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Multiple Distinct Neoplasms in a North African Hedgehog (*Atelerix algirus*)

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A B S T R A C T

This paper describes the clinical and pathological features of 4 different tumors, located in the integumentary, digestive, and endocrine systems, presenting in a North African hedgehog (*Atelerix algirus*). A 3.5-year-old female hedgehog was presented with a cutaneous mass on the right flank. The lesion consisted of a well-differentiated dermal mast cell tumor with no recurrence and metastasis after complete surgical excision. Six months later, the hedgehog developed a mass in the left lower jaw, lethargy, anorexia, and progressive weight loss. Clinical and radiographic evaluations revealed swelling, ulceration, displacement, and destruction of subjacent bone tissue, and the animal died 1 month after the onset of clinical signs. At necropsy, 2 neoplasms in the oral cavity (squamous cell carcinoma and histiocytic sarcoma) and multiple myelolipomas in the adrenal glands were detected. Metastasis of the oral squamous cell carcinoma was observed in the lungs. Although neoplasms are frequent in this species, and more than 1 type of tumor in a single individual has been occasionally reported, this is the first description of both myelolipoma and multiple concurrent neoplasms involving various organs and different cellular origins in a hedgehog.

Introduction

African hedgehogs are commonly kept as pets, as well as in zoologic collections and, consequently there is a demand for information about the health and disease aspects of this species. Neoplasia has been recognized as a leading cause of morbidity and mortality in captive individuals.[1-5] The average age for the appearance of neoplasms is 3.5 years; females are more predisposed, and neoplasia comprises 47% of all diseases reported in hedgehogs worldwide.[5-7] Neoplasia has been shown to account for 36% of all causes of death, and represents the primary cause of death for hedgehogs kept in zoos.[2] The main types of tumors documented in African hedgehogs include squamous cell carcinoma of the oral cavity, mammary gland adenocarcinoma, and intestinal, splenic, or cutaneous lymphomas.[8-13] The most common organ systems affected by neoplasms include the reproductive, digestive, endocrine, and hemolymphatic systems, and many of the tumors at these locations are reported to be malignant with poor prognosis in 56 to 85% of the cases.[10-13]

Case Presentation

This report describes the clinical and morphological features of 4 different neoplasms in a female, 3.5-year-old, North African hedgehog. The animal was kept outdoors in a garden in Gran Canaria, Spain, and was fed with cat food, crickets, and fruits. The animal was initially presented with a cutaneous mass on the right flank, which was removed. Six months after surgical excision, the hedgehog was represented with anorexia, lethargy, weakness, and progressive weight loss. An infiltrative mass in the left lower jaw was identified, and the hedgehog died 1 month after the onset of these clinical signs.

The initial cutaneous flank mass, and tissues obtained after complete necropsy, were submitted for histopathologic examination. Samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Representative microscopic sections of the cutaneous lesion were stained with toluidine blue (TB) stain.

The cutaneous flank lesion consisted of a circumscribed, but non-encapsulated, 6 cm, white to grey, ulcerated, mass (Fig 1a) with variable consistency. The lesion showed small hemorrhagic foci, but no changes were detected in the surrounded tissues or local lymph nodes. Histologically, the neoplasm was composed of round cells, with round central to slightly eccentric nuclei, arranged in cords and loose sheets and resembling normal granulated mast cells. Tumor cells showed distinct cell borders and numerous intracytoplasmic granules, and stained gray blue with H&E or purple with the metachromatic TB stain (Fig 1b). Cells revealed a single vesicular nucleus with inconspicuous nucleoli, without evidence of anisocytosis or anisokaryosis. Tumor cells showed low mitotic activity, with $<$ 2 mitoses per 10 high-power fields (2.37 mm^2). Although neoplastic cells infiltrated the surrounding dermis and the underlying adipose tissue and skeletal muscle, neoplastic mast cells did not extend to the surgical margins. Numerous eosinophils and, in a lesser extent, neutrophils, lymphocytes and histocytes were found throughout the tumor. Small foci of collagenolysis, sclerosis, edema, and necrosis were additional features.

The oral cavity contained 2 different neoplasms. One of them was in the left lower jaw, and was noted to have irregular margins, involvement of subjacent tissues, focal hemorrhages and necrosis of the surface (Fig 2a,b). The neoplasm was composed of solid, haphazardly arranged or in variably sized short streams and solid sheets, of large, pleomorphic, round to fusiform cells, with frequent multinucleated giant cells, numerous (17/2.37 mm^2) mitosis, marked cytological atypia and bizarre mitosis (Fig 3a). Nuclei were pleomorphic, elongated, or round, with coarse or granular chromatin, and prominent basophilic nucleoli. Neoplastic cells infiltrated perineuronal spaces and salivary glands. Extracellular matrix, including osteoid,

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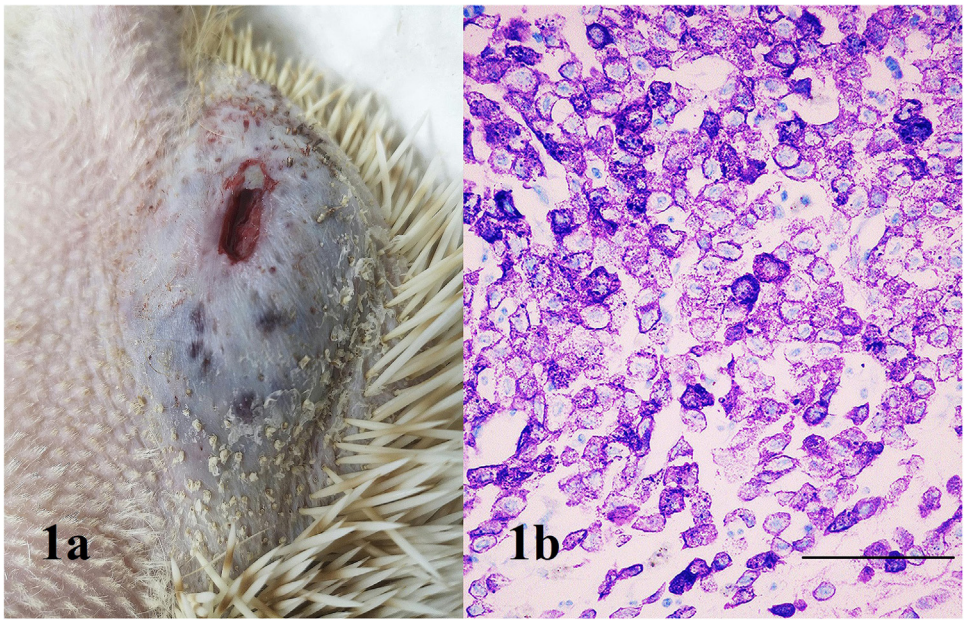

Fig 1. Appearance of the cutaneous neoplasm on the right flank (a) composed of dermal mast cells showing intracytoplasmic metachromatic granules (b) with the TB stain. Bar,150 μm. Color version of figure is available online.

chondroid, collagenous, mucinous, or myxoid matrix, was not observed. Immunohistochemistry was performed on deparaffinized sections using the avidin-biotin-peroxidase complex technique, according with the manufacture's datasheets (Table 1), for cytokeratins (AE1/AE3 and CK5/6), vimentin, S-100 protein, neuron-specific enolase, glial fibrillary acidic protein, Melan A, α-smooth muscle actin, desmin, CD18, CD31, and Iba1. A biotinylated rabbit anti-mouse or swine anti-rabbit IgG (Vector Laboratories), diluted 1:200, were applied as secondary reagents for 30 minutes at room temperature. An ABC complex (Vector) diluted 1:50 was applied as the third reagent. Sections were incubated for 5 minutes with 3,3-diaminobenzidine tetrahydrocloride (Sigma) 0.035% in TBS containing H_2O_2 0.1%. After rinsing in tap water, slides were lightly counterstained with Harris's hematoxylin and mounted under DPX mountant (BDH Laboratory Supplies) for microscopy. Appropriate positive controls (which included canine and feline histiocytic sarcoma, leiomyosarcoma, melanoma, squamous cell carcinoma, hemangiosarcoma, fibrosarcoma, and nerve sheath tumor), and a negative control of substitution of primary antibody by phosphate-buffered saline, were performed in parallel with each staining run, and internal controls were also present. Neoplastic cells showed strong cytoplasmic (vimentin, Fig 3b), membranous and cytoplasmic (Iba1, Fig 3c), or membranous (CD18) immunolabeling. Neoplastic cells were not immunoreactive to cytokeratins, S-100, neuron-specific enolase, glial fibrillary acidic protein, Melan A, smooth muscle actin, desmin, and CD31, which discharged an epithelial, neuronal, melanic, myogenic or vascular immunophenotype. The histopathologic features and immunohistochemical results were consistent with histiocytic sarcoma.

The other oral tumor was characterized by an ulcerated raised plaque affecting the right gingival mucosa and tongue, with prominent osteolysis revealed on radiographs. Histologically the plaque was composed of trabeculae and nests of epithelial cells extending into the submucosa. Cells at the periphery of the nests were smaller and more basaloid, showing areas of palisaded or acantholytic cells. Cells in central areas were larger and showed abundant eosinophilic cytoplasm, intercellular bridges and keratin pearl formation. Nuclei were large and vesicular, containing finely stippled or marginated chromatin, and prominent single or multiple nucleoli. There was moderate anisokaryosis and anisocytosis. Intense desmoplasia, emperipolesis (neoplastic cells containing intracytoplasmic neutrophils), marked cellular pleomorphism and numerous mitotic figures (11/2.37 mm^2), including multinucleated cells, bone invasion and osteolysis (Fig 4a), secondary neutrophilic infiltration, and surface exudate admixed with bacteria, were frequently seen. In the lungs, multiple pulmonary masses composed of solid nodules of large, variably sized neoplastic cells with abundant eosinophilic cytoplasm, intense anisokaryosis, and squamous differentiation were detected (Fig 4b). These findings were consistent with squamous cell carcinoma with metastases to the lungs.

Multiple, 1-2 mm. in diameter, well-defined, spherical, yellowish masses, were detected embedded in the parenchyma of both adrenal glands; these were more obvious in the left gland. The masses were expansile, demarcated but nonencapsulated from the normal adrenal tissue, and were composed of well-differentiated lipocytes admixed with immature and mature erythroid, myeloid, and megakaryocytic cells (Fig 5), consistent with myelolipomas.

Discussion

The cutaneous flank lesion was diagnosed as well differentiated dermal mast cell tumor which, in accordance with the behavior described in this and other animal species, is considered amenable to complete surgical excision.[13,14] In dogs, cats and ferrets, metastases usually occur first to regional lymph nodes, as well as to the spleen and liver. Contrary to dogs, there are no specific standards for determining the histologic grade of mast cell tumor in hedgehogs. Thus, to estimate malignancy and tumor grade, the classical criteria of cellular morphology, number of cytoplasmic granules, number of cell nuclei, number of mitotic figures, and infiltration of deeper tissues can be evauated.[3,14,15] Utilizing these parameters, the present described neoplasm in this hedgehog would be classified as well-differentiated mast cell tumor, but future studies including representative numbers of cases in hedgehogs, with clinical follow up information, would be needed to confirm this. In contrast with previous descriptions of poorly differentiated mast cell tumors in hedgehogs[15,16] in which lymph node and splenic metastasis were found, the current case did not show recurrence of disease after surgical excision, in spite of no additional radiotherapy or chemotherapy treatments being

Fig 2. Poorly defined mass in the lower jaw (a). Demarcation of the neoplasm (arrows), affecting subjacent alveolar and mandibular bone, with extensive deformation and ulceration (b). Color version of figure is available online.

administered, which would also be consistent with a well-differentiated, low grade mast cell tumor.

Mesenchymal tumors so far described in the hedgehog include histiocytic sarcoma, malignant fibrous histiocytoma, neurofibroma, neurofibrosarcoma, osteosarcoma, hemangioma, fibrosarcoma, and undifferentiated sarcoma.[1,5,17-21] In the present case, the histological and immunohistochemical features of the tumor cells were suggestive of a localized oral histiocytic sarcoma with highly aggressive and infiltrative behavior. Histiocytic sarcoma is an interstitial dendritic cell- or macrophage-derived malignant tumor.[14,22] Localized histiocytic sarcoma often originates in the skin and subcutaneous tissue. Other common primary locations in dogs and cats include spleen, tongue, lung, brainstem, nasal cavity, vertebral bone, epidural space, and joints of the limbs.[14,20-22] Histiocytic sarcoma in dogs and cats demonstrates locally invasive growth and metastasizes to draining lymph nodes and distant organs. In accordance with previous reports, localized histiocytic sarcoma in hedgehogs requires immunohistochemistry to reach a definitive diagnosis and tends to display aggressive local behavior, with infiltration and destruction of adjacent tissues,[13,19] consistent with observations in this case.

Oral squamous cell carcinoma is the most common tumor of the digestive tract in the hedgehog,[23] representing the third most common neoplasm of this species.[3,5] Clinical signs include swelling, ulceration, displacement and/or destruction of subjacent bone tissue and teeth, and weight loss. These findings are like those described in dogs

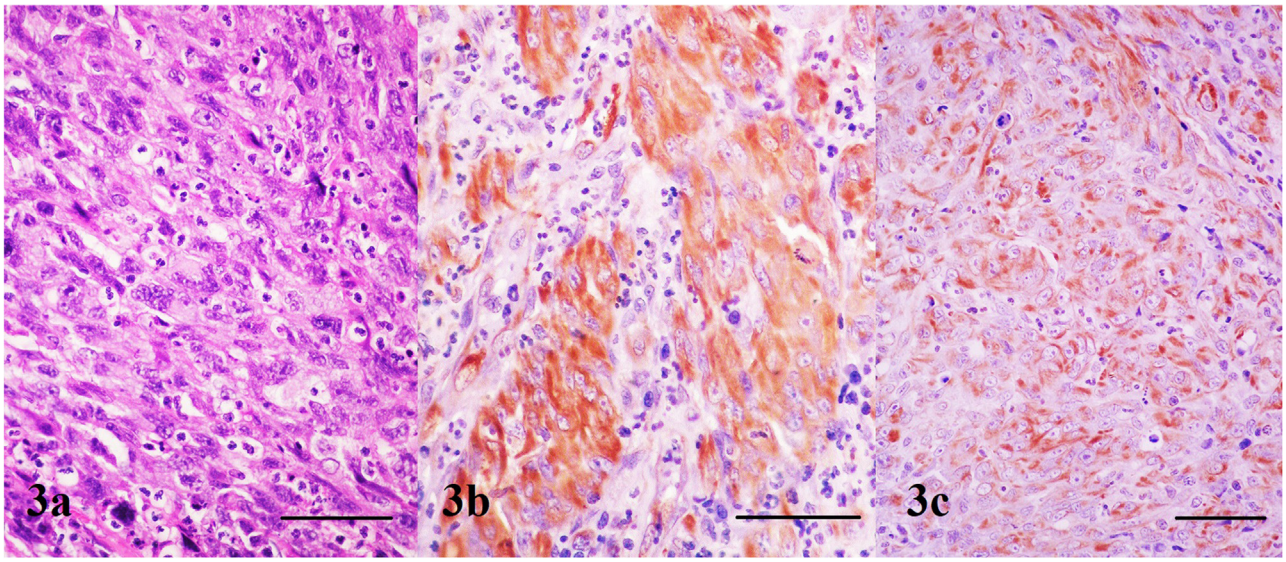

Fig 3. Localized histiocytic sarcoma in the oral cavity showing a solid pattern of pleomorphic large spindle or round cells, frequent multinucleation, with eosinophilic or slightly vacuolized cytoplasm, intense atypia and without evident extracellular matrix production (a), H&E. Histiocytic sarcoma showing strong cytoplasmic reaction to vimentin (b), and membranous and cytoplasmic immunolabeling to Iba1 (c), immunohistochemistry, avidin-biotin-peroxidase complex method. Bars,150 μm. Color version of figure is available online.

and cats, in which bone invasion is detected in 70% of the cases at diagnosis; regional lymphadenopathy or distant metastasis to the lung is very infrequently found in these species (15% and 5%, respectively).[14] Although some authors consider this tumor type as being more locally aggressive with lower metastatic potential,[3] this report agrees with Heatley et al[13] in which pulmonary metastasis seems to be more frequent in hedgehogs in comparison to other animal species.

The most frequent neoplasms in the adrenal gland of the hedgehog are adrenal cortical carcinoma and pheochromocytoma.[5,13] Myelolipomas are considered benign tumors that have been occasionally described in dogs and cats, and usually involve the spleen or liver, but are reported in other locations such as adrenals, omentum, epidural spinal canal, and eye.[14,24] The neoplastic, metaplastic, or hamartomatous origin of these masses is debated. Some grow to a large size, while others are small and multiple and are considered metaplastic or hamartomatous.[14] Although this neoplasm has been reported in dogs, cats, several bird species, non–human primates, ferrets, rats, and exotic large cats, this is the first description in hedgehogs. In accordance with those reports and in spite of its potential association with clinical signs of hyperadrenocorticism described in humans and dogs,[14,24] this neoplasm constituted an incidental finding in the current case, with no apparent clinical significance.

In veterinary literature, multiple primary malignancies are rare.[3,25,26] Since neoplasia appears to be unusually common in hedgehogs and their expected life span short, regular clinical evaluation, annually from one year of age and every six months from 3 years onwards, is recommend for early detection of possible neoplastic processes to try and improve therapy and prognosis of affected animals.[3] These evaluations should include full physical examination, complete blood count, serum biochemical profile, urinalysis, and thoracic and abdominal radiographs.[13] Neoplasia in this species may be amenable to complete surgical excision if diagnosed early, but this will not be effective in later stages of disease once tumors have spread locally or metastasized to distant sites.

Multiple primary neoplasms in hedgehogs have been previously documented in 3 animals, all of them involving the mammary gland and reproductive female tract.[5] As far as the authors know, this is the first description of multiple distinct primary neoplasms affecting 3 different organ systems (integumentary, digestive, and endocrine) with different cellular origins (mesenchymal, hematopoietic, and epithelial) reported in a hedgehog. The knowledge and proper diagnosis of this condition can have critical therapeutic and prognostic implications that will be helpful for clinician and pet owners in the process of decision-making. The authors underline the need for further studies to improve the knowledge of multiple neoplasias in this species. As such, for adequate treatment planning and evaluation of prognosis, more precise knowledge is needed regarding the biological behaviors of multiple neoplastic diseases occurring in hedgehogs.

Table 1
Summary of Antibodies and Procedures Used in the Immunohistochemical Techniques

Antibody	Clone	Antigen	Source	Dilution	AR
AE1/AE3	AE1/AE3	Cytokeratin	Dako	1:200	Pronase[a]
CK5/6	D5/16 B4	Cytokeratin	Dako	1:150	Pronase
Vimentin	V9	Vimentin	Dako	1:160	-
S-100	Polyclonal	S-100	Dako	1:500	-
NSE	BBS/NC/VI-H14	NSE	Dako	1:1000	Pronase
GFAP	Polyclonal	GFAP	Dako	1:100	Pronase
Melan A	A103	Melan A	Dako	1:100	HTAR[b]
SMA	1A4	α-SMA	Dako	1:150	-
Desmin	D33	Desmin	Dako	1:200	HTAR
CD18	CA1.4E9	Integrin-β2	Bio-Rad	1:150	HTAR
CD31	JC70A	PECAM-1	Dako	1:500	HTAR
Iba1	HL22	Iba1 protein	ThermoFisher	1:500	HTAR

Abbreviations: AR, antigen retrieval; NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; Melan A, melanosome-specific antigens; SMA, α-smooth muscle actin.
[a] Pronase: 5 minutes with 0.1% Protease E (Sigma Chemical Co., St. Louis, USA).
[b] HTAR: High-temperature antigen retrieval solution (10 minutes at 121°C in citrate buffer, pH 5).

Authors' Contributions

FR: methodology, writing original draft, review, and editing; JDH: methodology, resources, investigation; JO: conceptualization, methodology, investigation, resources, review and editing, supervision.

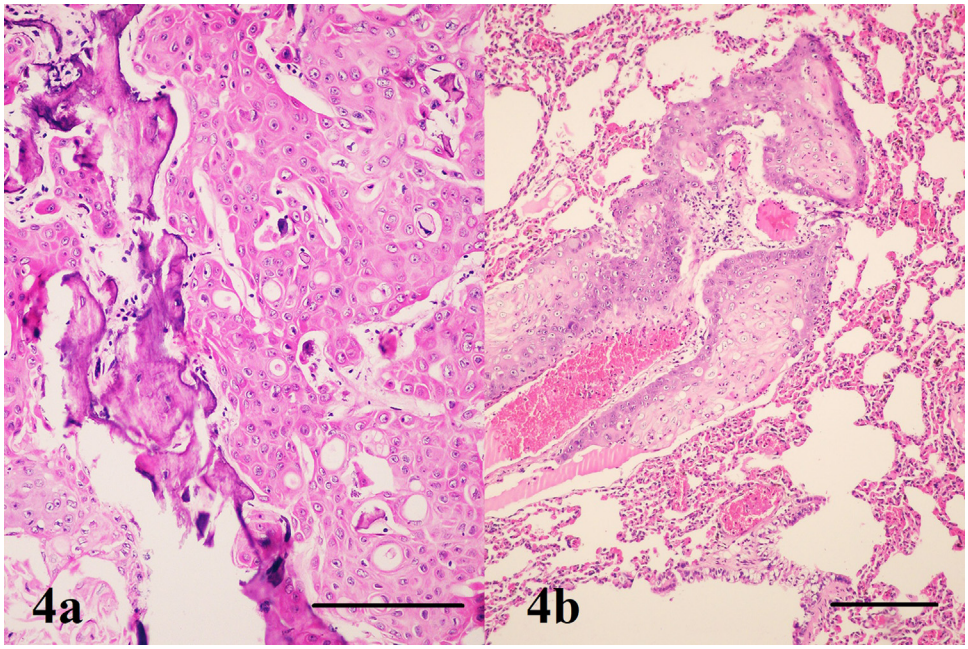

Fig 4. Primary squamous cell carcinoma in the oral cavity showing islands of well differentiated keratinizing neoplastic cells that invade the adjacent bony trabeculae (a). Metastasis of the oral squamous cell carcinoma in the lung, with concurrent neutrophilic infiltration (b), H&E. Bars,150 μm. Color version of figure is available online.

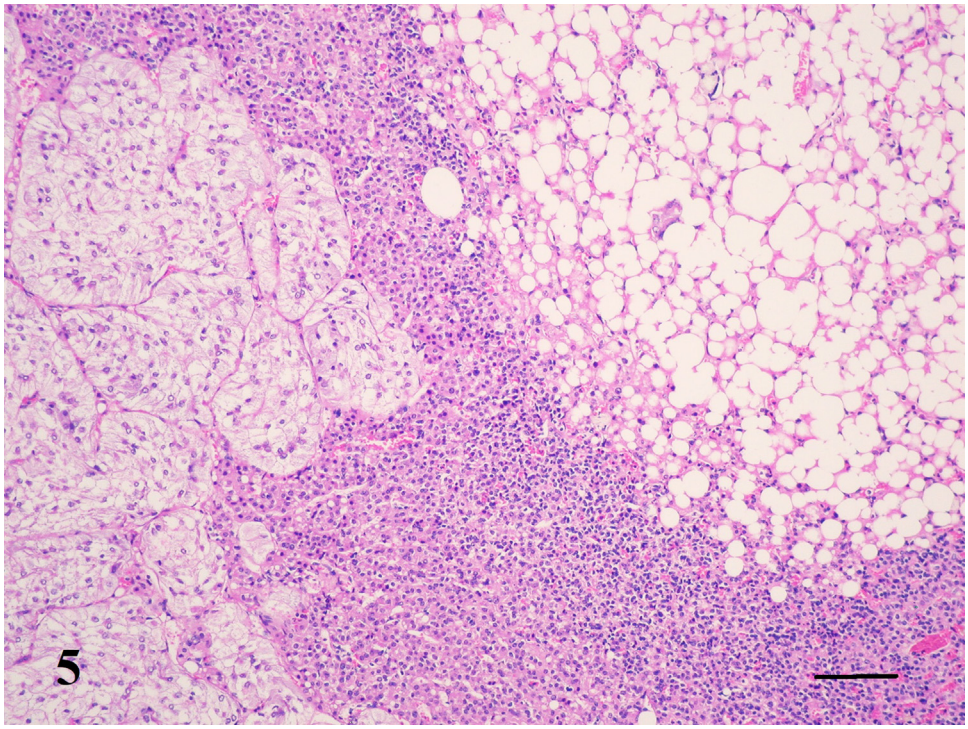

Fig 5. Expansile nodular lesion embedded in the adrenal parenchyma showing well−differentiated lipocytes with intermixed hematopoietic cells, H&E. Bar,150 μm. Color version of figure is available online.

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