



Hifumi T et al., Figure S1.

Figure S1. A—Immunohistochemical labeling with vimentin diffusely showed strong cytoplasmic immunoreactivity of neoplastic cells. Mayer's hematoxylin counterstaining. Bar = 20 μ m. B—Immunohistochemical labeling with S-100 protein diffusely showed strong cytoplasmic and nuclear immunoreactivity of neoplastic cells. Mayer's hematoxylin counterstaining. Bar = 20 μ m. C, D, E—Neoplastic cells were negative for panCK (C), Iba1-1 (D), melanoma marker (PNL2) (E). Additionally, immunohistochemical labeling with Iba-1 (D) diffusely showed strong cytoplasmic immunoreactivity of macrophages infiltrating between neoplastic cells. Mayer's hematoxylin counterstaining. Bar = 20 μ m. F—Immunohistochemical labeling with vimentin diffusely showed strong cytoplasmic immunoreactivity of fibrous connective tissues proliferating in the dermis of canine skin tissue. Mayer's hematoxylin counterstaining. Bar = 50 μ m. G—Immunohistochemical labeling with panCK diffusely showed strong cytoplasmic immunoreactivity of the epidermis and skin appendages in canine skin tissue. Mayer's hematoxylin counterstaining. Bar = 100 μ m. H—Immunohistochemical labeling with UCP1 diffusely showed strong cytoplasmic immunoreactivity of brown adipocytes in mouse tissue. Mayer's hematoxylin counterstaining. Bar = 20 μ m. I—Immunohistochemical labeling with myogenin sporadically showed nuclear immunoreactivity of regenerative striated muscles (black arrow) of canine skeletal muscle tissue. Mayer's hematoxylin counterstaining. Bar = 20 μ m. J—Immunohistochemical labeling with S-100 protein diffusely showed strong cytoplasmic and nuclear immunoreactivity of ganglion cells distributed in myenteric plexus of canine ileal tissue. Mayer's hematoxylin counterstaining. Bar = 20 μ m. K—Immunohistochemical labeling with Iba-1 diffusely showed strong cytoplasmic immunoreactivity of macrophages in medullary sinuses of canine inguinal lymph node tissue. Mayer's hematoxylin counterstaining. Bar = 50 μ m. L—Immunohistochemical labeling with melanoma marker (PNL2) sporadically showed strong cytoplasmic immunoreactivity of neoplastic cells of canine oral malignant melanoma tissue. Mayer's hematoxylin counterstaining. Bar = 20 μ m.

Table S1. Primary antibodies used for immunohistochemistry

Antibody	Host (Clone)	Dilution	Antigen retrieval	Source	Catalog no.	Positive cells**
Vimentin	Mouse monoclonal (3B4)	1:200	pH6.0, Autoclave	Dako, Glostrup, Denmark	M7020	Fibrous connective tissue
Keratin /cytokeratin (panCK) *	Rabbit polyclonal	Prediluted	Proteinase K	Nichirei Biosciences, Tokyo, Japan	422061	Epidermis and skin appendages
Uncoupling protein 1 (UCP1) *	Rabbit polyclonal	1:1,000	pH6.0, Autoclave	Abcam, Cambridge, UK	ab10983	Brown adipocyte of mouse
Myogenin	Mouse monoclonal (F5D)	1:200	pH6.0, Autoclave	Origene, Rockville, MD, USA	AM33347PU-T	Regenerative striated muscle
S-100 protein *	Rabbit polyclonal	1:300	pH6.0, Microwave	Spring Bioscience, California, USA	E2140	Ganglion cell
Ionized calcium-binding adapter molecule 1 (Iba-1)	Rabbit polyclonal	1:500	pH6.0, Microwave	Wako Pure Chemical Industries, Osaka, Japan	019-19741	Macrophage
Melanoma Marker	Mouse monoclonal (PNL-2)	1:100	pH6.0, Autoclave	Santa Cruz Biotechnology, California, USA	sc-59306	Malignant melanoma

pH6.0: 0.01 M citrate buffer solution

* 10% normal goat serum (Nichirei Biosciences, Tokyo, Japan) was applied for blocking before adding primary antibody.

** All primary antibodies except for UCP1 were tested on positive control tissues obtained from dogs.

Supplementary Figure 1 shows immunohistochemistry for antibodies except UCP1 and myogenin, and positive control tissues.

Table S2. Differentiation points between hibernoma and other diseases

	Rhabdomyoma	Granular cell tumor	Oncocytoma	Xanthogranuloma	Balloon cell melanoma	Liposarcoma	Hibernoma
Vimentin	+	+	−	+	+	+	+
UCP1	NR*	NR*	NR*	NR*	NR*	+/-	+
S-100 protein	−	+	+	−	+/-	+/-	+
Myogenin	−	−	−	−	−	+/-	+/-
PanCK	−	−	+	−	−	−	−
Iba-1	−	−	−	+	−	−	−
Melanoma marker (PNL-2)	−	−	−	−	+	−	−
Differentiation points other than immunohistochemistry from hibernoma	PTAH-positive cross striation	PAS-positive granules	Lacking lipid vacuoles	Lipid-laden foamy macrophages	Production of various amounts of melanin pigment	Higher cellularity, atypia, proliferative activity	Sudan Black B(+), PAS(-), PTAH(-) HE: Round to polygonal neoplastic cells having granular eosinophilic, multivacuolated, and univacuolated cytoplasm with mild nuclear atypia TEM: Numerous lipid droplets and mitochondria within the cytoplasm of neoplastic cells

NR*: Not reported

HE: Hematoxylin and eosin, PAS: Periodic acid–Schiff, PTAH: Phosphotungstic acid-hematoxylin

TEM: Transmission Electron Microscopy