CASE REPORT

Cytologic, histologic, and immunohistochemical features of lingual liposarcoma in a dog

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Abstract: A 9-year-old female spayed mixed-breed dog was presented to the referring veterinarian with a history of decreased appetite and difficulty with prehension and swallowing because of a firm oval mass in the tongue. On cytologic evaluation of a fine-needle aspirate of the mass there were numerous round to polygonal cells organized individually or in loose clusters with rare branching capillaries. The cells had eosinophilic granular cytoplasm, round to oval nuclei, and occasionally indistinct borders. The cytologic diagnosis was granular cell tumor (GCT) of the tongue. Impression smears of a biopsy sample of the lingual mass contained similar eosinophilic granular cells with variable numbers of clear vacuoles in the background, numerous perivascular arrangements, and occasional lipoblasts, suggestive of liposarcoma. On histologic examination the tumor was composed of numerous lipocytes with rare foci of round eosinophilic granular cells without evidence of vacuolation; occasionally, atypical mitotic figures were seen. Immunohistochemically, the cells were uniformly negative for periodic acid-Schiff and did not express smooth muscle actin, desmin, or cytokeratin but were immunoreactive for vimentin and S100. A diagnosis of well-differentiated liposarcoma was made on the basis of morphologic and immunohistochemical results. Eosinophilic granular cells may be a component of well-differentiated liposarcoma and are not limited to GCT. Liposarcoma should be considered in the differential diagnoses of lingual tumors in the dog when cytological evaluation reveals eosinophilic granular cells consistent with GCT.

Case Presentation

A 9-year-old female spayed mixed-breed dog was presented to the referring veterinarian with a history of decreased appetite and difficulty with prehension and swallowing. Physical examination revealed a nonpainful oval mass within the parenchyma of the tongue. Regional lymphadenopathy was not present, and there were no other clinical signs. The dog was anesthetized, and an extensive oral examination revealed a firm, well-circumscribed, nonulcerated oval mass approximately 5 × 2 cm in diameter located in the ventral body of the tongue (Figure 1). Smears made from fine-needle aspirates of the mass were air-dried, submitted to the San Marco Veterinary Diagnostic Laboratory and Hospital for cytologic examination, and stained with modified-Wright’s stain (Aerospray Slide Stainer 7120, Wescor, Logan, UT, USA). The specimen was highly cellular and the morphologic preservation of the cells was good. The background was clear and contained a few small clear vacuoles and rare RBCs. Nucleated cells were arranged individually or in loose sheets or clusters within which rare networks of capillaries and palisade-like arrangements were observed (Figure 2A). The cells had moderate N:C ratios and occasionally indistinct borders, pink finely granular cytoplasm, and round to oval nuclei that were usually centrally located and had coarse to stippled chromatin and, occasionally, 1–2 indistinct small round nucleoli (Figure 2B). Mild anisocytosis and anisokaryosis were evident. The cytologic interpretation was granular cell tumor (GCT) based on the presence of many large eosinophilic granular cells and location in the tongue. As GCTs comprise a heterogeneous group of
neoplasms, differential diagnoses included tumors of mesenchymal and neuroendocrine origin.

An incisional biopsy of the lingual mass was obtained, and impression smears of the biopsy sample were air-dried and stained with modified-Wright's stain (Aerospray Slide Stainer 7120, Wescor, Logan, UT, USA). Cytologic findings were similar to those for the first sample, except increased number of branching capillaries and clear vacuoles in the background. Moreover, moderate numbers of cells had clear vacuoles in the cytoplasm and resembled lipoblasts (Figure 2C). Cytologic findings were most consistent with mesenchymal neoplasia arising from adipose tissue, rather than muscular origin, which is the most common origin for GCTs. Thus, liposarcoma was considered likely.

The formalin-fixed biopsy sample was routinely processed for histopathologic examination and stained with H&E. The specimen contained round to polygonal cells arranged in large solid sheets crossed by fibrous septa and thin vascular channels. The cells had moderately abundant cytoplasm with clear vacuoles that varied in size and round to oval nuclei with irregular chromatin and 1–2 nucleoli. Pleomorphic nuclei, displaced to the periphery of the cell by large clear vacuoles, were also noted. These cells were interpreted to be lipocytes (Figure 3A). Symmetrical and atypical mitotic figures were seen (1–2 mitosis/× 400 field). In some areas, the cells had eosinophilic granular cytoplasm without evidence of vacuolation (Figure 3B). However, the predominance of large cells containing a single vacuole or multiple clear vacuoles was typical of adipose tissue, and a morphologic diagnosis of well-differentiated liposarcoma was made.

Figure 1. An oval mass in the parenchyma of the tongue of a dog.

Figure 2. Fine-needle aspirate (A, B) and imprint (C) of a lingual mass in dog. Modified–Wright’s stain. (A) Nucleated cells are arranged individually or in loose sheets or clusters among rare networks of capillaries and palisade-like arrangements. Variably sized clear vacuoles are noted in the background. × 20 objective. (B) Nucleated cells have indistinct cellular borders and pink finely granular cytoplasm; mild anisocytosis and anisokaryosis are noted. Numerous small clear vacuoles are present in the background. × 100 objective. (C) Numerous clear vacuoles in the background surround networks of capillaries and round to spindle-shaped cells. Some cells contain a single vacuole or multiple clear vacuoles and resemble lipoblasts. × 20 objective.
To confirm the diagnosis, replicate sections of the neoplastic tissue were stained with periodic acid-Schiff (PAS), and immunohistochemical analysis using antibodies obtained from DakoCytomation (Glostrup, Denmark) for vimentin (monoclonal, 1:300), desmin (monoclonal, 1:100), sarcomeric actin (monoclonal, 1:100), cytokeratin (polyclonal, 1:40), and S100 (polyclonal, 1:100) was performed. Frozen tissue was not available for lipid staining. Neoplastic cells were negative for PAS-staining, diffusely and strongly immunoreactive for vimentin and S100 protein (Figure 4), and did not express cytokeratin, desmin, or sarcomeric actin. Thus, epithelial and skeletal and smooth muscle tumors were excluded. Expression of vimentin and S100 and vimentin has been reported for both GCTs and liposarcomas of the tongue; however, negative PAS-staining and the histologic appearance supported a diagnosis of liposarcoma.

In the few days after the biopsy sample was obtained, the lingual mass increased in size and the clinical condition of the dog worsened. The owner elected euthanasia and declined postmortem examination.

Discussion

Lingual neoplasms are rare in dogs, accounting for only 2–4% of all oropharyngeal neoplasms. In 2 retrospective studies, melanoma and squamous cell carcinoma were the most common lingual malignant neoplasms, whereas benign neoplasms were less common and included mostly plasmacytoma and GCT. Liposarcoma has been described in only 2–3% of lingual neoplasms and typically involves the subcutis of extremities and the trunk, although it may arise in almost any site, including spleen, bone, abdominal organs, extradural spine, liver, heart, lung, thorax, and bone marrow. Liposarcomas are usually locally invasive but tend to have a low metastatic rate; lung, liver, spleen, and lymph node are the most common
liposarcomas in dogs,9 and in a Japanese macaque
with a myxoid liposarcoma the cells had abundant
eosinophilic granular cytoplasm in the tissue sections, with
no evidence of myxoid material or bizarre cells,
suggested well-differentiated liposarcoma with eosin-
ophilic granular cells. In people, histologic differen-
tiation between liposarcoma and hibernoma in
well-differentiated fatty tumors may be challenging.22
Hibernoma is a rare benign tumor of brown (hibernat-
ing) fat with pale-staining and eosinophilic multivac-
culated cells, mixed with univacuolated white fat.22 In
a study of 170 human hibernomas, 24 were represented
by the eosinophilic cell subtype, in which at least 50%
of the hibernoma cells had eosinophilic granular cyto-
plasm.22 Moreover, atypical features were not found in
any of the tissue sections. Thus, with the presence of
pleomorphic nuclei and atypical mitotic figures
observed in the dog reported here, hibernoma was
unlikely.

Immunohistochemical characterization of the
tumor was useful for definitive diagnosis. Expression
of S100 and vimentin has been reported for liposarco-
ma7 and GCT,1,2 although in an immunohistochemical
study of 11 GCTs, none had immunoreactivity for
S100.23 GCT usually stains strongly positive for PAS
sites for metastases.5,6 Malignant transformation of
lipomas to liposarcomas is not known to occur.8,9 An
association with glass foreign bodies and an implanted
microchip in dogs has been reported.8,10 Recurrent
trauma, suspected in a lingual liposarcoma reported in
a human patient, may be another cause.11 An under-
lying etiology was not found in the case reported here.

In domestic animals, liposarcomas have been clas-
sified as well differentiated, myxoid, and pleomorphic
subtypes based on a similar classification established
for human tumors. In a retrospective study of canine
liposarcomas, the pleomorphic subtype represented
77% of the tumors; myxoid and well-differentiated
liposarcoma subtypes were observed in 16% and 7%
of cases, respectively.6 Histologically, pleomorphic
liposarcomas have marked cellular atypia, bizarre mul-
tinucleated cells, collagenous stroma, and few intra-
cytoplasmic lipid vacuoles.5,12 In contrast, the myxoid
variant is distinguished by the presence of abundant
eosinophilic matrix and mild to moderate anisocytosis
and anisokaryosis among the tumor cells, with clear
cytoplasmic vacuoles found in some cells. The cells are
arranged in solid or loose sheets occasionally divided
by fibrovascular stroma.13,14 In well-differentiated
liposarcomas, the diagnosis is clear because the major-
tity of cells resemble normal adipocytes, having a single
clear lipid vacule and a peripheral nucleus. Other
cells have variably sized round to oval nuclei and
abundant cytoplasm that contains lipid droplets of
variable sizes.5

Cytologic findings for these variants have also
been reported in dogs. Perivascular arrangements and
spindle to oval cells with small to large cytoplasmic
vacuoles have been observed in all subtypes of canine
liposarcoma.12–14 In addition, well-differentiated liposar-
coma is characterized by abundant lipid in the back-
ground and numerous cells with large lipid droplets
that displace and flatten the nucleus, consistent with
lipocytes.12,15 The myxoid variant is distinguished by
abundant eosinophilic Alcian blue-positive matrix
associated with cell clusters.13,14 In contrast, pleomor-
phic liposarcomas have marked cellular atypia and
bizarre binucleated or multinucleated cells.12,16

The novel aspect of the tumor reported here was
the population of round cells with pink and finely
granular cytoplasm observed in both cytologic sam-
pies, resulting in the initial diagnosis of GCT. Cytolog-
ically, GCT is composed of single cells or cells in loose
sheets, separated by a network of stromal fibers,
with eccentric nuclei and abundant eosinophilic or
strongly PAS-positive cytoplasmic phagolysosomal
granules.17,18 Although GCT is considered a tumor of
uncertain histogenesis, precursor cell types most often
are Schwann cells and to lesser extent skeletal and
smooth muscle cells, histiocytes, and fibroblasts.19 In
a recent immunohistochemical study, reactivity of the
granular cells to a broad panel of antibodies did not
confirm any particular cell type as the histogenetic
origin of GCT.1 In people, cytoplasmic granularity is
not a feature unique to GCT and has been observed in a
wide variety of benign and malignant neoplasms, in-
cluding schwannoma, hibernoma, rhabdomyoma/
rhabdomyosarcoma, leiomyoma/leiomyosarcoma,
liposarcoma (round cell), carcinoma, oncocytoma,
melanoma, and malignant fibrous histiocytomas, as
well as in reactive lesions.1,18,20 It has been suggested
that granular cytoplasmic changes simply represent a
cytologic phenotype representing metabolic alteration
or reactive change not exclusively associated with
Schwann cell tumors.1,20

Eosinophilic granular cells have been described
histologically in liposarcomas in animals. Round to
polygonal cells with lightly eosinophilic and finely
granular cytoplasm were observed in pleomorphic
liposarcomas in dogs,9 and in a Japanese macaque
with a myxoid liposarcoma the cells had abundant
eosinophilic granular or microvacuolated cytoplasm.21
Similar cells have been also observed in a disseminated
liposarcoma in a dog, classified as round cell liposarco-
ma, although loose fibrous or myxoid tissue was
detected histologically.7 In the dog reported here, the
predominance of large cells with a single vacuole or
multiple clear vacuoles and rare sheets of round eosin-
ophilic granular cytoplasm in the tissue sections, with
no evidence of myxoid material or bizarre cells,
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due to the presence of lysosomes that confer cytoplasmic granularity to the GCT cells. The negative PAS stain observed in this case ruled out a GCT supporting the definitive diagnosis of liposarcoma. In the liposarcoma reported here, there were likely 2 distinct cell populations in the histologic specimen representing a spectrum of neoplastic cells undergoing differentiation from early to mature adipocytes, as described for myxoid liposarcoma. Alternatively, the different cell populations may represent a metabolic alteration or reactive change of the same cell type, as suggested previously by others.

The current report describes eosinophilic granular cells as a component of a well-differentiated liposarcoma, indicating that granular cells are not limited to GTC and that definitive diagnosis of GCT requires additional immunologic markers to characterize the cell type of origin. Liposarcoma should be considered in the differential diagnoses of lingual tumors in the dog when cytological evaluation reveals eosinophilic granular cells consistent with GCT.

References


