, 2000.
19. Bohr, V. A., and Anson, R. M. DNA damage, mutation and fine structure DNA repair in aging. Mutat. Res., *338:* 25–34, 1995.
20. von Zglinicki, T., Pilger, R., and Sitte, N. Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. Free Radic. Biol. Med., *28:* 64–74, 2000.
21. Greider, C. W., and Blackburn, E. H. Identification of a specific telomere terminal transferase activity in tetrahymena extracts. Cell, *43:* 405–413, 1985.
22. Shay, J. W., Wright, W. E., and Werbin, H. Defining the molecular mechanisms of human cell immortalization, Biochim. Biophys. Acta, *1072:* 1–7, 1991.
23. Harley, C. B., Kim, N. W., Prowse, K. R., Weinrich, S. L., Hirsch, K. S., West, M. D., Bacchetti, S., Hirte, H. W., Counter, C. M., Greider, C. W., and *et al.* Telomerase, cell immortality, and cancer. Cold Spring Harbor Symp. Quant. Biol., *59:* 307–315, 1994.
24. Counter, C., Avilion, A., LeFeuvre, C., Stewart, N., Greider, C., Harley, C., and Bacchetti, S. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. EMBO J., *11:* 1921–1929, 1992.
25. Lundblad, V., and Szostak, J. W. A mutant with a defect in telomere elongation leads to senescence in yeast. Cell, *57:* 633–643, 1989.
26. Blasco, M. A., Lee, H. W., Hande, M. P., Samper, E., Lansdorp, P. M., DePinho, R. A., and Greider, C. W. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell, *91:* 25–34, 1997.
27. Hastie, N. D., and Allshire, R. C. Human telomeres: fusion and interstitial sites. Trends Genet., *5:* 326–331, 1989.
28. von Zglinicki, T. Are the ends of chromosomes the beginning of tumor genesis? On the role of telomeres in cancer development. Fortschr. Med., *114:* 12–14, 1996.
29. Artandi, S. E., and DePinho, R. A. A critical role for telomeres in suppressing and facilitating carcinogenesis. Curr. Opin. Genet. Dev., *10:* 39–46, 2000.
30. Artandi, S. E., Chang, S., Lee, S. L., Alson, S., Gottlieb, G. J., Chin, L., and DePinho, R. A. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. Nature (Lond.), *406:* 641–645, 2000.
31. Hastie, N., Dempster, M., Dunlop, M., Thompson, A., Green, D., and Alshire, R. Telomere reduction in human colorectal carcinoma and with ageing. Nature (Lond.), *346:* 866–868, 1990.
32. Furugori, E., Hirayama, R., Nakamura, K. I., Kammori, M., Esaki, Y., and Takubo, K. Telomere shortening in gastric carcinoma with aging despite telomerase activation. J. Cancer Res. Clin. Oncol., *126:* 481–485, 2000.
33. Mehle, C., Ljungberg, B., and Roos, G. Telomere shortening in renal cell carcinoma. Cancer Res., *54:* 236–241, 1994.
34. Takagi, S., Kinouchi, Y., Hiwatashi, N., Chida, M., Nagashima, F., Takahashi, S., Negoro, K., Shimosegawa, T., and Toyota, T. Telomere shortening and the clinicopathologic characteristics of human colorectal carcinomas. Cancer (Phila.), *86:* 1431–1436, 1999.
35. Sommerfeld, H. J., Meeker, A. K., Piatyszek, M. A., Bova, G. S., Shay, J. W., and Coffey, D. S. Telomerase activity: a prevalent marker of malignant human prostate tissue. Cancer Res., *56:* 218–222, 1996.
36. Saunders, W. S., Shuster, M., Huang, X., Gharaibeh, B., Enyenihi, A. H., Petersen, I., and Gollin, S. M. Chromosomal instability and cytoskeletal defects in oral cancer cells. Proc. Natl. Acad. Sci. USA, *97:* 303–308, 2000.
37. Gisselsson, D., Pettersson, L., Hoglund, M., Heidenblad, M., Gorunova, L., Wiegant, J., Mertens, F., Dal Cin, P., Mitelman, F., and Mandahl, N. Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. Proc. Natl. Acad. Sci. USA, *97:* 5357–5362, 2000.
38. Rudolph, K. L., Millard, M., Bosenberg, M. W., and DePinho, R. A. Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. Nat. Genet., *28:* 155–159, 2001.
39. Engelhardt, M., Albanell, J., Drullinsky, P., Han, W., Guillem, J., Scher, H. I., Reuter, V., and Moore, M. A. Relative contribution of normal and neoplastic cells determines telomerase activity and telomere length in primary cancers of the prostate, colon, and sarcoma. Clin. Cancer Res., *3:* 1849–1857, 1997.
40. Meeker, A. K., Gage, W. R., Hicks, J. L., Simon, I., Coffman, J. R., Platz, E. A., March, G. E., and De Marzo, A. M. Telomere length assessment in human archival tissues: combined telomere fluorescence *in situ* hybridization and immunostaining. Am. J. Pathol., *160:* 1259–1268, 2002.
41. Henderson, S., Allsopp, R., Spector, D., Wang, S. S., and Harley, C. *In situ* analysis of changes in telomere size during replicative aging and cell transformation. J. Cell Biol., *134:* 1–12, 1996.
42. Harley, C. B. Telomeres and aging: fact, fancy, and the future. J. NIH Res., *7:* 64–68, 1995.
43. Nagele, R. G., Velasco, A. Q., Anderson, W. J., McMahon, D. J., Thomson, Z., Fazekas, J., Wind, K., and Lee, H. Telomere associations in interphase nuclei: possible role in maintenance of interphase chromosome topology. J. Cell Sci., *114:* 377–388, 2001.
44. Wang, N., Pan, Y., Heiden, T., and Tribukait, B. Fluorescence image cytometry for measurement of nuclear DNA content in surgical pathology. Cytometry, *22:* 323–329, 1995.
45. Cowden, R. R., and Curtis, S. K. Microfluorometric investigations of chromatin structure. I. Evaluation of nine DNA-specific fluorochromes as probes of chromatin organization. Histochemistry, *72:* 11–23, 1981.
46. De Marzo, A. M., Putzi, M. J., and Nelson, W. G. New concepts in the pathology of prostatic epithelial carcinogenesis. Urology, *57:* 103–114, 2001.
47. Reya, T., Morrison, S. J., Clarke, M. F., and Weissman, I. L. Stem cells, cancer, and cancer stem cells. Nature (Lond.) *414:* 105–111, 2001.
48. Verhagen, A. P., Ramaekers, F. C., Aalders, T. W., Schaafsma, H. E., Debruyne, F. M., and Schalken, J. A. Colocalization of basal and luminal cell-type cytokeratins in human prostate cancer. Cancer Res., *52:* 6182–6187, 1992.
49. De Marzo, A. M., Marchi, V. L., Epstein, J. I., and Nelson, W. G. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. Am. J. Pathol., *155:* 1985–1992, 1999.
50. Lee, W. H., Morton, R. A., Epstein, J. I., Brooks, J. D., Campbell, P. A., Bova, G. S., Hsieh, W. S., Isaacs, W. B., and Nelson, W. G. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis, Proc. Natl. Acad. Sci. USA, *91:* 11733–7, 1994.
51. Brooks, J. D., Weinstein, M., Lin, X., Sun, Y., Pin, S. S., Bova, G. S., Epstein, J. I., Isaacs, W. B., and Nelson, W. G. CG island methylation changes near the GSTP1 gene in prostatic intraepithelial neoplasia. Cancer Epidemiol. Biomark. Prev., *7:* 531–536, 1998.
52. Lin, X., Tascilar, M., Lee, W. H., Vles, W. J., Lee, B. H., Veeraswamy, R., Asgari, K., Freije, D., van Rees, B., Gage, W. R., Bova, G. S., Isaacs, W. B., Brooks, J. D., DeWeese, T. L., De Marzo, A. M., and Nelson, W. G. GSTP1 CpG island hypermethylation is responsible for the absence of GSTP1 expression in human prostate cancer cells. Am. J. Pathol., *159:* 1815–1826, 2001.
53. Koeneman, K. S., Pan, C. X., Jin, J. K., Pyle, J. M., Flanigan, R. C., Shankey, T. V., and Diaz, M. O. Telomerase activity, telomere length, and DNA ploidy in prostatic intraepithelial neoplasia (PIN). J. Urol., *160:* 1533–1539, 1998.
54. McNeal, J. E., Haillot, O., and Yemoto, C. Cell proliferation in dysplasia of the prostate: analysis by PCNA immunostaining. Prostate, *27:* 258–268, 1995.
55. Bonkhoff, H., Stein, U., and Remberger, K. The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate, *24:* 114–118, 1994.
56. van Heek, N. T., Meeker, A. K. Kern, S. E., Yeo, C. J., Lillemoe, K. D., Cameron, J. L., Offerhaus, G. J. A., Hicks, J. L., Wilentz, R. E., Goggins, M. G., De Marzo, A. M., Hruban, R. H., and Maitra, A. Telomere shortening is nearly universal in pancreatic intraepithelial neoplasia. Am. J. Pathol., in press, 2002.