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Title: What Is Your Diagnosis? Abdominal mass in a rat

Running title: Abdominal mass in a rat

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Summary

A pet rat (*Rattus novergicus*) was diagnosed with sarcoma after cytologic and histologic evaluation of a subcutaneous, ventrally-located abdominal mass. Immunohistochemistry revealed neoplastic cells positive for Iba-1 and CD204, indicating a final diagnosis of

histiocytic sarcoma. The tumor was aggressive and spread in a few weeks, leading to humane euthanasia.

Keywords: Mesenchymal tumor, histiocytic sarcoma, integument, subcutaneous, IHC

1 CASE PRESENTATION

A 1-year-8-month-old intact female dark brown rat (*Rattus novergicus*) was presented to Purdue University Veterinary Teaching Hospital (PUVTH) to evaluate a recently identified subcutaneous mass on her right lower abdomen and a nodule in her right armpit. The animal was otherwise healthy. The abdominal mass was approximately 2 x 1 cm, and the armpit nodule was judged to be an enlarged lymph node. The remaining physical examination was unremarkable. The cytologic evaluation of a fine-needle biopsy from the abdominal mass was performed (Figures 1 and 2).

Cytologic interpretation: Sarcoma

The specimen was moderately cellular, consisting of scattered mesenchymal cells in a markedly hemodiluted background. Overall, the cells were large, displaying marked anisocytosis, and were mostly individualized (Figure 1). The cells had variable amounts of blue cytoplasm, occasionally containing pinpoint pink granules. The nuclei were round to oval to occasionally reniform, with finely stippled to reticular chromatin pattern, and displayed marked anisokaryosis. Multinucleated cells with intracellular anisokaryosis, resembling crown cells, were observed (Figure 2). One to three large, round to irregularly shaped, prominent nucleoli were frequently noted. A few clumps of bright eosinophilic material were present (presumably collagen). There was no evidence of inflammation, and infectious agents were not found.

2 ADDITIONAL RESULTS

Eighteen days after the initial presentation, the patient returned for thoracic radiographs. With no evidence of pulmonary metastasis, CO_2 laser removal of the abdominal mass (4 x 3 x 1 cm) was performed (Figure 3A). Histologically, the subcutaneous mass was unencapsulated, poorly circumscribed, with evidence of invasion into the dermis. The neoplastic cells were arranged in dense sheets to interlacing bundles separated by a fibrovascular stroma. The cells were round to fusiform, with abundant pale eosinophilic cytoplasm. Irregularly oval to indented nuclei with one prominent nucleolus were present, in addition to several multinucleated cells with up to 10 nuclei and marked nuclear atypia. Sixteen mitotic figures in 10 consecutive fields at 400x magnification were identified. Multifocal lymphoplasmacytic infiltration was also seen. The morphologic diagnosis was pleomorphic sarcoma (Figure 4).

Immunohistochemistry (ICC) using CD31 (mouse anti-human, clone JC70A, Dako, Carpinteria, CA, USA), desmin (mouse anti-human, clone D33, Dako), Iba-1 (rabbit polyclonal, Biocare Medical, Pacheco, CA, USA), and CD204 (mouse anti-human, clone SRA-E5, TransGenic Inc., Kobe, Japan) showed that the neoplastic cells were moderately positive for Iba-1 and strongly positive for CD204 while being negative for the remaining markers. The positivity was diffuse, extending to fusiform, round, and giant cells, indicating a histiocytic sarcoma (Figure 5).

The surgical wound healed properly, with minor dehiscence. However, 26 days after surgery, a firm and irregular mass (6 x 2.5 x 2 cm) was identified attached to the body wall along the previous incision (Figure 3B). Due to the likely regrowth of sarcoma and poor prognosis, the patient was taken home for quality of life decisions. Forty days after

surgery, the mass was larger, necrotic, and ulcerated, and the rat underwent humane euthanasia.

3 DISCUSSION

Rats are essential laboratory animals for life sciences research, especially for carcinogenesis studies. With different breeds and strains available for research, it is important to record spontaneous tumors in these animals. In a study evaluating 930 Wistar rats (half males, half females) between 4 to 104 weeks of age, 83% were tumor-bearing animals, with a sum of 1,599 neoplasms identified on histologic evaluation. However, only 19% of these lesions were malignant. Tumors of endocrine origin are the most common in rats (42%), followed by neoplasms in the integumentary system (26%). Among all integumentary tumors, 55% are of mammary origin, being fibroadenoma the most common one.[1] Thus, a mammary epithelial tumor was the primary suspicion given the mass location in this case. No information regarding this pet rat's breed was available.

Based on the cytomorphology of the neoplastic cells, possible differential diagnoses included histiocytic sarcoma due to the multinucleation and marked anisokaryosis, and perivascular wall tumor (PWT), given the presence of crown cells. Histiocytic sarcomas are rare, representing 0.7% of neoplasms in rats. However, they are more frequently located in the hemolymphatic system instead of the integumentary system.[1] Perivascular wall tumor is a general classification of neoplasms that include hemangiopericytomas, myopericytomas, angioleiomyomas, angiofibromas, and angiomyofibrosarcomas.[2] Mesenchymal tumors are uncommon in rats, and the incidence varies from less than 1 to over 5%, with fibrosarcoma being the most common malignant form.[3]

The morphologic diagnosis of the mass on histopathology indicated pleomorphic sarcoma. This tumor is described in humans, dogs, cats, and rats and is believed to originate from pluripotent mesenchymal cells that can differentiate in multiple directions.[2,3,5] Pleomorphic sarcomas can be induced in rodents by foreign bodies of various metallic and synthetic materials.[3] It has been suggested that pleomorphic sarcomas are not one tumor type but a histologically and immunohistochemically diverse group of tumors, encompassing anaplastic sarcoma with giant cells and malignant fibrous histiocytoma (MFH).[2] According to the last World Health Organization classification of soft tissue tumors, the term MFH is now considered obsolete, and the term undifferentiated/unclassified pleomorphic sarcoma should be used whenever histologic, immunohistochemical, electron microscopic, and genetic techniques fail to demonstrate a well-defined cell line, which now represents a diagnosis of exclusion.[4,6] However, the use and nonuse of the term could cause difficulties in evaluating the literature for the incidence of the different tumors types.[6] For instance, if a neoplasm has the characteristic storiform appearance, the giant cells display a histiocytic phenotype, and the spindle cells show a fibroblastic phenotype; the tumor should be called fibrosarcoma with giant cells, not MFH or pleomorphic sarcoma. The importance of immunohistochemistry is reflected in this example and also in our case, where the staining for histiocytic markers was diffusely distributed among spindle, round, and multinucleated giant cells, thus, indicating the presence of histiocytic sarcoma.

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Figure Legends

Figure 1. Photomicrograph of a fine-needle aspirate from an abdominal mass in a rat. Modified Wright stain, x50 objective

Figure 2. Photomicrograph of two multinucleated cells observed on the same smear at higher magnification. Modified Wright, x100 objective

Figure 3. (A) Photograph of the abdominal mass before surgical removal (arrow). (B) A second mass (arrow, B), located caudally to the previous one, grew in the patient 26 days after removing the first mass (second intention healing of the previous incision is indicated by the arrowhead).

Figure 4. Photomicrograph of a histologic section of an abdominal mass from a rat. The densely arranged neoplastic cells contain pale eosinophilic cytoplasm. Multinucleation and nuclear pleomorphism are prominent. H&E, x40 objective