Lymphangiosarcoma in a Dog

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ABSTRACT. Lymphangiosarcoma was seen in the subcutis of right chest in a 11-year-old female Poodle. No metastasis was observed clinically. Tumor cells were vimentin positive and formed irregular space or slit without erythrocytes in the tumor tissue. Lymphocytic foci and edema were seen in the stroma. Only a few tumor cells had factor VIII-related antigen. Electronmicroscopically, tumor cells did not accompany with basement membrane and intercellular junctional complex.—KEY WORDS: canine, lymphangiosarcoma.
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Lymphangiosarcoma has been rarely encountered in dogs [5, 10, 19], cats [28], cattle [20] and rats [13]. This tumor reported in the past 50 years [11]. Stewart and Treves made the first report that radical mastectomy for breast cancer caused lymphangiosarcoma following persistent lymphedema and the condition has been referred to Stewart-Treves Syndrome (STS) [22]. However, lymphedema is not a constant feature in the patients with lymphangiosarcoma. There were five reported cases in dogs based on clinical and histopathological changes [5, 10, 19]. Present note describes histopathological, immunohistochemical and ultrastructural findings of canine lymphangiosarcoma.

A 11-year-old female Poodle dog was admitted to an animal clinic due to the subcutaneous tumor in the right breast. Six years ago, the owner found a nodule measuring 1.5 × 1.0 cm. This tumor mass had slowly grown up to 5.0 × 3.0 cm in size. Another mass measuring 3 cm in diameter located in the adjacent portion to the larger mass. X-ray examination revealed no metastatic lesions in the lung. The other organs had no detectable abnormalities.

Tumors were surgically excised, fixed in 10% neutral buffered formalin and sent to our laboratory. No recurrence and metastasis were seen during 8 months after excision. The skin overlying the larger mass was partially ulcerated. This solid and elastic neoplasm was grayish-white in color and mottled with hemorrhages and necroses.

The larger mass was consisted of spindle-shaped tumor cells often forming irregular lumens or slit-like spaces without erythrocytes (Fig. 1). Most lumens or spaces were lined by single layer of tumor cells with occasional piling up. Hemorrhage was present in the tumor tissue. The stroma was consisted of dense collagen bundles showing partial hyalinization (Fig. 1). Interstitial edema and focal aggregation of lymphocytes were also prominent (Fig. 2). Reticular fibers were inserted between tumor cells (Fig. 3). The tumor cells exhibited slight atypia and pleomorphism, varying in size (Fig. 4). In the highly cellular area, tumor cells loosely arranged and proliferated side by side (Fig. 4). Some intermingled tumor cell bundles were present in the other areas. A few tumor cells contained small number of lipofuscin granules. PAS stained sections proved discrete basement membrane around capillary vessels, but was extremely rare around the tumor cells. The tumor cell nuclei contained relatively rich heterochro-

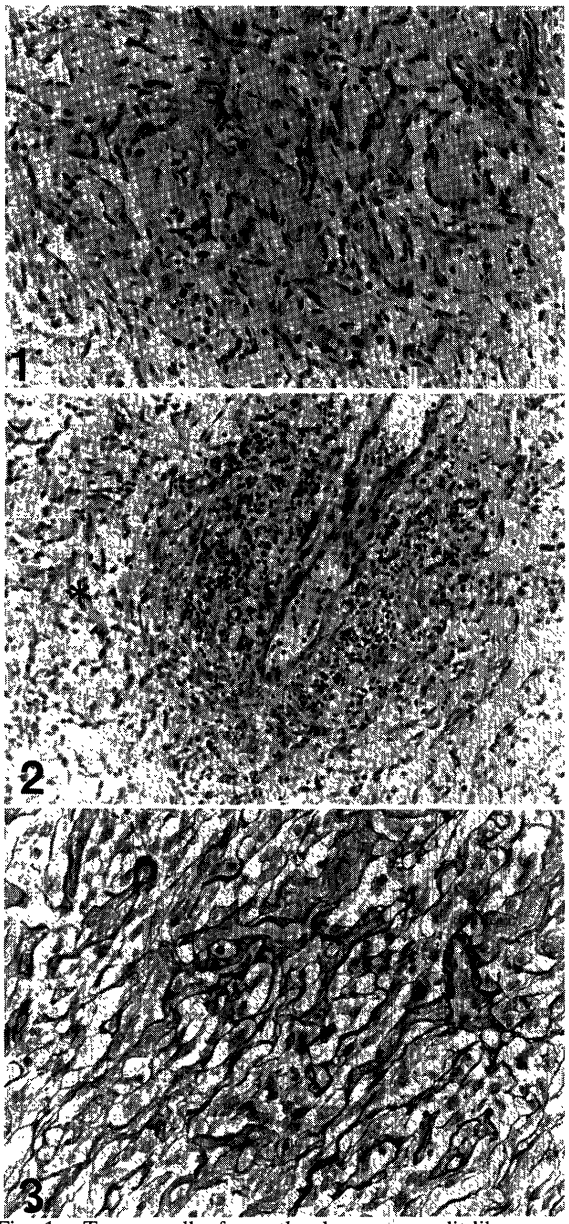


Fig. 1. Tumor cells form the lumens or slit-like spaces without erythrocytes. The stroma is consisted of abundant collagen fibers, occationally with hyalinization. H.E. stain. ×215.

Fig. 2. Focal aggregates of lymphocytes around the small vessel. The stroma is edematous (*). H.E. stain, × 215.

Fig. 3. Most tumor cells proliferate along reticulin fibers. Silver impregnation stain, × 430.

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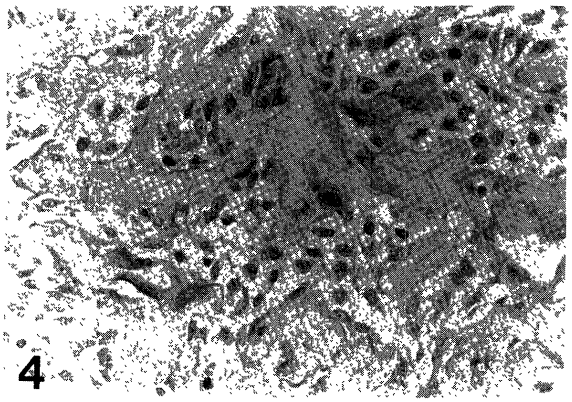


Fig. 4. Tumor cells showed slight atypia and pleomorphysm in the highly cellular area. Tumor cells had vesicular nuclei with distinct nucleoli. PAS reaction, × 430.

matin and indistinct nucleoli and frequent mitoses. The cytoplasm of tumor cells was basophilic. Most tumor cells reacted to anti-swine vimentin antibody (Dakopatts, Denmark) after microwave oven heating, while only a few cells were positive for factor VIII-related antigen (FVIIIRAg) (anti-human FVIIIRAg antibody, Dakopatts, Denmark) (Fig. 5). No reaction to anti-rat type IV collagen antibody (kindly supplied by Dr. M. Ito and Dr. K. Arai) was present around the tumor cells (Fig. 6), however, the basement membrane of blood capillaries scattered in the neoplastic tissue showed conspicuously positive reaction. Most of tumor cells expressed proliferating cell nuclear antigen (PCNA) (anti-PCNA antibody, Dako A/S, Denmark). Neither vascular endothelium nor tumor cells reacted to *Ulex europaeus* agglutinin-I (UEA-I: E-Y LABORATORIES, INC., U.S.A.), Peanut agglu-

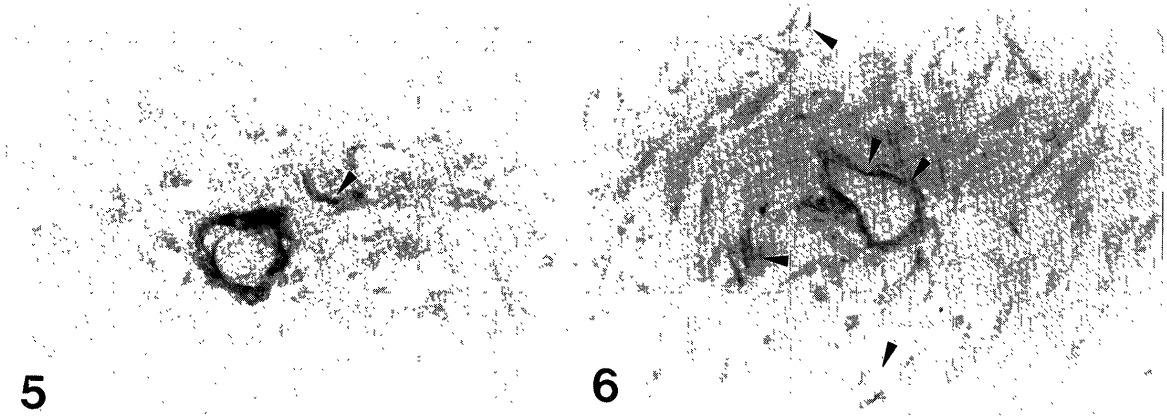


Fig. 5. A few tumor cells (arrowheads) react with factor VIII-related antigen. The endothelial cells of small vessel as a internal control are positive. Immunostaining with anti-human factor VIII-related antigen antibody, Methyl green counterstain. × 860.

Fig. 6. The wall of small vessels showing positive reaction to type IV collagen (arrowheads). No positive reaction around tumor cells. Immunostaining with anti-rat type IV collagen antibody. Methyl green counterstain. × 430.

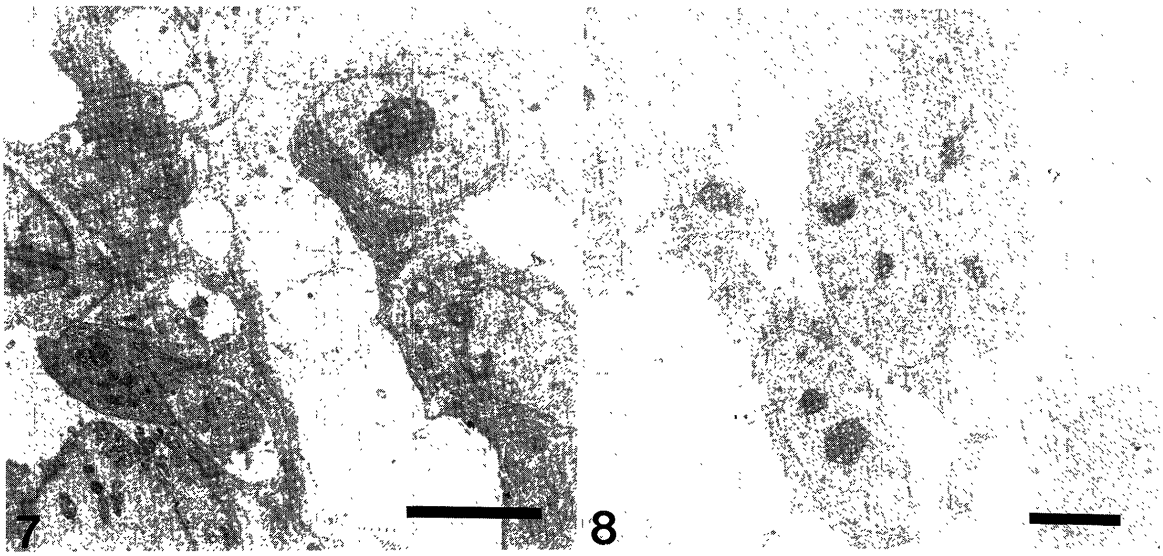


Fig. 7. Tumor cells with moderately developed organella showing luminal formation. Tumor cells lacked in basal lamina and intercellular junctional complex. Electron micrograph, Uranyl acetate and lead citrate, Bar = 5 μm.

Fig. 8. Closely attached tumor cells in edematous area. Electron micrograph, Uranyl acetate and lead citrate. Bar = 5 μm.

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tinin (PNA: E-Y LABORATORIES, INC., U.S.A.) anti-α-smooth muscle actin (SIGMA CHEMICAL CO., U.S.A.), desmin (Bio-science, Switzerland) and actin (ENZO DIAGNOSTIC INC., U.S.A.) antibodies. Tumor cells showed aggressive character in a part of the subcutaneous tissue and accompanied with myofibroblast proliferation. Electronmicroscopically, moderate number of mitochondria and rough endoplasmic reticula were present in the tumor cells, but no Weibel-Palade bodies (WPB) were seen (Figs. 7, 8). Neoplastic cells lacked in basal lamina and intercellular junctional complex (Figs. 7, 8). Some tumor cells had a few micropinocytic vesicles. The other mass was a mammary gland adenoma.

Most of human lymphangiosarcoma occurred in the site of chronic lymphedema following radical mastectomy. Lymphedema is considered to play an important role in the development of lymphangiosarcoma [24]. Present case lacked in edematous lesion around the tumor tissue and metastasis clinically. However, all canine cases with lymphangiosarcoma that have been reported in literature [5, 9, 19] bore preexisting edema and distant metastasis. Feline cases seldom showed metastasis, but recurrence following amputation or extensive excision was not uncommon [4], suggesting poor prognosis. The recurrence may take place after long silent period so that the present case should be followed up.

The present case should be differentiated from benign lymphangioma and hemangiosarcoma. It seemed to be difficult to differentiate lymphangioma from lymphangiosarcoma in dogs [26] and cats [4]. Peripheral dissection of collagen bundles by endothelial cells, blindly ending stromal trabeculae, and slight nuclear pleomorphysm are useful indicators of malignancy [26]. The previously reported four canine lymphangiomas [21] mainly consisted of cystic spaces lined by flattened endothelial cells. The present case was different from these canine lymphangiomas because of high cellularity, many mitoses, nuclear pleomorphysm/atypism and solid proliferation without lumen or cystic space formation.

Table 1. Immunohistochemical markers of the vascular and lymphatic endothelium in normal and neoplastic condition

Specimen from	FVIIIRAg	UEA-I	5'-N	CD31
Kaposi's sarcoma [2]	−	+	+	
Stewart-Treves syndrome [14]	+	+		
Canine lymphatic endothelium [16]	+			
Human lymphatic endothelium [27]		−		
Human lymphangioma [12]		+		
Human angiosarcoma [8]	−			
Human lymphangiosarcomatosis [18]	+			+
Canine lymphatic endothelium [25]	+			
Rat vascular endothelium [17]	+			
Rat lymphatic endothelium [17]	+			
Present case	(+)	−		

+:Positive, (+): Positive in a few tumor cells, −: Negative. FVIIIRAg: Factor VIII-related antigen, UEA-I: *Ulex europaeus* agglutinin-I, 5'-N: 5'-nucleotidase

Walder and Gross stated that stromal edema, lymphocytic infiltration, and the paucity of erythrocytes within vascular channels supported a diagnosis of lymphangiosarcoma [26]. The present case coincided with their descriptions. Many investigators had reported immunohistochemical markers for both vascular and lymphatic endothelium (Table 1). However, these results might depend on the employed fixatives, the duration of fixation and antibodies used. Furthermore, the phenotype of the endothelium might vary with the vascular size and malignant manifestation of tumors. FVIIIRAg expressed in the tumor cells of hemangioma and hemangiosarcoma in dogs [25] and cats [15]. Lectin UEA-I is useful marker of human vascular endothelium [27], however, it didn't bind to normal canine endothelium. Some malignant vascular tumors in dogs are likely to react to UEA-I [1]. PNA bound to vascular endothelium within the tumor tissue, but not to tumor cells. PNA is thought to be an appropriate marker for canine vascular endothelium in some tissues [7]. Actin was also a marker for vascular endothelium and was considered to be a cytoskeletal protein showing the widest variation with malignant differentiation. Many workers indicated that the morphological difference between vascular and lymphatic endothelium was the presence of basement membrane [6]. Extensive studies in rats to differentiate both endothelium revealed that immunohistochemical detection of laminin and type IV collagen was reliable and practical way by light microscopy [17]. The tumor cells of our case had no basement membrane and type IV collagen around the tumor cells and in the stroma, indicating that the origin was lymphatic endothelium. Numerous PCNA positive cells in this case suggested high proliferating activity.

WPB, the ultrastructural marker for vascular endothelium, seemed to disappear after malignant transformation [3]. Lymphatic endothelium was thought to lack in WPB, but some investigators claimed the presence of WPB in lymphatic endothelium [17, 23].

In a feline lymphangiosarcoma, malignant manifestation of hemangioma has been suggested [28]. The growth of the present case was very slow, suggesting a malignant transformation in the end stage of a benign counterpart.

Recently, Kindblom *et al.* indicated that angiosarcoma in STS originated from both vascular and lymphatic endothelium [11]. Angiosarcoma in STS seemed to show variegated figures histologically. However, in this case, histologic feature of the tumor was homogeneous.

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