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Case Report

A Collision Tumor Consisting of Granular Cell Tumor and Adenocarcinoma in the Uterus of an Aged Djungarian Hamster

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Abstract: A neoplastic nodular lesion consisting of an admixture of granular cell tumor and adenocarcinoma was found in the uterus of a 26-month-old Djungarian hamster. Neoplastic cells of the uterine adenocarcinoma showed an epithelial nature in their growth patterns and by cytokeratin-immunopositive reaction, exhibiting nuclear pleomorphism. The granular cells had an abundant amount of fine granular eosinophilic cytoplasm and eccentric or central nuclei with no nuclear atypia; the granular structures were positive for periodic acid-Schiff with diastase resistance and were confirmed as lysosomes/autophagosomes by electron microscopy; immunohistochemically, the cells reacted to desmin, vimentin and α-smooth muscle actin and negatively for neurogenic, histiocyte/macrophage or epithelial markers, indicating smooth muscle origin. Because these tumors were generated from different cell origins, a diagnosis of collision tumor was made. (DOI: 10.1293/tox.24.233; J Toxicol Pathol 2011; 24: 233–237)

Key words: adenocarcinoma, collision tumor, granular cell, hamster, uterus

A collision tumor is defined as a tumor consisting of two types of tumors derived from different origins[1]. Collision tumors are very uncommon in animals. We encountered a case of a uterine tumor consisting of an admixture of adenocarcinoma and granular cell tumor in a Djungarian hamster (*Phodopus sungorus*). Uterine adenocarcinomas have been reported in aged hamsters, with a frequency of 7%[2]. Granular cell tumors are considered to be a benign neoplasm of uncertain origin and have been reported in humans, dogs, cats, horses, cockatiels, parrots, rats, mice and hamsters; the affected anatomical sites involve the tongue, skin, heart, lungs, uterus, meninges and thyroid glands[3–6]. This report describes histological, immunohistochemical and ultrastructural characteristics of a collision tumor.

A 26-month-old (body weight of 49 g) Djungarian hamster kept as a pet was presented to a private animal hospital with a clinical history of bleeding from the vulva. Echographic examination detected unilateral swelling of the right uterine horn. Ovario-hysterectomy was surgically performed. The cut surface of the swollen uterine horn revealed a single nodular lesion that occupied the uterine lumen, and no other nodular lesions were found in the removed uterus (Fig. 1).

The uterine lesion was fixed in 10% neutral buffered formalin. According to routine processes, paraffin-embedded tissues were cut at a thickness of 4 μm. Deparaffinized sections were stained with hematoxylin and eosin (HE), phosphotungstic acid hematoxylin (PTAH) and periodic acid-Schiff (PAS) with or without diastase treatment. Additionally, the sections were immunolabeled with antibodies specific for smooth muscles (α-smooth muscle actin (α-SMA) and desmin), mesenchymal cells (vimentin), neuronal tissues (neuron-specific enolase (NSE), S-100 protein and glial fibrillary acidic protein (GFAP)), epithelial cells (cytokeratin (AE1/AE3)), proliferating cells (proliferating cell nuclear antigen (PCNA)) and histiocytes/macrophages (macrophage scavenger receptor (SRA-E5)). After pretreatments with heat (microwave in citrate buffer for 20 min), trypsin (0.1% trypsin in PBS for 30 min at 37 °C) or proteinase K (10 μg/ml proteinase K in PBS for 15 min) as shown in Table 1, sections were treated with 3% H_2O_2 in phosphate buffered saline (PBS) to quench endogenous peroxidase and then with 5% skimmed milk to inhibit nonspecific reactions. The sections were incubated with each primary antibody overnight at 4 °C and then reacted with the secondary antibody (Histofine Simple Stain MAX PO, Nichirei, To-

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Table 1. Primary Antibodies Used for Immunohistochemistry

Antibody	Clone, dilution	Pretreatment**	Source of antibody
Vimentin	V9, 1:200	Heat	Dako Corp., Glostrup, Denmark
Desmin	D33, 1:200	Heat	Dako
α-SMA	1A4, 1:100	Proteinase K	Dako
Cytokeratins	AE1/AE3, Predilution	Trypsin	Dako
S-100*	1:500	Proteinase K	Dako
NSE	BBS/NC/VI-H14, predilution	Heat	Dako
GFAP*	1:300	Proteinase K	Dako
MSR	SRA-E5, 1:100	Heat	TransGenic Inc., Kumamoto, Japan
PCNA	PC10, 1:600	Heat	Dako

* Rabbit polyclonal antibody; the rest are mouse monoclonal antibodies. ** Detailed methods are described in the text.

kyo, Japan). Positive reactions were visualized with 3, 3′-diaminobenzidine (DAB Substrate Kit, Vector Laboratories, Burlingame, CA, USA). Nonimmunized mouse or rabbit serum was used as a negative control. Sections were counterstained lightly with hematoxylin. Appropriate tissues obtained from other hamsters were used as positive controls. A portion of the formalin-fixed tissue was postfixed in osmium tetraoxide and embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and observed under an electron microscope (H-7500, Hitachi, Tokyo, Japan).

Histologically, the uterine nodular lesion was composed of two different types of tumors at the same site; one showed characteristics of adenocarcinoma, and the other consisted of granular cell tumor. There was no distinct border between these tumors (Fig. 2). Neoplastic cells of the adenocarcinoma revealed acinar, glandular, cystic or solid growth patterns, with nuclear atypia such as hyper-/heterochromatin and an indented structure; mitotic figures were often seen (Fig. 3). The tumor cells invaded into surrounding tissues in conjunction with necrosis and hemorrhage and expanded toward the perimetrium. These findings indicated a high malignancy of adenocarcinoma. The adenocarcinoma was intermingled with granular cells in the interstitium; in some areas, the granular cells were arranged in a compact sheet with clear cell borders. Morphologically, the granular cells had an abundant amount of fine granular eosinophilic cytoplasm and eccentric or central nuclei with prominent nucleoli (Fig. 2). Neither nuclear nor cellular atypia was evident. The cytoplasmic granules were positive for the PAS reaction and resistant to diastase digestion (Fig. 3); PTAH staining did not reveal any characteristic of striated muscles in the cytoplasm of granular cells.

Immunohistochemically, neoplastic epithelial cells of the adenocarcinoma reacted faintly to strongly to AE1/AE3, whereas granular cells did not react to the cytokeratin antibody (Fig. 4). The granular cells reacted to vimentin (Fig. 5), desmin (Fig. 6) and α-SMA (Fig. 7), although the cytoplasmic reactivity was diverse (faintly to strongly or fragmentally to homogeneously). The granular cells were negative for NSE, S-100 protein, GFAP and SRA-E5. PCNA-positive cells were seen not only in neoplastic epithelial cells but also in granular cells (Fig. 8); apparently, the frequency was greater in the epithelial element.

Ultrastructurally, there were numerous electron dense granules (approximate average diameter of 350 nm) packed

Fig. 1. Gross finding for a cross section of the uterine horn showing a nodular lesion (asterisk), resulting in a narrowed lumen (arrow). Bar=3 mm.

Fig. 2. The tumor consists of an admixture of neoplastic endometrial glandular epithelia (asterisks) and neoplastic granular cells (arrows); the granular cells have abundant eosinophilic granular cytoplasm with central or eccentric nuclei. HE. Bar=200 μm.

Fig. 3. The cytoplasm of neoplastic granular cells is positive for the periodic acid-Schiff (PAS) reaction after diastase digestion (arrows). The arrowhead indicates an epithelial cell of the uterine adenocarcinoma undergoing mitosis. PAS reaction, counterstained with hematoxylin. Bar=100 μm.

Fig. 4. The epithelia of the uterine adenocarcinoma show a positive reaction for cytokeratin (AE1/AE3). Granular cells are nonreactive for AE1/AE3 (arrows). Immunohistochemistry, counterstained with hematoxylin. Bar=100 μm.

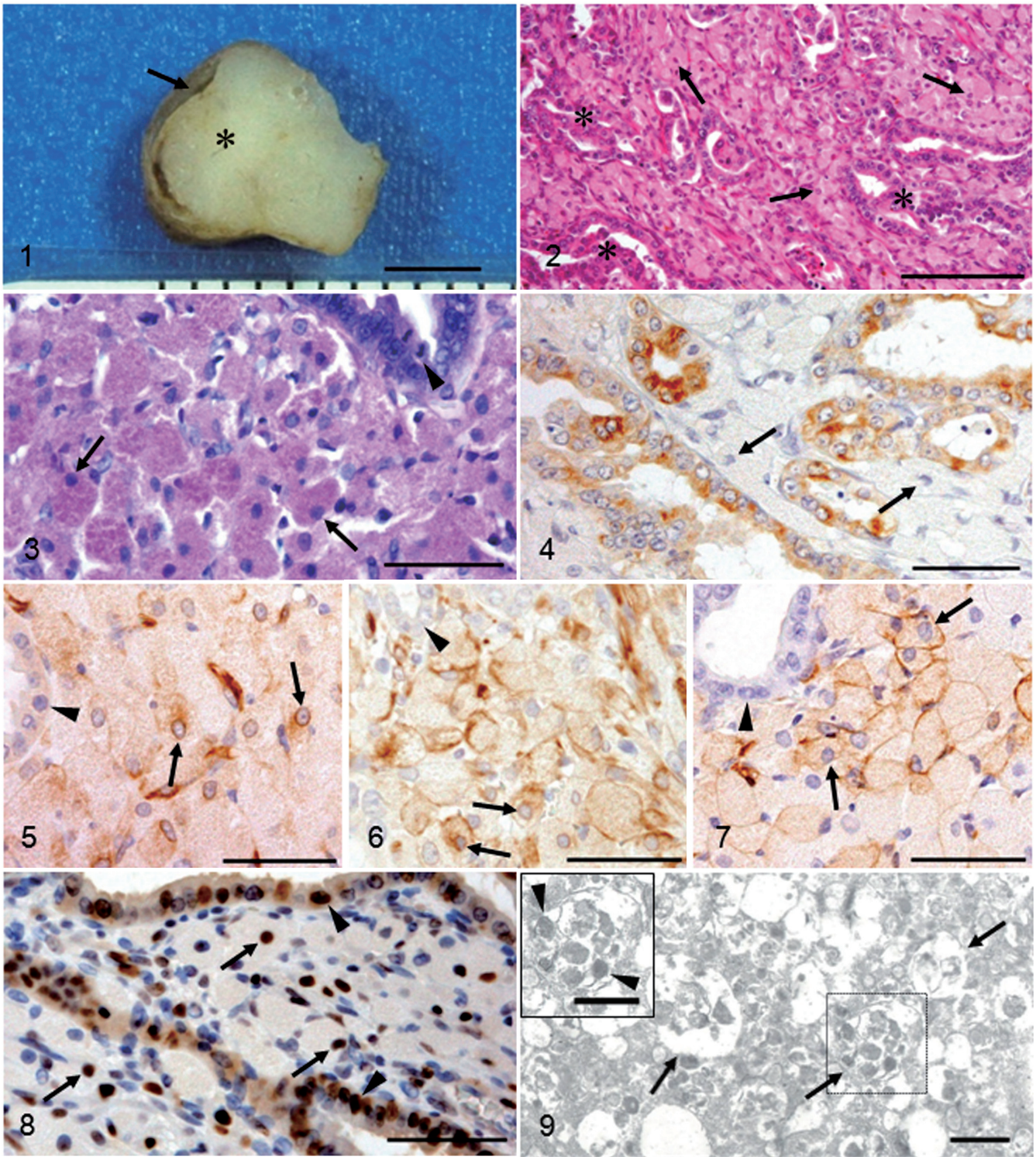
Fig. 5. Neoplastic granular cells react faintly or moderately to vimentin (arrows). Epithelial cells of the uterine adenocarcinoma are nonreactive to vimentin (arrowhead). Immunohistochemistry, counterstained with hematoxylin. Bar=130 μm.

Fig. 6. Neoplastic granular cells are moderately or strongly positive for desmin (arrows). Epithelial cells of the uterine adenocarcinoma are nonreactive for desmin (arrowhead). Immunohistochemistry, counterstained with hematoxylin. Bar=130 μm.

Fig. 7. Neoplastic granular cells show a weak or moderate reaction for α-smooth muscle actin (α-SMA) (arrows). Epithelial cells of the uterine adenocarcinoma are nonreactive for α-SMA (arrowhead). Immunohistochemistry, counterstained with hematoxylin. Bar=130 μm.

Fig. 8. There are neoplastic cells of the adenocarcinoma and granular cell tumor reacting to proliferating cell nuclear antigen (PCNA); arrows and arrowheads indicate a positive reaction to PCNA in granular cells and epithelial cells of the uterine adenocarcinoma, respectively. Immunohistochemistry, counterstained with hematoxylin. Bar=100 μm.

Fig. 9. An electron microscopic image of a granular cell having cytoplasmic granules (arrows). Bar=2.5 μm. Inset: arrowheads indicate lysosome/phagosome-like granules. Inset bar=1.25 μm.



in irregular membranous structures in the cytoplasm of the granular cells (Fig. 9). The granules were small and round, often with lamellar structures or a dense core surrounded by irregular single-layered boundary membranes with an outer halo resembling lysosomes/autophagosomes.

A collision tumor should be distinguished from a composite (complex) tumor. A composite tumor may be regarded as a tumor exhibiting coexistence of distinct components made up of different histologic features; important issues concerning a composite tumor are whether the histologically distinctive parts of the tumor reflect the ability of one tumor cell to proliferate and differentiate in two distinct lineages (such as complex carcinoma of canine mammary glands) and whether the tumor is really composed of two neoplastic clones that have arisen from different cell types in close proximity to each other[1]. Some pathologists may

consider the latter case to be a collision tumor. However, a collision tumor should be considered a tumor in the same area consisting of two different types of neoplasms derived from clearly different origins. In the uterus, combination tumors such as carcinosarcoma have been reported as malignant Müllerian mixed tumor generated from the ontogenetically same Müllerian duct[7], the concept of which differs from that of a collision tumor.

In the present collision tumor, neoplastic cells of adenocarcinoma obviously showed an epithelial cell nature in their morphology and by cytokeratin immunoreaction; based on the anatomical location, the adenocarcinoma was considered to originate from endometrial glands[2,8]. When the origin is not identified, tumors formed by cells with morphological characteristics comprising round cells with fine granular eosinophilic cytoplasm arranged in a compact sheet are termed as a granular cell tumor, and these tumors have been reported in various animals, affecting a wide range of anatomical sites[3,4,6]. Recently, it was reported that the metrial glands, which are formed in pregnancy or the decidual reaction of pseudopregnancy in mice and rats, might show morphologic features similar to those of granular cells[9]. The metrial gland-constituting cells are derived from granulated metrial gland cells or decidualized endometrial stromal cells and are characterized by immunopositive reactions to NSE, S-100 protein and macrophages, as well as the appearance of frequent binucleated cells. The present granular cells did not exhibit such characteristics.

The origin of granular cells is still a matter of debate[10]. On the basis of immunohistochemical and ultrastructural evaluations, granular cell tumors have been proposed to originate from neural tissues such as Schwann cells[11,12] or nonneural tissues including smooth muscle cells[6], histiocytes[13], endothelial cells[14] or undifferentiated mesenchymal cells[15]. Immunohistochemically, granular cells, which are considered to originate from Schwann cells, are positive for neuronal markers (S-100 protein, NSE and myelin related protein) and negative for epithelial, myogenic and histiocyte/macrophage markers[10,12,16,17]; additionally, the granular cells of Schwann cell origin may possess a basal lamina in electron microscopy[10]. On the other hand, granular cells of nonneural origin are positive for the respective markers of smooth muscles (desmin and α-SMA), histiocytes/macrophages (CD68) and mesenchymal cells (vimentin) and show negative reactions to neuronal markers[13]. However, the common finding of granular cells is a fine structure characterized by abundant lysosomes and autophagic organelles in the cytoplasm, which has been considered to be the hallmark of granular cell tumors[10]; similar fine structures were seen in the present granular cells. Furthermore, the present granular cells showed positive reactions for vimentin, desmin and α-SMA, and were negative for neurogenic and epithelial markers. These findings were consistent with granular cell tumors of smooth muscle origin found in the subcutis and gingiva[18,19]. Uterine granular cell tumors have been reported in mice and rats; these cells were considered to be of myogenic origin in mice and neurogenic origin in rats[6,20].

Generally speaking, collision tumors reported so far consist of two different types of malignant tumors[21]. The present case of collision of a malignant adenocarcinoma and a benign granular cell tumor may be unique. In humans, the coexistence of granular cells as a part of tumors has been reported in three cases including ductal carcinomas of the breast[5], intramuscular adenocarcinoma of the esophagus[22] and adenocarcinoma-malignant lymphoma composite of the stomach[23]. To our knowledge, there has been no report in animals of a collision or composite tumor including granular cells as one of the elements of the tumor tissues.

Hamsters have been used in carcinogenicity studies in toxicologic pathology. It is important to report pathological characteristics of spontaneously occurring tumors in hamsters to establish background data. There are no published reports on uterine granular cell tumor in hamsters, although leiomyomas (10%) and endometrial adenocarcinomas (7%) of the uterus have been reported[2].

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