Published in final edited form as:

Cancer Genet Cytogenet. 1996 January ; 86(1): 50–53.

Chromosome Telomere Integrity of Human Solid Neoplasms

Merlin G. Butler,

Department of Orthopedics, Vanderbilt University Medical Center. Nashville. Tennessee

Department of Pediatrics, Vanderbilt University Medical Center. Nashville. Tennessee

Department of Pathology, Vanderbilt University Medical Center. Nashville. Tennessee

Marcus Sciadini,

Department of Orthopedics, Vanderbilt University Medical Center. Nashville. Tennessee

Lora K. Hedges, and

Department of Orthopedics, Vanderbilt University Medical Center. Nashville. Tennessee

Herbert S. Schwartz

Department of Orthopedics, Vanderbilt University Medical Center. Nashville. Tennessee

Department of Pathology, Vanderbilt University Medical Center. Nashville. Tennessee

Abstract

Eukaryotic chromosomes contain specialized structures at the termini called telomeres. This region of DNA is required for replication and stability of the chromosome. Telomere reduction can contribute to genetic instability and has been described in certain malignancies (e.g., colon, leukemia, giant cell tumor of bone). To determine whether telomere reduction is a generalized phenomenon in malignancies, the telomere integrity of genomic DNA isolated from tumor cells was determined from 39 individuals with 15 different malignancies categorized as musculoskeletal, epithelial, cranial, or other, and peripheral blood leukocytes from the same patient, when possible, or age-matched controls. Significant telomere reduction occurred randomly across histopathologic groups including giant cell tumor of bone, glioblastoma, colon cancer, and Wilms' tumor while telomere elongation occurred in chondroma. The other remaining 10 malignancies did not show significant differences in telomere lengths compared with controls.

INTRODUCTION

The telomere is that region of DNA at the end of linear chromosomes required for replication and stability of the chromosome. DNA at the terminal ends of all chromosomes has a special structure to avoid binding to the ends of DNA from other chromosomes, thus preventing end-to-end fusions or telomeric associations (*tas*). Cytogenetic studies of certain tumors such as giant cell tumor of bone (GCT) have *tas* demonstrated repeatedly in about 75% of CCT patients [1–3]. In addition, telomeric fusions have been reported in Wilms' tumor cells [4].

Telomeric DNA consists of terminal repeat arrays (TRAs) of base pairs characterized by clusters of G residues in the 3 prime strand (TTAGGG)_n and have been isolated from telomeres in humans [5, 6]. The length of the telomere is variable and influenced by the age of an individual. Recently, Schwartz et al. [7] reported loss of telomere size in peripheral blood cells from healthy individuals with advancing age. The greatest rate of telomere reduction was observed in the younger individuals (e.g., less than 20 years of age). The average telomere length for healthy individuals less than 10 years of age was approximately 15,000 base pairs while approximately 5,000 base pairs were reported for those healthy individuals between 60 and 70 years of age. In addition, telomere shortening during aging of human fibroblasts has also been reported [8].

TRAs are highly conserved in nature and are found in the termini of linear chromosomes from plants, animals, protists, and fungi [9]. The maintenance of telomere integrity is fundamental for a cell to repeatedly undergo mitosis and for a neoplastic cell to propagate its transformed phenotype. Telomere reduction can contribute to genetic instability and has been observed in malignancies (e.g., colon, leukemia, giant cell tumor of bone) [7, 10, 11].

To determine if telomere reduction is a general phenomenon of oncogenesis, we studied the telomere length in a wide variety of human solid neoplasms and peripheral blood leukocytes from the same patient, when possible, or from age-matched controls. Representative tumors were analyzed from differing histopathologic groups.

MATERIALS AND METHODS

Tumor samples (about 1 cm³) were obtained intraoperatively from 39 individuals with solid tissue neoplasms. These individuals and tumor types were as follows: four with breast, average age = 60 years, age range 46 to 71 years; three with lung, average age = 54 years, age range 44 to 63 years; two with ovarian, average age = 52 years, age range 49 to 55 years; two with colon, average age = 64 years, age range 59 to 68 years; one with renal, age of 71 years; eight with giant cell tumor of bone, average age = 36 years, age range 20 to 58 years; one with chondrosarcoma, age of 11 years; four with chordoma, average age = 55 years, age range 36 to 71 years; four with glioblastoma, average age = 54 years, age range 47 to 61 years; one with medulloblastoma, age of 17 years; one with astrocytoma, age of 2 years; four with neuroblastoma, average age = 7 years, age range 2 to 27 years; two with Wilms' tumor, average age = 16 years, age range 3 to 30 years; one with pheochromocytoma, age of 46 years; and one with malignant melanoma, age of 43 years. The above tumors were confirmed histopathologically.

Genomic DNA was isolated from the tumor and from peripheral blood samples from the same patient when possible or age-matched controls following standard protocols [7, 12]. Five micrograms of DNA were digested from each sample with *HinfI* enzyme at 37°C for 4 hours. A minigel electrophoresis was performed to check for completeness of digestion. DNA was then loaded onto a 0.8% agarose gel and electrophoresis performed for 5 hours at 58 volts. The gel was then blotted to Gene Screen Plus nylon membrane and hybridized with the ³²P-nick-labeled (TTAGGG)₅₀ telomeric probe synthesized by polymerase chain reaction and available commercially from Oncor, Inc. (Gaithersburg, MD). Hybridization

was performed at 42°C for 2 days and the filter washed in an SSC/SDS mixture at 42°C. The filters were then exposed to high-performance autoradiographic film. The exposed autoradiographs were analyzed using an LBK soft laser spectrophotometer to determine the maximum DNA peak, length of hybridization signal, and the total area under the DNA absorption curve. Several tumor and matched blood DNA samples are shown in Figure 1. Peak intensity and migration distance were then analyzed from the DNA isolated from the tumors and leukocytes. DNA fragments migrating farther than control DNA, as detected by the hybridized radiolabeled telomere probe, represented shortened telomeres (telomeric reduction) and those not migrating as far represented elongated telomeres. Average telomere size was determined by the peak migration distance and compared with DNA markers of known size. For statistical analysis, matched t-test values were calculated for the telomeric data.

RESULTS

The 15 different human neoplasms from 39 individuals were categorized into musculoskeletal, epithelial cranial, and “other” histopathologic groups. The number of subjects, ages, peak migration distances, telomere size, and matched t-test values of both tumor and control specimens are given in Table 1. Autoradiographs of the radio labeled hybridized filters were densitometrically scanned, producing peaks for each DNA lane. Peak migration distance was calculated from each DNA lane. Graphic representation of the Southern blot hybridizations are shown in Figure 1 from several patients with different types of tumors and controls. Optical density recorded by the scanner signifies radio labeled probe hybridization at a known kilobase (kb) migration distance. The kb migration length was established using markers of known size. A mean peak intensity was calculated for each of the tumor DNA and matched leukocyte DNA. Tumor DNA from most tumor patients showed telomeric reduction of the tumor DNA when compared with DNA isolated from the patient’s respective leukocytes when available or from age-matched controls (e.g., using DNA densitometry peak location, glioblastoma DNA showed significantly greater telomere reduction [matched t-test = 3.23; $\mu < 0.05$; one-tailed test] than control leukocyte DNA from the same patients [tumor DNA average peak migration = 57.1 mm $\pm$ 16.8 [S.D.]; control DNA average peak migration = 30.2 mm $\pm$ 4.1 [S.D.]; average telomere size = 5.0 kb $\pm$ 2.6 [S.D.]; average control telomere size = 10.9 kb $\pm$ 1.8 [S.D.]) (See Table 1). There were no differences observed in telomere size between leukocyte DNA from GCT patients and leukocyte DNA from age-matched controls without a history of tumor development (data not shown). Significant telomere reduction ($p < 0.05$; matched t-test) occurred in giant cell tumor of bone, glioblastoma, colon cancer, and Wilms tumor while telomere elongation ($p < 0.05$; matched t-test) occurred in chorionoma (See Table 1). The other malignancies did not demonstrate significant differences in telomere lengths.

DISCUSSION

Telomere length is variable and influenced by age and neoplasm [7, 10]. As the age of a subject increases, the length of the telomere decreases, with an average loss of 40 base pairs per year [7]. The telomere size is also altered in tumors when compared to telomere DNA from age-matched controls [7]. Telomeric length alteration is likely a result rather than the

inciting event in tumorigenesis but, as our study indicated, not all neoplasms showed telomere reduction. Specifically, chordomas showed telomeric elongation and not reduction. No consistent chromosome abnormalities were seen in chordoma cells from our chordoma patients or from the tumor cells from the patients with Wilms' tumor or renal carcinoma. However, chromosomal telomeric associations were observed in 60 % of our giant cell tumor of bone patients and in 10–30% of tumor cells [7]. In addition, Nurnberg et al. [13] reported that among 60 intracranial tumors 42% showed telomere elongation, 22 % showed telomere reduction, and 37% exhibited equal telomere length when compared with the patient's peripheral blood leukocytes. Thus, there appeared to be variability in telomere size in cells from different intracranial tumors. Similarly, 25% of our previously studied giant cell tumor of bone patients did not show telomere reduction compared with their leukocyte DNA [7] and there appeared to be no significant correlation between telomere size and telomeric associations in these patients. Overcoming a dysfunctional telomere is a necessary prerequisite for a transformed cell to propagate.

We also found variability in the size of the telomere compared with controls in our 39 individuals with 15 different human neoplasms categorized into musculoskeletal, epithelial, cranial, and "other" groups. A majority of our 39 patients' tumor telomeric DNA showed deletions or reductions in relationship to control DNA; however, chordoma showed significant telomeric elongation (telomeric DNA migration distance was approximately 50 % of that seen in its matched control DNA; average DNA peak migration = 16.8 mm in chordoma and 30.7 mm in control DNA) compared with control telomeric DNA. Thus, telomeric reduction is not a generalized phenomenon in human cancers. When telomeric reduction was observed there appeared to be variability in the size of the reduction. For example, marked telomeric reduction of approximately a threefold difference in DNA migration distance was seen in Wilms' tumor DNA (average DNA peak migration = 73.8 mm in Wilms' tumor and 26.7 mm in control DNA) compared with control DNA, while approximately a 10% difference in telomeric DNA migration distance was observed in giant cell tumor of bone compared with controls (average DNA peak migration = 32.7 mm in giant cell tumor of bone and 29.3 mm in control DNA). Similarly, there appeared to be more variation (i.e., larger standard deviation) in the average length of DNA migration and the telomeric DNA size in neoplasms compared to control DNA, which may indicate heterogeneity of the tumor sources (e.g., multiple populations of tumor cells) or chromosomal aneuploidy in malignant cells.

Telomere length reduction destabilizes chromosomes and, in addition, telomere reductions have been reported to correlate with a poor prognosis in neuroblastoma [14]. The accumulated karyotypic changes within a cell occurs until a crisis period in which cell replication is balanced by cell death [15, 16]. Cells that escape this crisis period require reactivation of the telomerase gene and production of telomerase. This ribonucleoprotein enzyme synthesizes the addition of new TTAGGG repeats on the telomeres, restoring their integrity.

Identifying those tumors with altered telomeric lengths may then be important in developing chemotherapeutic treatment strategies aimed at critical enzymes such as telomerase. Telomerase may be overly active in those tumors with little or no reduction of their telomere

size. Studies to explore the relationship of telomere size and telomerase activity in several tumors, including giant cell tumor of bone and musculoskeletal tumors in our laboratory, are currently underway.

We conclude that telomeric reduction is not a generalized phenomenon in human cancer, but when telomere alteration in tumor cells is observed they are more often reduced rather than elongated. Our preliminary data on 15 different tumors showed that significant telomere reduction occurred in four tumor sources. In contrast, chordoma cells demonstrated telomere length alteration via elongation relative to its control. Further characterization of telomeric alteration in human cancer should provide insight into which tumors may be responsive to enzymatic manipulation (e.g., antitelomerase drug targeted for malignant cells which should allow for cell death of the tumor cells) or may be helpful in determining the biologic aggressiveness of certain tumors.

Acknowledgments

We thank Peggy Perkinson for expert preparation of the manuscript. This research was supported by the Journal of Bone and Joint Surgery. Orthopedic Research, and Educational Foundation Grant (H.S.S.).

References

1. Bridge JA, Neff JR, Bhatia PS, Sanger WG, Murphey MD. Cytogenetic findings and biological behavior of GCT of bone cancer. *Cancer*. 1990; 65:2097–2703.
2. Schwartz HS, Butler MG, Jenkins RB, Miller DA, Moses HL. Telomeric associations and consistent growth factor over expression detected in GCT. *Cancer Genet Cytogenet*. 1991; 50:203–276.
3. Bridge JA, Neff JR, Movron BJ. GCT: Chromosomal analysis of 48 specimens and review of the literature. *Cancer Genet Cytogenet*. 1992; 58:2–13. [PubMed: 1728946]
4. Fett-Conte AC, Liedtke H Jr, Chavey H, Thome EH. Telomeric fusions in Wilms' tumor. *Cancer Genet Cytogenet*. 1993; 69:141–145. [PubMed: 8402554]
5. Allshire RC, Dempster M, Hastie ND. Human telomeres at least three types of G-rich repeats distributed non-randomly. *Nucleic Acids Res*. 1989; 17:4611–4627. [PubMed: 2664709]
6. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RF, Wu J-R. A highly conserved repetitive DNA sequence (TTAGGG)_n present at the telomeres of human chromosome. *Proc Natl Acad Sci USA*. 1988; 85:6622–6626. [PubMed: 3413114]
7. Schwartz HS, Dahir GA, Butler MG. Telomere reduction in giant cell tumor of bone and with aging. *Cancer Genet Cytogenet*. 1993; 71:132–138. [PubMed: 8281516]
8. Harley CB, Futcher AB, Greider CW. Telomeres shorten during aging of human fibroblasts. *Nature*. 1990; 345:458–460. [PubMed: 2342578]
9. Blackburn EH. Telomeres and their synthesis. *Science*. 1990; 249:489–490. [PubMed: 2200120]
10. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with aging. *Nature*. 1990; 346:806–868.
11. Adamson DJA, King DJ, Haites NE. Significant telomere shortening in childhood leukemia. *Cancer Genet Cytogenet*. 1992; 61:204–206. [PubMed: 1638505]
12. Butler MG, Dahir GA, Schwartz HS. Molecular analysis of transforming growth factor beta in giant cell tumor of bone. *Cancer Genet Cytogenet*. 1993; 60:108–112.
13. Numberg P, Thiel G, Weber F, Epplen JT. Changes of telomere lengths in human intracranial tumors. *Hum Genet*. 1993; 90:190–192.
14. Hiyama E, Hiyama K, Yokoyama T, Ichikawa T, Matsura Y. Telomeric repeats in neuroblastoma: correlation with prognosis and other biological characteristics. *Jpn J Oncol Res*. 1992; 83:159–164.

15. Allsopp RC, Vaziri H, Patterson I, Goldstein S, Younglai EV, Futcher AB, Greider CW, Horley CB. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci USA*. 1992; 89:10114–10118. [PubMed: 1438199]
16. Wright WE, Pereira-Smith DM, Shay JW. Reversible cellular senescence. Implications for immortalization of normal human diploid fibroblasts. *Mol Cell Biol*. 1989; 9:3088–3092. [PubMed: 2779554]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 1.

Autoradiograph of radiolabeled telomere probe (TTAGGG)₅₀ to *HindIII*-digested genomic DNA (5 µg per lane) from 20 individuals with a variety of malignancies and their control leukocytes. DNA from smaller telomere regions migrate farther by electrophoresis and thus show a longer signal length. Peak migration distance was determined by densitometry and average kilobase size of the telomeric fragments determined by comparison to the migration distance of fragments of a known DNA marker [lambda DNA digested with *HindIII*). The appropriate locations of the high-weight marker fragments (in kilobases) are shown on the vertical axis for the known markers. Lane 1, glioblastoma; Lane 2, control for glioblastoma; Lane 3, chordoma; Lane 4, control for chordoma; Lane 5, medulloblastoma; Lane 6, control for medulloblastoma; Lane 7, astrocytoma; Lane 8, control for astrocytoma; Lane 9, colon cancer; Lane 10, control for colon cancer; Lane 11, Wilms' tumor; Lane 12, control for Wilms' tumor; Lane 13, lung cancer; Lane 14, control for lung cancer; Lane 15, giant cell tumor of bone; Lane 16, control for giant cell tumor of bone; Lane 17, malignant melanoma; Lane 18, control for malignant melanoma; Lane 19, breast cancer; and Lane 20, control for breast cancer.

Table 1

Clinical and telomere data from 39 individuals with malignancies

Malignancy	N	Age		Average peak migration mm (± SD)		Average telomere size kb (± SD)		Matched t-test value	average peak migration
		Years	Mean	Tumor	Control	Tumor	Control		
Musculoskeletal									
Giant cell tumor of bone	8	20-58	36	32.7 (1.2)	29.3 (2.2)	5.6 (0.8)	6.9 (2.1)	1.91 ^{a,-}	
Chondrosarcoma	1	11	11	26.3	23.9	13.3	15.0	NT	
Chordoma	4	36-71	55	16.8 (12.1)	30.7 (2.3)	22.1 (12.7)	9.2 (1.0)	2.86 ^{a,+}	
Epithelial									
Breast	4	46-71	60	24.9 (18.8)	19.8 (1.4)	20.8 (10.9)	18.9 (1.6)	0.50	
Lung	3	44-63	54	35.7 (1.4)	37.3 (2.3)	8.9 (0.5)	8.5 (0.7)	0.78	
Ovary	2	49-55	52	32.6	36.8	10.2 (2.3)	8.6 (0.1)	1.20	
Colon	2	59-08	63	26.2	20.3	16.0 (1.4)	25.2 (4.6)	8.49 ^{a,-}	
Renal	1	71	71	41.3	37.7	7.5	8.4	NT	
Cranial									
Glioblastoma	4	47-01	54	57.1 (16.8)	30.2 (4.1)	5.0 (2.6)	10.9 (1.8)	3.23 ^{a,-}	
Medulloblastoma	1	17	17	33.0	26.3	9.7	13.8	NT	
Astrocytoma	1	2	2	35.6	28.3	8.8	12.0	NT	
Other									
Neuroblastoma	4	1-27	7	12.6 (2.7)	10.0 (3.6)	32.8 (7.8)	40.2 (10.0)	1.39	
Wilms' tumor	2	3-30	16	73.8	26.7	3.2 (0.8)	13.6 (4.7)	20.43 ^{a,-}	
Pheochromocytoma	1	46	46	35.3	35.8	9.4	8.8	NT	
Malignant melanoma	1	43	43	10.8	19.9	26.0	26.0	NT	

abbreviations:

^a neoplasm telomere elongation;⁻ neoplasm telomere reduction; NT not tested.^a $p < 0.05$ (t-tailed) for matched t-test value.