A cohort study of telomere and telomerase biology in cats

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Objective—To investigate telomere lengths in tissues of domestic shorthair (DSH) cats of various ages, evaluate the relationship between telomere length and age of cats, and investigate telomerase activity in the somatic tissues of cats.

Sample Population—Tissues obtained from 2 DSH cats and blood samples obtained from 30 DSH cats.

Procedure—DNA isolated from blood cells and somatic tissue samples was subjected to terminal restriction fragment (TRF) analysis to determine mean telomere repeat lengths. Protein samples were subjected to analysis by use of a telomeric repeat-amplification protocol to assess telomerase activity.

Results—Mean TRF values of cats ranged from 4.7 to 26.3 kilobase pairs, and there was significant telomeric attrition with increasing age of cat. Telomerase activity was not found in a wide range of normal tissues obtained from 2 cats.

Conclusions and Clinical Relevance—Analysis of these results clearly indicates that telomeres are shorter in older cats, compared with young cats; therefore, telomeres are implicated in the aging process. The analysis of telomerase activity in normal somatic tissues of cats reveals a pattern of expression similar to that found in human tissues.

Impact for Human Medicine—Fundamental differences in the biological characteristics of telomeres and telomerase exist between humans and the other most widely studied species (ie, mice). The results reported here reveal similarities in telomere and telomerase biological characteristics between DSH cats and humans. Hence, as well as developing our understanding of aging in cats, these data may be usefully extrapolated to aging in humans. (Am J Vet Res 2003;64:1496–1499)

Telomeres are nucleoprotein caps found at the ends of all eukaryotic chromosomes. Their structure is highly conserved among species and based on a hexanucleotide repeat sequence (ie, TTAGGG) found in all vertebrate species analyzed thus far, which constitutes the bulk of the telomere along with various telomere-associated proteins. Telomeres allow DNA-repair complexes to differentiate between chromosomal ends and breaks in double-stranded DNA, which protects the genome from recombination, fusion, and sequence loss.

In human somatic cells, telomeres normally shorten with each cell division. This telomeric attrition is attributable to the end-replication problem, a term used to describe the sequence loss associated with the semiconservative nature of DNA replication. This phenomenon is the result of the unidirectional (5’ to 3’) action of DNA polymerases and their requirement for an RNA primer to initiate DNA synthesis. As a result, terminal bases (50 to 100 bp/cell division) from the lagging strand are lost during each round of replication, because the requisite primer cannot base-pair to allow their replication. There is also evidence for loss of the terminal sequence involving additional mechanisms, including oxidative damage.

It has been proposed that telomeric attrition is the mitotic clock that counts down and acts as the trigger mechanism for the phenomenon of replicative senescence; this concept was first described > 30 thirty years ago. This phenomenon is of great importance, because replicative senescence has been linked to the processes of aging and cancer progression through influences on gene expression and cellular excretory phenotypes or via a powerful replicative block. It is believed that replicative senescence evolved primarily as an anticancer mechanism, and it has been described as an example of antagonistic pleiotropy. This model for a mitotic clock has been supported by in vivo evidence of a negative correlation between age and telomere length in humans.

Germ-line cells, cells with markedly high turnover rates, and cells without normal growth-control mechanisms (eg, cancer cells) overcome the end-replication problem by upregulating activity of the ribonucleoprotein complex telomerase, which is capable of replacing telomeric sequences lost during DNA replication. Activity of this enzyme complex is at baseline values or totally lacking in most other normal human cell types investigated so far.

Little research has focused on telomeres and telomerase biological characteristics in cats; however, telomeric attrition can occur with increasing age in dogs, and telomerase activity has been identified in tumor tissues obtained from dogs. Considering the importance attached to this area for research on aging and cancer in humans, it is important to expand this potentially beneficial area of research in cats. Thus, the objective of the study reported here was to assess basic telomere and telomerase biological characteristics in cats and determine whether a significant in vivo correlation could be identified between age of a cat and mean terminal restriction fragment (TRF) value for a panel of health-screened cats.
Materials and Methods

Sample population—Tissue samples were obtained from 2 client-owned cats; owners provided informed consent for tissue collection. These were neutered male domestic shorthair (DSH) cats that were < 2 years old. The cats had been euthanatized at our facility after they were severely injured by vehicles. Blood samples were obtained from 30 cats that were client-owned or housed at the Waltham Centre for Pet Nutrition. These DSH cats ranged from 1 to 17 years of age and were considered healthy at time of sample collection on the basis of a routine health examination performed by a veterinarian. None of the cats used for blood samples were related to each other or to the cats that provided post-mortem tissue samples. The protocol used for collection of blood samples complied with published guidelines. Informed consent for collection of blood samples was obtained from each owner.

Collection and processing of samples—Tissues obtained from the 2 euthanatized cats included samples of liver, cardiac muscle, lung, brain (cerebral cortex), and skeletal muscle from the pelvic limb. After overnight digestion of tissues in digestion buffer (100mM NaCl, 10mM Tris HCl [pH, 8.0], 25mM EDTA [pH, 8.0], 0.5% SDS, and 0.1 mg of proteinase K/mL), DNA was isolated by use of phenol-chloroform extraction and ethanol precipitation in accordance with standard protocols. Quality of DNA was verified by use of spectrophotometry, and each sample was evaluated after agarose gel electrophoresis to ensure that all DNA samples used were free from degradation.

Blood samples (5 mL) were collected into potassium EDTA. The DNA was isolated by use of a commercially available DNA isolation kit in accordance with the manufacturer’s instructions. Quality of DNA was verified by use of spectrophotometry and evaluation after agarose gel electrophoresis.

Analysis of telomere length—Mean length of telomere restriction fragments for each DNA sample was determined by use of Southern blotting techniques and a commercially available kit. Briefly, 2 µg of DNA/sample was digested overnight at 37°C with Rsal and Hinfl enzymes at a concentration of 4 U of enzyme/mg of DNA. Agarose gel electrophoresis of a small aliquot of the sample was used to confirm digestion, and DNA fragments in the remainder of the sample were then separated by use of agarose gel electrophoresis (0.6% agarose gel electrophoresed at 110 V for 6 hours). Digested DNA was transferred by Southern blotting techniques from the gel to a nylon membrane during overnight incubation and hybridized to a digoxigenin (DIG)-labeled DNA probe (TTAGGG)7 by incubation for 3 hours at 42°C. A DIG chemiluminescence detection kit was then used to detect telomeric smears and generate autoradiographs for analysis. Analysis of autoradiographs was performed with a densitometer by use of commercially available software.

Mean TRF value was calculated by use of the following equation:

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TRF = \frac{\Sigma (OD_i) \times L_i}{\Sigma (OD_i/L_i)}
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where ODi is the chemiluminescent signal, and Li is the length of the TRF fragment at position i. The calculation accounted for the higher signal intensity from larger TRF fragments that resulted from multiple hybridization of the telomere-specific hybridization probe. All samples were analyzed in duplicate.

Assay of telomerase activity—Tissues used in the telomerase assay were digested as described previously, and protein concentrations were then determined by use of a standard Bradford assay. All samples were analyzed in duplicate, and 5 µg of total protein was used for each tissue sample analyzed.

Telomerase activity was assayed by use of a commercially available telomeric repeat-amplification protocol6 that was conducted in accordance with the manufacturer’s recommendations. Briefly, telomerase in the samples added telomeric repeats to the 3’ end of a biotin-labeled synthetic primer. These products, as well as an internal standard contained within the same reaction vessel, were amplified by a polymerase chain reaction (PCR) assay. The PCR products were divided into 2 aliquots, denatured, and hybridized separately to DIG-labeled detection probes specific for vertebrate telomeric repeats or the 216-bp internal standard, respectively. The resulting products were immobilized via the biotin label to a streptavidin-coated microtiter plate. Detection was performed against DIG by use of a specific antibody conjugated to horseradish peroxidase and the sensitive peroxidase substrate, tetramethylbenzidine. An aliquot (1 µL) of a control template with high telomerase activity was used as the positive-control sample and provided telomerase activity between 2.0 and 4.0. A sample that contained only lysis buffer was used as a negative-control sample, and analysis of a heat-inactivated version of each unknown sample provided sample-specific, negative-control values. Heat inactivation was performed at 85°C for 10 minutes. Telomerase activity in each unknown sample was expressed numerically as the relative telomerase activity (RTA), which was calculated by use of the following equation:

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RTA = \frac{\left( \frac{A_{S0}}{A_{S}} - \frac{A_{S0}}{A_{S,IS}} \right)}{\left( \frac{A_{TS8,0}}{A_{TS8}} - \frac{A_{TS8,0}}{A_{TS8,IS}} \right)} \times 100
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where As is absorbance of the unknown sample, As0 is absorbance of the heat-inactivated sample, As,IS is absorbance of the internal standard of the unknown sample, As,TS8,0 is absorbance of the positive-control sample, and As,TS8,IS is absorbance of the negative-control sample. Samples were considered positive for telomerase when the mean RTA value was greater than twice the mean background activity of the sample.

Statistical analysis—Standard linear regression was performed by use of a statistical program. Significance was set at values of P < 0.05.

Results

Autoradiographs were used for analysis (Fig 1). Mean TRF values were measured in peripheral blood leukocytes obtained from 30 DSH cats that ranged from 1 to 17 years of age. Mean TRF values in these samples ranged from 4.7 to 20.6 kilobase pairs (kbp). A significant correlation (R²; 0.33; P = 0.001) was detected between reduced telomere length and advanced age of cats (Fig 2).

Mean TRF values were measured in samples of various normal tissues collected from 2 euthanatized DSH cats. Values ranged from 12.60 to 26.3 kbp (Fig 3). We did not detect significant differences in TRF values among the tissues evaluated. Telomerase activity was measured in the same postmortem tissue samples, and negative results were found for all samples by use of the guidelines described previously. Telomerase activity was detected in the positive-control sample (value of 3.09).
Discussion

The objective of the study reported here was to investigate telomere and telomerase biological characteristics in DSH cats. The TRF analysis of DNA isolated from blood samples and somatic tissues revealed mean TRF lengths that ranged from 4.7 to 26.3 kbp. Telomere lengths identified in this study are similar to those reported for humans and dogs but are significantly shorter than those measured in another study by investigators who used a technique of fluorescence in situ hybridization and flow cytometry. Analysis of telomere dynamics and age revealed a significant correlation ($R^2$, 0.33; $P = 0.001$) for in vivo telomeric attrition with increased age of the cats. Most of the samples were from old or young cats (Fig 2). Use of animals from the tail ends of the age distribution is a powerful tool to investigate the relationship between age and telomere length, because this experimental design emphasizes the contribution of age to the variation in telomere length. Analysis of the results indicated that old cats have shorter telomeres than young cats. This implies that telomere length decreases with increasing age; however, additional studies will be required to reveal whether this relationship is linear or more complex, as has been identified in human peripheral blood leukocytes.

To our knowledge, this is the first study to document telomeric attrition with increasing age in cats, which suggests an important role for telomeres in aging in cats. This pattern matches that identified in studies performed in humans and dogs and is in contrast to results for mice, providing further evidence of species variation.

A fundamental difference between murine and human telomere biological characteristics is the differing role of telomerase in normal somatic tissues. Telomerase activity is widespread in normal tissue samples obtained from mice; therefore, telomeric attrition will not lead to senescence in the same manner as for tissues obtained from humans. Telomerase-knockout mice can also develop tumors, and cells derived from these mice immortalize with identical frequency to that of their wild-type (ie, telomerase-positive) counterparts. It is postulated that such evolutionary differences are linked to the small physical size of mice, which, when coupled with long telomeres, may cause a system of growth arrest (based on telomeric attrition) that is ineffectual. In contrast, the study reported here has revealed that telomerase activity is lacking from a broad range of somatic tissues in cats, a situation that is mirrored in samples obtained from humans. This corroborates with results of another study in which telomerase activity in DSH cats was found to be largely confined to malignant tissues. Furthermore, it is possible the extremely long telomeres of laboratory mice are, in fact, an artifact caused by extrinsic selection pressure to produce individuals of high fecundity, which in turn has selected strongly against reproductive senescence. This hypothesis is supported by results of a study in which inbred strains of mice had significantly lengthened telomeres, compared with the length of telomeres in outbred mice from which the inbred strains were derived. Coordination of senescence among organs, such that no single organ is responsible for limiting the life span of an animal, will then lead to the production of an animal whose telomeres are overall much longer than those found in outbred individuals.
from the same species. This has led to effective disabling of the telomeric-replicative arrest mechanism in laboratory mice that may render them unsuitable for use as models in human cancer and aging, because tests performed in these laboratory mice are likely to overestimate cancer risks and underestimate tissue damage. 23

The TRF lengths of somatic tissues obtained from cats and examined in the study reported here (12.6 to 26.3 kbp) are more comparable in size to human telomeres than those for strains of laboratory mice that have been examined in another study. 28 We did not detect unusually long or short telomeres in any particular organ of either euthanatized cat, despite the likelihood that cell turnover rates are lower in the cerebral cortex and myocardial tissues, compared with turnover rates in the other organ tissues examined. 27 However, both of those cats were juveniles (< 2 years old) at the time they were euthanatized and samples collected, and because telomere reduction rates in individual organs are relatively slow (29 to 60 bp/y in human liver), it is not surprising that the impact of varying rates of cell turnover were not discernible in such young cats. We did not detect significant differences between the TRF values of the non-replicative tissues investigated and the TRF values of the blood samples, and none of the cats used in the study had a medical history that should have influenced their telomeres. For example, there were no known incidences of chronic inflammation that may have resulted in increased replicative demand and associated telomere loss. Although the tissue samples used in the study were mostly from tissue types that do not undergo postembryonic replication, the distinction between feline and murine tissues and the similarities with human tissues are worthy of mention. Restricted availability of postmortem samples precluded the use of a broader range of tissues from a greater number of cats. It would be interesting to investigate whether telomerase activity could be identified in tissues with mitotic potential, such as bone marrow, and whether this activity would have any bearing on telomere dynamics and increasing age.

Analysis of telomere length and telomerase activity indicated a much closer link between the biological characteristics of telomeres and telomerase in DSH cats and humans than between those 2 species and mice. We suggest that additional studies are necessary to elucidate whether there is exact duplication in the control mechanisms that govern telomere dynamics in human cells and cells from DSH cats.

References