NATURAL DISEASE

Spontaneous Equine Pulmonary Granular Cell Tumors: Morphologic, Histochemical, and Immunohistochemical Characterization

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Abstract. Spontaneous equine pulmonary granular cell tumors were diagnosed in six mature horses at slaughter. These tumors were grossly recognized as multiple (5/6) or single (1/6) creamy white, firm nodules. The tumors, located adjacent to bronchi and bronchioles, often invaded airways, resulting in partial to complete occlusion of the lumina. Neoplastic cells were rounded to polyhedral with numerous eosinophilic cytoplasmic granules that reacted uniformly positive with S-100 and neuron-specific enolase antibodies and multifocally with glial fibrillary acidic protein antibodies. These cells were negative for muscle-specific actin, lysozyme, cytokeratin, chromogranin A, and myelin basic protein antigens and did not stain with silver by the Grimelius technique. Uniformly blue-green and scattered pink intracytoplasmic granules were evident with luxol fast blue and periodic acid-Schiff (PAS) counterstain for myelin and myelin breakdown products. Histochemical and immunohistochemical staining results of these tumors suggest that they are composed primarily of myelinating Schwann cells with lesser numbers of scattered nonmyelinating Schwann cells. The morphologic features of the equine pulmonary granular cell tumors are strikingly similar to those of endobronchial granular cell tumors of human beings.

Key words: Granular cell tumor; horses; immunohistochemistry; lung.

Equine granular cell tumors are rare primary lung tumors, and reports in the literature have usually been limited to single case studies. Granular cell tumors in human beings have been described in the tongue, skin, biliary system, central nervous system, gastrointestinal system, respiratory system, and reproductive tracts. Granular cell tumors also have been reported in the tongue, skin, heart, brain, and thorax of dogs, and uterus of aging rats, in the tongue, vulva, and digits of cats, and in the periocular tissue of a cockatiel.

The objectives of this study were to describe pulmonary granular cell tumors in six horses, to characterize the histochemistry, intermediate filament, and cytoplasmic enzymes in the cells of these tumors, and to compare the tumors with those of human beings and other animals.

Materials and Methods

Tissues were submitted to the US Department of Agriculture Food Safety and Inspection Service Eastern Laboratory because of gross lesions detected during routine slaughter and inspection procedures. The six cases of equine granular cell tumors originated from 530 equine tissues submitted from October 1988 through April 1992. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), luxol fast blue counterstained with cresyl echt violet, luxol fast blue counterstained with PAS-hematoxylin, and the Grimelius technique for examination by light microscopy.

Serial histologic sections of formalin-fixed tissues from the tumors were used for immunohistochemical staining with streptavidin-biotin-alkaline phosphatase (BioGenex Supersensitive Kit, San Ramon, CA). The tumors were fixed in formalin for approximately 2–5 days before being embedded in paraffin. The sections were incubated separately with polyclonal antibodies to neuron-specific enolase (NSE), S-100 protein, lysozyme, and myelin basic protein and with monoclonal antibodies to vimentin clone V9, glial fibrillary acidic protein clone GA-5 (GFAP), cytokeratin clone Lu-5 (CKL5), muscle-specific actin clone 1A4 (SMA), chromogranin A clone (A-11), and myelin basic protein (BioGenex primary antibodies for use with the Supersensitive Kits). Peripheral nerve, brain, skeletal muscle, lung, lymph node, and spleen from a normal horse were used as positive controls. Positive control tissues were incubated for 3 days in formalin before being embedded in paraffin. Vimentin was used as an internal positive control for tissue processing. Control incubations using nonimmune sera as the first antibody resulted in the absence of filament or enzyme-specific staining in the tumors and in control tissues.
Fig. 1. Lung; horse No. 5. Granular cell tumor centered around bronchus, displacing epithelium luminally with partial occlusion of the airways and disruption of peribronchial cartilage. HE. Bar = 200 μm.

Results

No abnormalities were found on any of the six horses at antemortem examination. Gross lesions from horse No. 1 were described as multiple firm, white 1-cm-diameter masses occurring within a 6 × 6-cm focus of the lung. Mucopurulent exudate was present in obstructed bronchioles. The tumor in horse No. 2 was a single 4-cm-diameter cream-colored firm mass in the left lung lobe. Gross lesions in horse No. 3 were multiple coalescing masses within one circumscribed 8 × 8-cm focus of lung parenchyma. In horse No. 4, the entire left lung lobe was infiltrated by nodules from 1 cm to 5 cm in diameter. Horse No. 5 was described as having several growths and cystic lesions 2–4 cm in diameter in the left lung lobe. In horse No. 6, numerous nodules 1–8 cm in diameter were scattered throughout both lung lobes. Regional lymph nodes were described as normal in all horses. No other gross lesions were detected at postmortem examination in any of the horses. The pulmonary masses from all six horses were similar histologically. The circumscribed, expansile masses often compressed the surrounding pulmonary parenchyma and were usually centered around bronchi and bronchioles. Tumor cells replaced normal peribronchial structures and displaced bronchial epithelium luminally, often completely occluding the airways (Fig. 1). Disruption of peribronchial cartilage into disorganized chondroid islands was seen in horse No. 6. Neoplastic cells were large and rounded or polyhedral, with poorly defined cytoplasmic margins (Fig. 2). Nuclei were ovoid or round and sometimes eccentrically placed, with stippled, usually margined chromatin and a central nucleolus. Mitotic figures were not observed. The cytoplasm of neoplastic cells was usually filled with coarse eosinophilic granules. The cells were grouped into either nests or rows and separated by sparse stroma composed of slender hyalinized collagen fibers or were arranged in large uninterrupted sheets.
with little stroma. The results of histochemical and immunohistochemical staining of tumor granular cells are summarized in Tables 1 and 2. Cytoplasmic granules in the equine granular cell tumors were variably PAS positive, lacked silver staining with the Grimelius technique, and were uniformly blue-green with luxol fast blue with cresyl echt violet counterstain (Fig. 3). When luxol fast blue was counterstained with PAS-hematoxylin, various ratios of granules stained blue-green or pink. All granular cells reacted strongly and uniformly to S-100 (Fig. 4) and NSE (Fig. 5). None of the granular cells were positive for CKL5, SMA, lysozyme, A-11, or myelin basic protein. Rare scattered clusters of five or six granular cells reacted with antibodies to GFAP (Fig. 6).

Single small cells, interpreted to be normal neuroendocrine cells, located at the base of bronchial mucosal epithelial cells and in pulmonary interstitial tissue exhibited cytoplasmic NSE, S-100, A-11, and silver staining with the Grimelius technique. All control tissues stained as expected.

### Discussion

All granular cell tumors reported in horses have been limited to the lungs, as were all the tumors in this study. As in previous reports, five of the six horses in this study had multiple tumors. The propensity for the right lung lobe suggested by other authors was not evident in our study. In the four horses in which lung field was stated, three had lesions involving the left lobe and the other had bilaterally distributed lesions. All horses were mature, as were horses in earlier case reports. Involvement of the bronchi with partial or complete occlusion in all six horses of this study is similar to results in previous reports.

Earlier descriptions of pulmonary nodules bordered on several sides by normal to flattened bronchial or bronchiolar epithelium were also characteristic of the tumors in this study. Tumor cells were similar in appearance to those previously described, but angulate bodies described in one equine case and in some human tumors were not evident in our study. All tumors in this study were histologically benign, which is consistent with previous reports of equine granular cell tumors.

Equine granular cell tumors are remarkably similar to the rare form of endobronchial granular cell tumor of human beings. These tumors occur in the lower trachea and bronchi, are usually multiple, and are often associated with airway obstruction. Human endobronchial granular cell tumors also infiltrate the submucosa of the bronchus, elevating the intact mucosa, and occasionally displace the bronchial cartilage plates as did the equine tumors. Cells of equine and human tumors are histologically similar.

The granular cell tumor is a tumor of uncertain histogenesis, but it has been considered most often to have either a striated muscle or a Schwann cell origin. The cytoplasmic granules of all tumors in this study stained positively for S-100, as had been described previously in equine, canine, and rat tumors. The additional detection of NSE in all equine granular cell tumors in this study and in one previous study also suggests a potential neural origin for these tumors. Two possible origins of these peri- and intrabronchiolar tumors would include Schwann cells, as previously suggested, and neuroendocrine cells.

In contrast to previous reports of equine granular cell tumors, intracytoplasmic granules were not uniformly PAS positive. Only 10–30% of the cells contained PAS-positive granules in most cases, with a majority of the granules staining positive in only one case. However, granules in most cells of all six granular cell tumors stained brightly blue-green with luxol fast blue–cresyl echt violet, which demonstrates the presence of choline-based phospholipids and suggests the presence of a myelinlike product. This result contrasts with the only previously published report of an equine gran-
Fig. 4. Pulmonary granular cell tumor; horse No. 3. Granular cells react positively to S-100 antibody. Streptavidin–biotin–alkaline phosphatase complex method, Mayer’s hematoxylin counterstain. Bar = 20 μm.

Fig. 5. Pulmonary granular cell tumor; horse No. 3. Neoplastic cells stain positively for neuron-specific enolase. Streptavidin–biotin–alkaline phosphatase complex method, Mayer’s hematoxylin counterstain. Bar = 20 μm.

Fig. 6. Pulmonary granular cell tumor; horse No. 1. Rare scattered clusters of granular cells stain positively for cytoplasmic glial fibrillary acidic protein. Streptavidin–biotin–alkaline phosphatase complex method, Mayer’s hematoxylin counterstain. Bar = 20 μm.

Pulmonary granular cell tumor stained with luxol fast blue–PAS in which the granules stained pink. When we repeated the luxol fast blue stain and counterstained with PAS rather than with cresyl echt violet, the PAS-positive granules did tend to mask the greenish blue staining in some cells, but the green-blue staining was quite evident in many other cells. Luxol fast blue with PAS counterstain is considered an excellent stain for detecting both myelin (bluish green) and myelin breakdown products (pink). Sphingomyelin is PAS positive if the amide linkage is split leaving the 1-amide-2 hydroxyl group of sphingosine available for reaction with periodic acid to form aldehyde. The results of these stains seem to indicate that the granules of equine granular cell tumors are composed of sphingomyelin-like material and its breakdown products. Several studies of neural origin granular cell tumors in human beings also have documented that the cytoplasmic granules contain in part myelin, autophagecytized myelin, or myelin basic protein. These findings suggest that the cells of equine granular cell tumors are phenotypically Schwann cells. Granular cell variants of Schwannomas have been reported in human beings and rats. The lack of reactivity of equine granular cell tumors with antimyelin basic protein antibodies parallels the lack of detectable amounts of myelin basic protein in Schwann cell tumors and oligodendrogliomas of human beings.

The multifocal reactivity to GFAP in five of the six equine tumors most likely represents clusters of less differentiated nonmyelinating Schwann cells. Cytoplasmic accumulation of GFAP-immunoreactive intermediate filaments have been documented in human Schwann cells when removed from axonal contact, resulting in loss of myelinating function. Dual production of myelin and GFAP in the equine granular cell tumors therefore appears to result from assumption of a less differentiated phenotype of neoplastic Schwann cells and expression of typically nontranscribed DNA. Nine of 17 granular cell brain tumors in Wistar rats also contained GFAP-positive cells, which the authors interpreted as suggesting potential astrocytic origin in these centrally occurring tumors. Astrocytes exhibit immunohistochemical reactivity characteristics similar to those of Schwann cells but would be a less likely cell of origin for the peripherally occurring equine pulmonary granular cell tumors.

Negative immunoreactivity with anticytokeratin and antichromogranin antibodies as well as lack of silver staining by the Grimelius technique tends to rule out the possibility of any neuroendocrine origin or differentiation. The lack of actin immunoreactivity suggests that equine pulmonary granular cell tumors are not derived from myogenic cells in contrast to those tumors reported from cats and a cockatiel.
Electron microscopic examination of equine pulmonary granular cell tumors has been described in two cases. The presence of membranous whorls (or myelin figures) described in one case is in agreement with the positive staining with luxol fast blue in the present tumors. The lack of muscle filaments detected in both cases correlates well with the absence of MSA immunoreactivity in the current study.

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