

Disseminated transmissible venereal tumor in a dog

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Abstract. Transmissible venereal tumor (TVT) is a well-documented transplantable tumor in dogs, with no breed or sex predilection and a low metastatic rate. In this report, a 2-year-old intact female Mastiff that had numerous, rapidly growing masses throughout the subcutis mainly at the dorsal body plane, the caudal half of the ventral abdomen, and around the vulva was euthanized due to poor prognosis. Neoplastic nodules similar to those seen in the subcutis were also noted in the lung, anterior mediastinum, liver, spleen, kidney, and superficial and deep lymph nodes in both abdominal and thoracic cavities. The neoplastic nodules from the subcutis as well as metastatic foci revealed similar cytologic and histologic features, which were consistent with canine TVT. By immunohistochemical staining, the neoplastic cells were positive for lysozyme and vimentin but were negative for cytokeratin, desmin, CD3, and CD79a. The diagnosis of the TVT was further supported by the identification and analysis of long interspersed nuclear elements (LINE) from paraffin-embedded tumor tissue. This case is a rare example of TVT with multiorgan metastasis. In this case, the polymerase chain reaction technique was useful in differential diagnosis of canine round cell tumors because this technique can be applied in retrospective as well as future study.

Key words: Dog; LINE; lysozyme; metastasis; PCR; transmissible venereal tumor.

Canine transmissible venereal tumor (TVT), also known as canine transmissible sarcoma, has been reported from many regions of the world and is a naturally occurring contagious round cell tumor of dogs.⁵ It is the only known naturally occurring tumor that can be transplanted as an allograft across major histocompatibility barriers within the same species, and even to other members of the canine family, such as coyotes, foxes, and wolves.⁷ Due to the unique nature of transmission by sexual contact, the external genitalia of either sex are most commonly affected. Less commonly, the tumor may also be transmitted to the nasal or oral cavities, skin, and the rectum by sniffing or licking.¹⁰ More rarely, they may be found in other areas, including the lips, oral mucosa, and peritoneum, or in organs such as the tonsils, eye, liver, spleen, kidney, lung, and musculature.^{10,11,13} In this report, we describe a TVT that is unique in that it is accompanied by metastasis to multiple organs in a young Mastiff. The diagnosis was supported by cytology, histopathology, immunohistochemical staining, as well as polymerase chain reaction technique.

A 2-year-old intact female Mastiff was presented to

a local veterinary hospital. On physical examination, multiple dermal masses that had been rapidly growing for the last 3 weeks were palpated throughout the body surface. The masses were firm and freely movable. Because of the dog's deteriorating health and the poor prognosis, the owner elected euthanasia. The dog was necropsied at the Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University.

At necropsy, multiple round to oval, firm nodules, ranging from 3 cm to 12 cm in diameter, were noted throughout the subcutis, mainly at the caudal half of the ventral abdomen and around the vulva, where the neoplastic masses often formed chains (Fig. 1). The masses were often encapsulated and attached to the subcutaneous plane. On cut section, the masses were tan with areas of necrosis and hemorrhage. The mucosal wall of the vulva was also thickened. Neoplastic nodules similar to those in the subcutis were also present in the lung, anterior mediastinum, liver, spleen, kidney, and many lymph nodes in both abdominal and thoracic cavities. Impression smears were made from several neoplastic masses, air dried, and stained with Dip-quick stain.

The neoplastic masses from different anatomical locations and other representative tissues of the dog were collected, fixed in 10% neutral buffered formalin, routinely processed, embedded in paraffin, sectioned at 4 μ m, and stained with hematoxylin and eosin (HE) for microscopic examination. Replicate sections from the neoplastic masses were also stained with toluidine blue to rule out canine mast cell tumor. The presence of

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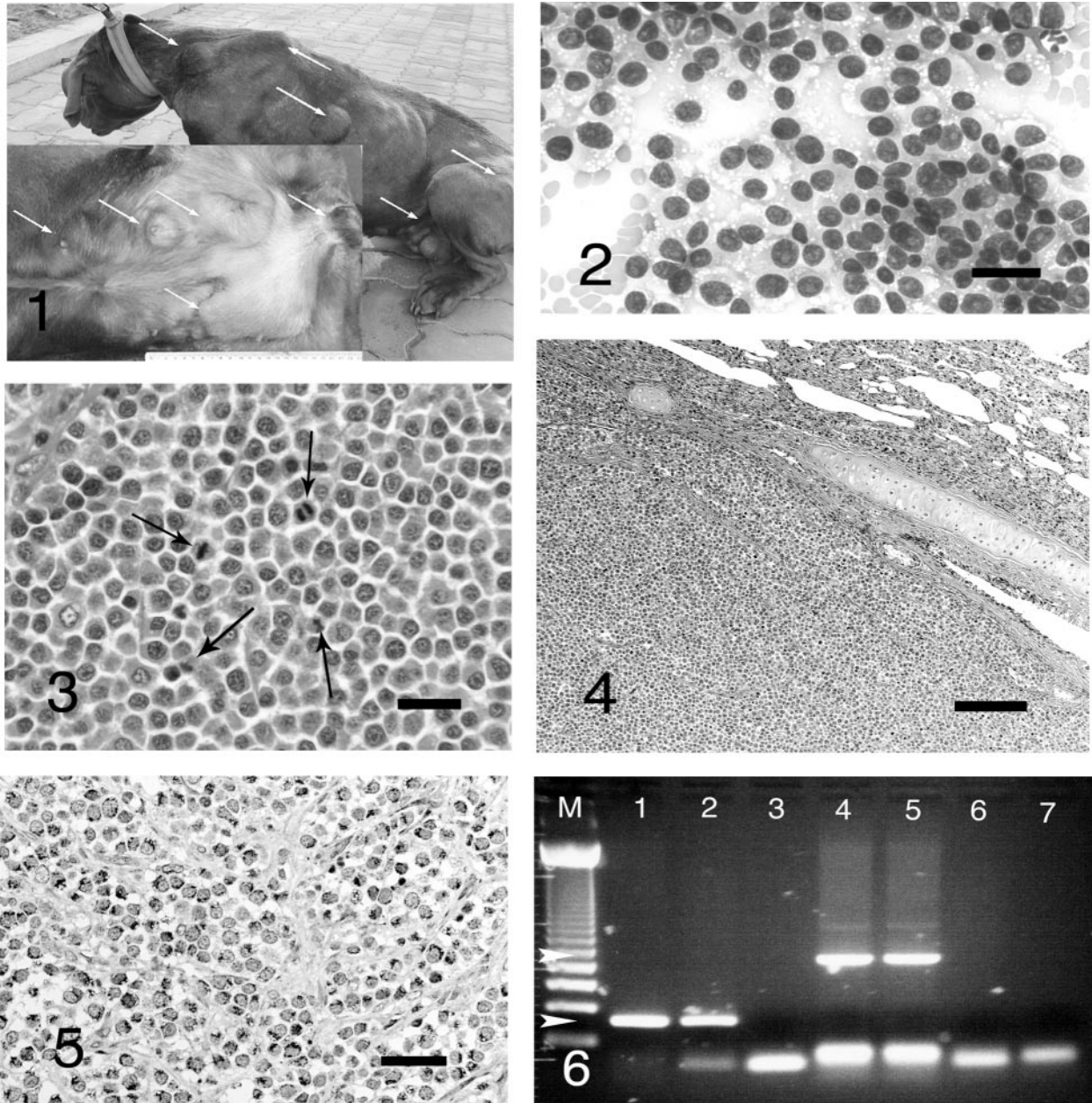


Figure 1. Mastiff dog. There are multiple round to oval white neoplastic nodules ranging from 3 cm to 12 cm in diameter, scattered throughout the subcutis of trunk, mainly at the caudal half of the ventral abdomen, where the neoplastic masses often formed chains.

Figure 2. Lymph node; Mastiff dog. Impression smear. Tumor cells were present individually or in sheets. They were round with distinct cytoplasmic borders. The cytoplasm was slightly blue or clear, finely granular, and contained clear, distinct vacuoles arranged along the periphery of the cells. Oval to round nuclei were usually centrally located and had a prominent nucleolus and finely stippled chromatin. Neucleocytoplasmic ratio was high. Dip-quick stain. Bar = 450 μ m.

Figure 3. Vulvar mass; Mastiff dog. The tumor is composed of diffuse sheets of round cells and scant amounts of connective tissue stroma. Individual neoplastic cells had a round hyperchromatic nucleus with a distinct eosinophilic nucleolus and stippled chromatin. The cytoplasm was moderate and granular, with distinct cytoplasmic borders. Anisokaryosis was mild to moderate. Note frequent mitotic figures. HE. Bar = 40 μ m.

Figure 4. Lung; Mastiff dog. Diffuse sheets of neoplastic tissue displaced and replaced normal architecture of the lung. Note normal cartilage of the bronchiole at one side. HE. Bar = 300 μ m.

Figure 5. Vulvar mass; Mastiff dog. Cytoplasm of tumor cells are positively labeled with lysozyme antibody. Avidin–biotin Complex method with DAB. Bar = 80 μ m.

Figure 6. Gel electrophoresis of the first (lanes 1–3) and second (lanes 4–7) pairs of primer PCR products from paraffin-embedded tumor tissue and normal tissue. PCR products were loaded on a 2% agarose gel. Note 0.2-kb bands on lanes 1 and 2, and 0.55-kb bands on lanes 4 and 5. M: 123-bp ladder molecular markers (upper arrowhead: 550 bp; lower arrowhead: 200 bp). Lanes 1, 4, and 5, tumor tissue; lanes 2 and 6, normal tissue; lanes 3 and 7, water.

tumor markers was evaluated by immunohistochemistry (IHC) using Vectastain Elite ABC Kit.^a Primary antibodies used were vimentin,^b cytokeratin AE1/AE3,^c desmin,^b CD3,^b neuron specific enolase (NSE),^c lysozyme,^d S-100,^b and CD79a^b. Diaminobenzidine (DAB)^a was used as a chromogen to demonstrate the antigens.

To further characterize the tumor, the polymerase chain reaction (PCR) technique was used to identify long interspersed nuclear elements (LINE) in tumor tissues. The methods for DNA isolation have been described.¹⁴ Briefly, DNA was isolated from paraffin-embedded sections containing neoplastic foci using a DNAeasy tissue kit.^e DNA was amplified by the PCR with primers for LINE. The PCR procedure has been reported previously.⁸ DNA extracted from the normal areas and no-DNA controls were included in all sets of reactions. The PCR products were sequenced using BigDye Terminator v 1.1 Cycle Sequencing Kit^f and ABI PRISM 3100 sequencer.^f Prior to sequencing, PCR products were purified using a QIAquick Gel Extraction Kit.^e The amplification primers also served as sequencing primers.

The impression smears of the neoplasm were cellular and had similar features both in the genital and extragenital tumor. Tumor cells were present in sheets with occasional individual cells (Fig. 2). They were round with distinct cytoplasmic borders. The cytoplasm was slightly blue or clear, finely granular, and contained clear, distinct vacuoles arranged along the periphery of the cells (Fig. 2). Oval to round nuclei were usually centrally located and had a prominent nucleolus and finely stippled chromatin. Nucleocytoplasmic ratio ranged from 1:1 to 4:1. Anisokaryosis and anisocytosis were mild to moderate and a few mitotic figures were present.

Histologically, the neoplastic masses from genital areas as well as metastatic foci revealed similar findings (Fig. 3). The neoplastic foci were composed of diffuse sheets of round cells and scant amounts of connective-tissue stroma. In some areas, neoplastic cells were arranged in pseudoalveolar pattern with intervening delicate fibrous stroma. Individual neoplastic cells had a round hyperchromatic nucleus with coarsely stippled chromatin and a distinct eosinophilic nucleolus. The cytoplasm was moderate and granular with indistinct cytoplasmic borders. Anisokaryosis was mild to moderate, and mitotic rates were high, ranging from 3 to 7 mitoses per 400 \times microscopic fields. Necrosis and hemorrhage were present in some areas. There was minimal to mild perivascular infiltration of lymphocytes, plasma cells, and rare macrophages. Neoplastic cells within the submucosa of the vagina elevated the overlying mucosa with attenuated epithelial cells. The neoplastic cells within the extragenital

areas, including lungs (Fig. 4), kidneys, and liver displaced and replaced the preexisting normal architecture. In some areas, tumor cell foci were well demarcated and surrounded by compressed parenchyma. In the liver, the neoplastic cells were present within the sinusoids, expanding the lumen and attenuating the adjacent hepatic cells cords.

By IHC staining, the neoplastic cells were positive for lysozyme (Fig. 5) and vimentin and negative for cytokeratin, desmin, NSE, S-100, CD3, and CD79a. Toluidine blue staining did not reveal metachromatic granules in the tumor cells. The anatomical location of the tumor, cytologic and histopathological features strongly suggested a diagnosis of TVT, and the IHC staining profile was consistent with that reported in canine TVT.^{3,10,12} The diagnosis of TVT was further supported by identification and analysis of LINE element by molecular techniques (Fig. 6). One pair of primers (T1 and P3) did not amplify the 1.6-kb segment of genomic DNA but produced a nonspecific 0.2-kb band from all tissues tested, including normal tissues (Fig. 6). The other set of primers (Myc S2 and LINE AS-1) amplified the 0.55-kb segment from the tumor genomic DNA only. The nucleotide sequence of the 0.55-kb product was identical to the sequence reported previously (data not shown).⁸

Differential diagnoses for this case included lymphoma, canine cutaneous histiocytoma, anaplastic mast cell tumor, amelanotic melanoma, and poorly differentiated carcinomas.⁹ The negative reactivity to CD3, CD79 used as markers for T and B lymphocytes contributed to the differentiation of TVT from lymphoma, which was further supported by positive staining against vimentin. Positive staining for vimentin also excluded undifferentiated carcinoma, which was corroborated by the negative staining for cytokeratin. Negative S-100 protein allowed differentiation of TVT from amelanotic melanoma. The neoplastic cells did not have metachromatic granules when stained with toluidine blue, which excluded anaplastic mast cell tumor. The possibility of canine cutaneous histiocytoma was excluded based on the anatomical location of the tumors and the cytologic features.⁵

Consistent with previous reports, the neoplastic cells in this case revealed the expression of lysozyme. Despite many studies, the cellular origin of TVT remains unidentified. However, the expression of lysozyme and alpha-1-antitrypsin suggests a histiocytic origin of this neoplasm.⁹ In some reports, tumor cells contained *Leishmania* amastigotes within the cytoplasm, further suggesting histiocytic origin of TVT.^{1,2}

Canine transmissible venereal tumors grow rapidly at first and then remain static for a time, with eventual spontaneous regression after several months.⁶ Regression is associated with increased numbers of tumor-

infiltrating lymphocytes and is characterized by increased apoptotic tumor cells and fibrosis.⁶ In this dog, the tumor appeared to be in progressive growth stage, as suggested by minimal involvement of tumor-infiltrating lymphocytes and high numbers of mitotic figures.

The biological behavior of TVT is quite variable and depends on the host immune response. Metastasis of TVT usually occurs in suboptimal physiological conditions of the dog, such as immunosuppression, malnutrition, or young age. There was no evidence of immunosuppression or malnutrition in the present case. Metastasis is uncommon in TVT but is most often seen in the skin and lymph nodes.¹⁰ The involvement of visceral organs is a rare event in naturally occurring TVT and the metastasis is probably by hematogenous or lymphatic routes.¹¹ A few cases having brain/eye and hepatic metastasis have been reported.^{4,12}

This report presents a canine TVT with metastasis to multiple visceral organs with a concurrent detectable genital focus of the tumor. Impression smears, IHC staining, as well as identification of LINE element by molecular techniques aided in the differentiation from other round cell tumors. Furthermore, this case proved that the identification and analysis of the LINE element could be done in retrospective study using paraffin-embedded tissues for differential diagnosis of canine round cell tumors.

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Sources and manufacturers

- a. Vector Laboratories, Inc., Burlingame, CA.
- b. Dako, Carpinteria, CA.
- c. Biogenex, San Ramon, CA.
- d. Biomedica, Frost city, CA.
- e. QIAGEN Inc., Valencia, CA.
- f. Applied Biosystems, Foster City, CA.

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