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Telomere Lengths and Telomerase Activity in Dog Tissues: A Potential Model System to Study Human Telomere and Telomerase Biology

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Abstract

Studies on telomere and telomerase biology are fundamental to the understanding of aging and age-related diseases such as cancer. However, human studies have been hindered by differences in telomere biology between humans and the classical murine animal model system. In this paper, we describe basic studies of telomere length and telomerase activity in canine normal and neoplastic tissues and propose the dog as an alternative model system. Briefly, telomere lengths were measured in normal canine peripheral blood mononuclear cells (PBMCs), a range of normal canine tissues, and in a panel of naturally occurring soft tissue tumours by terminal restriction fragment (TRF) analysis. Further, telomerase activity was measured in canine cell lines and multiple canine tissues using a combined polymerase chain reaction/enzyme-linked immunosorbent assay method. TRF analysis in canine PBMCs and tissues demonstrated mean TRF lengths to range between 12 and 23 kbp with heterogeneity in telomere lengths being observed in a range of normal somatic tissues. In soft tissue sarcomas, two subgroups were identified with mean TRFs of 22.2 and 18.2 kbp. Telomerase activity in canine tissue was present in tumour tissue and testis with little or no activity in normal somatic tissues. These results suggest that the dog telomere biology is similar to that in humans and may represent an alternative model system for studying telomere biology and telomerase-targeted anticancer therapies. *Neoplasia* (2001) **3,** 351–359.

Keywords: telomere, telomerase, dog, cancer, model.

Introduction

Telomeres are specialised DNA–protein complexes that cap the ends of linear chromosomes. In vertebrates, telomeres consist of tandem repeats of the sequence TTAGGG and a number of telomere-associated proteins. Telomeres maintain genomic integrity by protecting chromosome ends from recombination, fusion, and from being recognised as DNA damage [1–4]. In normal human somatic cells, telomeres shorten with each cell division [5,6]. This telomeric attrition has been attributed to the inability of the DNA replication machinery to efficiently replicate the 5′ ends of linear chromosomes (end replication problem) [7,8]. Thus, telomeres shorten progressively with cell division, and telomere shortening to a critical length has been proposed to limit the lifespan of somatic cells in humans and play an important role in the process of cellular senescence. Consistent with this model, telomere shortening has been observed *in vivo* during ageing of normal somatic tissues as well as *in vitro* cultured human fibroblasts [9–11]. In addition, cells from patients with premature ageing syndromes show evidence of premature telomeric erosion [12,13].

In cells that proliferate indefinitely, such as germline cells and transformed cells, the enzyme telomerase provides a mechanism for the maintenance of telomere length. Telomerase is a ribonucleoprotein reverse transcriptase [4] capable of synthesising terminal TTAGGG telomeric repeats, thereby extending telomere length and compensating for telomeric attrition during replication.

In adult human somatic tissues, telomeres are approximately 10 to 15 kbp [14] and telomerase activity is low or undetected in normal tissues but present in germ cells, activated leukocytes, and stem cells from a variety of organs [15–17]. Telomerase activity has been also detected in more than 80% of cancer tissues *in vivo* and immortal cell lines [18], suggesting that such tumours are dependent on telomerase activity for their continued proliferation. In mice, however, the situation is quite different; mice possess much longer telomeres, and telomerase activity appears to be less stringently regulated in somatic tissues [19–21].

Few studies have addressed telomere and telomerase biology in the dog. The aim of this study was to investigate

Abbreviations: ELISA, enzyme-linked immunosorbent assay; kbp, kilobase pair; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; TRF, terminal restriction fragment

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telomere lengths and telomerase activity in a range of canine tissues including normal and tumour tissues. Terminal restriction fragment (TRF) analyses were employed to study telomeric lengths in 63 canine peripheral blood mononuclear cell (PBMC) samples from dogs of varying breeds and ages including samples from puppies and geriatric dogs. This demonstrated mean TRFs in the range of 12 to 23 kbp. TRF analysis in 18 canine mesenchymal tumours identified two groups; one with a mean TRF of 22.2 kbp and the other with a mean TRF of 18.2 kbp. TRFs were also evaluated in a panel of somatic tissues from the same subject and demonstrated TRF heterogeneity among different tissues. Further, telomeric attrition was demonstrated during the *in vitro* culture of canine primary fibroblasts. Using a combined polymerase chain reaction (PCR)/enzyme-linked immunosorbent assay (ELISA) method, telomerase activity was analysed in multiple adult canine tissues derived from both tumour-bearing and non–tumour-bearing dogs. Telomerase activity was detected in tumour and gonadal tissues with little or no activity present in normal somatic tissues. Telomerase activity was also assessed in a panel of canine immortalised cell lines. High levels of telomerase activity were detected in several canine cell lines. One cell line (MDCK) was negative for enzyme activity, suggesting that an alternative pathway for telomere maintenance may operate in these cell types. Based on our finding, we propose that the dog may represent an alternative model system to study telomere biology in humans and investigate the efficacy of telomerase-targeted therapeutics for cancer.

Materials and Methods

TRF Analysis

Canine blood samples PBMC-derived DNA was obtained from EDTA and heparinised blood samples from canine subjects using standard protocols. A total of 63 dogs of various breeds were studied, ranging in age from 2 months to 15 years of age.

Normal somatic/tumour tissues from the same canine subject Genomic DNA samples were isolated from a canine subject which presented with multicentric lymphoma (case B) and euthanised for medical reasons. Genomic DNA was isolated from several tissues including testis, forelimb muscle, spleen, stomach, liver, adrenal gland, and lymphoma tissue taken at post-mortem. Haematoxylin/eosin sections of normal tissues confirmed them to be tumour-free (data not shown).

Canine soft tissue sarcomas (STS) A total of 18 STS samples were retrieved from the pathology archives of the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, University of Utrecht. Samples had originally be taken either at biopsy, surgical

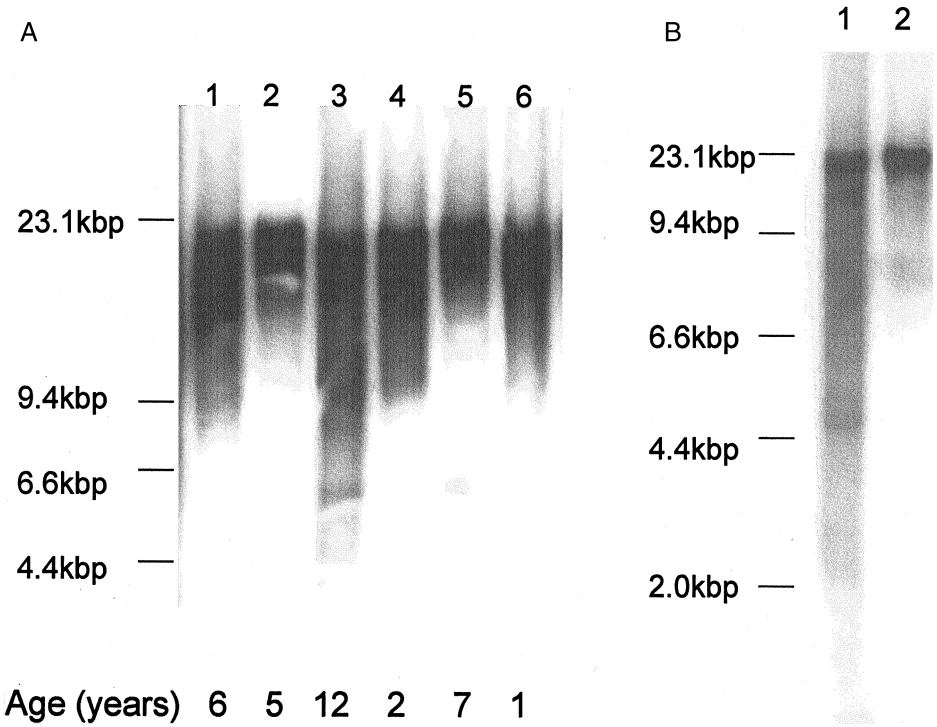

Figure 1. *TRF distribution in canine DNA samples prepared from PBMCs. (A) TRF distribution in a panel of Retrievers of varying ages. The size and position of the molecular weight markers are indicated on the left. Lane 1 mean TRF = 17.9 kbp; lane 2 mean TRF = 20.6 kbp; lane 3 mean TRF = 15.1 kbp; lane 4 mean TRF = 17.4 kbp; lane 5 mean TRF = 21.3 kbp; lane 6 mean TRF = 18.0 kbp (these data are an average of two analyses). (B) TRF distribution showing short telomere lengths. Lane 1, 1-year-old Border Collie (mean TRF = 12 kbp); lane 2, 1-year-old crossbreed (mean TRF = 17.9 kbp).*

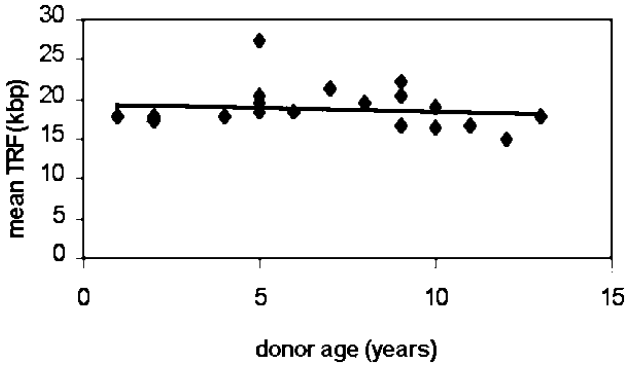

Figure 2. *Plot and regression curve of the mean TRF against donor age in canine Retrievers.*

excision, or at post-mortem and archived at −70°C. Diagnosis was based upon histological analysis using haematoxylin/eosin staining.

Cell culture Dermal fibroblasts were prepared from skin biopsy tissue isolated from an 11-year-old dog at post-mortem. Primary fibroblasts were cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Paisley, UK) supplemented with 10% foetal calf serum and in the presence of 5% CO_2. Population doublings (PDs) were determined by cell counts upon passage.

TRF analysis TRF analysis was performed by Southern Blot Hybridisation using the Telomere Length Kit (BD PharMingen, Oxford, UK). Briefly, high-molecular-weight genomic DNA was isolated from blood and tissue samples by digestion with proteinase K and extraction with phenol/chloroform and verified by gel electrophoresis. Three micrograms of genomic DNA was digested with *Hin*fI and *Rsa*I enzymes at 37°C for 16 hours and subjected to 0.6% agarose gel electrophoresis for 3 hours at 160 V. Digested DNA was then transferred to Hybond N^+ nylon membrane (Amersham Pharmacia, Buckinghamshire, UK) and hybridised with a biotinylated $(TTAGGG)_7$ probe for 16 hours at 55°C. Filters were washed in 2×SSC/0.01% SDS and subjected to chemiluminescence detection and exposed to autoradiography film for 30 seconds. Biotinylated DNA ladders and digested genomic DNA with known mean telomeric repeat lengths were used as size markers. The mean TRF length was calculated following image scanning by integrating the signal intensity above background over the entire TRF distribution as a function of TRF length using the formulae: $L=\sum(OD_lL_l)/\sum(OD_l)$, where OD_l and L_l are the signal intensity and TRF length, respectively, at position I on the gel image. The peak telomere length was taken as being the molecular weight corresponding to peak intensity. The shortest TRF was estimated as the smallest signal that could be detected.

Analysis of Telomerase Activity

Canine tissues Telomerase activity was assessed in a range of tissues derived from three dogs that had been euthanised for medical reasons. Cases A and B were canine subjects which presented with lymphoma and case C was a canine subject which presented with a non-malignant condition. Canine samples examined included thyroid, stomach, spleen, liver, kidney, heart, adrenal gland, forelimb muscle, lymph node, testis, and lymphoma tissues.

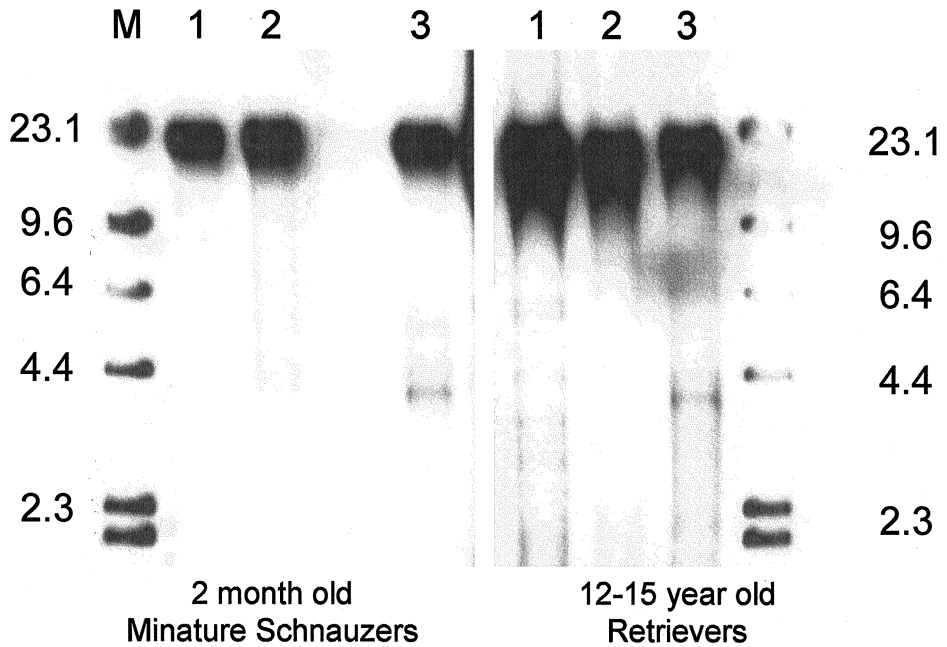

Figure 3. *TRF patterns in PBMC-derived DNA samples from 2-month-old Minature Snauzers pups and geriatric Retrievers (the mean TRFs for each sample are shown in Table 1).*

Table 1. TRF Lengths in PBMC-Derived DNA Samples from Dogs of Varying Ages and Breeds.

Breed	Age	Mean TRF	Peak TRF	Short TRF
(a) TRF lengths in medium-sized canine breeds				
Retriever	1	18	20.7	8.1
Retriever	2	17.8	19.5	8
Retriever	2	17.7	15.3	5
Retriever	2	17.4	23.1	9.6
Retriever	4	17.9	21.7	5
Retriever	4	17.8	23.4	11.8
Retriever	5	27.5	24.9	9
Retriever	5	20.6	21.8	11.5
Retriever	5	19.7	19.3	9.6
Retriever	5	18.4	24.9	9.8
Retriever	6	18.5	21.3	6.9
Retriever	7	21.3	22.8	10.6
Retriever	8	19.7	19.5	12.5
Retriever	9	20.4	21.8	8.4
Retriever	9	16.7	14.9	5
Retriever	9	22.1	29.7	11.8
Retriever	10	19.1	23.4	9.5
Retriever	10	16.4	24.4	11.5
Retriever	11	16.7	19.5	6.1
Retriever	12	15.1	19.2	5.5
Retriever	13	18.02	18.7	10.9
German Shepherd	1	16	20.5	8.9
German Shepherd	1	20	20	7.8
German Shepherd	7	21.2	20.6	11
German Shepherd	8	20.9	23.2	9.9
German Shepherd	9	21.9	20.6	12
Rottweiller	1	19.8	22.8	13.7
Rottweiller	2	19.4	26.1	10.6
Rottweiller	4	24.4	22.8	8.6
Border Collie	1	12	23.6	2.6
Border Collie	7	20.8	30	13.5
Greyhound	9	23	23	9.7
Greyhound	8	21.8	23.4	11.3
Greyhound	9	21.5	23.6	9.7
Greyhound	9	21.6	21	10.1
Crossbreed	1	17.9	26.7	10
Crossbreed	2	16.9	21.4	6.2
Crossbreed	4	19.1	24.1	12.0
Crossbreed	6	19.2	22.9	9.5
Crossbreed	7	27.6	23.8	14.5
Crossbreed	10	19.9	26.7	8.4
Crossbreed	12	19.4	22.9	4.4
Crossbreed	15	17.3	18.2	7
Bull Terrier	3	16.3	18.3	12.2
German Short Hair Pointer	7	20.4	21.5	10
English Setter	5	21.7	22.4	16.9
Lurcher	10	21.9	23.4	12.9
(b) TRF lengths in large-sized canine breeds				
Great Dane	1	14	16.6	5.2
Bull Mastiff	10	21.72	23	16.473
Bermese Mountain Dog	8	23.5	24.5	20
(c) TRF lengths in small-sized canine breeds (ages are given in years, unless stated otherwise)				
Minature Schnauzer	2 months	20.8	20.8	10.2
Minature Schnauzer	2 months	18.5	20.8	9.6
Minature Schnauzer	2 months	19.3	20	10.4
Beagle	4	21.7	21.9	15.2
Beagle	12	18.7	18.5	9.4
Spaniel	1	19.2	22.4	16.5
Spaniel	7	14.8	26.6	6.4
Spaniel	11	23.5	23.5	19.8
Spaniel	12	16	20.2	7
West Highland White Terrier	2	12.3	13.5	5.4
West Highland White Terrier	10	22	30	15
Poodle	8	20.9	23.2	10.4
Papillon	5	19.2	19.7	13.6

Cell lines Telomerase activity was assessed in several canine cell lines: CML10 (melanoma), MDCK (normal kidney), D17 (osteosarcoma), A72 (fibroblastoid tumour), GHK (normal kidney), CMT3 (osteosarcoma), CMT7 (osteosarcoma), and CMT8 (osteosarcoma). In addition, the human telomerase-positive SV40-transformed cell line 293T and the human telomerase-negative GM437 cell lines were used as controls.

Assay for telomerase activity Telomerase activity was measured using the Telomerase PCR/ELISA assay (Roche, East Sussex, UK), following the manufacturer's recommended protocol. Tissue samples/cells were homogenised in 200 μL of ice-cold lysis buffer and incubated for 30 minutes on ice. After centrifugation at 16,000$\times g$ for 20 minutes at 4°C, the supernatant was collected, frozen in liquid nitrogen, and stored at -80°C. Protein concentration was measured using the Bradford assay (Sigma, Dorset, UK). Protein samples were incubated with reaction buffer containing a biotin-labelled P1-TS primer and P2 primer, telomerase substrate, and *Taq* polymerase for 30 minutes at 25°C in a final volume of 50 μL. To each PCR reaction, an internal standard (IS) was included to detect the presence of PCR inhibitors. After further incubation at 94°C for 5 minutes, the resulting mixture was subjected to the PCR for 30 cycles of 30 seconds at 94°C, 30 seconds at 50°C, and 90 seconds at 72°C. The amplification products were denatured and hybridised with a digoxigenin-labelled, telomeric repeat-specific detection probe. The resulting product was immobilised through the biotin-labelled TS primer to a streptavidin-coated microtiter plate and detected with an antidigoxigenin antibody

Table 2. TRFs Values in Somatic and Tumour Tissues from the Same Canine Subject.

Tissue Type	Mean TRF	Peak TRF	Shortest TRF
Metastatic tumour	19.1	20.2	10.3
Lymphoma	22.8	20.9	14.6
Testis	14.4	19.8	4.1
Liver	13.5	14.4	6.9
Adrenal gland	17.8	22.2	9.6
Spleen	18.2	16.6	7.1
Forelimb muscle	21.1	22.8	11.1
Stomach	18.6	20.7	9.3

Table 3. TRF Lengths in Canine Soft Tissue Sarcomas.

Tumour Histological Type	Mean TRF	Peak TRF	Shortest TRF
Narrow TRF range			
Spindle cell tumour	22.2	21.8	16
Liposarcoma	21.4	21.4	13
Malignant fibrous histiocytoma	22.2	21.7	16.8
Fibrosarcoma	20.9	21.7	15.1
Lymphoma	19.1	21.4	13.1
Synovial cell sarcoma	22.2	22.2	13.4
Broad TRF range			
Spindle cell tumour	19.5	20.1	10
Spindle cell tumour	12.8	20.2	3.8
Nephroblastoma	14	19.1	7.6
Leiomyosarcoma	14.7	19.8	7.8
Leiomyosarcoma	17.5	20.8	7
Leiomyosarcoma	19.4	20.1	7
Leiomyosarcoma	16.7	21.8	6.1
Anaplastic sarcoma	18.3	22.2	6.6
Hemangiosarcoma	20	22.4	3.9
Chondrosarcoma	17.6	18.9	11.5
Neurofibrosarcoma	7.2	21.4	3.4
Rhabdomyosarcoma	15.5	19.1	3.5

conjugated with peroxidase. Absorbance values were measured using a microtiter reader at 450 nm with a reference wavelength of 690 nm. Samples were regarded as telomerase-positive if the absorbance was higher than 0.2 arbitrary units ($A_{450\ nm} - A_{690\ nm}$). The absorbance reading obtained with the positive control supplied with the kit was always higher than 2.0 U. All extracts were initially tested at 10 μg/μL of protein. In cases where telomerase activity was undetectable at this concentration, extracts were also tested at various concentrations ranging from 0.02 to 10 μg/μL of protein. All assays were performed in duplicate. To evaluate the presence of PCR inhibitors in protein extracts, samples that were negative for telomerase activity were reassayed for telomerase activity using the TeloTAGGG Telomerase PCR/ELISA^{PLUS} assay (Roche) using an IS following the manufacturer's recommended protocol.

Results

Mean TRFs in Canine Normal Tissues Range Between 12 and 23 kbp

TRFs were examined in a panel of 63 canine peripheral blood samples derived from subjects of varying ages and breeds by Southern blot hybridisation. A representative blot is shown in Figure 1*A*. The results of this study are presented in Table 1 and subjects are grouped according to breed and breed size. The mean TRFs of all the samples analysed ranged between 12 kbp and at least 23 kbp and the shortest telomeric length detectable was approximately 2.6 kbp (Figure 1*B*). These data did not demonstrate any statistically significant correlation between mean or peak TRF with donor age. However, within the Retrievers (representing the largest group analysed), a trend indicating telomeric attrition with increasing donor age was observed (Figure 2) (see Discussion section). No obvious differences in TRFs were evident between different breeds; however, Figure 3 shows TRF analyses in three 2-month-old Minature Schanuzer pups and three geriatric Retrievers aged 11 to 13 dogs (TRF data are presented in Table 1). This demonstrates that samples from older animals generate a greater smear pattern than from young pups, suggesting that breed-specific differences in telomere lengths may exist.

To evaluate whether tissue-specific differences in telomere lengths exist in the dog, TRF analysis was performed in a range of tissues derived from a dog, which presented with multicentric lymphoma (case B). Normal somatic canine tissues (testis, forelimb muscle, spleen, stomach, liver, adrenal gland) and lymphoma tissues were analysed. Considerable variation in telomere lengths was demonstrated among different tissues from the same subject with mean TRF values ranging between 13.5 kbp and approximately 23 kbp (Table 2). The highest mean TRF value was detected in multicentric lymphoma sites. The shortest mean TRF values were detected in testis and liver tissue.

Two Groups of Canine STS are Identified by TRF Analysis

In order to evaluate the length of telomeres of STS, mean TRFs, peak TRFs, and the shortest detectable telomere lengths were estimated in a panel of 18 STS (Table 3). All samples showed the presence of a single peak. Six of the samples examined showed the presence of a narrow TRF smear with mean TRFs ranging between 19.1 and 22.2 kbp and an average mean TRF of 22.2 kbp. The smallest telomere length detected in this group was 13.1 kbp. The remaining 15 samples showed the presence of variable heterogeneous TRFs ranging from as

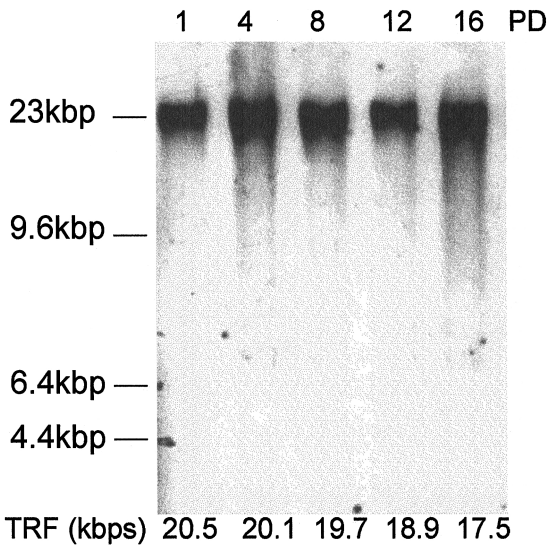

Figure 4. *Telomere length analysis of canine dermal fibroblasts in culture. The size and position of the markers are indicated on the left and the PDs and mean TRFs are shown.*

little as 3.4 kbp to approximately 23 kbp with mean TRFs between 7.2 and 19.5 kbp and an average mean TRF of 18.2 kbp.

Telomeric Attrition Occurs During In Vitro Aging of Canine Dermal Fibroblasts

Canine dermal fibroblasts were cultured *in vitro* for 16 PDs. DNA was isolated as various passages and subjected to TRF analysis, and the results are shown in Figure 4. The mean telomere length decreased with *in vitro* aging from 20.5 kbp at passage 1 to 17.5 kbp at passage 16.

Telomerase Activity is Confined to Germline and Tumour Tissue in Dogs

Telomerase activity was assessed in a range of canine tissue samples taken from three dogs (cases A, B, and C) and the results are presented in Figure 5. No telomerase activity could be demonstrated in thyroid, spleen, liver, kidney, heart, adrenal gland, and forelimb muscle samples; however, strong activity was observed in testis tissue and in multicentric lymphoma samples. The levels of telomerase activity in both tumour and testis material were similar in case B. An absorbance value of 0.2 was taken as positive for telomerase activity. Of the normal somatic tissues examined, weak telomerase activity ($A_{450\ nm}$=0.18) was detected in stomach tissue from case B. However, no activity was detectable in stomach tissue taken from cases A and C. Since it is possible that some tissue samples contain *Taq* polymerase inhibitors, lack of telomerase activity was confirmed by reassaying at various different protein concentrations and further confirmed by reassaying for telomerase activity using the TRAP-PCR/ELISA method with an IS PCR control.

Telomerase Activity is Demonstrated in Certain Canine Cell Lines

Several canine cell lines were examined for telomerase activity as shown in Figure 6. The highest levels of activity (>0.7 U) were detected in CMT3 (1.12 U), CMT7 (0.82 U), and CMT8 (0.71 U) cell lines. Telomerase activity >0.2 U was also identified in CML10 (0.65 U), D17 (0.3 U), A72 (0.3 U), and GHK (0.20) cell lines, although the levels of activity were lower than in the CMT3-8 lines. Telomerase activity levels <0.2 U were detected in both MDCK (0.1 U) and the human GHK cells (0.1 U), and these lines were considered to be telomerase-negative.

Correlation Between Telomere Lengths and Telomerase Activity

Telomere lengths were analysed in several tissues from case B, which were also subjected to telomerase activity assessment (Figure 5, Table 2). These tissues included: two multicentric lymphoma sites, adrenal gland, stomach, liver, spleen, testis, and forelimb muscle. The largest mean TRFs were detected in multicentric lymphoma sites and both these tissues showed high levels of telomerase activity, suggesting that telomerase activity is associated with telomeric elongation. Although the mean TRFs varied among normal somatic tissues including adrenal gland, liver, spleen, kidney, and

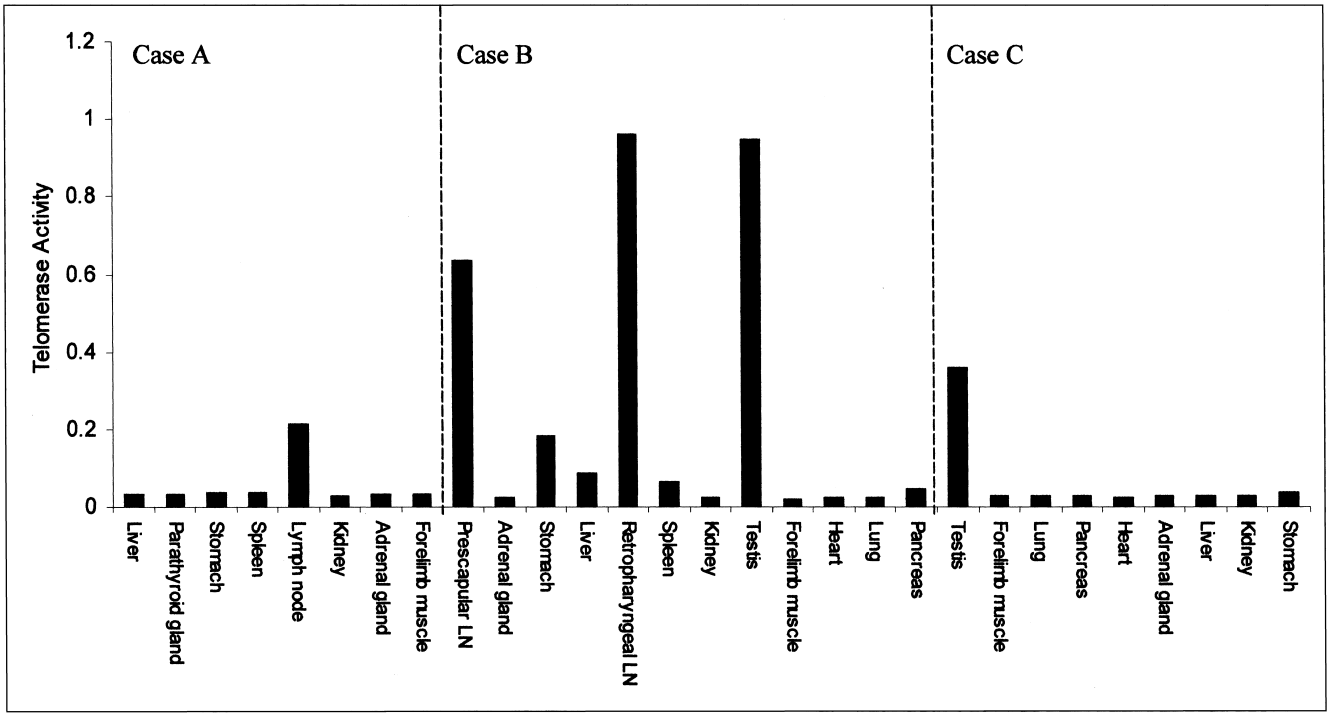

Figure 5. *Telomerase activity in various canine tissues from three subjects (A, B, C). An absorbance ($A_{450\ nm}$) >0.2 is taken as positive for telomerase activity. LN refers to lymph node. Cases A and B were canine subjects which presented with multicentric lymphomas and case C was a canine subject which presented with a non-malignant condition.*

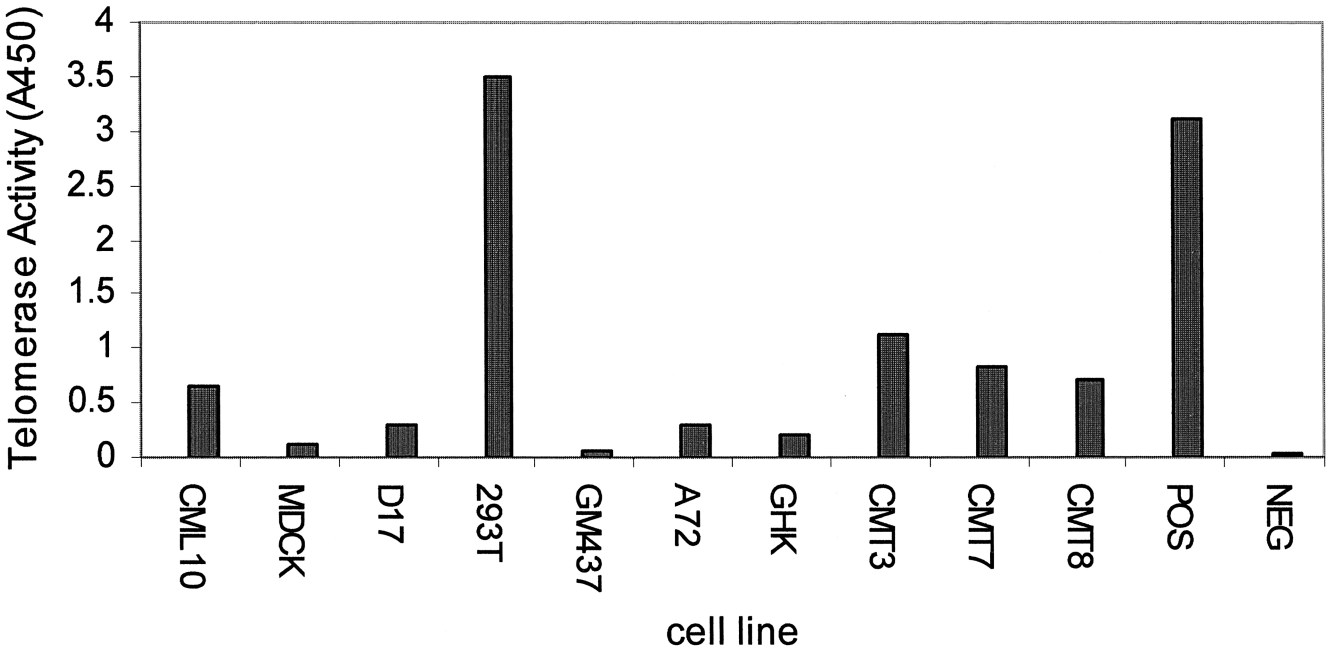

Figure 6. *Telomerase activity in various canine cell lines. An absorbance ($A_{450\ nm}$) >0.2 is taken as positive for telomerase activity. The GM437 line is a human telomerase-negative cell line and the 293T cell line is a human-positive line.*

forelimb muscle (ranging between 14.4 and 8.6 kbp), no telomerase activity could be detected in these samples. A high level of telomerase activity was identified in gonadal tissue and this tissue demonstrated the smallest mean TRF length.

Discussion

Telomeres play a critical role in determining the lifespan of somatic cells. It has been proposed that telomeric attrition upon successive cell divisions in somatic cells is circumvented in tumours and immortalised cells through activation of telomerase, and the presence of telomerase activity may be associated with the ability to proliferate indefinitely (reviewed in Refs. [22,23]). To evaluate the lengths of telomeres in dogs, we analysed telomere lengths in canine blood samples and a range of normal somatic tissues. Our data indicate that canine telomeres consist of large heterogeneous telomeres with a mean TRF length ranging between 12 and 23 kbp.

The data presented in this paper do not demonstrate any statistically significant correlation between the mean and peak TRF lengths and donor age and/or breed. However, in the Retriever group (the largest group analysed), the trend is towards telomeric attrition with increasing age, although not statistically significant with the number of animals studied. To further support age-related differences in telomere lengths, the youngest dogs analysed in this study were 2 months of age, demonstrating an average mean TRF of 19.3 kbp. In comparison, the geriatric dogs (age range 11 to 13 years) demonstrated an average mean TRF value of 17.1 kbp. Although sample number prevents detailed statistical analysis, these data indicate that further investigation is warranted with larger sample sizes. It is well documented that lifespan among dogs is shortest in the large and giant breeds, and longest in the small breeds. It is interesting to note that the Great Dane, which is regarded as a large breed, in this study was only 1 year of age and had a mean TRF value of 14 kbp. Within the small breeds studied, one subject (a West Highland White Terrier) had a mean TRF value of only 12.3 kbp at 2 years, compared to a dog of the same breed with a mean TRF of 22 kbp at an age of 10 years. Analysis of the TRF gel for this subject suggests that this dog had a range of TRF lengths between 5.4 and 23 kbp. However, the gel demonstrated marked heterogeneity in telomere lengths in this subject, which is reflected in a low mean TRF value compared to the 10-year-old subject of the same breed. Although this result does not fit well with the concept of age-related telomeric attrition or interbreed variation in telomeric lengths, one explanation could be that this subject has had a medical condition or treatment that has affected telomeric lengths. A similar observation was found in the Border Collies.

In PBMCs from humans of different ages, inverse correlations between mean TRF length and donor age have been reported [24–26] with a TRF length reduction at a rate of approximately 20 to 40 bp per year [25]. The lack of correlation demonstrated in the present study may reflect the sample size and may be explained by genetically determined interindividual heterogeneity in telomere lengths or breed-specific differences in telomere lengths that may mask any age-related correlation in telomere lengths. Telomere lengths are most commonly measured as the mean TRF by Southern blot hybridisation encompassing both the telomeric and subtelomeric regions. In humans, subtelomeric sequences consist of approximately 2.5 kbp [27];

however, the size of subtelomeric sequences in dogs was not elucidated in the present study. Analysis of a larger number of canine breeds, using techniques such as quantitative fluorescent *in situ* hybridisation and flow cytometry methods [28,29], may identify variations in telomere lengths and/or subtelomeric lengths between different breeds. Although we were unable to demonstrate a clear correlation between telomere loss and donor age, telomere shortening was clearly evident during culture propagation of canine dermal fibroblasts.

In this study, we were able to detect considerable variation in the telomere lengths between different tissues; testis and liver samples showed the lowest mean TRF with the highest mean TRF detected in forelimb muscle. The significance of low TRFs in testis and liver is uncertain, but may have implications regarding the toxicity of telomerase inhibitors. However, the low mean value for these tissues may again reflect the high level of heterogeneity in TRFs in these individual samples.

Telomere lengths were also studied in a panel of canine STS. All samples showed the presence of a single peak with little variation between peak TRFs. Based on the hybridisation patterns and TRF values, we identified two subgroups: a narrow TRF range subgroup and a broad range TRF subgroup. The mean TRFs of each group were 22.2 and 18.2 kbp, respectively. The latter subgroup demonstrated a mean TRF value 4 kbp, less that identified in the remainder of the STS. Although preliminary, these data indicate that the significance of telomeric lengths in canine STS requires further investigation by analysing comparative normal tissue samples. Telomeric shortening and elongation occur frequently in human tumours and have been reported by a number of groups [30–32], although it appears this is not uniform for all tumours, even for a particular histopathological subtype. In addition, we examined telomere lengths in two multicentric lymphoma sites and these tissues demonstrated the presence of mean TRFs greater than those detected in normal somatic tissues from the same subject. Indeed, the multicentric lymphomas from this patient also showed the presence of telomerase activity, further supporting telomere lengthening in these tumours. This study provides evidence to support telomeric attrition and elongation in canine tumours and requires further validation in a larger cohort of tumour types.

To further study telomere biology, we assessed telomerase activity in a wide range of normal somatic tissues from dogs to evaluate the organismal distribution of enzyme activity. Telomerase activity was detected in canine tumour tissues with no evidence for activity in normal somatic tissues, with the exception of testis. Telomerase activity has previously been demonstrated in over 95% of canine tumour tissues in a broad range of tumour types [33,34]; however, the distribution of telomerase activity in a wide range of normal somatic tissues has not been evaluated. This paper provides the first comprehensive study of telomerase activity in normal somatic tissues and demonstrates that the distribution of telomerase activity among tissue in dogs is similar to humans, i.e., germinal tissues and tumour tissues have significant telomerase, whereas other somatic tissues show low or no activity. In humans, telomerase activity can also been detected in cell renewal somatic cells, albeit at low levels [15–17]. In the present study, a low level of telomerase activity was detected in stomach tissue, but absent from other somatic tissues. The results of this study therefore suggest that telomerase activity in canine tissues is limited to cells with high proliferative potential and telomerase may therefore represent a useful tumour marker.

Several canine cell lines were also examined for telomerase activity. The highest levels of activity were detected in osteosarcoma cell lines (CMT3, CMT7, and CMT8). Telomerase activity units was also identified in melanoma (CML10) and a canine kidney cell line (GHK), although the levels of activity were lower than in the CMT3-8 lines. Telomerase activity levels <0.2 U were detected in MDCK cells and this line was considered to be telomerase-negative.

Telomerase/telomere biology is complicated by studies which show that telomerase expression and telomere lengths differ between humans and mice. Firstly, human telomeres range between 5 and 15 kbp [14], whereas in mice, telomeres are much larger, in the range of 40 to 60 kbp [19]. Although telomeric attrition occurs in mice and generation six telomerase-deficient mice show the expected conditions associated with shortened telomeres including atrophy of organs, infertility, reduced stress responses, and increased apoptosis [35–37], the regulation of telomerase activity appears to differ between mice and humans [38]. Secondly, while telomerase expression is largely absent in human somatic tissues, expression is evident in adult murine somatic tissues [37], and mouse cells spontaneously immortalize, while human cells do not [39]. These differences will have profound implications for cancer research using murine models, as it is clear that clinical applications involving the telomerase system cannot be evaluated convincingly in the mouse. Further species variation has been found in studies on chicken telomeres. Venkatesan and Price [40] found that, although chicken telomeres resemble human telomeres (8 to 20 kbp), the distribution of telomerase activity in chickens resembles that found in mice. However, decrease in telomerase activity in adult chicken somatic tissues with age was evident. In this study, we have clearly identified similarities in telomere and telomerase biology in dogs and humans. Although several important parameters remain to be elucidated including telomere loss with ageing, telomerase regulation in different tissues, and the identification of canine telomerase gene sequences, our study proposes the dog as an ideal model organism for *in vivo* telomerase studies. The dog provides a naturally occurring outbred model for many cancer types found in humans, and the advantages of using the dog as a model have recently been reported [41]. The dog may provide a situation that could be exploited for studies on novel therapeutic strategies involving telomerase targeting or inhibition and telomerase-based gene therapy protocols.

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