Pleomorphic Rhabdomyosarcoma in a Cow

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ABSTRACT. In a 5-year-old Holstein cow, a neoplasm composed of a large intramuscular mass and multiple metastases in the lungs and lymph nodes was diagnosed as a pleomorphic rhabdomyosarcoma. This neoplasm was characterized by marked variation in tumor cell size and giant cells with single bizarre nuclei. Although the presence of cross striations and myoglobin could be confirmed, expression of alpha-smooth muscle actin (SMA) was also recognized in a few cells. Neoplastic cells showing intense staining for desmin, vimentin and proliferating cell nuclear antigen irrespective of their size differed from those in an embryonal rhabdomyosarcoma that exhibited a wide spectrum of differentiation, reminiscent of normal skeletal myogenesis. The cellular pleomorphism and SMA expression seemed to be characteristic of deviation from normal muscle cells or satellite cells in adult muscle.

KEY WORDS: bovine, giant cell, pleomorphic rhabdomyosarcoma.

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In human beings, pleomorphic rhabdomyosarcoma is considered to be exceedingly rare or nonexistent [14]. Although canine rhabdomyosarcomas are divided into three subtypes, most of the recorded cases with indubitable evidence of skeletal muscle differentiation were embryonal or alveolar [5, 11]. These two forms have also been reported in cattle [4, 8]. Here we describe a pleomorphic rhabdomyosarcoma in a cow that was clearly distinguished from an embryonal rhabdomyosarcoma by immunohistochemistry.

A 5-year-old Holstein cow had a 10 cm diameter tumor mass on the left side of the brisket 6 weeks after her third delivery. Two weeks later, the tumor became enlarged to 20 cm in diameter, and the animal showed loss of appetite. Because of the large size of the tumor and severe emaciation, the animal was euthanized at 81 days *post partum*.

Macroscopically, the tumor mass, which measured 30 cm in diameter, was situated beneath the cutaneous muscle and extended deeply into the underlying skeletal muscles. Although the fascia overlying the tumor was detectable, the tumor was very infiltrative into the surrounding musculature in the deeper parts. The cut surface was pale yellowish white to pale pink in color and had a fatty appearance. It was somewhat friable. The trachea and esophagus appeared intact. The retropharyngeal, cervical and axillary lymph nodes were greatly enlarged. In the lungs, there were multiple spherical masses, up to 6 cm in diameter, which were well demarcated. The bronchial and mediastinal lymph nodes were enlarged, and the internal iliac lymph nodes were also large.

Tissue specimens were fixed in 10% buffered formalin, processed and embedded routinely in paraffin, and sectioned at 4 μ m. Sections were stained with hematoxylin and eosin (HE), trichrome and phosphotungstic acid-hematoxylin (PTAH) stains. Cryostat sections of fixed tissue were stained with Sudan III for fat. The avidin-biotin-peroxidase

complex (ABC) method was applied to selected paraffin sections. The following were used as primary antibodies: rabbit polyclonal antibodies to myoglobin and S100 protein (prediluted, Nichirei Corporation, Tokyo, Japan), and mouse monoclonal antibodies to desmin (1:10, Bio-Science Products, Emmenbrücke, Switzerland), alpha-smooth muscle actin (1:25, SMA, Dako A/S, Glostrup, Denmark), vimentin (prediluted, Dako Corporation, Carpinteria, CA, U.S.A.) and proliferating cell nuclear antigen (prediluted, PCNA, BioGenex Laboratories, San Ramon, CA, U.S.A.). For a detection of PCNA, a pretreatment with microwave oven heating was performed. An ABC kit (BioGenex) was used in the subsequent processes. Small pieces taken from formalin-fixed tissue were post-fixed in 1% osmium tetroxide and prepared for transmission electron microscopy (TEM).

Histologically, there were widespread areas of necrosis in the large tumor mass, and the tumor tissue, composed of sheets of neoplastic cells, invaded surrounding muscular and adipose tissues. The enlarged lymph nodes were almost entirely displaced by neoplastic tissue. In the lungs, most of the neoplastic lesions were partially encapsulated by fibrous tissue.

The tumor cells were round, oval or polygonal in shape. Their size varied greatly, and mononuclear giant cells were seen at times (Fig. 1). The nuclei were round or ovoid and vesicular with nucleoli of various sizes, but larger cells tended to show extremely irregular nuclear contours (Fig. 2). The neoplastic cells had small to moderate amounts of cytoplasm, where fat-positive vacuoles were detected occasionally. Despite the presence of several cells with eosinophilic myofibrils in places (Fig. 3), cells exhibiting cross striations were exceedingly rare. Mitotic figures were numerous.

In immunohistochemistry for all antigens examined, the staining intensity or the frequency of positive cells was unre-

Fig. 1. Lung. Neoplastic cells vary enormously in size. The nucleus of a giant cell looks slightly irregular, but nuclear pockets are detectable (arrows). HE. $\times 400$.

Fig. 2. Intramuscular tumor. Bizarre, mononuclear giant cells predominate in this field. HE. $\times 400$.

Fig. 3. Intramuscular tumor. This area consists of small neoplastic cells, sometimes with eosinophilic myofibrils (arrows). HE. $\times 400$.

Fig. 4. Intramuscular tumor. Various sized neoplastic cells display positive or negative reactivity for desmin. ABC. $\times 400$.

Fig. 5. Intramuscular tumor. Irrespective of the size, neoplastic cells are largely positive for vimentin. ABC. $\times 400$.

Fig. 6. Intramuscular tumor. Positive nuclear staining for PCNA is observed in many tumor cells including giant forms (arrows). ABC. $\times 200$.

Fig. 7. Intramuscular tumor. Arrows indicate SMA-positive neoplastic cells. More intensely stained are vascular smooth muscle cells (arrowheads). ABC. $\times 400$.

lated to the cell size (Fig. 4). Many neoplastic cells were positive for desmin in some areas, while few cells were in other areas. Most neoplastic cells revealed positive reactivity for vimentin (Fig. 5), and many were strongly positive for

PCNA (Fig. 6). Myoglobin- or S100-positive cells were sometimes observed, and a few SMA-positive cells were found (Fig. 7).

Ultrastructurally, the organelles were slightly to poorly

Fig. 8. Intramuscular tumor. There are osminophilic Z lines, some of which are organized (arrows) and probably correspond with light microscopical cross striations. Scattered glycogen particles and a lipid droplet (arrowhead) are also visible. TEM. $\times 18,000$.

developed, and there were glycogen particles or lipid droplets in the cytoplasm of some tumor cells. Cytoplasmic filaments were inconspicuous, but formed aggregates in a minority of cells. The presence of both thick and thin filaments was confirmed. Almost all Z lines were haphazardly distributed, but only a few cells contained aligned sarcometric structures (Fig. 8), which would have appeared as cross striations by light microscopy.

Rhabdomyosarcoma is a malignant tumor of the skeletal muscle, and is characterized by the presence of cross striations and positive immunoreactivity for myoglobin and desmin [10]. Since these features could be discerned, our case was categorized as a rhabdomyosarcoma. In contrast to embryonal rhabdomyosarcoma originating from undifferentiated mesenchyme of young children [16] and a young cow [4], human pleomorphic rhabdomyosarcoma is a tumor of adults that typically occurs within large voluntary muscles [2] and is composed almost exclusively of large pleomorphic rhabdomyoblasts [16]. The present tumor arising from the pectoral musculature of a 5-year-old cow showed severe pleomorphism and nuclear atypia, and was diagnosed as a pleomorphic rhabdomyosarcoma.

Vimentin is known to be present in the early stages of normal skeletal muscle development and, later in the myogenesis, vimentin is partially or completely replaced by desmin [13]. Synthesis of myoglobin occurs still later than that of desmin, and desmin tends, therefore, to be more sensitive in detecting immature myoblasts [1]. Human pleomorphic

rhabdomyosarcoma was believed to be one of the better-differentiated types of rhabdomyosarcoma, based on the positive reaction to anti-desmin and anti-myoglobin in many neoplastic cells. Similarly, a rhabdomyosarcoma in the laryngeal wall of an 11-year-old dog is interpretable as a highly differentiated type, because most tumor cells stained positively for desmin and myoglobin and several cells had cross striations [7]. Moreover, vimentin was observed in some giant cells alone, and mitotic figures were few [7]. The present neoplasm, in which a fair number of tumor cells were strongly positive for desmin, may be considerably differentiated, but intense positivity for vimentin was seen in most tumor cells.

In an immunohistochemical study of human striated muscle tumors, an anti-vimentin antibody did not produce any positive staining of normal cardiac and skeletal striated muscle or an adult rhabdomyoma [13]. In a bovine embryonal rhabdomyosarcoma, multinucleated giant cells, displaying intense immunoreactivity for desmin and weak or negative reactivity for vimentin, were thought to be terminally differentiated cells, and were weakly positive or negative for PCNA [4]. On the other hand, small immature cells, intensely positive for PCNA and vimentin and weakly positive for desmin, were regarded as imitating replicable myoblasts [4]. In the present case, the immunohistochemical staining pattern in giant tumor cells was similar to that in smaller ones, and these tumor cells apparently differed from those in the embryonal type [4]. Thus, pleomorphic rhab-

domyosarcoma, unlike embryonal rhabdomyosarcoma, is not a mimic or caricature of developing fetal skeletal muscle, as has been suggested by Gaffney *et al.* [2]. The cellular pleomorphism in the pleomorphic type seemed to be due to malignant neoplastic transformation and not to be relevant to the stage of differentiation in myogenesis. Similarly, the presence of SMA in a few tumor cells may represent an immunohistochemical aberration associated with neoplastic change [15]. On the basis of transient expression of alpha-cardiac actin (CA) and SMA in embryonic skeletal muscle, human rhabdomyosarcomas of all histological types, which expressed CA and SMA messenger RNAs, were considered to follow normal skeletal myogenesis [12], but strong or moderate immunostaining of neoplastic cells for SMA was observed only in the pleomorphic type [12, 15].

In the present case, a pleomorphic liposarcoma [9] was considered in the differential diagnosis, because occasional tumor cells had lipid droplets or showed S100 positivity. A few lipid droplets may be present in normal myofibers [3], and the excessive deposition of lipid presumably occurred during neoplastic transformation. Lipid-rich cells simulating lipoblasts [6, 14] or S100-positive cells [10] have been observed in human rhabdomyosarcomas, although the significance of S100 positivity is unknown.

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