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Primary neuroblastoma in the skin of an adult shih tzu dog

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Abstract

A subcutaneous mass arising in the right gluteal area of an 11-year-old female shih tzu dog was surgically excised. Histologically, the mass was composed of small round or ovoid neoplastic cells that were arranged in nests of various sizes. The neoplastic cells generally had hyperchromatic nuclei and scanty eosinophilic cytoplasm, and were surrounded by a pale pink fibrillar area. Immunohistochemically, the neoplastic cells were positive for vimentin, S-100 protein, neurone-specific enolase and synaptophysin, but negative for cytokeratin, neurofilament protein, glial fibrillary acidic protein and chromogranin A. On ultrastructural observation, aggregates of thin cytoplasmic processes were frequently seen among the neoplastic cells. Based on these features, the tumour was diagnosed as a neuroblastoma. To the authors' knowledge, this is the first description of a neuroblastoma originating from the skin in an adult dog.

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Neuroblastomas are derived from primitive neuroectodermal cells, which are potentially capable of differentiating into both neuronal and glial cells.[1] In humans, neuroblastoma is one of the most common paediatric neoplasms occurring in the adrenal glands and central nervous system. It exhibits occasional metastasis to the skin, resulting in multiple nodules, whereas primary neuroblastoma in the skin is rarely seen.[1–3] In dogs, reports of neuroblastoma are restricted to tumours of the central nervous system and peripheral nervous tissues, including the adrenal medulla and sympathetic ganglia.[4–8] So far, neuroblastoma arising primarily in the skin has not been reported in domestic animals. To the authors' knowledge, this is the first description of a neuroblastoma arising as a primary tumour of the skin of a dog.

An 11-year-old female shih tzu dog was admitted to an animal hospital, presenting with a mass in the skin of the right gluteal area, which had rapidly grown to a size of 2 × 2 × 1 cm in 2 months. The mass was removed surgically at the owner's request. Apart from the mass, no other abnormalities were observed on physical examination, complete blood count or routine serum biochemical profile. Detailed ultrasonographic and radiographic examinations revealed that no mass suggestive of tumour was present in the thoracic or abdominal cavities.

The excised mass was fixed in 10% neutral buffered formalin and routinely embedded in paraffin wax for histopathological examination. Sections were cut 4 μm thick, and subjected to haematoxylin and eosin, silver impregnation, Fontana-Masson's argentaffin, Grimelius' argyrophil and Nissl's stains, and the periodic acid-Schiff (PAS) reaction. Immunohistochemistry was performed using a labelled streptavidin-biotin-peroxidase technique with mouse antibodies against cytokeratin AE1/AE3 (DAKO, Glostrup, Denmark; 1 : 50), melan A (Novocastra, Newcastle, UK; 1 : 25), neurofilament protein (DAKO; 1 : 200), neurone-specific enolase (NSE, DAKO; 1 : 200), BLA36 (BioGenex, San Ramon, CA, USA; 1 : 300), smooth muscle actin (SMA, DAKO; 1 : 400) and vimentin (DAKO; 1 : 200), and rabbit antibodies against CD3 (DAKO; 1 : 300), chromogranin A (DAKO; 1 : 150), glial fibrillary acid protein (GFAP, DAKO; 1 : 500), S-100 protein (DAKO; 1 : 1500), synaptophysin (DAKO; 1 : 200) and desmin (DAKO, Carpinteria, CA, USA; 1 : 100). The antibodies used were validated by a positive reaction with their corresponding normal tissues, and by a negative reaction on replacement with normal mouse or rabbit serum. For electron microscopy, small pieces of the formalin-fixed mass were post-fixed in 1% osmium tetroxide, embedded in epoxy resin, and stained with uranyl acetate and lead citrate.

The mass was located in the subcutis and was well circumscribed by fibrous tissues. The cut surface was greyish white, and reddish brown in an area of haemorrhage. Histologically, the mass was composed of neoplastic cells arranged in nests of various sizes separated by delicate fibrovascular septa. The neoplastic cells generally had small round or ovoid hyperchromatic nuclei and scanty eosinophilic cytoplasm, and were surrounded by a pale pink fibrillar area. The frequency of mitotic figures was 0–3 per high-power field. The border between the cells was indistinct (Figure 1). Variably sized nests of neoplastic cells were clearly demarcated by reticular fibres (Figure 2). The neoplastic cells were negative with Fontana-Masson, Grimelius' and Nissel's stains, and for the PAS reaction. An additional

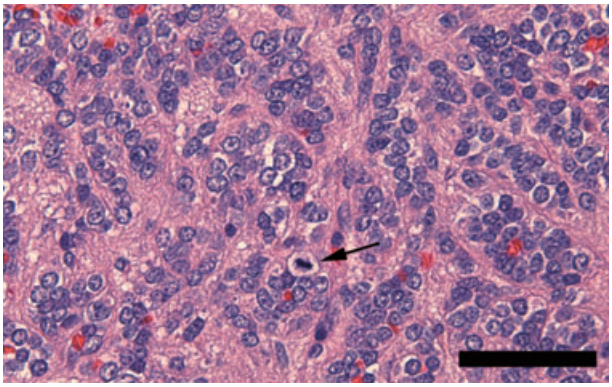

Figure 1. Variously sized nests of neoplastic cells surrounded by a pale pink fibrillar area. Arrow indicates mitotic figure. H&E. Bar = 50 μm.

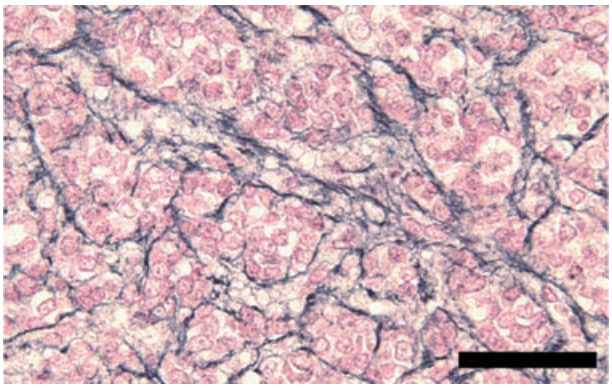

Figure 2. Variously sized nests of neoplastic cells outlined by reticular fibres. Silver impregnation. Bar = 50 μm.

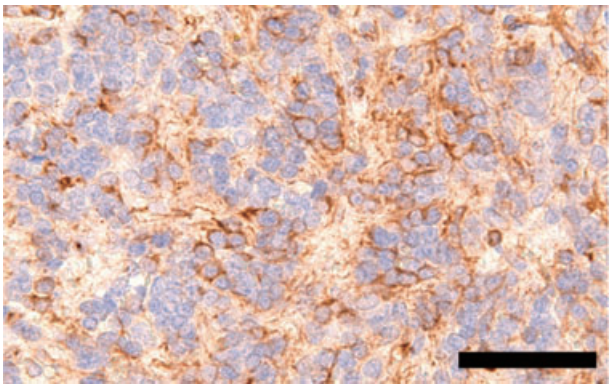

Figure 3. The cytoplasm of almost all neoplastic cells is positive for vimentin in varying degrees. Immunohistochemistry for vimentin with haematoxylin counterstain. Bar = 50 μm.

finding was moderate haemorrhage among nests of the cells.

Immunohistochemically, the neoplastic cells were strongly positive for vimentin (Figure 3) but not for cytokeratin. Scattered cells (about 30% of the neoplastic cells) showed granules that were positive for synaptophysin, especially in the pale pink fibrillar area (Figure 4). The neoplastic cells were also strongly positive for S-100 protein and weakly positive for NSE, whereas they were negative for neurofilament protein and GFAP. The pale pink fibrillar area showed the same immunostaining as the perinuclear area. For the other markers, such as chromogranin A, melan A, SMA, desmin, CD3 and BLA36, no immunostaining was detected in the neoplastic cells.

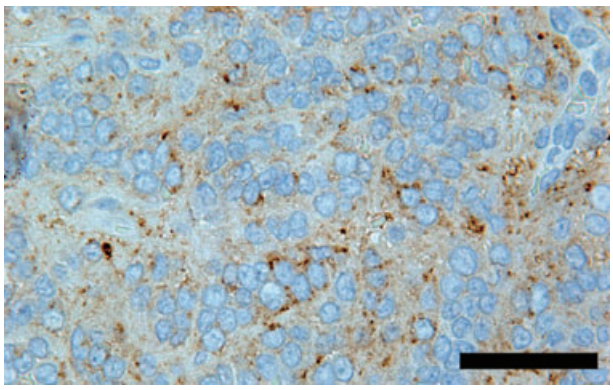

Figure 4. Neuropil-like and perinuclear area of the neoplastic cells are partially positive for synaptophysin. Immunohistochemistry for synaptophysin with haematoxylin counterstain. Bar = 50 μm.

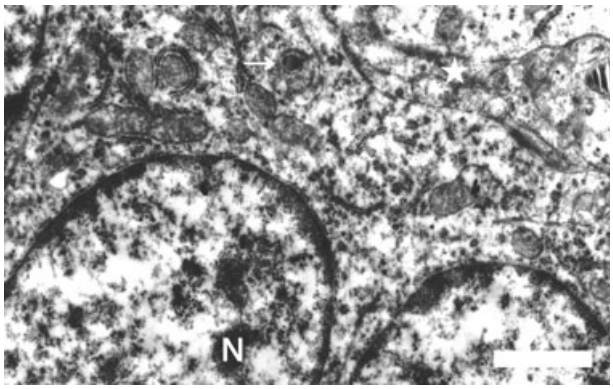

Figure 5. Electron micrograph showing two neoplastic cells with round nuclei (N) and abundant mitochondria in the cytoplasm. A dense-core granule is seen in the cytoplasm (arrow). The asterisk indicates aggregates of thin cytoplasmic processes. Uranyl acetate and lead citrate stain. Bar = 500 nm.

On ultrastructural examination, the neoplastic cells showed round to oval nuclei and diffusely distributed chromatin material (Figure 5). The scanty cytoplasm contained abundant mitochondria, and rarely showed a few dense-core granules with a peripheral halo (Figure 5, open arrow). Additionally, aggregates of thin cytoplasmic processes, including empty vesicles, were frequently seen at the margin of the neoplastic cells and corresponded to the pale pink fibrillar areas.

Based on the positive immunostaining for S-100, NSE and synaptophysin, and the existence of aggregates of thin cytoplasmic processes with the appearance of neuropil, the tumour was diagnosed as a neuroblastoma. According to the international classification of neuroblastoma pathology in humans,[1] neuroblastomas are subdivided into undifferentiated, poorly differentiated and differentiating types, depending on the percentage of large cells differentiating towards ganglion cells, which are characterized by prominent Nissl substance. The morphological features of this case were similar to those of the undifferentiated type. Homer Wright rosettes formed by tumour cells, which are one of the distinctive features of neuroblastoma,[3] were not observed in the present case. Similarly, less differenti-

ated type of peripheral neuroblastomas in two dogs[7,8] did not show such rosettes. Neurofilament protein, NSE, chromogranin A and synaptophysin are known to be useful markers in making a diagnosis of neuroblastoma.[9] The present tumour showed a positive immunoreactivity for NSE and synaptophysin, as did a canine peripheral neuroblastoma that was previously reported.[7] A weak staining of NSE and negative staining of chromogranin A and neurofilament protein must be due to the undifferentiated nature of this tumour. This case of neuroblastoma should be distinguished diagnostically from round cell tumours, particularly Merkel cell tumours and glomus tumours that arise in the skin. Merkel cell tumours in domestic animals express not only neuroendocrine markers, including chromogranin A, synaptophysin and NSE, but also the epithelial marker cytokeratin, whereas neuroblastoma is positive for vimentin but not cytokeratin.[10,11] On the other hand, glomus tumours retain the expression of vimentin and SMA.[12] Other round cell tumours, including lymphoma and embryonal rhabdomyosarcoma, arising in the skin could be ruled out by the results of the positive immunostaining for NSE and synaptophysin, and the negative immunostaining for desmin, BLA36 and CD3.

Further support for a diagnosis of neuroblastoma can be obtained from ultrastructural findings such as the presence of cytoplasmic processes, granules and microtubules. However, microtubules were not found in our case, although the former two were recognized. This may have been because the tumour was of the undifferentiated type of neuroblastoma, in which microtubules are not necessarily identified,[13] or because the fresh tumour sample was not processed with a glutaraldehyde fixative suitable for electron microscopy.

With regard to the cellular origin of this tumour, development from ectopic neural crest cells or neuronal stem cells in the skin may be hypothesized. Ectopic neural crest cells that aberrantly migrated to the skin during organogenesis may have transformed into the neoplasm; a canine ganglioneuroblastoma that occurred in the oral mucosa was considered to be of this origin.[14] Another possibility is that this neoplasm may have developed from neuronal stem cells, which are located in the hair follicles and are capable of differentiating into neuronal and glial cell populations.[15,16] Walton *et al.* have suggested that neuroblastoma may arise from a rare small population of these stem cells.[17]

In adult humans, neuroblastoma is a rare neoplasm of the skin and exhibits a poor prognosis because of its tendency to metastasize or recur after surgery.[3] Skin metastases of neuroblastoma at another primary location, which shows as multiple nodules has been well documented in children but not in adults.[3] Primary neuroblastoma of the skin seems to be indistinguishable from metastatic disease by detailed microscopic observation alone, because the histopathological features are almost the same.[3] The tumour in the present case was located only in the skin of the gluteal region, and there was no recurrence or metastasis 22 months after surgical excision, suggesting that this tumour was of cutaneous origin and benign in its biological behaviour despite the appearance of an undifferentiated phenotype. This suggests that the biological behaviour of neuroblastoma of the skin in dogs may be different from that in humans.

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Résumé Une masse sous-cutanée apparue dans la région du fessier droit d'une chienne shih tzu de 11 ans a été chirurgicalement excisée. Histologiquement, la masse était composée de petites cellules rondes à ovoïdes et arrangées en faisceaux de tailles variables. Les cellules néoplasiques présentaient généralement un noyau hyperchromatique, un cytoplasme faiblement éosinophilique et étaient entourées par une fine bande fibrillaire. Immunohistochimiquement, les cellules tumorales étaient positives pour la vimentine, la protéine S-100, l'énolase neurone spécifique et la synaptophysine, mais négatives pour les cytokératines, la protéine de neurofilament, la protéine fibrillaire acide gliale et la chromogranine A. L'examen ultrastructural a montré fréquemment dans les cellules néoplasiques de minces agrégats cytoplasmiques. A partir de ces données, la tumeur a été diagnostiquée comme un neuroblastome. A la connaissance des auteurs, c'est la première description d'un neuroblastome d'origine cutanée chez un chien adulte.

Resumen Se extirpó quirúrgicamente una masa subcutánea de la región glútea de una perra shih tzu de once años de edad. Histológicamente la masa estaba formada por células redondeadas a ovaladas organizadas en nidos de varios tamaños. Las células neoplásicas tenían en general un núcleo hipercromático y un citoplasma eosinofílico escaso, y estaban rodeadas de un área fibrilar rosada pálida. Mediante inmunohistoquímica las células neoplásicas fueron positivas para vimentina, proteína S-100, citoqueratina, proteína de neurofilamentos, proteína fibrilar glial acídica y cromogranina A. En el estudio ultraestructural, se observaron frecuentes agregados de procesos citoplásmicos delgados entre las células neoplásicas. Basados en estas características, el tumor se diagnosticó como un neuroblastoma. A nuestro entender, esta es la primera descripción de un neuroblastoma originado en la piel de un perro adulto.

Zusammenfassung Eine subkutane Masse, die sich aus der rechten Glutealgegend eines 11 Jahre alten weiblichen shih tzu's erhob, wurde chirurgisch entfernt. Histologisch bestand die Masse aus kleinen runden bis ovalen neoplastischen Zellen, die in Nestern unterschiedlicher Größe angeordnet waren. Die neoplastischen Zellen hatten allgemein hyperchromatische Kerne und ein spärliches eosinophiles Zytoplasma, und waren von einer blassrosaroten fibrillären Zone umgeben. Immunhistochemisch waren die neoplastischen Zellen positiv für Vimentin, S-100 Protein, Neuron-spezifische Enolase und Synaptophysin, aber negativ für Cytokeratin, Neurofilament Protein, gliales fibrilläres Säureprotein und Chromogranin A. Bei der ultrastrukturellen Untersuchung sah man zwischen den neoplastischen Zellen häufig Aggregate von dünnen zytoplasmatischen Fortsätzen. Der Tumor wurde aufgrund dieser Charakteristika als Neuroblastom diagnostiziert. Nach dem besten Wissen des Autors handelt es sich hierbei um die erste Beschreibung eines Neuroblastoms, das aus der Haut eines adulten Hundes stammt.

