Molecular cloning of the canine telomerase reverse transcriptase gene and its expression in neoplastic and non-neoplastic cells

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Objective—To perform molecular cloning of the canine telomerase reverse transcriptase (TERT) gene and determine its expression in neoplastic and non-neoplastic cells.

Sample Population—9 canine tumor cell lines derived from various neoplasms, 16 primary canine tumors, and tissues from 15 normal canine organs.

Procedure—Tumor cell lines were derived from canine tumors that included osteosarcoma, mammary gland adenocarcinoma, melanoma, acute lymphoblastic leukemia, lymphoma, and mastocytoma and a canine primary fibroblast culture. Canine TERT complementary DNA (cDNA) was amplified by use of polymerase chain reaction (PCR) and sequenced. Expression of TERT mRNA was examined by reverse transcription (RT)-PCR assay. Telomerase activity was measured by use of the telomeric repeat amplification protocol assay.

Results—The canine TERT cDNA clone was 237 base pairs in length and contained a central region encoding the reverse transcriptase motif 2. Expression of TERT mRNA was detected in canine tumor cell lines that had telomerase activity but not in telomerase-negative canine primary fibroblasts. The TERT mRNA was detected in 13 of 16 canine tumor tissues and several normal tissues such as liver, ovary, lymph node, and thymus. A significant correlation between TERT expression level and telomerase activity was noted.

Conclusions and Clinical Relevance—Expression of TERT mRNA was closely associated with telomerase activity in neoplastic cells as well as some non-neoplastic cells from dogs. In addition to telomerase activity, expression of TERT mRNA can be used as a marker of tumor cells. (*Am J Vet Res* 2003;64:1395–1400)

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] 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. Decrease in telomere length results in the inability of cells to divide and triggers cell senescence.[4-6] 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.[7]

Telomerase consists of an essential RNA template (**telomerase RNA component [TERC]**) and protein components including a form of reverse transcriptase (**telomerase reverse transcriptase [TERT]**).[7-10] Telomerase binds to the 3' end of the single-stranded region of chromosomal DNA. Then, the sequence repeats are added to the end of the DNA by use of TERC as a template.[7-10] In humans, TERC is present in all cells, whereas the expression of TERT gene is confined to cells that have telomerase activity.[9,10] The ectopic expression of the TERT gene enables telomerase-negative cells to have telomerase activity,[11] indicating that TERT is the major determinant of telomerase activity.

Telomerase activity is detected in many naturally occurring tumors in humans,[12,13] dogs,[14-18] and cats.[19] Because telomerase activity in normal canine somatic cells is undetectable or very low but specifically high in tumor cells, telomerase inhibitors may have potential as new anticancer drugs.[20-22]

Telomerase activity has been measured by a **polymerase chain reaction (PCR)**-based telomerase activity assay called the **telomeric repeat amplification protocol (TRAP)** assay. This method is sensitive, but some extracts from cells or tissues contain inhibitors of *Taq* polymerase.[23] Furthermore, the procedures used to examine telomerase activity with the TRAP assay are complicated, making its application to clinical specimens difficult.

The purpose of the study reported here was to perform partial molecular cloning of the canine TERT gene and determine its expression in canine neoplastic and non-neoplastic cells to compare it with telomerase activity.

Received December 23, 2002.
Accepted May 1, 2003.
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Materials and Methods

Cell culture—We used normal canine primary cultured fibroblasts and various canine tumor cell lines derived from osteosarcoma (HOS[24] and OOS[24]), mammary gland adenocarcinoma (CHMp and CIPm), melanoma (KMeC[25] and CMeC[25]), acute lymphoblastic leukemia (GL-1[26]), lymphoma (CL-1[27]), and mastocytoma (VI-MC[28]). The primary cultured fibroblasts were obtained from a skin biopsy specimen from a 4-year-old healthy Beagle. The CHMp and CIPm cell lines were established from the mammary gland adenocarcinomas of a 10-year-old and 12-year-old dog, respectively. The canine primary fibroblasts were cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin sulfate (100 μg/mL) at 37°C in a humidified atmosphere of 5% CO_2. The primary fibroblasts were passaged 4 times and stored. The tumor cell lines, HOS, OOS, CHMp, CIPm, KMeC, CMeC, GL-1, CL-1, and VI-MC, were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin sulfate (100 μg/mL) at 37°C in a humidified atmosphere of 5% CO_2. The passage numbers of the cell lines, HOS, OOS, CHMp, CIPm, KMeC, CmeC, and VI-MC used in this study were 35, 35, 127, 126, 101, 51, and 60. The GL-1 and CL-1 cell lines had been passaged in culture for 5 years before use in this study. The cells were washed twice with PBS solution and divided into 2 specimens, 1 for telomerase activity assay and the other for TERT mRNA detection to be carried out with identical specimens. These specimens were rapidly frozen in liquid nitrogen and stored at –80°C until analyzed.

Tissue specimens—Tissue specimens were obtained by surgical resection at the Veterinary Medical Center, University of Tokyo, from 16 dogs with tumors including osteosarcoma (n = 1), renal adenocarcinoma (1), leiomyosarcoma (1), leiomyoma (2), hemangiosarcoma (1), mammary gland adenoma (1), sebaceous epithelioma (1), ceruminous gland adenocarcinoma (1), myxofibrosarcoma (1), fibrosarcoma (1), perianal gland adenoma (2), squamous cell carcinoma (1), and mast cell tumor (2). Specimens of normal tissues were obtained from 5 healthy dogs that were kept for experimental purposes after euthanasia via IV administration of a pentobarbital overdose. Normal tissues examined in the study included liver (n = 5), kidney (5), spleen (4), pancreas (5), adrenal gland (5), stomach (5), small intestine (3), colon (5), urinary bladder (5), ovary (4), uterus (4), lung (4), skin (4), lymph node (5), and thymus (2). After removal, all the tissue specimens were placed on ice, washed with cooled (4°C) physiologic saline (0.9% NaCl) solution, frozen in liquid nitrogen within 15 minutes, and stored at –80°C until analyzed. The frozen tissue specimens immersed in liquid nitrogen were homogenized with a mortar and pestle and divided into 2 aliquots, 1 for telomerase activity assay and the other for TERT mRNA detection to be carried out with identical samples.

Cloning and sequence analysis of canine TERT cDNA—Template mRNA for **reverse transcription (RT)**-PCR was extracted from GL-1 cells by use of affinity binding to oligo(dT) cellulose,[a] because this cell line had high telomerase activity in a preliminary experiment. A cDNA sample was synthesized from the mRNA template with a commercially available kit.[b] Oligonucleotide primers to amplify a central region of canine TERT cDNA were designed on the basis of the sequence of human TERT cDNA[10,c]: forward primer, 5′-GACTCCGCTTCATCCCCAAG-3′ (**nucleotides [nt]** 1,859 to 1,878 in human TERT cDNA) and reverse primer, 5′-CATCCACCTTGACAAAGTACAG-3′ (nt 2,137 to 2,116 in human TERT cDNA). Using the primer pair, canine TERT cDNA was amplified from canine GL-1 cDNA with *Taq* polymerase.[d] The PCR amplification was performed with 35 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and polymerization at 72°C for 1.5 minutes. The DNA fragment generated by the PCR was cloned into a plasmid vector[e] and sequenced by the dideoxy chain termination method.[f]

Determination of telomerase activity—Telomerase activity was measured by use of a TRAP assay with a commercially available kit,[g] as described,[15] with slight modifications. Briefly, frozen cell- or tissue-specimens were homogenized in a lysis buffer containing 0.5% 3-([3-cholamidopropyl] dimethylammonio)-1-propanesulfonate, 0.1mM benzamidine, 5mM β-mercaptoethanol, 10mM Tris-HCl (pH, 7.5), 1mM magnesium chloride, 1mM **ethylene glycol bis(β-aminoethyl ether) N,N,N′,N′-tetraacteic acid (EGTA)**, and 10% glycerol. After incubation on ice for 30 minutes, the homogenized specimens were centrifuged, and the supernatant (cell extract) was rapidly frozen in liquid nitrogen and stored at –80°C. The protein concentration in the cell extract was measured by use of Lowry method.[h] Two microliters of the cell extract (equivalent to 50 ng) was incubated in a reaction mixture[g] consisting of 1× reaction buffer (20mM Tris-HCl [pH, 8.3], 1.5mM MgCl2, 63mM KCl, 0.05% Tween 20, and 1mM EGTA), deoxynucleoside triphosphate (dNTP) mixture, 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 internal control in the next PCR step), TSK1 template (this oligonucleotide serves as the internal control in the next PCR step), and 2 units of *Taq* polymerase[i] at 30°C for 30 minutes. During the first 30-minute incubation, telomerase in samples acted to add telomeric repeats to the TS primer. This mixture was then subjected to 30 cycles of PCR consisting of denaturation at 94°C for 30 seconds and annealing and polymerization at 59°C for 30 seconds. During the amplification process, the extended TS primer was amplified by use of 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)** that was used to estimate the degree of contamination with inhibitors of *Taq* polymerase or the efficiency of PCR amplification for each sample. At the same time, the TRAP assay was performed by use of TSR8 control template (TS primer + 8 telomeric repeats [5′-AATCCGTCGAGCGAGAGTTAG {GGTTAG}7-3′]) instead of the tissue extract as a control to assess the PCR amplification step. The PCR products were separated by electrophoresis in a 12.5% nondenaturing polyacrylamide gel and stained with SYBR Gold[j] for visualization. The gel images were captured with a CCD camera,[k] and the densitometric intensities of the TRAP assay products were analyzed with an image analysis program.[l] Telomerase activity (**total product generated [TPG]**) in tissue extract was determined according to the formula:

$$\text{TPG (U/50 ng of protein)} = [(X-X0)/C]/[(r-r0)/CR] \times 100$$

where X, X0, r, and r0 are the signal intensities of the oligonucleotide ladder derived from the tissue extract, heat-inactivated tissue extract, TSR8 control template, and lysis buffer, respectively, and C and CR are the signal intensities of ITAS derived from the tissue extract and TSR8 control template, respectively.

RNA sample preparation for detection of TERT mRNA—Total RNA samples were extracted by use of a silica-gel membrane method.[m] To avoid contamination of genomic DNA, the RNA samples were treated with deoxyribonuclease I. The concentration of RNA was examined by measuring the

absorbance in water at 260 nm in a spectrophotometer.[n] The purity of RNA was determined by measuring the absorbances in 10mM Tris-Cl [pH, 7.5] at **260 nm (A260)** and **280 nm (A280)**. The integrity of RNA was checked by denaturing agarose gel electrophoresis (1.2% agarose gel in 1X MOPS buffer [20mM 3-{N-morpholino}propanesulfonic acid, 5mM sodium acetate, 1mM EDTA {pH was adjusted to 7.0 with NaOH} and 0.246M formaldehyde]) and gold staining.[j]

Detection of TERT mRNA by RT-PCR—An equal amount of RNA (500 ng) in each sample was used for RT-PCR analysis to compare the expression level of TERT mRNA among the tissue and cell samples. Single-strand cDNA was synthesized from 500 ng total RNA in a reaction primed with oligo(dT) by use of a commercially available kit according to the manufacturer's manuals.[o] To amplify canine TERT cDNA, a primer pair was prepared: forward primer, 5′-AGCATCT-CACCTCACAACTG-3′ (nt 77 to 96 in canine TERT cDNA; the sequence was determined in this study), and reverse primer, 5′-CGGATTCTGGGCCCGTATGC-3′ (nt 209-228 in canine TERT cDNA). A PCR was performed by use of a commercially available *Taq* polymerase.[p] After denaturation at 95°C for 2 minutes, 30 cycles of the reaction (95°C for 1 minute [denaturation] and 60°C for 1 minute [annealing and polymerization]) were performed, followed by a final extension procedure at 72°C for 7 minutes. The PCR products were electrophoresed through a 4% agarose gel and stained with gold[j] for visualization. The gel images were captured with a charge-coupled device camera,[k] and the densitometric intensities of the RT-PCR products were analyzed with an image analysis program.[l] The expression level of TERT mRNA was estimated by comparing the band intensity of the sample with that of OOS cell RNA samples of different amount (5, 50, or 500 ng). The RT-PCR signals that were less than that of 5-ng OOS cell RNA were tentatively designated as not detected.

Statistical analyses—Correlation between telomerase activity determined by use of the TRAP assay and TERT gene expression level was assessed by use of simple linear regression analysis. A value of $P \leq 0.05$ was considered significant.

Results

Molecular cloning and sequencing of canine TERT gene—The canine TERT cDNA clone obtained in this study was 237 base pairs in length and encoded 79 amino acid residues,[q] with 82.7 and 69.2% nucleotide sequence similarities and 77.2 and 62.0% amino acid sequence similarities with human[c] and mouse[29,r] TERT cDNAs, respectively. The same nucleotide sequence was obtained from the HOS cell line. A BLAST search[30] revealed that the probabilities of these similarities occurring by chance were 2×10^{-31} and 3×10^{-23}, respectively. This clone contained a reverse transcriptase motif 2 (amino acids 630 to 649 in human TERT[10]).

Quality of RNA samples—All RNA samples had an A260:A280 ratio of 1.9 to 2.1 in 10mM Tris-Cl (pH, 7.5). The respective ribosomal bands appeared as sharp bands, and 28S rRNA bands appeared with greater intensity than that of the 18S rRNA band (**Fig 1 – 3**).

Expression of TERT mRNA and telomerase activity in canine cultured cells—The RT-PCR analysis was performed to examine the expression of TERT gene in canine cultured cells (Fig 1). The TERT mRNA was detected in all 9 canine tumor cell lines but not in normal canine fibroblasts. The TERT mRNA in the normal fibroblasts was not detected even when the number of PCR cycles was increased to 35 (data not shown). A TRAP assay was performed to evaluate the telomerase activity in these canine cultured cells. Telomerase activity was detected in all of the 9 tumor cell lines and ranged from 10 to 606 U/50 ng of protein. However, no telomerase activity was detected in the normal canine primary fibroblasts (Fig 1), even with use of an increased amount of protein (200 ng).

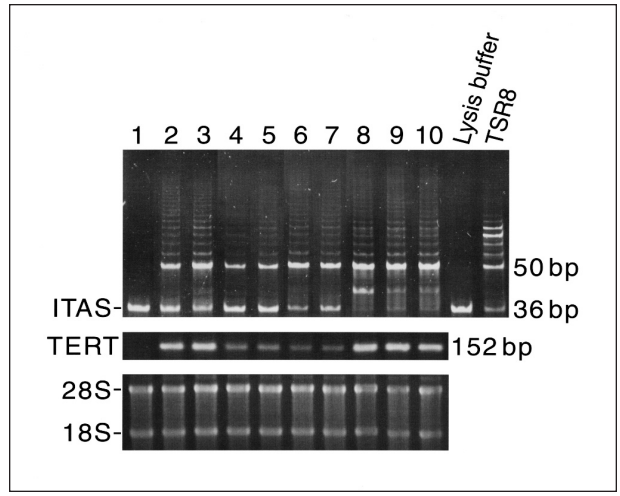

Figure 1—Agarose gel electrophoretograms of telomerase activity and telomerase reverse transcriptase (TERT) mRNA expression in cultured canine cells. Upper panel—Telomerase activity was examined by use of a telomeric repeat amplification protocol (TRAP) assay. Middle panel—Expression of TERT mRNA was detected by use of reverse transcription (RT)-polymerase chain reaction (PCR) assay. Lower panel—Total RNA samples stained with SYBR Gold[j]. Bands for 28S ribosomal RNA (rRNA; 28S) and 18S rRNA (18S) are indicated. For all panels, lane 1 = canine primary fibroblast culture; lanes 2 and 3 = osteosarcoma cell lines, HOS and OOS, respectively; lanes 4 and 5 = mammary gland adenocarcinoma cell lines, CHMp and CIPm, respectively; lanes 6 and 7 = melanoma cell lines, KMeC and CMeC, respectively; lane 8 = acute lymphoblastic leukemia cell line, GL-1; lane 9 = lymphoma cell line, CL-1; and lane 10 = mastocytoma cell line, VI-MC. TSR8 = Control template. ITAS = Internal standard for telomerase amplification. bp = Base pairs.

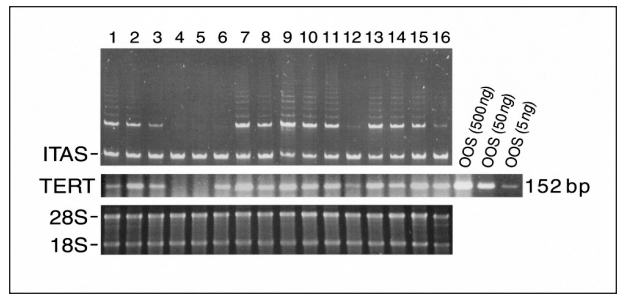

Figure 2—Agarose gel electrophoretograms of telomerase activity and TERT mRNA expression in various canine tumor tissues. Upper panel—Telomerase activity examined by use of a TRAP assay. Middle panel—Expression of TERT mRNA detected by use of an RT-PCR assay. Lower panel—Total RNA samples stained with SYBR Gold[j]. For all panels, lane 1 = osteosarcoma; lane 2 = renal adenocarcinoma; lane 3 = leiomyosarcoma; lanes 4 and 5 = leiomyoma; lane 6 = hemangiosarcoma; lane 7 = mammary gland adenoma; lane 8 = sebaceous epithelioma; lane 9 = ceruminous gland adenocarcinoma; lane 10 = myxofibrosarcoma; lane 11 = fibrosarcoma; lanes 12 and 13 = perianal gland adenoma; lane 14 = squamous cell carcinoma; and lanes 15 and 16 = mast cell tumor.

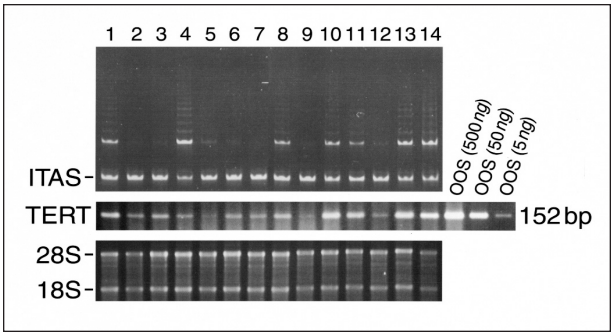

Figure 3—Agarose gel electrophoretograms of telomerase activity and TERT mRNA expression in various normal canine tissues. Upper panel—Telomerase activity examined by use of a TRAP assay. Middle panel—Expression of TERT mRNA detected by use of an RT-PCR assay. Lower panel—Total RNA samples stained with SYBR Gold®. For all panels, lane 1 = liver; lane 2 = kidney; lane 3 = spleen; lane 4 = pancreas; lane 5 = adrenal gland; lane 6 = stomach; lane 7 = small intestine; lane 8 = colon; lane 9 = urinary bladder; lane 10 = ovary; lane 11 = uterus; lane 12 = lung; lane 13 = lymph node; and lane 14 = thymus.

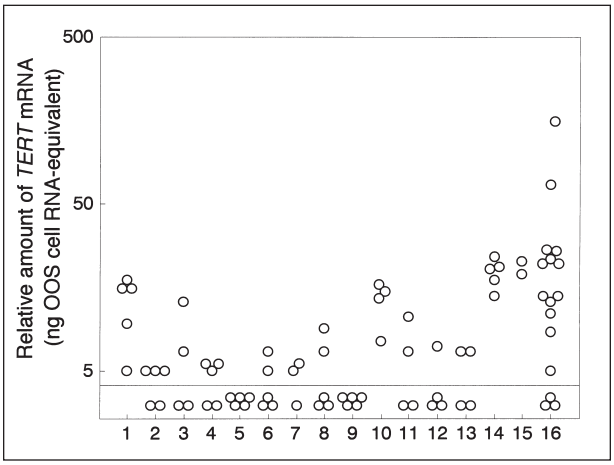

Figure 4—Relative amount of TERT mRNA in canine neoplastic and non-neoplastic tissues. Two to 5 normal tissue specimens from each organ and 16 tumor tissue specimens were examined for TERT mRNA expression by use of an RT-PCR assay. The TERT expression level is expressed relative to that in OOS cells. Lane 1 = liver; lane 2 = kidney; lane 3 = spleen; lane 4 = pancreas; lane 5 = adrenal gland; lane 6 = stomach; lane 7 = small intestine; lane 8 = colon; lane 9 = urinary bladder; lane 10 = ovary; lane 11 = uterus; lane 12 = lung; lane 13 = skin; lane 14 = lymph node; lane 15 = thymus; and lane 16 = various tumor specimens.

Expression of TERT mRNA and telomerase activity in canine primary tumor tissues and normal organ specimens—The TERT mRNA was detected in 13 of the 16 tumor specimens including osteosarcoma, renal adenocarcinoma, leiomyosarcoma, hemangiosarcoma, mammary gland adenoma, sebaceous epithelioma, ceruminous gland adenocarcinoma, myxofibrosarcoma, fibrosarcoma, perianal gland adenoma, squamous cell carcinoma, and mast cell tumor (Fig 2–4). In 2 leiomyoma specimens and a perianal gland adenoma specimen (lanes 4, 5, and 12, respectively, in Fig 2), TERT mRNA was not detected. Fairly high-level expressions of TERT mRNA were detected in the liver, ovary, lymph node, and thymus (Fig 3 and 4). Telomerase activity in the 30 tissue specimens (16 tumor tissues and 14 normal tissues)

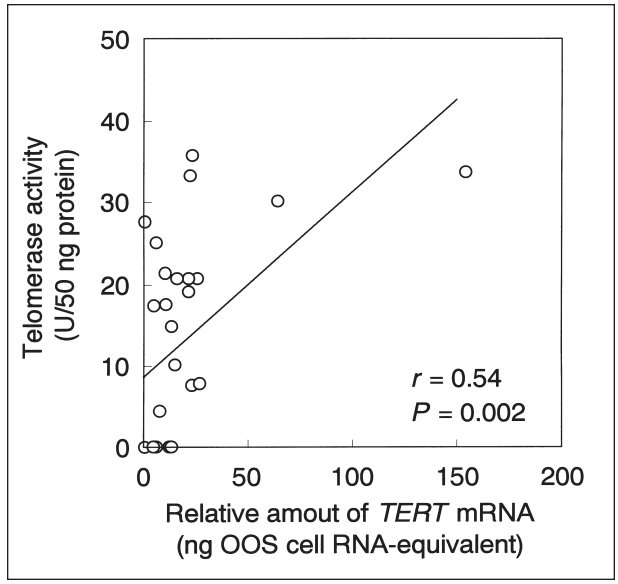

Figure 5—Comparison between telomerase activity and TERT mRNA expression level in 30 canine tissue specimens (14 normal tissues and 16 tumor tissues). Telomerase activity and TERT mRNA were examined by TRAP assay and RT-PCR assay, respectively. The amount of TERT mRNA was determined relative to that in OOS cells.

was quantified, and the results were compared with those obtained by use of RT-PCR for TERT mRNA expression. The relative amounts of TERT mRNA of the tissue specimens were determined in comparison to those of OOS cells of different RNA amounts. A positive correlation was detected between the relative TERT mRNA amount and telomerase activity (r = 0.54; P = 0.002; **Fig 5**). However, in some tissues, there was a discrepancy between the telomerase activity and TERT mRNA level. For example, a hemangiosarcoma specimen and a mast cell tumor specimen (lanes 6 and 16, respectively, in Fig 2) had high levels of TERT expression but did not have detectable telomerase activity. Conversely, normal pancreas (lane 4 in Fig 3) had a high level of telomerase activity but a relatively low level of TERT mRNA.

Discussion

In this study, canine TERT cDNA was partially cloned, and the TERT mRNA expression was examined in canine neoplastic and non-neoplastic cells for comparison with their telomerase activity. Expression of TERT mRNA was detected in 9 telomerase-positive canine tumor cell lines but not in a telomerase-negative canine primary fibroblasts culture. Furthermore, a significant correlation between TERT mRNA expression and telomerase activity was noted in canine tissue specimens comprising 16 tumor specimens and 14 normal tissues in dogs. The results were consistent with those in humans.[9,10] Therefore, TERT expression was considered to be a primary determinant regulating telomerase activity in canine cells.

Telomerase activity has been measured by a PCR-based telomerase activity assay called the TRAP assay. In this method, telomerase activity is measured in vitro in 2 steps. In the first step of the reaction, telomerase in a cell or tissue extract adds telomeric repeats

onto the oligonucleotide primers. In the second step, the extended products are amplified by use of PCR. The TRAP assay requires relatively complicated procedures and high-quality materials to obtain accurate results. There may be several problems when the TRAP assay is used for examination of clinical specimens. Because the RT-PCR assay for TERT mRNA provided essentially the same results as the TRAP assay in our study, it will also be applicable to examination of clinical specimens. As in other laboratory examination systems used to assess the amount of telomerase in cells, in situ hybridization for TERT mRNA or immunohistochemistry for TERT protein will have an advantage regarding revealing the cell types that contain telomerase.

In some tissues, there was a discrepancy between the telomerase activity and the TERT mRNA expression level. Two tumor specimens from a hemangiosarcoma and a mast cell tumor, respectively, had high levels of TERT expression but did not have detectable telomerase activity. Coversely, normal pancreas had high telomerase activity but a relatively low level of TERT mRNA. Factors other than TERT expression (eg, TERC expression) might have a role in regulating the telomerase activity in these tissues.

Quantification of the mRNA expression level by use of RT-PCR assay depends on the quality and quantity of the RNA sample. Housekeeping genes such as β-actin or glyceraldehyde-3-phosphate dehydrogenase have often been used as controls for RT-PCR assays. In humans, rats, and mice, however, neither β-actin nor glyceraldehyde-3-phosphate dehydrogenase is appropriate for the control RNA molecule when comparing the expression level of some gene among different tissues[31] because of the variation of their expression levels. Therefore, in our study, equal amounts of RNA samples verified by spectrophotometery and electrophoresis were used for RT-PCR analysis to compare the expression levels of TERT mRNA among various tissues. The TERT mRNA was detected in various normal tissues, and high-level expression was detected in the liver, ovary, lymph node, and thymus. In humans, it has been reported that gonadal cells and lymphocytes have distinct telomerase activity; therefore, TERT mRNA expression in canine ovary, lymph node, and thymus detected in our study was conceivably derived from these cells. Interestingly, a relatively high TERT mRNA level was detected in the canine liver specimens in this study. Hepatocytes that have a capacity for regeneration or lymphoid and mononuclear phagocytic cells in the liver might have TERT mRNA expression; however, we could not determine which cell expressed TERT mRNA in our study. To assess the TERT expression at the cell level in canine tissues, in situ hybridization or immunohistochemical staining should be carried out.

Our data provide fundamental knowledge of telomerase in neoplastic and non-neoplastic cells and tissues in dogs. Further experiments, such as quantitative measurement of TERT mRNA expression and in situ detection of TERT, will advance the study of telomerase in dogs and may support development of novel diagnostic and therapeutic procedures.

[a]Fast Track mRNA isolation kit, Invitrogen, Carlsbad, Calif.
[b]cDNA synthesis kit, Amersham Biosciences, Piscataway, NJ.
[c]GenBank/EMBL/DDBJ accession No. AF015950.
[d]Takara, Kyoto, Japan.
[e]pCR2.1 vector, Invitrogen, Carlsbad, Calif.
[f]ABI Prism Big Dye Primer Cycle Sequencing Ready Reaction Kit, Applied Biosystems, Foster City, Calif.
[g]TRAPeze telomerase detection kit, Intergen, Purchase, NY.
[h]Bio-Rad DC protein assay kit, Bio-Rad Laboratories, Hercules, Calif.
[i]AmpliTaq, Applied Biosystems, Foster City, Calif.
[j]Molecular Probes, Eugene, Ore.
[k]XC-75 video camera module, SONY, Tokyo, Japan.
[l]NIH Image, version 1.62, US National Institute of Health, Bethesda, Md.
[m]RNeasy Mini Kit, Qiagen, Hilden, Germany.
[n]Ultrospec 3000 pro, Amersham Biosciences, Piscataway, NJ.
[o]GeneAmp RNA PCR kit, Applied Biosystems, Foster City, Calif.
[p]AmpliTaq Gold, Applied Biosystems, Foster City, Calif.
[q]GenBank/EMBL/DDBJ accession No. AB094677.
[r]GenBank/EMBL/DDBJ accession No. AF051911.

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