J Vet Diagn Invest 15:399–406 (2003)

Identification of canine transmissible venereal tumor cells using in situ polymerase chain reaction and the stable sequence of the long interspersed nuclear element

Kuang-Wen Liao, Zei-Yi Lin, Hai-Nie Pao, Sook-Yee Kam, Fun-In Wang, Rea-Min Chu

Abstract. Canine transmissible venereal tumor (CTVT) is a unique tumor that can be transplanted across the major histocompatibility complex (MHC) barrier by viable tumor cells. In dogs, CTVT grows progressively for a few months and then usually regresses spontaneously. A long interspersed nuclear element (LINE) insertion is found specifically and constantly in the 5′ end of the CTVT cell c-myc gene, outside the first exon. The rearranged LINE–c-myc gene sequence has been used with polymerase chain reaction (PCR) to diagnose CTVT. However, in CTVT cells, the total length of the inserted LINE gene is not constant. In this experiment, variation in the inserted LINE gene was studied to determine which parts of the LINE sequence can be used as primers to identify CTVT cells with in situ PCR (IS PCR). The LINE gene was inserted between the TATA boxes in the promoter region of c-myc. In CTVT cells, deletions of different lengths are frequent in this gene. However, the 550-bp segment at the 5′ end of the LINE–c-myc gene was stable. Thus, primers were designed to cover the stable 0.55-kb segment from the 5′ end outside the first exon of the c-myc gene to the 5′ end of LINE gene stable segment. With these primers and IS PCR, individual CTVT cells in formalin-fixed tissue sections and CTVT cultures were identified. Cells from other canine tumors were negative for this gene. In addition, the CTVT-specific, 0.55-kb segment was not found in any spindle-shaped cells from progressive or regressive phase CTVT. The IS PCR technique also did not detect any positive spindle-shaped cells in CTVT cell cultures. Thus, fibroblastic terminal differentiation is less likely to be a mechanism for spontaneous regression of CTVT cells.

Canine transmissible venereal tumor (CTVT) is a naturally occurring tumor transmitted by viable tumor cells. CTVT cells are small and round, with a large nucleolus and prominent cytoplasm in which cytoplasmic vacuolization is common. Although the origin of CTVT cells has not been well defined, studies using immunohistochemical staining indicate that the tumor origin is histiocytic.[25,27] The normal chromosome number for dogs is 78, and only the sex chromosomes are metacentric. In CTVT cells, the number of chromosomes ranges from 57 to 59, and 16 to 18 chromosomes are metacentric.[28,31]

Transposable elements (TE), or transposons, are a group of discrete genes that transport themselves directly from one site to another within the genome.[26] Transposon activity has been associated with chromosomal rearrangements, such as deletions, duplications, inversions, and translocations, and the recombination of host genomes. A 1.6-kb-long interspersed nuclear element (LINE),[20] a TE sequence or retroposon, was found in CTVT cells, inserted in the 5′ end of the c-myc gene, outside the first exon. The truncated LINE-1 element has been sequenced,[7] and it contains a 1,378-bp insert flanked by a 10-bp direct repeat upstream of the c-myc gene. The DNA sequence of the LINE gene is similar to the repetitive element of primates.[20] This unique insertion has been used to diagnose CTVT with polymerase chain reaction (PCR).[9] However, the PCR products from the primers varied in length. Therefore, the stable portion of the LINE gene insertion yielded a constant result. In addition, in situ PCR (IS PCR) was developed to identify the LINE gene insertion in individual cells in formalin-fixed tissue and tumor cell smears. A 0.55-kb segment, which began at the 5′ end, outside the first exon of c-myc, was stable and was excellent for identifying CTVT cells with IS PCR.

After transmission to a susceptible host, CTVT grows progressively for 4–6 months and then usually regresses spontaneously.[8,12] The spontaneous regression of CTVT may be caused by host humoral and cellular immune responses against CTVT[10,13] and overexpression of major histocompatibility complex (MHC) molecules.[19,36] During the progression phase (P phase) when the tumor mass continuously grows, CTVT expresses very small amounts of MHC molecules because of a lack of β2-microglobulin.[11] During tumor regression (R phase), as it decreases in size, CTVT increases the expression of MHC molecules,[19,36] which may increase the cytotoxic susceptibility of CTVT cells to tumor-infiltrating lymphocytes or peripheral blood lymphocyte. In other studies, the number of spindle-shaped cells increased during CTVT re-

From the Department of Veterinary Medicine, National Taiwan University, Taipei, Taiwan 106, ROC. Current address (Chu): 142 Chou-San Road, Taipei, Taiwan 106, ROC.

gression, which suggests that these cells were a transitional stage in the differentiation of fibroblasts.[18,22] Using anti-ferritin/antitumor-associated antigen (TAA, with a molecular weight of 70 kD) hybrid antibodies against CTVT, specific ferritin grains were detected on the fibroblast-like cells in CTVT with an electron microscope.[17] There have been no further studies with this particular TAA. In this study, the IS PCR technique and primers associated with the LINE insertion were used to investigate whether the fibroblastic cells in the tumor mass or in tumor cell cultures were positive for LINE insertion.

Materials and methods

Sample preparations

Suspensions of 1×10^8 viable tumor cells from a spontaneous case of CTVT were injected subcutaneously into each of the 10 sites in the back of 3 mixed-breed dogs. After inoculation, 1 tumor mass was excised from each dog every 3 wk during the P phase or every 1 to 2 wk during the R phase. Part of each tumor mass was mechanically crushed with a stainless steel mesh and passed through a 2-layered metal filter (pore size: 190 μm). The CTVT cells were isolated with a 42% Percoll gradient as described previously.[19] The isolated CTVT cells were cultured at 37 C in Dulbecco modified Eagle medium[a] containing 10% fetal bovine serum.[a] Another portion of each tumor was fixed in 10% buffered formalin, embedded in paraffin, and cut into 4-μm-thick sections. The IS PCR technique was used to test for the LINE–c-myc insertion in cultured CTVT cells, tissue sections, in formalin-fixed and paraffin-embedded sections of other spontaneous canine tumors, including cutaneous histiocytoma (3), cutaneous mast cell tumor (3), cutaneous lymphoma (2), cutaneous squamous carcinoma (2), and mammary gland adenocarcinoma (3). These tumors were obtained from the National Taiwan University Veterinary Teaching Hospital, Taipei, Taiwan.

Primers

Primers P1 and T3 were designed to cover 1.6 kb of the LINE–c-myc insertion,[20] between the 5′ end of c-myc, outside the first exon, and the 3′ end of the LINE gene.[9] Another set of primers, Myc S-2 and LINE AS1, was designed to cover a 553-bp segment extending from the 5′ end of the first c-myc exon of the LINE insertion (Fig. 1).

T1: ATGCACCAAGATTTTCTTCACTGC

P3: GCCATGAGATTCAAAACCAAGGCGG

MycS-2: ATTCCTACGAATGAATGATTGGCCAGA

LINE AS-1: CAGACACATAGATCAGTGGAACAGAAT.

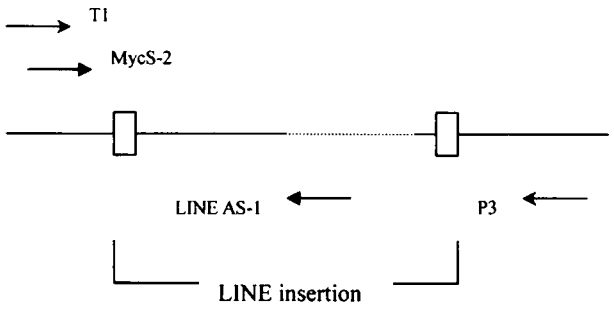

Figure 1. Design of PCR primers for CTVT cell identification.

PCR detection of the LINE–c-myc insertion

The CTVT tissue (200 mg) was ground and mixed with 1.2 ml digestion buffer (10 mM Tris–Cl, pH 8; 100 mM NaCl; 25 mM ethylenediamine-tetraacetic acid [EDTA], pH 8; 0.5% sodium dodecyl sulfate; and 0.1 mg/ml Proteinase K). Tissues were shaken in a water bath (50 C) for at least 12 hr and then separated by sequentially reacting them with phenol, phenol–chloroform, and chloroform. Supernatants were collected and mixed with 0.8 volume of isopropanol and 0.1 volume of 3 M sodium acetate (pH 5.2). After centrifugation, the DNA was washed, air dried, and dissolved in sterilized water. The DNA concentration was measured (OD at 260 nm). The amount of DNA in sterilized water was determined by the formula: total DNA (μg) = 50 × (OD at 260) × (dilution fold) × (total volume in milliliters). The PCR was performed as described previously.[9] In brief, the following reagents were added to 1 μg canine genomic DNA to make 50 μl of PCR solution[b]: 50 mM KCl, 10 mM Tris–HCl pH 9.0, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 100 μM each of dATP, dCTP, dGTP, dTTP, 5 U *Taq* DNA polymerase, and 0.8 μM of each primer. The samples were processed in a Mastercycler[c]: 1 cycle at 94 C for 5 min; 35 cycles at 94 C for 1 min, 64 C for 50 sec, 72 C for 1 min, and 72 C for 5 min. Positive and negative controls were included.

Each 10 μl of PCR product was gel electrophoresed (2.0% agarose gel prepared with Tris–borate–EDTA buffer) at 100 volts for 20 min. The size of the PCR products was evaluated with a DNA ladder marker.[d] The products were stained with ethidium bromide solution (0.5 μg/ml) and viewed at 260 nm with a UV illuminator. The PCR products were sequenced with an automatic DNA sequencer.[e]

Fifty CTVT cells suspended in 10 μl phosphate-buffered saline (PBS) were used as a template, mixed with PCR reagents (20 μM LINE-AS1, 2 μl; MycS-2, 2 μl; 10× buffer, 5 μl; 15 mM Mg, 10 μl; 25 mM diethylnitrophyenyl thiophosphate [dNTP], 4 μl; *Taq*, 1 μl; and H_2O, 16 μl), and PCR was performed with a PCR device[f] as described above. After PCR, the samples were eletrophoresed on 1.5% agarose gel. The gel

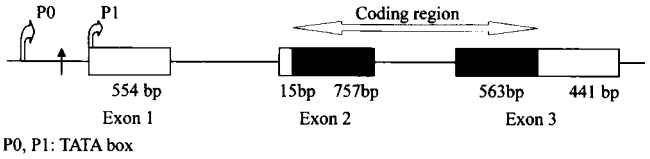

Figure 2. Insertion site of the LINE gene (arrow) at the 5′ end of the first exon of CTVT c-myc.

was observed with a UV box and photographed. The PCR products were sequenced using an automatic DNA sequencer.[e]

In situ PCR

The CTVT suspension cells and spindle-shaped cells. Suspensions of freshly isolated CTVT cells were smeared and air-dried on glass slides. The CTVT cells were washed gently and collected for the first passage. Fibroblasts that remained at the bottom of the culture flask were discarded. Fibroblasts grown in the first and second passages were collected for further experiments. The CTVT smears and fibroblasts were fixed with 4% paraformaldehyde for 15 min at room temperature. Slides were washed 3 times in PBS and then rinsed with 0.1 M glycine in PBS for 30 min to block free aldehyde groups. Slides were treated with 0.05% Tween 20 in PBS for 20 min at room temperature and washed 3 times with PBS. The samples were covered with in situ flame,[c] overlaid with 65 μl of the PCR reagent (MycS-2, 3 μl; LINE AS-1, 3 μl; 10× buffer with Mg^{2+}, 6.5 μl; dNTP-dig, 0.65 μl; double distilled water (DDW), 33.8 μl; *Taq*, 0.65 μl) and sealed with cover slips.[c] The PCR was performed as described above.

After PCR, the samples were stained with AP-conjugated anti-dig antibody (Ab) mixture[g] (0.1% fragment ab (FAB)–anti-dig = 500:1) for 1 hr at room temperature and washed 3 times (5 min each time) with PBS. Samples were developed in 15 ml of alkaline phoshatase (AP) substrate[h] (25× AP color development buffer 0.6 ml, H_2O 14.4 ml, 150 μl AP color reagent A, 150 μl AP color reagent B). The reaction was stopped with water, and the samples were examined under a light microscope.

The CTVT sections. The CTVT sections (4-μm thick) were treated with xylene for 20 min at 37 C and rinsed in series of ethanol solutions (100%, 95%, and 75%) for 1 min each. The IS PCR technique was performed as described above using the sections as templates. The IS PCR technique was also performed on sections from 2 to 3 cases of each of the canine tumors, including cutaneous histiocytoma, cutaneous mast cell tumor, cutaneous lymphoma, squamous cell carcinoma, and canine mammary gland adenocarcinoma.

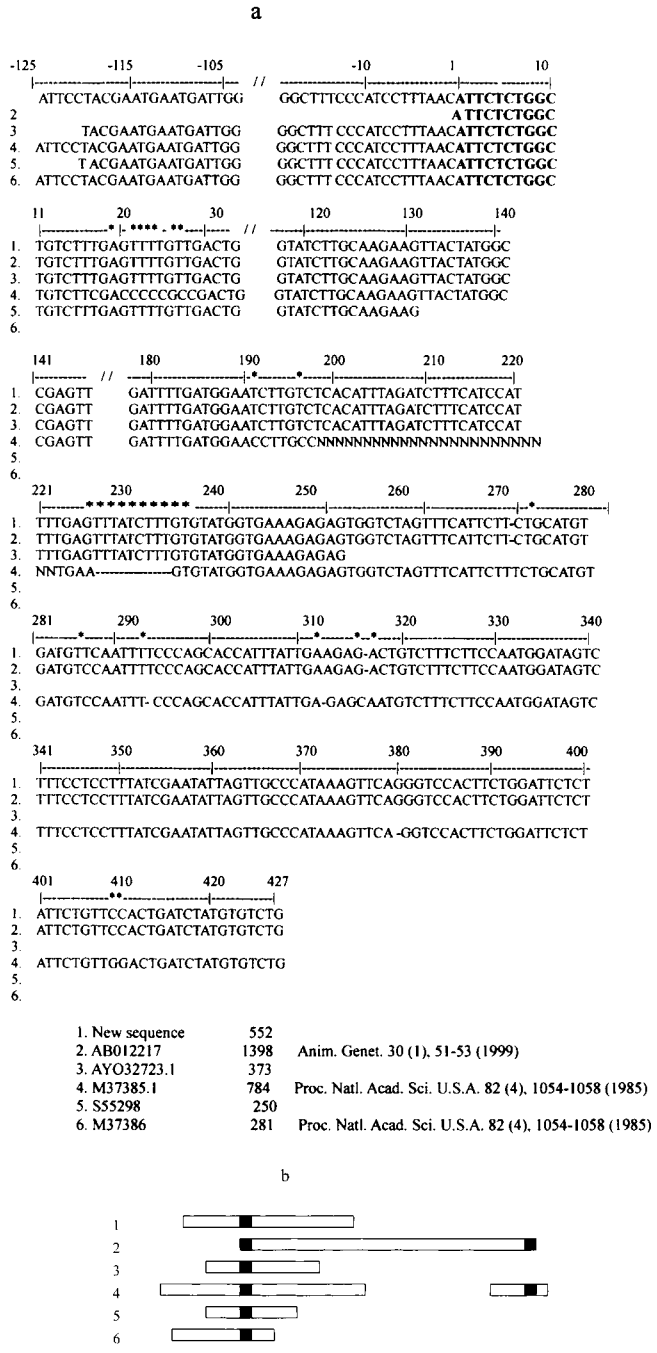

Figure 3. Nucleotide sequence homology of the 0.55-kb segment and 5 previously published sequences. **a**, the nucleotide sequence of the 0.55-kb segment obtained using PCR with the second set of primers (Myc S-2 and LINE AS1) is shown with 5 other published sequences. The 10 bp shown in bold is the direct repeats outside the 1 exon of c-myc at the boundary of the LINE gene insertion. **b**, diagramatic representations of the 6 sequences. The black blocks represent the 10-bp direct repeat (ATTCTCTGGC) of the c-myc sequence. The blank blocks are sequenced segments, and the empty spaces in between the 10-bp direct repeats in 5′ and 3′ ends are segments that are not being sequenced or are missing.

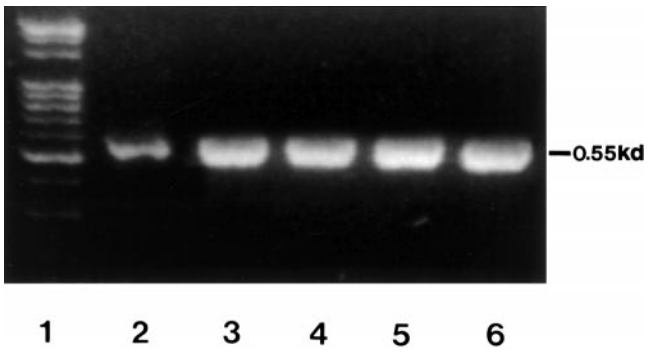

Figure 4. Gel electrophoresis of the second pair of primer PCR products from isolated CTVT cells from 5 different P phase tumors. Lane 1, 100-bp ladder molecular markers; Lanes 2–6, CTVT cells from P phase tumors.

Results

Primer selection

The first pair of primers (T1 and P3) did not always amplify the 1.6-kb sequence of CTVT cell genomic DNA. However, there were a variety of PCR products from 1.6 to 0.55 kb; most were shorter than 0.7 kb. Sequencing the DNA of these PCR products revealed that many lacked a sequence in the 0.88-kb segment upstream of the 3′ end of the LINE insertion. By comparing the c-myc promoter sequences between humans and dogs, LINE insertions were always found between the TATA boxes of the c-myc gene of CTVT (Fig. 2). Therefore, a second set of primers (Myc S-2 and LINE AS1) was designed to target the 0.55-kb sequence from outside the 5′ end of the first exon of c-myc to the 5′ end of the LINE insertion. The nucleotide sequence of the 0.55-kb PCR product was compared with published sequences (Fig. 3a). The sequence was

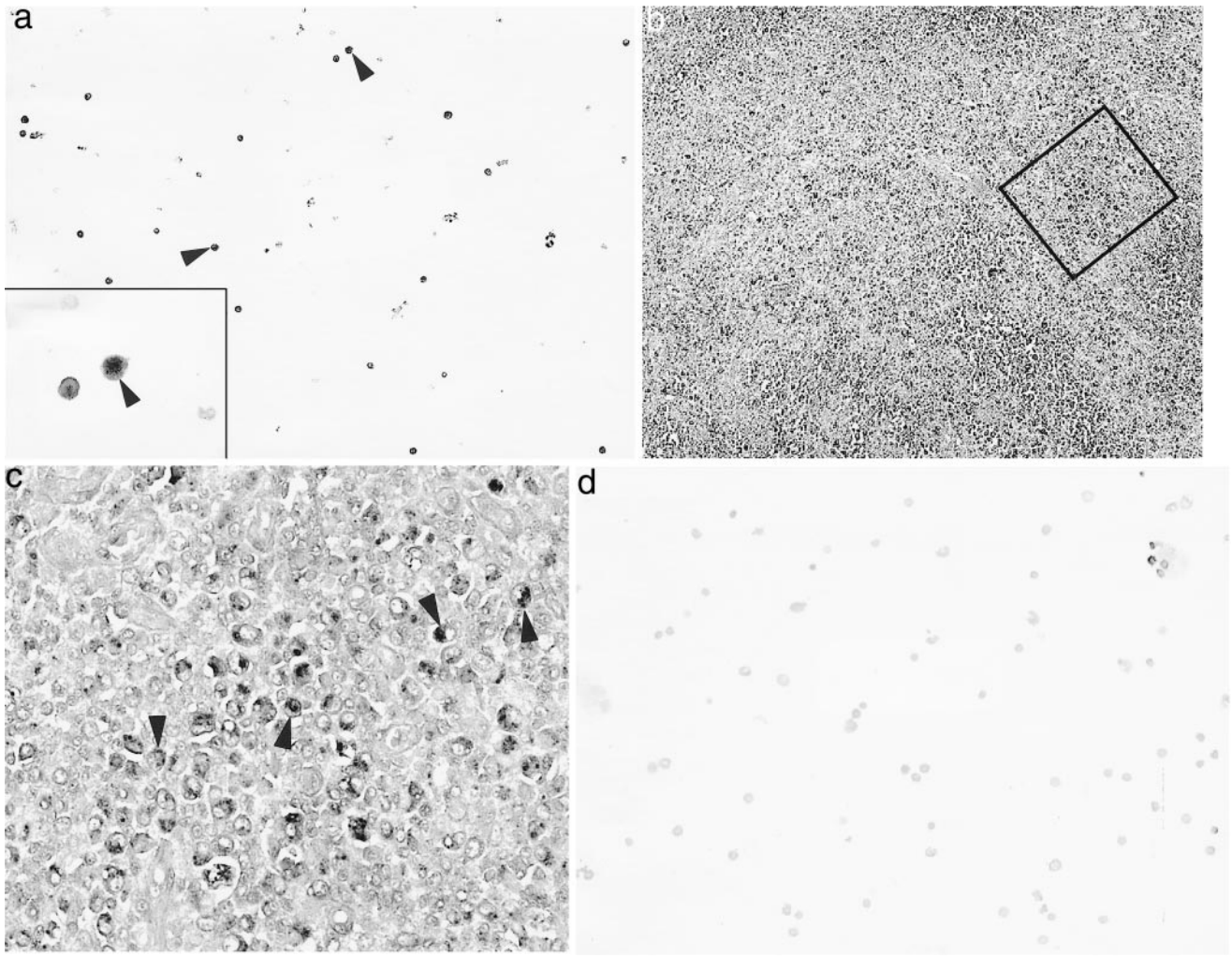

Figure 5. In situ PCR results for cell suspensions and tumor tissue sections. **a**, positive cells were found in the suspension of CTVT cells and a higher power view insert (arrowheads), and **b**, in the cells of CTVT tissue sections. **c**, positive cells (arrowheads) in a higher power view of the marked area of **b**. **d**, positive cells were not found in white blood cells of a normal dog blood.

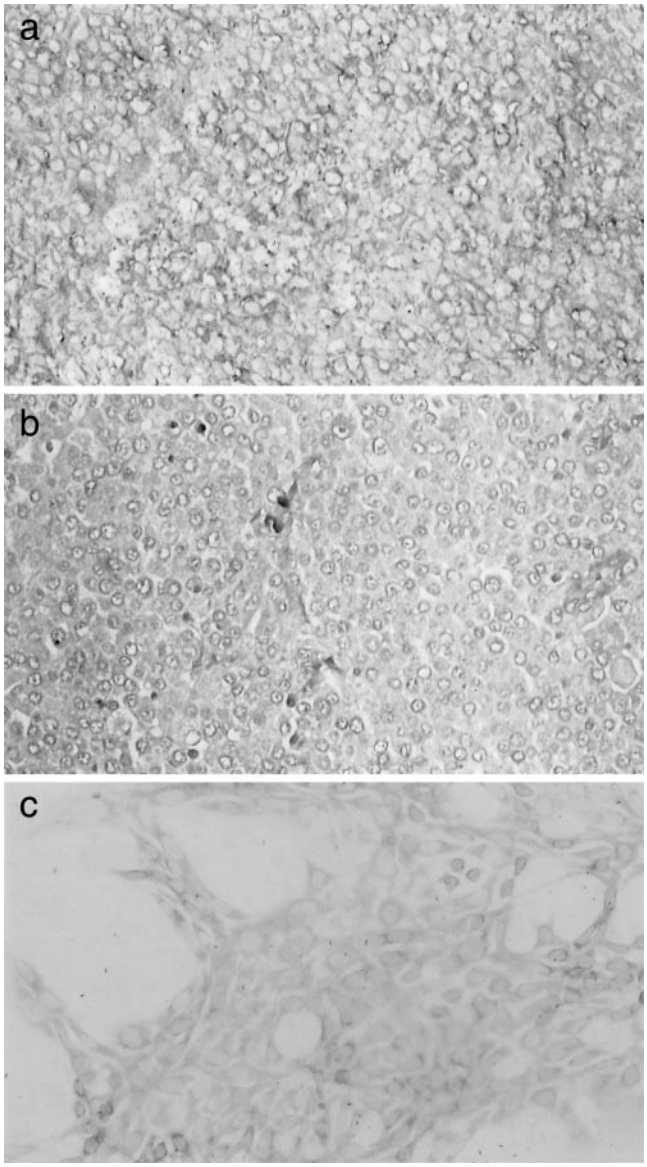

Figure 6. Tissue samples, including **a**, cutaneous lymphoma, **b**, cutaneous histiocytoma, **c**, and squamous carcinoma, were negative.

identical to the sequence reported previously[9] and was 99.8% and 97.5% homologous to the sequences reported by others.[7,20] On comparing the published sequences, it was seen that the 5′ end of the inserted LINE gene was always present, on the contrary, most of the 3′ end sequencing data were missing (Fig. 3b). The PCR product of the second set of primers was specific because it was always 0.55 kb, and a nonspecific, 0.2-kb band was absent (Fig. 4). Therefore, the second pair of primers was used in the development of IS PCR.

The IS PCR technique

The IS PCR technique readily detected the LINE–c-myc sequence in the nucleus of cultured CTVT cells in suspension (Fig. 5a). With this technique, the gene could be detected in as few as 3 cells. However, in suspensions with more than 39 cells, definitive detection was not possible because of excess cell components. The same results were obtained using IS PCR on tumor cells smeared on a glass slide and tumor tissue sections (Fig. 5b, 5c). No LINE–c-myc sequence was detected in the white blood cell from a normal dog (Fig. 5d) or in the cells of any of the other tested canine tumor sections (Fig. 6a–6c). The IS PCR technique did not detect the 0.55-kb LINE–c-myc sequence in any of the spindle-shaped cells from deparaffinized, formalin-fixed CTVT sections or from the first and second CTVT passages (Fig. 7).

Discussion

There are 2 kinds of TE. Retroposons (Class 1 TE) code for a reverse transcriptase that makes DNA from their RNA transcript and integrates the DNA at new sites. Transposons (Class 2 TE) code for a transposase and directly manipulate DNA.[16] Known retroposons (class 1 elements) include *Ali-*, *Ty-*, *copia*-like elements, and LINE or L1. Known transposons (class 2 elements) include *IS 10*, *IS 50*, *P*, *Ac*, *Tam*, and *Spm.* Retroposons are classified further by the presence (LTR) or absence (non-LTR) of long terminal repeats. Transposable elements are ubiquitous in prokaryotes and eukaryotes and make up about 10% of the *Drosophila* genome,[14] 50% of the maize genome,[6] and 40% of the mammalian genome.[35]

The LINE gene insertion has been found in CTVT cells collected in many countries around the world.[1,9] The insertion always begins at the 5′ end, outside the first exon of c-myc gene.[1,9,20] Because the insertion is specific, a PCR method was developed to diagnose this tumor.[9] The primers (T1 and P3) used with this PCR method[8] obtained a specific, 1.6-kb segment. However, a nonspecific 0.2-kb band was present in all tissues tested, including normal canine tissue. Furthermore, PCR with primers T1 and P3 did not always detect the 1.6-kb segment. After repeated PCR and sequencing, it was found that part of the LINE gene insertion near the 3′ end of the 1.6-kb segment was frequently missing, and the missing sequence usually was less than 0.88 kb. The missing sequences occurred in the 0.88-kb segment upstream of the 3′ end of the LINE gene insertion and not in the remaining 0.55-kb segment. Thus, the 0.55-kb segment is a stable region, and the 0.86-kb is an unstable region. Similar results were found on comparing the DNA sequences of LINE gene insertions in CTVT cells described in other published studies (Fig. 3b). Therefore, a pair of primers (MycS-2 and LineAS-1) was designed to target the 0.55-kb segment. The new primers were superior to the old primers in that PCR results were constant and the non-

Figure 7. In situ PCR results for spindle-shaped cells. No spindle-shaped cells from the first passage of CTVT culture were positive.

specific 0.2-kb band was no longer present in any of the samples. Thus, in this study, IS PCR was developed using MycS-2 and LineAS-1.

The new primers were first tested, successfully, in CTVT cell cultures derived from tumors in the experimental dogs. Then the primers were applied to formalin-fixed, deparaffinized tissue sections. Positive reactions were specific to the CTVT cell nuclei. No positive reactions were obtained from normal dog tissues, other kinds of canine tumors, or human gastric adenocarcinoma. Thus, IS PCR system using primers MycS-2 and LineAS-1 specifically identifies CTVT cells, including those in formalin-fixed tissue sections.

It is interesting that in CTVT cells the 3′ end of the LINE sequence is partly deleted. In studies on other mammals, LINE insertions were usually severely truncated at the 5′ end.[35] Other types of retroposons, such as Kpn, also suffer from truncations, inversions, or deletions.[34] The role of LINE deletions in CTVT cells is not known. Retroposons were thought to be selfish DNA that provided no benefits to their host. However, retroposons can affect the function of the host genome in a variety of ways. The LINE gene mutations in 2 loci defective for RNA interference will desilence tandem repeats in the *Caenorhabditis elegans* germline.[5] In humans, 14 different disease-producing retrotransposition events involving diseases, such as hemophilia A and B and muscular dystrophy, are now known.[21] Only a few studies have demonstrated an association between retroposons and carcinogenesis. Insertion of the intracisternal A particle retroposon genome in c-mos in mouse plamacytoma has transforming activity.[32] Transposon insertion can alter the regulation and expression of flanking genes.[15] In addition, c-myc mRNA of CTVT cells was more abundant than that of normal liver cells and suggested that the inserted LINE sequence affects c-myc transcription level.[20] The effect of the LINE gene insertion on CTVT cell transformation is not known, but c-myc is involved in the tumorigenic transformation of primary embryo fibroblasts.[23] Sustained activation of c-myc in adult suprabasal epidermis induces papillomatosis with angiogenesis, and deactivation of the oncogene spontaneously regresses the premalignant changes.[30]

Terminal differentiation usually results in tumor regression.[2,24] During the stable and regression phases, the morphology of CTVT cells observed with an electron microscope was transitional between oval and spindle shaped.[18,22] The spindle-shaped cells contained cytoplasmic vacuolar collagens, which were taken as evidence that the CTVT cells were undergoing fibroblastic differentiation. In addition, TAAs were present on transitional CTVT cells and fibroblastic cells.[17] The molecular weight of the TAA was over 70 kd,[29] but this TAA has not been characterized further. Using IS PCR, the CTVT-specific, LINE gene insertion at the

5′ end, outside the first exon of c-myc, was not found in the spindle-shaped cells in tumor sections or in the CTVT cultures. Therefore, fibroblastic terminal differentiation is less likely to be responsible for CTVT regression. However, the possibility that loss of the LINE gene insertion could result in terminal differentiation cannot be ruled out.

Other oncogenes and differentiation-inducing agents are involved in the terminal differentiation process. Neu was found to be a paracrine differentiation factor for prostate gland cancer.[24] Neu differentiation factor (heregulin) upregulates expression of p53 by stabilizing the protein and upregulates p53-inducible gene $p21^{WAF1/CIPI}$ in cell lines.[3] erbB apparently induces tumor cell differentiation.[2] Chemicals such as theophylline[33] and butyrate[4] can also induce growth inhibition and differentiation. Little is known about the oncogenes associated with terminal differentiation of canine cancers. Detailed research on canine molecular genetics is needed to gain a fuller understanding of the mechanisms.

Sources and manufacturers

a. GIBCO-BRL, Life Technologies, Paisley, Scotland, UK.
b. Promega, Madison, WI.
c. Eppendorf, Hamburg, Germany.
d. Bio-100™, Protech, Taipei, Taiwan, ROC.
e. Model 377, PE-ABI, Foster city, CA.
f. Mastercycler, Eppendorf, Hamburg, Germany.
g. Roche Molecular Biochemicals, Mannheim, Germany.
h. Bio-Rad Laboratories, Hercules, CA.

References

1. Amariglia EN, Hakim I, Simoni FB, et al.: 1991, Identity of rearranged LINE/c-MYC junction sequences specific for the canine transmissible venereal tumor. Proc Natl Acad Sci USA 88: 8136–8139.
2. Bacus SS, Huberman E, Chin D, et al.: 1992, A ligand for the erbB-2 oncogene product (gp30) induces differentiation of human breast cancer cells. Cell Growth Differ 3:401–411.
3. Bacus SS, Yarden Y, Oren M, et al.: 1996, Neu differentiation factor (Heregulin) activates a p53-dependent pathway in cancer cells. Oncogene 12:2535–2547.
4. Barnard JA, Warwick G: 1993, Butyrate rapidly induced growth inhibition and differentiation in HT-29 cells. Cell Growth Differ 4:495–501.
5. Birchler JA, Bhadra MP, Bhadra U: 2000, Making noise about silence: repression of repeated genes in animals. Curr Opin Gen Dev 10:211–216.
6. Chen M, San Miguel P, De Oliveira AC, et al.: 1997, Microcolinearity in sh2-homologus regions of the maize, rice and sorghum genomes. Proc Natl Acad Sci USA 94:3431–3435.
7. Choi Y, Ishiguro N, Shinagawa M: 1999, Molecular structure of canine LINE-1 elements in canine transmissible venereal tumor. Anim Genet 30:51–53.
8. Chu RM, Lin JY, Liu CG, et al.: 2001, Proliferation characteristics of canine transmissible venereal tumor. Anticaner Res 21: 4017–4028.
9. Chu RM, Sun TJ, Yang HY: 2001, Heat shock proteins in canine transmissible venereal tumor. Vet Immunol Immunopathol 82: 9–21.
10. Cohen D: 1980, In vitro cell-mediated cytotoxicity and antibody-dependent cytotoxicity to the transmissible venereal tumor of the dog. J Natl Cancer Inst 64:317–321.
11. Cohen D, Shalev A, Krup M: 1984, Lack of beta 2-microglobulin on the surface of canine transmissible venereal tumor cells. J Natl Cancer Inst 72:395–401.
12. Epstein RB, Bennett BT: 1974, Histocompatibility typing and course of canine venereal tumors transplanted into unmodified random dogs. Cancer Res 34:788–793.
13. Fenton MA, Yang TJ: 1988, Role of humoral immunity in progressive and regressive and metastatic growth of the canine transmissible venereal sarcoma. Oncology 45:210–213.
14. Finnegan DJ, Fawcett DH: 1986, Transposable elements in Drosophila melanogaster. *In:* Oxford surveys on eucaryotic genes, ed. Maclean N, vol. 3, pp. 1–62. Oxford University Press, Wellington Square, Oxford, UK.
15. Girard L, Freeling M: 1999, Regulatory changes as a consequence of transposon insertion. Dev Genet 25:291–196.
16. Gray YHM: 2000, It takes two transposons to tango. Trends Genet 16:461–468.
17. Hill DL, Yang TJ: 1985, Immunoelectron microscopic localization of a tumor-associated antigen (TAA) in the canine transmissible venereal sarcoma by anti-TAA-antiferritin hybrid antibodies. J Natl Cancer Inst 75:725–732.
18. Hill DL, Yang TJ, Wachitel A: 1984, Canine transmissible venereal sarcoma: tumor cell and infiltrating leukocyte ultrastructure at different growth stages. Vet Pathol 21:39–45.
19. Hsiao YW, Liao KW, Hung SW, Chu RM: 2002, Effect of tumor infiltrating lymphocyte on the expression of MHC molecules in canine transmissible venereal tumor cells. Vet Immunol Immunopathol 87:19–28.
20. Katzir N, Rechave G, Cohen JB, et al.: 1985, ‘‘Retroposon’’ insertion into the cellular oncogene *c-myc* in canine transmissible venereal tumor. Proc Natl Acad Sci USA 82:1054–1058.
21. Kazazian HH Jr: 2000, L1 retrotransposons shape the mammalian genome. Science 289:1152–1153.
22. Kennedy JR, Yang TJ, Alien PL: 1977, Canine transmissible venereal sarcoma: electron microscopic changes with time after transplantation. Br J Cancer 36:375–385.
23. Land EJ, Parada LF, Weinberg RA: 1983, Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature 304:596–602.
24. Lyne JC, Melhem MF, Finley GG, et al.: 1997, Tissue expression of neu differentiation factor/heregulin and its receptor complex in prostate cancer and its biologic effects on prostate cancer cells in vitro. Cancer J Sci Am 3:21–30.
25. Marchal T, Chabanne L, Kaplanski C: 1997, Immunophenotype of the canine transmissible venereal tumour. Vet Immunol Immunopathol 57:1–11.
26. McClintock B: 1947, Cytogenetic studies of maize and Neurospora. Carnegie Inst Wash Year Book 46:146–152.
27. Mozos E, Mendez A, Gomez-Villamandos JC, et al.: 1996, Immunohistochemical characterization of canine transmissible venereal tumor. Vet Pathol 33:257–263.
28. Murray M, James ZH, Martin WB: 1969, A study of the cytology and karyotype of the canine transmissible venereal tumour. Res Vet Sci 10:565–568.
29. Palker TJ, Yang TJ: 1981, Identification and physicochemical characterization of a tumor-associated antigen from canine transmissible venereal sarcoma. J Natl Cancer Inst 66:779–787.
30. Pelengaris S, Littlewood T, Khan M, et al.: 1999, Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. Mol Cell 3:565–577.
31. Prier JE: 1966, Chromosome pattern of canine transmissible sarcoma cells in culture. Nature 212:724–726.
32. Rechavi G, Givol D, Canaani E: 1982, Activation of a cellular

oncogene by DNA rearrangement: possible involvement of an IS-like element. Nature 300:607–611.
33. Sandlund JT, Neckers LM, Schneller HE, et al.: 1993, Theophylline induced differentiation provides direct evidence for the deregulation of c-myc in Burkitt's lymphoma and suggests participation of immunoglobulin enhancer sequences. Cancer Res 53:127–132.
34. Shafit-agardo B, Brown FL, Zavodny PJ, Naio JJ: 1983, Transcription of the *Kpn*I families of long interspersed DNAs in human cells. Nature 304:277–280.
35. Smit AFA: 1999, Interspersed repeats and other mementos of transposable elements in mammalian genomes. Curr Opin Gen Dev 9:657–663.
36. Yang TJ, Chandler JP, Dunne-Anway S: 1987, Growth stage dependent expression of MHC antigens on the canine transmissible venereal sarcoma. Br J Cancer 55:131–134.