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Characterization of Uterine Granular Cell Tumors in B6C3F1 Mice: A Histomorphologic, Immunohistochemical, and Ultrastructural Study

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Abstract

The granular cell tumor is most often a benign neoplasm of uncertain origin. Four uterine granular cell tumors in control and treated female B6C3F1 mice were identified in chronic studies at the National Toxicology Program. Two tumors occurred in untreated control animals and 2 in treated animals receiving different compounds. Tissue sections were evaluated histologically and stained with hematoxylin and eosin, periodic acid–Schiff with diastase resistance, Masson's trichrome, toluidine blue, phosphotungstic acid–hematoxylin, and stained immunohistochemically with a panel of antibodies to muscle (desmin, alpha smooth muscle actin), neural (S-100, neuron specific enolase), epithelial (wide-spectrum cytokeratin), and macrophage (F4/80) markers. The main histomorphologic feature of tumor cells was the presence of abundant cytoplasmic eosinophilic granules that stained positive for periodic acid–Schiff with diastase resistance. Tumors varied in appearance and were comprised of sheets and nests of round to polygonal cells with distinct borders. Nuclei were hyperchromatic, pleomorphic, and centrally to eccentrically located and often contained single nucleoli. Occasional multinucleated giant cells were observed. Tumors were pale pink and homogeneous with trichrome stain and negative with toluidine blue. Three tumors had positive to weakly positive immunoreactivity for desmin, and 1 was positive for alpha smooth muscle actin. Expression of S-100, wide-spectrum cytokeratin, and neuron-specific enolase was negative for all tumors. Ultrastructurally, prominent electron-dense cytoplasmic granules were abundant and contained secondary lysosomes with heterogeneous lysosomal contents. The characteristics of these uterine granular cell tumors were suggestive of a myogenic origin.

Keywords

B6C3F1 mouse; cytoplasmic granules; granular cell tumor; immunohistochemistry; secondary lysosomes; ultrastructure; uterus

Introduction

In humans, the granular cell tumor is described as an uncommon tumor of nerve sheath origin with a predilection for the skin, soft tissue, mucosa of the upper respiratory tract, tongue, and vocal cords.²⁰ Granular cell tumors of the skin occur in the dermis and subcutis of the chest

and upper extremities. They occur as solitary, well-circumscribed, yellow nodules less than 3 cm in diameter; most granular cell tumors are benign.⁷ Fewer than 30 reported cases of malignant granular cell tumors exist in the literature.¹¹ The histogenesis has been a source of controversy. Granular cell tumors were first identified by Abrikossoff.¹ Initially, these tumors were thought to originate from striated muscle and were therefore termed “myoblastic myomas.” It was believed that the cytologic characteristics as well as topographic relationship of the tumors to muscle implied a common origin.¹ These were subsequently renamed “granular cell myoblastomas.” Identical tumors were observed at sites adjacent to peripheral nerves, and these were considered to be of perineural origin and renamed “granular cell neuromas.”¹⁰

There is no known normal cell counterpart of granular cell tumors. Several cellular origins have been proposed, including muscle, fibroblastic, neural, and histiocytic.^{13,53} In general, most human granular cell tumors are considered to originate from Schwann cells, based on evidence from electron microscopic findings indicating that granular cells share morphologic features with Schwann cells and undifferentiated mesenchymal cells.^{25,41,46,50} There is additional evidence for a Schwann-cell origin from immunohistochemical studies showing positive immunoreactivity with S-100 protein, neuron-specific enolase (NSE), myelin-related protein, vimentin, and negative reaction to epithelial markers, muscle antigens, and macrophage markers.^{24,30,43,48,49,52} Ultrastructurally, the cytoplasmic granules have been described as lysosomes or intracytoplasmic autophagic vacuoles that are filled with fragmented cell organelles, including mitochondria and endoplasmic reticulum, and are considered the hallmark of granular cell tumors.^{7,13} Another ultrastructural feature described is the presence of angulate bodies that are fibrillar and oval in shape, limited by a single membrane, and often contain dense droplets consistent with lipid.⁵¹ Angulate bodies are described as lysosomes of angulate profile composed of rigid parallel arrays of microtubules of varying lengths.³

Granular cell tumors are usually benign and rarely infiltrative. They are composed of sheets and nests of large, round to polygonal cells separated by fine fibrovascular stroma. The tumor cells have distinct cell borders and abundant, finely granular, pale eosinophilic cytoplasm. The granules are periodic acid–Schiff–positive with diastase resistance (PAS-D) and Alcian blue positive, indicating the presence of lysosomes. The small, round, and hyperchromatic nuclei are usually centrally located but are occasionally eccentric. Nuclear pleomorphism is minimal, and a nucleolus is often prominent. Mitotic figures are uncommon.²⁹

Immunocytochemical studies of granular cell tumors in animals are limited.^{45,56} Granular cell tumors occur among different species in a wide range of sites involving multiple organ systems (Table 1). Their distribution is species specific and appears to be an example of heterogeneous tumor cell differentiation despite apparent homogeneous morphological appearance.^{16,23,26,54} Based on morphologic and electron microscopic findings in dogs, cats, and rats, a neural origin is supported, although other cell origins are possible.^{9,12,16,26,45,53,56} In the rat, tumors are closely associated with the meninges of the brain and spinal cord, and are derived from meningeal arachnoid cells.^{18,22,26} Granular cell tumors are the most common primary central nervous system tumor in the Wistar rat.⁵⁸ The first uterine granular cell tumor in a rat was characterized in 1991.^{5,34} A review of the 2007 National Toxicology Program (NTP) historic control database of granular cell tumors of the uterus and vagina of the rat found 11 (10 in F344, 1 in Sprague-Dawley). The incidence of granular cell tumors in mice is low. A review of the 2007 NTP historic control database of granular cell tumors of the uterus and vagina of the B6C3F1 mouse found 9 (<https://ntp-apps.niehs.nih.gov/iacf/demo/CBDS/SearchTDMSCBDSMorph.cfm>).

In female mice, granular cell tumors arise most commonly at the junction of the uterus and cervix and are usually too small to be detected grossly, but can reach 1 cm in diameter.¹⁷ The

incidence of granular cell tumors in the B63CF1 mouse may not be accurate since the junction of the uterus and cervix is not routinely sampled during NTP studies. The predilection for the uterine cervix has not been reported in other species, including humans.¹⁹ In the female mouse reproductive tract, they have been primarily reported following estrogen treatment.^{8,15,31} There are no published data on the incidence of spontaneous cervical granular cell tumors, but these tumors are thought to occur. In male mice, granular cell tumors occur spontaneously in the seminal vesicles as nodules.¹⁷

Although granular cell tumors appear to originate in the same location in the uterine cervix, the varied histomorphologic features bring their cell of origin into question. In the current study, granular cell tumors were evaluated from B6C3F1 mice from NTP chronic studies. Four uterine granular cell tumors in control and treated female B6C3F1 mice were selected for further study from a total of 6 tumors identified from 183 chronic mouse studies at the NTP dating back to 1982. Tissues were collected and were characterized histochemically, immunohistochemically and ultrastructurally to better define their origin.

Materials and Methods

Histochemical, immunohistochemical staining, and light microscope evaluation

Tissue samples were fixed in 10% neutral buffered formalin. Paraffin sections, cut from original paraffin blocks of 4- μ m thickness were stained with HE, PAS-D, Masson's trichrome, toluidine blue, and phosphotungstic acid-hematoxylin (PTAH). Table 2 lists the immunohistochemistry antibodies and procedures used in our study. Endogenous peroxidases were quenched with 3% hydrogen peroxide for 15 minutes. Visualization was achieved by treatment with liquid 3,3'-diaminobenzidine chromogen for 6 minutes, which in the presence of peroxidase produces a brown precipitate insoluble in alcohol. Slides were rinsed in tap water and counterstained with modified Harris hematoxylin, a regressive nuclear stain, and dehydrated through graded ethanol and xylene.

Ultrastructural evaluation

Tissue samples from 2 animals were examined with electron microscopy (Tecnai Bio-Twin microscope, FEI Company, Hillsboro, OR). Formalin-fixed tumor tissues were refixed in Modified Karnovsky's (2% paraformaldehyde plus 2.5% glutaraldehyde in 0.1 molar Millonig's phosphate buffer). Thin sections were placed on carbon-coated copper grids for observation under the electron beam.

Results

Histomorphologic findings

Granular cell tumor lesions were space occupying and expansive, and were composed of typical granular cells with minimal amounts of interstitial collagen. Three granular cell tumors contained sheets of polygonal cells with hyperchromatic, pleomorphic, and centrally to eccentrically located nuclei with eosinophilic granular cytoplasm typical of those previously reported. One of the granular cell tumors had atypical characteristics, which included enlarged tumor cells with vesicular nuclei and numerous multinucleated giant cells. Additional observations included collagen between cells, neutrophils, mast cells, and scattered macrophages. Two of the 4 tumors were in situ at the uterine cervical junction. Mouse No. 1 was the best example of a typical granular cell tumor in situ (Fig. 1). Peripheral tumor cells had more granules, and the cells appeared to arise from muscle bundles. In mouse No. 2, the tumor from an untreated animal was large and more varied in appearance than in mouse No. 1. Nuclei were pleomorphic, more eccentrically located, and the nucleoli were more prominent. Two multinucleated giant cells were observed. In mouse No. 3 the tumor was atypical,

composed of granular cells mixed with numerous multinucleated giant cells filled with cytoplasmic granules (Fig. 2). The tumor from an untreated animal in mouse No. 4 was similar in appearance to that in mouse No. 2.

Histochemical and immunohistochemical findings

The histochemical and immunohistochemical staining results of granular cell tumors in the uterus of 4 B6C3F1 female mice are summarized in Table 3. Immunoreactivity was characterized as +, positive reaction, +/-, weakly positive, and 0, no reaction. All 4 tumors stained positive in a diffuse pattern for periodic acid–Schiff with diastase resistance (PAS-D) (Fig. 3, Fig. 4). In mouse No. 1, the tumor stained positive in a diffuse pattern for alpha smooth muscle actin (Fig. 5). Three of the tumors stained weakly positive to positive for desmin (Fig. 6, Fig. 7). In mouse No. 1, desmin was expressed in peripheral tumor cells (Fig. 6). All 4 tumors were negative for PTAH, a histochemical stain that detects cross striations in muscle cells.

None of the 4 tumors reacted positively with the antibodies S-100, NSE, or wide-spectrum cytokeratin. Corresponding sections of brain from each animal stained positively with S-100 and NSE, and there was positive-stained nerve tissue at the periphery of 2 of the tumors. Tumors from mice Nos. 1 and 4 were negative for F4/80, a mouse macrophage marker; however, macrophages within the unaffected section of the tissue were positive and served as internal positive controls. F4/80 staining of tumors in mice Nos. 2 and 3 was negative, although the sections were mostly comprised of tumor tissue and contained no peripheral macrophages. Attempts to stain with luxol fast blue and vimentin were unsuccessful, possibly because of long-term exposure to formalin during archival storage.

Ultrastructural findings

Electron micrographic analyses were completed on tissues from mice Nos. 2 and 3. Ultrastructurally, secondary lysosomes with heterogeneous contents were seen inside individual granules in tumor cells in mouse No. 2 (Fig. 8). Nuclei were centrally and eccentrically located in the cells, and there was margination of nuclear chromatin. Nuclei varied in shape from round to irregular to indented. Sparse mitochondria and scattered rough endoplasmic reticulum were observed. There was amorphous electron-dense material in the nucleus and cytoplasm. The cell membrane was uniformly smooth, clearly outlining the granule-laden cytoplasm. Electron micrographs from mouse No. 3 (not shown) revealed a similar population of neoplastic cells with a round to polygonal shape, round to oval nuclei, and cytoplasm packed with heterogeneous electron-dense granules.

Discussion

Granular cell tumors are reported in a wide range of species and in several anatomic locations. Regardless of species and anatomic location, the majority of tumors have a homogeneous morphologic appearance and benign clinical course.^{35,38,54} In our study, the immunohistochemical and ultrastructural findings support a heterogeneous population despite their homogeneous light microscopic appearance. As granular cell tumors grow, they may differentiate and take on characteristics that vary between neoplasms. Granular cell tumors were once thought to be myoblastomas because of their relationship to muscle. To date, there is no consensus as to their pathogenesis, and conflicting reports regarding immunostaining of granular cell tumors from any site are common.

In the present study, 3 tumors reacted positively to muscle markers and none of the 4 reacted to the neural or epithelial markers. This suggests a possible myoblastic origin of these cervical granular cell tumors in mice. To address the issue of skeletal muscle origin, PTAH was used for detection of cross striations in the tumor cells compared with a skeletal muscle–positive

control. All 4 granular cell tumors were negative when stained with PTAH and did not show cross striations. These data show that the tumors are not striated and suggest a smooth muscle origin.

Our results were similar to previous studies. For example, in 2 avian studies and a canine study there was a positive reaction for the muscle marker desmin.^{39,40} In a report on cervical granular cells in mice, the Schwann-cell origin was questioned because of the absence of S-100 staining and lack of basal lamina. Another study demonstrated no reactivity to S-100 in the tumor cells of the meninges in Wistar rats.²⁷ In contrast to our findings, uterine cervical and vaginal granular cell tumors in 2 female aged (110-week-old) Wistar rats stained positively for S-100 and negatively for desmin.⁵ Our results were not completely consistent with the description that Cooper and Valentine reserved for the diagnosis of granular cell tumors in multiple species. They described neoplasms composed of cells containing abundant eosinophilic, PAS-positive and diastase-resistant granules. These neoplasms were immunohistochemically negative for muscle markers and positive for NSE or S-100.⁴ We verified the negative S-100 and NSE staining of the granular cell tumors in our study using positive control brain sections from each animal. This demonstrated the integrity of these stains despite prolonged formalin fixation of the tissues. Moreover, small amounts of nerve tissue peripheral to 2 of the tumors stained positive for S-100 (mice Nos. 1 and 4) and NSE (mice No. 4), acting as a positive internal control.

In our study, multinucleated giant cells were observed in 2 granular cell tumors and were numerous in 1 of the tumors. The mouse macrophage stain F4/80 was negative in all 4 tumors. Positive internal control macrophages were found in mice Nos. 1 and 4, whereas in mice Nos. 2 and 3 the entire section, consisting mainly of tumor tissue, was negative. Therefore, the multinucleated cells found rarely in mice No. 2 and frequently in mice No. 3 were negative either because they were not macrophage-derived, or because they failed to stain because of long-term exposure to formalin during archival storage.

Two of the tumors in our study were found in untreated control animals from 2 NTP chronic inhalation studies.^{32,33} Prior to these studies, granular cell tumors in mice were most often observed in the uterine cervix of animals treated with estrogen.¹⁷ The data indicate that uterine granular cell tumors are rare in untreated mice and can be produced by treatment with estrogens. The NTP chronic inhalation studies were the first reported studies in which spontaneously occurring granular cell tumors were reported. Subsequently, another study described 9 cases of granular cell tumors in treated B6C3F1 mice from 16 carcinogenicity studies, and the authors considered the tumors to have occurred spontaneously since there was no estrogenic effect.²⁷ However, it was noted these mice were not control animals. Spontaneous uterine granular cell tumors have been described in 1 female Fischer 344 rat and 2 female Wistar rats.^{5,34} It has been reported that up to 23% of control female rats in carcinogenicity studies have granular cell lesions in the distal reproductive tract.²³

In a previous study, the ultrastructure of the granules were described to have amorphous dense material, microvesicles, or lamellae, all representing disorganized material suggestive of digestion as observed in secondary lysosomes.⁶ Secondary lysosomes are sites of current or past digestion. In mouse No. 2 the cytoplasm was dense with variably sized cytoplasmic granules, each containing heterogeneous electron-dense material. These secondary lysosomes were a distinguishing characteristic of the granular cell tumors evaluated.

The diagnostic criteria of a schwannoma rely on the ultrastructural demonstration of an external basal lamina around neoplastic Schwann cells and their cell processes, as well as desmosomes.⁶ In schwannoma cells, the basal lamina is reported to be thickened and often folded into long redundant loops. The lack of a distinct basal lamina and absence of desmosomes, in addition

to immuno-histochemical findings, supports the contention that these mouse granular cell tumors are not of Schwann cell origin. We did not observe angulate bodies in our study, which have been described in human granular cell tumors.³ Another study found no basement membranes around the tumor cells of meningeal granular cell tumors of rats. The authors concluded that a Schwann cell origin was unlikely, and their findings were more consistent with a meningeal arachnoid cell origin.²⁶

Immunohistochemical and ultrastructural characterizations of a series of granular cell lesions from the distal reproductive tract of female rats did not result in new insights as to the pathogenesis of the lesion. However, additional data were provided in support of these lesions being similar to those of humans, and most likely of neural origin.²³ In contrast, the granular cell tumors from the reproductive tracts of mice in our study showed no immunohistochemical (reaction to S-100 or neuron-specific enolase) or ultrastructural characteristics to support a neural origin.

Ultrastructural findings for mice Nos. 2 and 3 also support a nonhistiocytic lineage for these tumors, despite multinucleated giant cells observed in mouse No. 3. This rules out histiocytic sarcoma as an alternative diagnosis for these neoplasms. Histiocytes, especially activated macrophages, might be expected to have numerous secondary lysosomes, but unlike the neoplastic cells described here, the cytoplasm would likely also contain increased numbers of mitochondria, pinocytotic vesicles, enlarged Golgi, and have an undulating membrane. None of these characteristics was seen in these cells.

No ultrastructural characteristics of smooth muscle cells such as spindle shape, bundles of cytoplasmic filaments, or membrane-associated dense bodies were seen. This challenges our suggestion of smooth muscle histogenesis from a strictly ultrastructural view. However, the histochemical and immunohistochemical findings were strongly supportive of smooth muscle origin, and therefore immunohistochemistry may be a better tool for exploring the histogenesis of these tumors.

In conclusion, our study is in agreement with previous findings confirming that granular cell tumors have a large degree of variation despite their homogeneous morphologic appearance and provides additional data to support a possible myogenic origin for these tumors.

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Fig. 1.
Uterus; B6C3F1 mouse No. 1. Granular cell tumor at uterine cervix with hyperchromatic nuclei. HE. Bar = 75 μ m.

Fig. 2.
Uterus; B6C3F1 mouse No. 3. Atypical granular cell tumor with multinucleated giant cells filled with granules. HE. Bar = 75 μ m.

Fig. 3. Uterus; B6C3F1 mouse No. 1. Granular cell tumor at uterine cervix with PAS positive granules in cytoplasm of tumor cells. PAS-D. Bar = 75 μ m.

Fig. 4.
Uterus; B6C3F1 mouse No. 3. Atypical granular cell tumor. PAS-D. Bar = 75 μ m.

Fig. 5. Uterus; B6C3F1 mouse No. 1. Granular cell tumor at uterine cervix. Alpha smooth muscle actin expressed diffusely in tumor cells. Note positive staining bundles of smooth muscle cells (arrows) adjacent to tumor cells. DAB chromogen, hematoxylin counterstained. Bar = 75 μ m.

Fig. 6. Uterus; B6C3F1 mouse No. 1. Granular cell tumor at uterine cervix. Desmin expressed in peripheral tumor cells. Note positive staining of smooth muscle cells (arrows). Also note, fibroblasts (FB) and polymorphonuclear cells (arrowhead) admixed in a collagenous stroma adjacent to tumor cells. DAB chromogen, hematoxylin counterstained. Bar = 75 μ m.

Fig. 7. Uterus; B6C3F1 mouse No. 3. Atypical granular cell tumor. Desmin expressed weakly. DAB chromogen, hematoxylin counterstained. Bar = 75 μ m.

Fig. 8. Uterus; B6C3F1 mouse No. 2. Ultrastructural view of a granular cell tumor cell with eccentrically placed nucleus. Arrows = individual cytoplasmic granules. *Inset:* Secondary lysosomes (arrowhead) within individual cytoplasmic granule. Bar = 2 μ m.

Table 1

Anatomical sites of granular cell tumors among different species.

Species	Site	Reference
Dog	Cerebral cortex	Parker et al. ³⁶
	Meninges	Sharkey et al. ⁴⁷
		Higgins et al. ¹⁴
	Intracranial	Liu et al. ²¹
	Pituitary gland	Barnhart et al. ²
	Spine	Montoliu et al. ²⁸
	Lip, gingiva, subcutaneous tissue	Patnaik ³⁸
	Tongue	Rallis et al. ⁴⁰
		Van de Gaag and Walvoort ⁵⁵
	Thyroid gland	Wyand and Wolke ⁵⁷
	Intrathoracic	Foley ¹²
	Heart	Sanford et al. ⁴⁴
Cat	Ventral tongue, digit	Patnaik ³⁸
	Tonsil	Wilson et al. ⁵⁶
	Vulva	Robertson et al. ⁴²
Horse	Lung	Turk and Breeze ⁵³
		Parker et al. ³⁷
Cockatiel	Periocular	Patnaik ³⁸
Parrot	Subcutaneous tissue	Quist et al. ³⁹
Rodents	Female reproductive tract	Courtney et al. ⁵
	(uterine, cervix, and vagina)	Yoshida et al. ⁵⁸
	Meninges	Markovits and Sahota ²³
	Subcutaneous tissue	Mitsumori et al. ²⁶
		Miyajima et al. ²⁷
		Nyska et al. ³⁴
		Morton and Tekeli ²⁹

Table 2
Immunohistochemistry reagents and procedures for evaluation of granular cell tumors in female mice.*

Antibody	Species	Specific Reactivity	Source	Dilution	Pretreatment	Incubation [†]
Desmin	Rabbit	Smooth, striated muscle cells	Accurate	1 : 500	Microwave in citrate buffer, 5 minutes	1 hour
SMA	Mouse	Smooth muscle cells	Dako	1 : 100	Decloaker in 200 ml EDTA, 5 minutes	32 minutes
S-100	Rabbit	Schwannomas, benign, malignant melanomas	Neomarkers	1 : 150	None	30 minutes
Cytokeratin	Rabbit	Epithelial cells	Dako	1 : 200	Decloaker in citrate buffer, 5 minutes	30 minutes
NSE	Mouse	Neural, neuroendocrine tissue	Lab Vision	1 : 800	Decloaker in citrate buffer, 5 minutes	1 hour
F4/80	Rat	Macrophages	Caltag	1 : 25	0.02% trypsin digestion	1 hour

* SMA = alpha smooth muscle actin; EDTA = ethylene diamine tetraacetic acid; NSE = neuron-specific enolase.

[†] of primary antibody, at room temperature.

Table 3
Histochemical and immunohistochemical results of granular cell tumors of the uterus in B6C3F1 mice.*

Case Nos.	Chemical Study	Dose mg/kg	Histochemistry				Antibodies				
			PAS(D)	PTAH	Trichrome	T-blue	Desmin	SMA	S-100	CK	NSE F4
1	Mercuric chloride	10	+	0	Pale pink	0	+	+	0	0	0
2	Glutaraldehyde	0†	+	0	Pale pink	0	0	0	0	0	0
3	Tert-butyl alcohol	2.0%	+	0	Pale pink	0	+/-	0	0	0	0
4	Tetrafluoroethylene	0†	+	0	Pale pink	0	+/-	0	0	0	0

* + = positive reaction; +/- = weak positive; 0 = no reaction; SMA = smooth muscle actin; NSE = neuron-specific enolase; (D) = diastase resistance; CK = cytokeratin.
† control (untreated).