# Telomere length and telomerase activity in canine mammary gland tumors

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**Objective**—To measure telomere length and telomerase activity in naturally occurring canine mammary gland tumors.

**Sample Population**—27 mammary gland tumor specimens obtained during resection or necropsy and 12 mammary gland tissue specimens obtained from healthy (control) dogs.

**Procedure**—Telomere length in tissue specimens was measured by use of restriction endonuclease digestion and Southern blot analysis. Telomerase activity was measured by use of a telomeric repeat amplification protocol assay.

**Results**—Telomere length in mammary gland tumors ranged from 11.0 to 21.6 kilobase pairs (kbp; mean ± SEM, 14.5 ± 0.5 kbp) but did not differ among tumor types. Telomeres in mammary gland tumors were slightly shorter than in normal tissue specimens, but telomere length could not be directly compared between groups, because mean age of dogs was significantly different between groups. Age was negatively correlated with telomere length in control dogs but was not significantly correlated with length in affected dogs. Telomerase activity was detected in 26 of 27 mammary gland tumors and in 4 of 12 normal tissue specimens. However, telomerase activity and telomere length were not correlated in tumor specimens.

**Conclusions and Clinical Relevance**—Telomere length is maintained in canine mammary gland tumors regardless of the age of the affected dog. Measurement of telomere length may be a useful tool for monitoring the in vivo effects of telomerase inhibitors in dogs with tumors. (*Am J Vet Res* 2001; 62:1539–1543)

Telomeres are specific structures at the end of linear chromosomes that protect chromosomes from DNA degradation, end-to-end fusion, and rearrangement.[1] In vertebrates, telomeres consist of highly conserved tandem repeats of guanine-rich sequences such as TTAGGG.[2,3] At the end of telomeric DNA, there is a 3' overhang comprising a variable number of guanine residues. The length of this overhang is 150 to 200 nucleotides in humans.[4] The overhang inserts into double-stranded DNA, and the double-stranded DNA is then looped back at the end of the telomere.[5] Telomeric structure and the presence of telomeric binding proteins are considered important for telomere function.[5-7]

Conventional DNA polymerases synthesize DNA in the 5' to 3' direction and require an RNA primer for initiation. This means that DNA polymerase cannot synthesize a complete daughter strand of DNA at the end of lagging strands. Therefore, telomeres become progressively shorter with repeated cell divisions. This decrease in telomere length results in the inability of cells to divide and triggers cell senescence.[8-12] For these reasons, normal somatic cells die after a certain number of cell divisions. However, tumor cells can continue to divide because of telomere-maintenance mechanisms (eg, telomerase) that add new telomeric sequences to the end of chromosomal DNA.[13-15] Telomerase activity is detected in many naturally occurring tumors in dogs[16,17] and humans[18,19] but is undetectable or low in most somatic cells. Results of some studies[20-22] indicate that telomerase inhibitors may have potential as new anticancer drugs. If telomerase inhibitors are to be used to treat tumors, measurement of telomere length may be a useful technique to monitor drug efficacy.

Mammary gland tumors are the most common neoplasm in female dogs.[23-25] The incidence of mammary gland tumors in dogs is higher than that in any other domestic animal and is 3 times higher than that in humans.[23,25] Approximately half of mammary gland tumors in dogs are malignant, and by the time of diagnosis, approximately half of the malignant tumors have metastasized.[23-25] Resection is an effective treatment if metastasis has not occurred, but prognosis is poor when metastatic disease is detected. Although several chemotherapeutic drugs have been used for the treatment of metastatic mammary gland tumors in dogs, their efficacy is limited.[23-25] We and others have recently described telomerase activity in mammary gland and other tumors in dogs,[16,17,26] but to our knowledge, telo mere length in canine tumors has not been reported.

The telomere systems of humans and rodents differ. For example, murine somatic cells normally have telomerase activity[27,28] and murine telomeres are > 30 **kilobase pairs (kbp)**, whereas human telomeres are typically 5 to 15 kbp.[29,30] Moreover, 2 cooperating oncogenes, *ras* or *myc* and large T antigen, can transform rodent cells,[31-33] whereas expression of a telomerase cat-

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alytic subunit (hTERT) is required in addition to these oncogenes to transform human cells.[34] These differences between murine and human cells make the murine telomeric system an unlikely animal model for the human system. Therefore, another model is required for the continued study of telomere function and telomerase activity in humans and other animals. The purpose of the study reported here was to measure telomere length and telomerase activity of naturally occurring mammary gland tumors in dogs.

## Materials and Methods

**Tissue samples**—Specimens were obtained during resection or necropsy at the Veterinary Medical Center, University of Tokyo from 27 dogs with mammary gland tumors. Tumor types included benign mixed tumors (n = 8), adenoma (7), malignant mixed tumors (6), adenocarcinoma (5), and malignant myoepithelioma (1). As controls, specimens of normal mammary gland tissue were obtained from 12 healthy dogs of various ages that were used in other unrelated research protocols at Central Research Laboratories, Nippon Zenyaku Kogyo. All tissue specimens were washed with saline (0.9% NaCl) solution immediately after collection, rapidly frozen in liquid nitrogen, and stored at –80 C until analyzed.

**Preparation of genomic DNA**—Frozen tissue specimens immersed in liquid nitrogen were homogenized with a mortar and a pestle, and the samples were incubated at 37 C overnight in lysis buffer (10 m*M* Tris-HCl [pH 8.0], 1 m*M* EDTA [pH 8.0], 0.5% SDS, 20 mg of proteinase K/ml, and 10 mg of RNase A/ml). After phenol-chloroform extraction, genomic DNA was precipitated with ethanol, dissolved in TE buffer (10 m*M* Tris-HCl [pH 8.0] and 1 m*M* EDTA [pH 8.0]), and stored at 4 C for less than 1 month until used for Southern blot analysis.

**Measurement of telomere length**—Telomere length was measured by use of Southern blot analysis as described by Harley et al[11] with a slight modification. In a preliminary study, telomere length of normal canine genomic DNA was evaluated by use of this method after restriction endonuclease digestion with various combinations of endonucleases that recognize 4-base sequences[a] (single digestion: *Hin*fI, *Msp*I, and *Rsa*I; double-digestion: *Hin*fI/*Alu*I, *Hin*fI/*Mbo*I, *Hin*fI/*Rsa*I, *Msp*I/*Alu*I, *Msp*I/*Mbo*I, *Msp*I/*Rsa*I, *Rsa*I/*Alu*I, and *Rsa*I/*Mbo*I). Sizes of the telomere-specific hybridization signals were similar regardless of endonuclease used, although the size and intensity of nonspecific signals varied among the digests. Genomic DNA digested with *Hin*fI/*Rsa*I yielded distinct telomere signals apart from the nonspecific signals. Therefore, to measure telomere length in the present study, we digested 5 µg of genomic DNA with *Hin*fI/*Rsa*I for Southern blot analysis. Digested genomic DNA was then extracted with phenol-chloroform, precipitated with ethanol, and separated by use of pulse-field gel electrophoresis[b] (1.0% agarose gel in 0.5X TBE buffer [0.045*M* Tris-borate and 0.001*M* EDTA]). Electrophoresis was performed, using a 180-V forward and 120-V reverse field strength, at 14 C for 16 hours with a ramped pulse time from 0.1 to 0.8 seconds. After electrophoresis, DNA was transferred to a nylon filter membrane,[c] and the membrane was incubated with a $^{32}$P-labeled telomeric DNA probe ($[TTAGGG]_4$) at 40 C for 16 hours in hybridization solution (5X SSC [1X SSC, 0.15*M* NaCl and 0.015*M* sodium citrate], 1% SDS, 50 m*M* Tris-HCl [pH 7.6], 100 µg of salmon testis DNA/ml, and 5X Denhardt solution). After hybridization, membranes were washed at 40 C with a solution containing 0.4X SSC and 0.1% SDS and exposed to x-ray film with an intensifying screen. The peak hybridization signal was detected by use of densitometric software,[d] and the mean length of telomeres that yielded a positive signal was determined. Mean telomere length for genomic DNA from each tumor or normal tissue specimen was determined from results of 3 independent Southern blot analyses.

**Determination of telomerase activity**—Telomerase activity in tissue specimens was measured by use of a commercially available **polymerase chain reaction (PCR)**-based assay referred to as the **telomeric repeat amplification protocol (TRAP)** assay.[18,e] This assay was originally developed for measurement of telomerase activity in human tissue specimens. However, because the nucleotide sequence added by telomerase is conserved in vertebrates,[2] we used this assay to measure canine telomerase activity.

Briefly, frozen tissue specimens were homogenized in buffer containing 0.5% 3-[(3-cholamidopropyl) dimethylammonio)]-1-propanesulfonate (CHAPS), 0.1 m*M* benzamidine, 5 m*M* β-mercaptoethanol, 10 m*M* Tris-HCl (pH 7.5), 1 m*M* magnesium chloride, 1 m*M* egtazic acid (EGTA), and 10% glycerol. After incubation on ice for 30 minutes, homogenized specimens were centrifuged, and the supernatant (tissue extract) was rapidly frozen in liquid nitrogen and stored at –80 C. Protein concentration in tissue extract was measured by use of a Bradford assay,[f] and 2 µg of protein was subsequently used for each TRAP assay.

Two microliters of tissue extract (2 µg of protein) was incubated at 30 C for 30 minutes in 50 µl of the reaction mixture supplied by the manufacturer, which contained all 4 dNTP, $^{32}$P-labeled TS primer (5'-AATCCGTCGAGCAGAGTT-3'), RP primer (this primer anneals to the TTAGGG repeats in the next PCR step), K1 primer (this primer is used for amplification of the internal control in the next PCR step), TSK1 template (this oligonucleotide serves as the internal control in the next PCR step), and *Taq* polymerase.[g] During the first 30-minute incubation, telomerase in tissue extracts acted to add telomeric repeats to the 3'-end of the labeled TS primer. This mixture was then subjected to 27 cycles of amplification consisting of denaturation at 94 C for 30 seconds and annealing and polymerization at 60 C for 30 seconds. During the amplification process, the extended TS primer was amplified, using the TS and RP primers. Amplification generated a ladder of oligonucleotides. The TSK1 template was amplified with TS and K1 primers. Amplification of control reagents resulted in production of an **internal telomerase assay standard (ITAS)**, which was used to estimate the degree of contamination with inhibitors of *Taq* polymerase or the efficiency of PCR amplification for each sample. As a negative control, an additional aliquot of each tissue extract was heat-inactivated at 85 C for 10 minutes prior to the TRAP assay. Because telomerase is a heat-sensitive enzyme, heating the tissue extract eliminates telomerase activity, and assuming that there is no contamination, amplification products will not be detected.

To quantitate telomerase activity, the TRAP assay was performed, using the TSR8 control template (TS primer + 8 telomeric repeats; 5'-AATCCGTCGAGCGAGAGTTAG(GGTTAG)$_7$-3') instead of the tissue extract. Amplification products were separated by use of electrophoresis (12.5% nondenaturing polyacrylamide gel). The gel was dried on a filter paper and exposed to an x-ray film with an intensifying screen. Signal intensity of amplified products was determined by use of a densitometer,[d] and telomerase activity (**total product generated [TPG]**) in tissue extracts was determined according to the formula:

$$TPG\ (U/2\ \mu g\ of\ protein) = [(X - X_0)/C]/[(r - r_0)/CR] \times 100$$

where X, $X_0$, r, and $r_0$ are the signal intensities of the oligonucleotide ladder derived from the tissue extract, heat-inactivated tissue extract, TSR8 control template, and lysis



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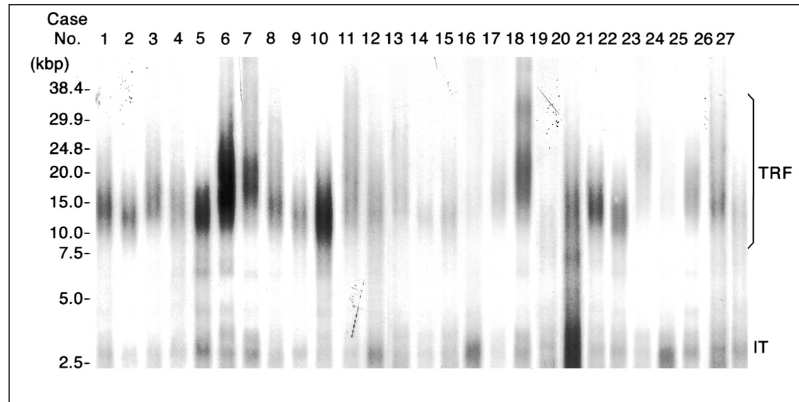


Figure 1—Southern blot of genomic DNA from 27 canine mammary gland tumor specimens after restriction endonuclease digestion with *Hin*fI and *Rsa*I. Digested DNA was separated by use of pulse-field gel electrophoresis, transferred to a nylon membrane, and hybridized with a $^{32}$P-labeled telomeric DNA probe. Hybridization signals of telomeres (TRF) and telomere-like internal sequences (IT) are indicated on the right side of blot; lengths in kilobase pairs (kbp) are indicated on the left.

buffer, respectively, and C and CR are the signal intensities of ITAS derived from the tissue extract and TSR8 control template, respectively. Telomerase activities in 19 of the 27 mammary gland tumor specimens were reported in a previous study.[17]

**Statistical analyses**—Correlations between telomere length and dog's age and between telomere length and telomerase activity were assessed by use of linear regression. Mean age of dogs with mammary gland tumors was compared with that of healthy dogs by use of an unpaired Student *t*-test. For all tests, $P \leq 0.05$ was considered significant.

## Results

Telomere length in the 27 mammary gland tumor specimens ranged from 11.0 to 21.6 kbp (mean ± SEM, 14.5 ± 0.5 kbp; **Fig 1 and Table 1**), whereas length in the 12 normal tissue specimens ranged from 15.4 to 20.6 kbp (18.0 ± 0.5 kbp; **Table 2**). Telomerase activity was detected in 26 of 27 mammary gland tumors and ranged from 6 to 546 U/2 µg of protein. However, telomerase activity was detected in only 4 of 12 normal tissue specimens. Three of these specimens yielded low activity (2 to 30 U/2 µg of protein), and 1 specimen from a dog in late gestation yielded high activity (556 U/2 µg of protein).

Mean length of telomeres in mammary gland tumors was less than that in normal mammary gland tissue. However, mean age was significantly ($P < 0.001$) different between groups. Dogs with tumors ranged from 3 to 14 years old (mean ± SEM, 10.3 ± 0.5 years old), and healthy dogs ranged from 1 to 7 years old (3.8 ± 0.62 years old). Therefore, length of telomeres in normal mammary gland tissue specimens could not be directly compared with that of telomeres in mammary gland tumors. In normal mammary gland tissue specimens, age was negatively correlated ($r = -0.69$, $P < 0.02$) with telomere length, such that for every 1-year increase in age, telomere length decreased by 0.6 kbp. In contrast, age of dogs with mammary gland tumors was not significantly ($r = -0.22$; $P = 0.28$) correlated with telomere length **(Fig 2)**.

Mean telomere lengths in benign mixed tumors, adenomas, malignant mixed tumors, and adenocarcinomas were 14.5, 13.4, 14.7, and 15.8 kbp, respectively. Telomere length did not significantly differ among the types of mammary gland tumors evaluated.

Telomerase activity was detected in all but 1 of the mammary gland tumor specimens; however, activity varied among specimens. No significant ($P = 0.19$) correlation was detected between telomere length and telomerase activity in mammary gland tumor specimens (Table 1).

Table 1—Telomere length and telomerase activity in canine mammary gland tumor specimens

| Tumor type | Telomere length (kbp) | Telomerase activity (U/2 µg of protein) |
|---|---|---|
| Benign mixed | | |
| 8-year-old (F)* | 13.4 | 225† |
| 14-year-old (F) | 11.3 | 37† |
| 12-year-old (F) | 15.2 | 28† |
| 11-year-old (F) | 14.1 | 91† |
| 12-year-old (F) | 12.7 | 72† |
| 7-year-old (F) | 17.6 | 28† |
| 8-year-old (F) | 17.7 | 196† |
| 9-year-old (F) | 14.7 | 61† |
| Adenoma | | |
| 9-year-old (F) | 12.9 | 145† |
| 12-year-old (SF) | 12.4 | 57† |
| 11-year-old (F) | 15.6 | 6† |
| 11-year-old (F) | 13.6 | 238† |
| 3-year-old (M) | 15.6 | 546 |
| 11-year-old (F) | 12.4 | 430 |
| 8-year-old (F) | 11.4 | 461 |
| Malignant mixed | | |
| 7-year-old (F) | 16.8 | 38† |
| 14-year-old (F) | 14.8 | ND† |
| 9-year-old (F) | 20.0 | 146† |
| 12-year-old (F) | 10.9 | 95 |
| 9-year-old (F) | 13.1 | 45 |
| 7-year-old (F) | 12.7 | 250 |
| Adenocarcinoma | | |
| 13-year-old (F) | 11.3 | 390† |
| 12-year-old (F) | 21.6 | 148† |
| 12-year-old (SF) | 15.5 | 9† |
| 14-year-old (SF) | 16.1 | 38† |
| 10-year-old (F) | 14.2 | 204 |
| Myoepithelioma | | |
| 14-year-old (F) | 12.7 | 481 |

*Age (sex) of dog from which tumor specimens were obtained. †Values were reported in a previous study.[17]

kbp = Kilobase pairs. F = Female. SF = Spayed female. M = Male. ND = Not detected (ie, < 1 U/2 µg of protein).



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Table 2—Telomere length and telomerase activity in mammary gland tissue specimens obtained from healthy female dogs

| Age (y) | Telomere length (kbp) | Telomerase activity (U/2 µg of protein) |
|---|---|---|
| 1 | 16.8 | 6 |
| 1 | 19.8 | 30 |
| 2 | 19.8 | ND |
| 2 | 19.9 | ND |
| 3 | 20.6 | ND |
| 3 | 19.2 | ND |
| 4 | 19.3 | ND |
| 4 | 16.9 | ND |
| 5 | 16.4 | 556 |
| 6 | 15.5 | ND |
| 7 | 15.4 | ND |
| 7 | 16.9 | 2 |

*See* Table 1 for key

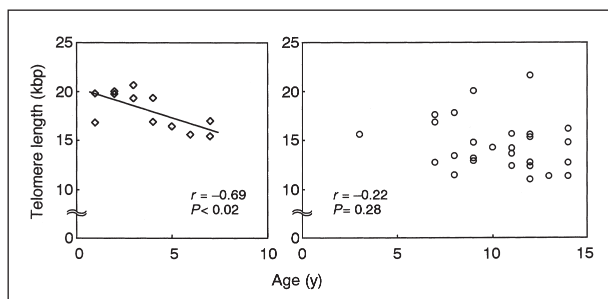


Figure 2—Telomere length in specimens of mammary gland tissue from 12 healthy dogs (left) and mammary gland tumors from 27 dogs (right) versus age of dog. In healthy dogs, age was negatively correlated with telomere length, such that for every 1-year increase in age, telomere length decreased by 0.6 kbp.

## Discussion

We found that telomere length in the canine mammary gland tumors ranged from 11.0 to 21.6 kbp. To examine the association of telomeres with tumorigenesis, telomere length should be compared between affected and unaffected tissue specimens from the same animal. However, because most of the resected mammary gland tissue from the dogs described in this report was infiltrated with tumor cells, control tissue specimens could not be obtained. Therefore, we measured telomere lengths of mammary gland tissue specimens obtained from healthy dogs maintained for experimental purposes unrelated to the present study. Because these dogs are seldom maintained until they reach ages similar to those of dogs with mammary gland tumors, our control group comprised relatively young dogs. Mean age was significantly different between groups, so we could not directly compare telomere length in normal mammary gland tissue specimens with that in mammary gland tumors. Telomere length in normal mammary gland tissue became progressively less with age. In contrast, telomere length in the mammary gland tumors was not correlated with age. Because tumor specimens were likely composed of cells after multiple cell divisions, these findings suggest that telomere length is maintained in canine mammary tumors by some mechanism. Telomerase activity was detected in most of the tumor specimens in this study, indicating that telomerase may play an important role in the maintenance of telomere length in canine mammary gland tumors.

Although maintenance of telomere length in mammary gland tumors may be mediated by telomerase activity, other mechanisms should also be considered, because we did not detect a significant correlation between telomere length and telomerase activity in tumor specimens. Several telomeric binding proteins such as **telomeric repeat binding factor (TRF)** 1 and TRF2 have been isolated, and expression of these proteins has been shown to affect telomere length.[6,7] The structure of telomeric DNA may also affect the length.[5] Thus, it is conceivable that high telomerase activity is not directly associated with telomere elongation. In fact, although telomerase activity was not detected in 1 tumor specimen and was low (< 10 U/2 mg of protein) in 2 specimens, telomere lengths in these specimens were maintained. A telomerase-independent mechanism for maintaining telomere length may have been operational in these specimens: such a mechanism has been described in some human tumors and tumor cell lines.[35] In human cells, the extreme ends of chromosomes are tucked into the double-strand repeat thus forming a loop.[5] Therefore, it is conceivable that embedded single-strand DNA can be elongated by conventional DNA polymerase, using the double-strand repeat as a template. Also, telomere recombination in yeast is considered a mechanism for maintaining telomere length.[36,37]

Although telomerase activity was not detected in 8 of the 12 normal mammary gland tissue specimens, low activity was detected in 3 specimens and high activity in 1 specimen. Low telomerase activity may have resulted from activity of telomerase in mononuclear cells within these specimens. However, the presence of mononuclear cells does not explain the high activity in 1 specimen. The normal tissue specimen with high telomerase activity was obtained from a pregnant dog, and we believe that the proliferation of mammary gland epithelial cells during gestation resulted in increased expression of telomerase. Telomerase has been shown to be up-regulated in lymphocytes and hematopoietic cells after mitogenic stimulation.[38]

Because telomerase activity has been detected in a diverse range of tumor types in dogs[16,17] and humans,[18,19] telomerase inhibitors may have potential as novel anticancer drugs.[20-22] The effect of telomerase inhibitors on arrest of cell proliferation or on cell death cannot be evaluated until the telomere is short enough to interfere with cell division. Therefore, measurement of telomere length may be a useful tool for monitoring the in vivo effects of telomerase inhibitors.

The telomere lengths in normal canine mammary gland tissue (range, 15.4 to 20.6 kbp) and mammary gland tumors (11.0 to 21.6 kbp) that we detected were greater than those reported in humans.[39] In humans, telomeres in normal mammary gland tissue ranged from 8 to 15 kbp, and those in mammary gland tumors were of similar length or slightly shorter. Humans are unique in terms of telomere length, because telomeres are shorter, compared with any other mammal.[40] In dogs as in humans, high telomerase activity is typically only detected in neoplastic cells,[39,41] whereas in rodents, somatic cells have high telomerase activity[27,28] and much longer telomeres (> 30 kbp) than human



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somatic cells.[29,30] Moreover, the relationship between telomere length, telomerase activity, and tumorigenesis is clearly different between humans and rodents.[34,42] Therefore, dogs may be a more desirable model than rodents for the study of the telomere and telomerase systems in humans.

[a]New England Biolabs, Beverly, Mass.
[b]FIGE Mapper System, Bio-Rad Laboratories, Hercules, Calif.
[c]Biodyne B membrane, Pall BioSupport, East Hills, NY.
[d]Molecular Analyst, Bio-Rad Laboratories, Hercules, Calif.
[e]TRAPeze telomerase detection kit, Intergen, Purchase, NY.
[f]Bio-Rad protein assay kit, Bio-Rad Laboratories, Hercules, Calif.
[g]Takara, Kyoto, Japan.

## References


1. Blackburn EH. The molecular structure of centromeres and telomeres. *Annu Rev Biochem* 1984;53:163–194.
2. Meyne J, Ratliff RL, Moyzis RK. Conservation of the human telomere sequence (TTAGGG)n among vertebrates. *Proc Natl Acad Sci U S A* 1989;86:7049–7053.
3. Moyzis RK, Buckingham JM, Cram LS, et al. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci U S A* 1988;85:6622–6626.
4. Makarov VL, Hirose Y, Langmore JP. Long G tails at both ends of human chromosomes suggest a C strand degradation mechanism for telomere shortening. *Cell* 1997;88:657–666.
5. Griffith JD, Comeau L, Rosenfield S, et al. Mammalian telomeres end in a large duplex loop. *Cell* 1999;97:503–514.
6. Broccoli D, Smogorzewska A, Chong L, et al. Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2. *Nat Genet* 1997;17:231–235.
7. van Steensel B, Smogorzewska A, de Lange T. TRF2 protects human telomeres from end-to-end fusions. *Cell* 1998;92:401–413.
8. Allsopp RC, Vaziri H, Patterson C, et al. Telomere length predicts replicative capacity of human fibroblasts. *Proc Natl Acad Sci U S A* 1992;89:10114–10118.
9. Bodnar AG, Ouellette M, Frolkis M, et al. Extension of lifespan by introduction of telomerase into normal human cells. *Science* 1998;279:349–352.
10. Counter CM, Avilion AA, LeFeuvre CE, et al. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J* 1992;11:1921–1929.
11. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990;345:458–460.
12. Zakian VA. Telomeres: beginning to understand the end. *Science* 1995;270:1601–1607.
13. Feng J, Funk WD, Wang SS, et al. The RNA component of human telomerase. *Science* 1995;269:1236–1241.
14. Morin GB. The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. *Cell* 1989;59:521–529.
15. Nakamura TM, Morin GB, Chapman KB, et al. Telomerase catalytic subunit homologs from fission yeast and human. *Science* 1997;277:955–959.
16. Biller BJ, Kitchell BE, Cadile CD. Evaluation of an assay for detecting telomerase activity in neoplastic tissues of dogs. *Am J Vet Res* 1998;59:1526–1529.
17. Yazawa M, Okuda M, Setoguchi A, et al. Measurement of telomerase activity in dog tumors. *J Vet Med Sci* 1999;61:1125–1129.
18. Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994;266:2011–2015.
19. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997;33:787–791.
20. Fletcher TM, Salazar M, Chen SF. Human telomerase inhibition by 7-deaza-2'-deoxypurine nucleoside triphosphates. *Biochemistry* 1996;35:15611–15617.
21. Norton JC, Piatyszek MA, Wright WE, et al. Inhibition of human telomerase activity by peptide nucleic acids. *Nat Biotechnol* 1996;14:615–619.
22. Strahl C, Blackburn EH. Effects of reverse transcriptase inhibitors on telomere length and telomerase activity in two immortalized human cell lines. *Mol Cell Biol* 1996;16:53–65.
23. Dorn CR, Taylor DO, Schneider R, et al. Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. *J Natl Cancer Inst* 1968;40:307–318.
24. Kurzman ID, Gilbertson SR. Prognostic factors in canine mammary tumors. *Semin Vet Med Surg (Small Anim)* 1986;1:25–32.
25. Priester WA, Mantel N. Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. *J Natl Cancer Inst* 1971;47:1333–1344.
26. Spangler EA, Rogers KS, Thomas JS, et al. Telomerase enzyme activity as a diagnostic tool to distinguish effusions of malignant and benign origin. *J Vet Intern Med* 2000;14:146–150.
27. Prowse KR, Greider CW. Developmental and tissue-specific regulation of mouse telomerase and telomere length. *Proc Natl Acad Sci U S A* 1995;92:4818–4822.
28. Wright WE, Piatyszek MA, Rainey WE, et al. Telomerase activity in human germline and embryonic tissues and cells. *Dev Genet* 1996;18:173–179.
29. Kipling D, Cooke HJ. Hypervariable ultra-long telomeres in mice. *Nature* 1990;347:400–402.
30. Kipling D. Telomere structure and telomerase expression during mouse development and tumorigenesis. *Eur J Cancer* 1997;33:792–800.
31. Land H, Parada LF, Weinberg RA. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 1983;304:596–602.
32. Rassoulzadegan M, Cowie A, Carr A, et al. The roles of individual polyoma virus early proteins in oncogenic transformation. *Nature* 1982;300:713–718.
33. Ruley HE. Adenovirus early region 1A enables viral and cellular transforming genes to transform primary cells in culture. *Nature* 1983;304:602–606.
34. Hahn WC, Counter CM, Lundberg AS, et al. Creation of human tumour cells with defined genetic elements. *Nature* 1999;400:464–468.
35. Bryan TM, Englezou A, Dalla-Pozza L, et al. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat Med* 1997;3:1271–1274.
36. Lundblad V, Blackburn EH. An alternative pathway for yeast telomere maintenance rescues est1- senescence. *Cell* 1993;73:347–360.
37. Nakamura TM, Cooper JP, Cech TR. Two modes of survival of fission yeast without telomerase. *Science* 1998;282:493–496.
38. Hiyama K, Hirai Y, Kyoizumi S, et al. Activation of telomerase in human lymphocytes and hematopoietic progenitor cells. *J Immunol* 1995;155:3711–3715.
39. Hiyama E, Gollahon L, Kataoka T, et al. Telomerase activity in human breast tumors. *J Natl Cancer Inst* 1996;88:116–122.
40. Kakuo S, Asaoka K, Ide T. Human is a unique species among primates in terms of telomere length. *Biochem Biophys Res Commun* 1999;263:308–314.
41. Tsao J, Zhao Y, Lukas J, et al. Telomerase activity in normal and neoplastic breast. *Clin Cancer Res* 1997;3:627–631.
42. Blasco MA, Lee HW, Hande MP, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. *Cell* 1997;91:25–34.




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